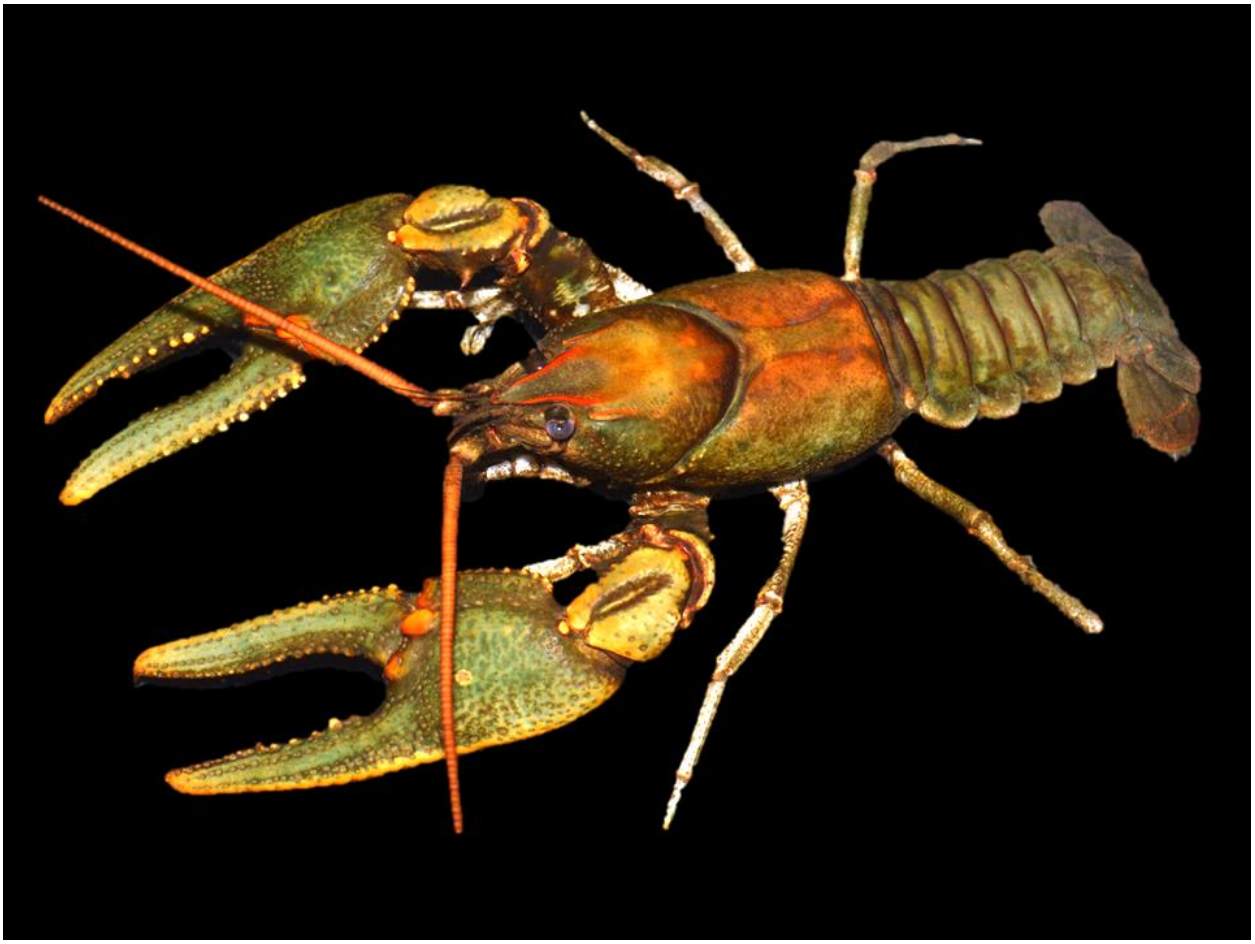
Cambarus callainus Range Wide Conservation Status Survey



Report prepared for the

United States Fish and Wildlife Service

Regions 4 and 5:

Fall 2015

Report prepared by:

Zachary J. Loughman, Ph.D.
Associate Professor of Zoology/Ecology
Department of Natural Sciences and Mathematics
West Liberty University,
West Liberty WV, 26074

INTRODUCTION

During June, July and September of 2015, surveys were performed across the Upper Big Sandy River basin's Lower Levisa, Tug Fork, Upper Levisa Fork's Russell Fork (ULF-Russell Fork) and Upper Levisa Fork's Levisa Fork (ULF-Levisa Fork) watersheds for *Cambarus callainus* (Big Sandy River Crayfish) to determine the current distribution and conservation standing of the species. The following is a description of methods, results, and a discussion of survey findings.

MATERIAL AND METHODS

Site selection

Site selection was based on historic sites of occurrence. Prior to field work, all historic *C. callainus* locations were plotted in a Geographic Information System (GIS) to create a historic occurrence map. Because all previous surveys indicate *C. callainus* is endemic to wadeable streams in the Big Sandy River basin's Lower Levisa Fork, Tug fork, ULF-Russell Fork, and ULF-Levisa Fork watersheds in eastern Kentucky, southwestern Virginia, and Southwestern West Virginia (Taylor and Schuster 2004; Thoma 2009; Thoma 2010; Thoma et al. 2014; Loughman 2013; Loughman and Welsh 2013; Loughman 2014), the USGS National Hydrography Stream layer was used to focus survey efforts. Streams depicted in this layer are large, wadeable streams, and are the most likely to maintain habitat with high velocity riffles and large slab boulders conducive to *C. callainus* presence (Jezerinac et al. 1995, Loughman 2014, Thoma et al. 2014). Potential sample reaches on these streams were selected to ensure sites were spaced relatively equidistant to each other and within *C. callainus'* preferred habitat. Using this approach, 153 sites were identified for sampling across the aforementioned watersheds (Fig. 1). In the field, all historic and newly identified sample sites were surveyed as close to the digitally-identified locations as site access allowed.

Crayfish collection and site covariates

A sample site consisted of a 125-m stream reach in riffle, run, or both riffle and run habitats. At each site, crayfish were sampled using seine nets (2.4 x 1.3-m seine with 3.2-mm mesh) hauled at 10 locations within the 125m stream reach. Because *C. callainus* associates with slab boulders (Taylor and Schuster 2004; Thoma 2009;

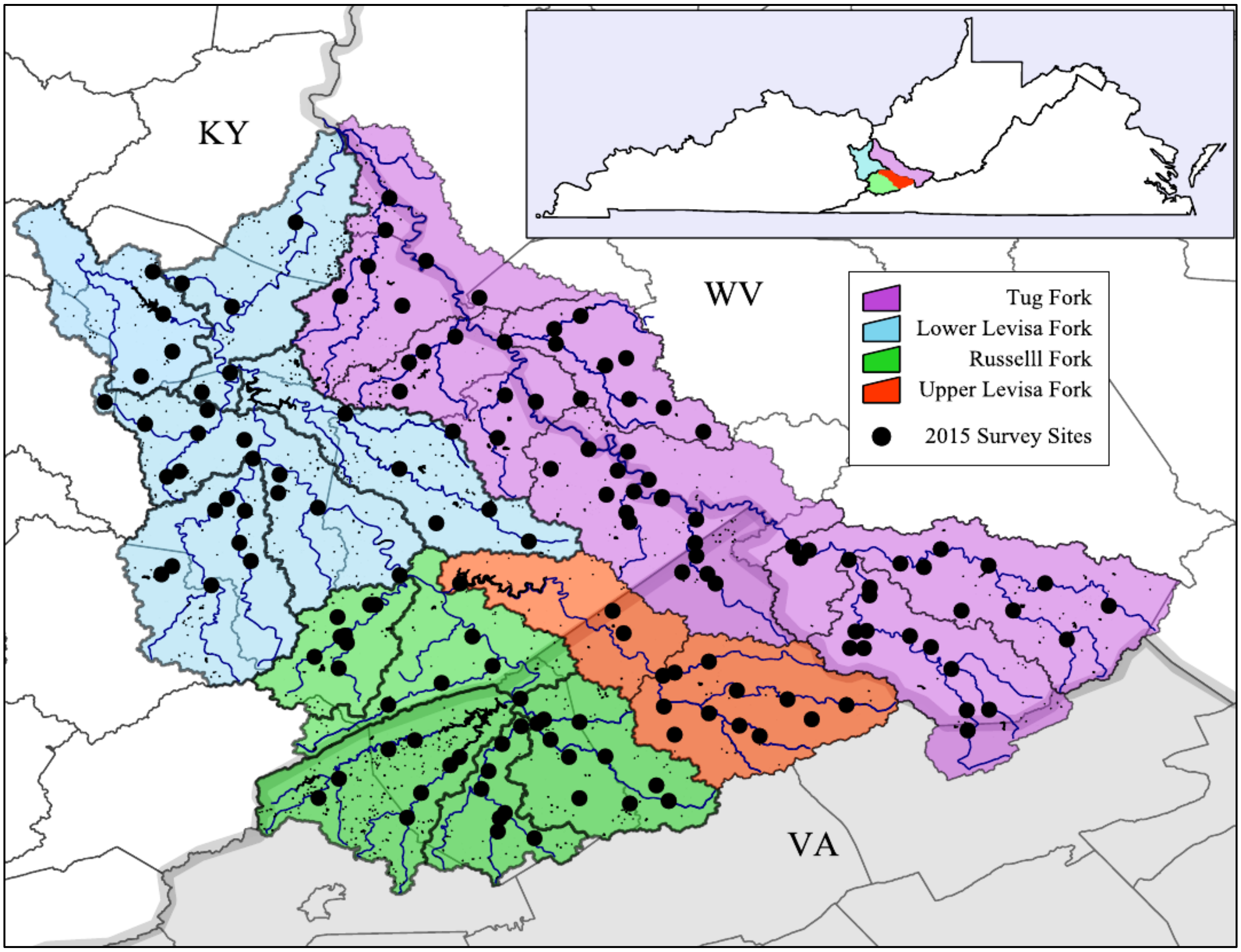


Figure 1: *Cambarus callainus* 2015 survey sites

Thoma 2010; Thoma et al. 2014; Loughman 2013; Loughman and Welsh 2013; Loughman 2014), if slab boulders were present within the stream reach they were given sampling priority. That is, if 10 slab boulders were present in a 125 m reach, all 10 slab boulders would be sampled. If the stream reach did not contain sufficient slab boulders, the following other bottom features were given sampling priority: boulders, large cobble, course woody debris and artificial cover respectively.

When collected, each individual *C. callainus* specimen was measured (total carapace length, TCL mm) and sexed. Third walking legs on the right side of adults were preserved for future genetic analyses. Each animal's life stage was recorded (young of the year; juvenile; form I male; form II male; non-reproductive female; glared female; ovigerous female). If collected in sufficient numbers, one or two adult males were vouchered, and all other individuals released at their point of capture. All crayfish of other species collected were combined into a single sample and preserved in 80% ethanol in the field (if any *C. callainus* voucher specimens were collected, they were also included). Specimens were subsequently identified to species in the laboratory, and assessed into the West Liberty University Crayfish Collection.

Water quality and physical habitat quality data were also collected at each site for use in ecological modeling. At each sampling site a Yellow Springs Instrument (YSI) 6920V2 data sonde was used to measure pH, temperature, percent dissolved oxygen, turbidity, and conductivity. Water samples (250 ml) were also collected for sulfate (SO_4) determination in the laboratory. These samples were collected from 10 cm under the stream surface, mid-channel, at each sampling site. Sulfate concentrations were determined using a turbidity method on a Hach DR 2800 (wavelength 450 nm) spectrophotometer. The maximum detection limit was 70 ppm (mg/L). Sample concentrations that exceeded the maximum detection limit were diluted to the appropriate concentration that was within the method detection limits. Each sample was subjected to four replicate trials to determine an average and standard deviation. All sulfate concentrations are reported at the 95% confidence limit.

In addition to water quality, physical habitat quality was evaluated through use of the Qualitative Habitat Evaluation Index (QHEI; OEPA 2006). The QHEI is a commonly accepted method of stream physical habitat assessment (Rankin, 1995; Burskey & Simon, 2010; Gazendam et al., 2011), and provided inference—specifically for crayfish in central Appalachia in previous investigations (Thoma 2009; Thoma 2010; Welsh and Loughman 2014; Loughman unpublished data). QHEI overall score and sub-scores, specifically substrate type and quality, in-stream cover type and amount, channel morphology (sinuosity, development, channelization, and stability), riparian zone (width, quality, and bank erosion), pool quality (maximum depth, current, and morphology), riffle quality (depth, substrate stability, and substrate embeddedness), and stream gradient were used as physical habitat covariates for modeling.

Overall physical habitat quality was assessed by summing sub-scores obtained from seven categories, thus resulting in a QHEI score, where the maximum possible QHEI score was 100. The QHEI scores were categorized separately for headwater and larger streams following OEPA (2006). Categories for streams sampled based on total QHEI score were: excellent (≥75), good (60-74), fair (45-59), poor (30–44), and very poor (<30). Site elevation (m) was also recorded to help determine if elevation plays a role in *C. callainus* presence (or absence).

Logistic and Linear Regression Modeling - Logistic regression and linear regression models were used to analyze presence/absence and catch per unit effort (CPUE) data of *C. callainus*, respectively, by fitting 40 candidate models with single covariates or additive-effects of stream habitat, water quality, and presence/absence (or CPUE) of other crayfish species. Models for the logistic regression analyses were fit with a binomial distribution and a logit link function; whereas those for the linear regression analyses were fit with a normal distribution and an identity link function (SAS 9.4, PROC GENMOD). Before analysis, water quality and physical habitat covariates were examined for near collinearity using Pearson Correlation coefficients.

For both analyses, the candidate model set included single covariate models of physical habitat metrics (instream cover, riffle/run, channel morphology, pool/current, substrate, riparian zone, stream gradient, and QHEI), water quality (conductivity, SO_4, water temperature), and elevation. The logistic regression analysis also included models with covariates of presence/absence data of *C. hatfieldi*, *C. theepiensis*, and O. *cristavarius*; whereas the linear regression analysis included models with covariates of CPUE for those three species. Also, for both analyses, we fit four additive-effects models with two covariates (conductivity + QHEI, elevation + QHEI, SO_4 + QHEI, and QHEI + water temperature). The 19 previously described models were also repeated with an additive basin effect, where four basins were represented: lower Levisa Fork basin, Levisa Fork and Russell Fork basins of the upper Levisa Fork drainage, and Tug Fork River basin. We fit an intercept model for both analyses. For the logistic regression analysis, we also fit a global model (additive effects) of basin, QHEI, conductivity, SO_4, water temperature, elevation, *C. hatfieldi* presence/absence, *C. theepiensis* presence/absence, and *O. cristavarius* presence/absence. For the regression analysis, we fit a global model (additive effects) of basin, QHEI, conductivity, SO_4, water temperature, elevation, *C. hatfieldi* CPUE, *C. theepiensis* CPUE, and O. *cristavarius* CPUE.

For the logistic regression analysis (presence/absence data) and linear regression analysis (CPUE data), we used an information-theoretic approach for model selection and inference, where each model represented an alternative hypothesis. Selection of the best approximating model (or set of competing models) was based on the small sample size correction of the Akaike Information Criterion (AIC_c; Burnham & Anderson, 2002). We reported AIC_c, the AIC_c distance between each model and the best approximating model (ΔAIC_c), and AIC_c model weights (wt; Burnham & Anderson, 2002). For the logistic regression analysis, global model fit was tested with the Hosmer-Lemeshow Goodness-of-Fit statistic. Receiver operating characteristic (ROC) curves were used to graphically depict predictive accuracy of each supported model. A steep rise to the ROC curve and a large area under the curve (AUC) supports a model with high predictive accuracy (Hosmer & Lemeshow, 2000). We used descriptive statistics (means and standard errors) for model-weighted covariates to aid interpretation of modeling results. For descriptive statistics, QHEI metric scores were scaled between 0 and 1 based on the maximum score of each metric, which allowed for comparison between sites with presence and absence of *C. callainus*.

RESULTS

Cambarus callainus was present at 39 of 153 sampling sites (25.4 % of sites sampled) in 23 streams. Within the Big Sandy River basins sub-watersheds, *C. callainus* was found at 2 of 15 sites (13% of sites sampled) in ULF-Levisa Fork, 21 of 39 sites in ULF-Russell Fork (53.8% of sites sampled), and 16 of 65 sites in Tug Fork (24.6% of sites sampled). *Cambarus callainus* was not found at any of the 32 sites surveyed in the lower Levisa Fork. Site descriptions, description of physical habitat and physiochemical values, as well as crayfish assemblages for each Big Sandy River basin sub-watershed are provided in the following paragraphs.

Lower Levisa Fork – Historic records for *C. callainus* within the Lower Levisa Fork are limited to the Levisa Fork in Georges Creek and Mud Creek sub-watersheds (Taylor and Shuster 2004; Thoma 2010). In the current effort, 32 sites were sampled across the Lower Levisa Fork resulting in zero *C. callainus* captures (Fig. 2). Given the lack of records, each sub-watershed is treated as a cohesive sampling unit in the following paragraphs.

Five sample reaches (Sites 1, 3, 4, 8, & 9; Fig. 2 &; Table 1) were surveyed in the Georges Creek watershed. All Georges Creek watershed sites were impacted by riparian corridor elimination, extensive bank erosion, stream channelization and excessive sedimentation, reflected in the watershed's average QHEI of 57.8 (n = 5 sites; SD ± 23.1 QHEI). Habitats affiliated with *C. callainus* occurrence (riffles and runs coursing over slab boulders) were noticeably absent within the Georges Creek watershed. When slab boulders, boulders, and large cobbles were encountered, all were concreted into the substrate and encased in sediment. Boulders may be naturally rare within Georges Creek system, given their overall paucity at sites

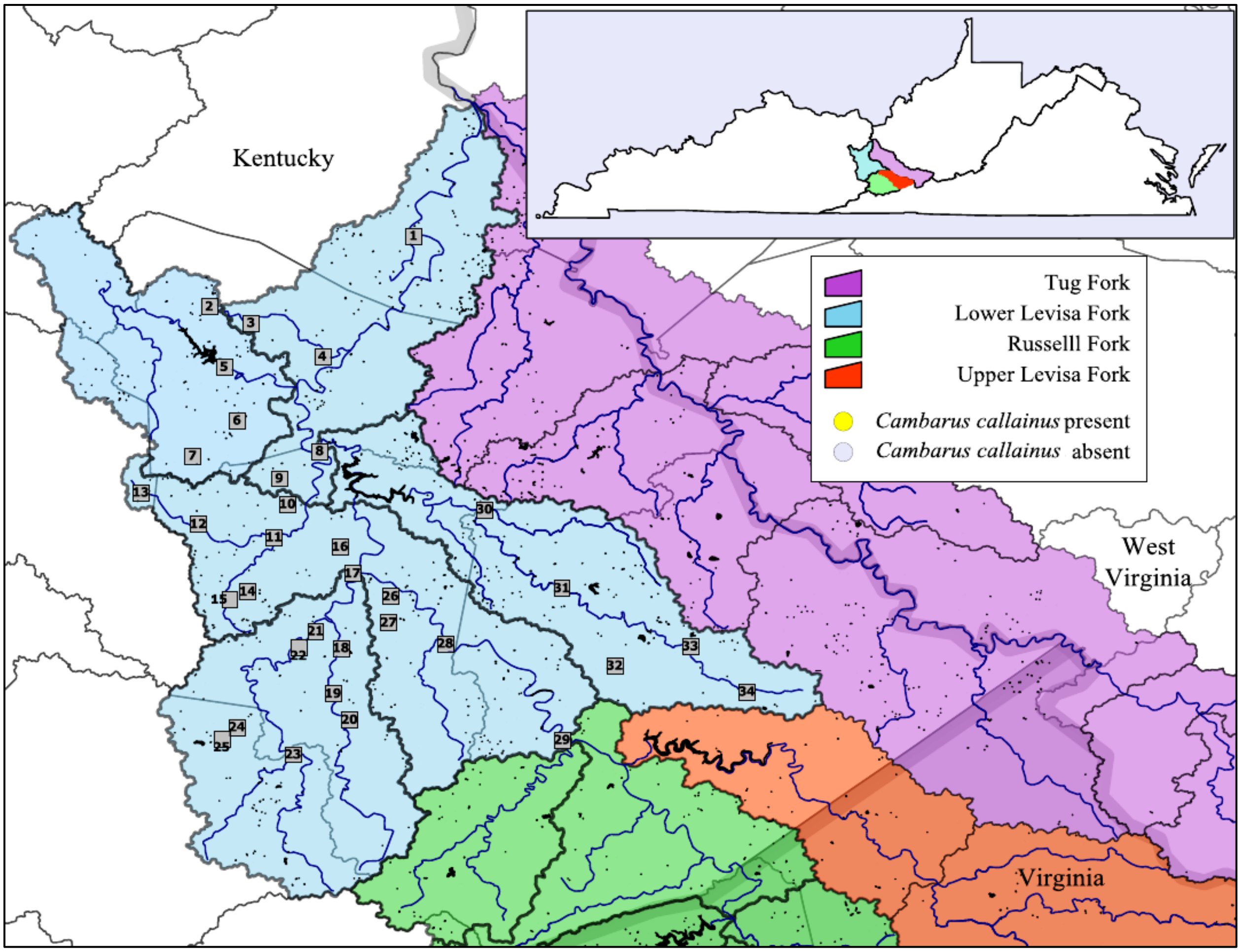


Figure 2: Lower Levisa Fork 2015 *Cambarus callainus* survey sites

sampled within the watershed and the prevalence of sand at all Georges Creek sites. It is possible that naturally occurring boulders and slabs have been covered by sediment falling out of the water column as water velocity decreased in the Lower Levisa Fork watershed responsive to the lower gradient observed in the basin.

Average conductivity and sulfate levels were lower at the Georges Creek sites ($\bar{X}$ sulfate = 249.0 ppm; n = 21 sites; SE ± 151.2 ppm) compared to sites in the other Big Sandy basin subwatersheds that maintained *C. callainus* ($\bar{X}$ conductivity = 689.0 µS; n = 21 sites; SD ± 254.0 µS). *Orconectes cristavarius* ($\bar{X}$ CPUE = 11 crayfish/h; n = 5 sites; SD ± 15 crayfish/h) and *Cambarus theepiensis* ($\bar{X}$ CPUE = 11 crayfish/h; n = 5 sites; SD ± 15 crayfish/h) compose the Georges Creek tertiary burrowing crayfish community. Average overall crayfish CPUE was 10.0 crayfish/h (n = 5 sites; SD ± 12 crayfish/h). Three streams were sampled at four locations (Sites

Table 1: Site information for 2015 *Cambarus callainus* Lower Levisa Fork watershed collection locales. Site #'s correspond to sites depicted in Figure 2.

Site #	Stream Name	County	Elevation	QHEI Score	Conductivity	Sulfate	Crayfish CPUE	*O. cristivarius* CPUE	*C. theepiensis* CPUE	*C. hatfiledi* CPUE
1	Georges Creek	Lawrence	562	32	87	110	0	0	0	0
2	Mudlick Cr.	Johnson	763	67.5	232	88	21	12	9	0
3	Toms Cr.	Johnson	702	64	181	57.8	9	4	5	0
4	Tutors Cr.	Johnson	594	35	302	31.3	0	0	0	0
5	Paint Cr.	Johnson	622	66.5	126	25.4	38	22	0	16
6	Jenny Cr.	Johnson	634	56	154	42.5	37	30	7	0
7	Jenny Cr.	Johnson	700	65	199	35.3	1	1	0	0
8	Johns Cr. Levisa Fk. Con.	Pike	1032	76.5	526	134.5	13	5	0	8
9	Little Paint Cr.	Floyd	640	81.5	170	20.3	28	20	8	0
10	Abbott Cr.	Floyd	577	58.3	118	13.1	8	5	3	0
11	Middle Cr.	Floyd	641	51.5	314	68.4	13	13	0	0
12	Middle Cr.	Floyd	656	72	383	94.5	14	10	4	0
13	Middle Cr.	Magoffin	779	71	201	10.9	16	13	0	3
14	Caney Cr.	FLoyd	722	56.5	949	369.3	56	55	1	0
15	Left Fk. Middle Cr.	FLoyd	677	57	737	262	5	5	0	0
16	Bull Cr.	Floyd	642	54	435	68.9	45	40	5	0
17	Beaver Cr.	Floyd	654	57	944	366.6	14	13	1	0
18	Left Fk. Beaver Cr.	Floyd	625	53	874	97.5	5	4	1	0
19	Left Fk. Beaver Cr.	FLoyd	681	47	407	76.8	1	1	0	0
20	Hall Fk.	FLoyd	709	55	670	212.5	53	42	11	0
21	Right Fk. Beaver Cr.	Floyd	671	45.5	511	146.4	1	1	0	0
22	Right Fk. Beaver Cr.	Floyd	671	44	504	130.8	19	18	1	0
23	Right Fk. Beaver Cr.	Knott	745	75	394	104.7	46	35	11	0
24	Jones Fk.	Knott	731	65	725	254.4	14	12	2	0
25	Jones Fk.	Knott	628	65.5	729	271.2	53	43	10	0
26	Prater Cr.	Floyd	653	72	495	152.5	121	119	2	0
27	Prater Cr.	Floyd	690	62.5	573	151.5	54	31	23	0
28	Levisa Fk.	Floyd	673	33.5	880	318	15	15	0	0
29	Levisa Fk.	Pike	684	73.5	850	265.6	11	11	0	0
30	Brushy Cr.	Pike	637	42.5	630	200.3	22	22	0	0
31	Johns Cr.	Pike	718	48.5	585	196	1	1	0	0
32	Racoon Cr.	Pike	784	76.5	382	89.3	22	16	0	6
33	Johns Cr.	Floyd	618	72.5	666	205.3	14	14	0	0
34	Johns Cr.	Pike	827	71.5	461	142.5	11	10	0	1

2, 5-7; Fig. 2; Table 1) within the Paint Creek watershed. Unlike Georges Creek, substrates of streams in the Paint Creek watersheds were composed of boulders and large cobbles, as well as sand, fines, and silt. Paint Creek (proper) substrate possessed slab boulders, boulders, and isolated boulders, as well as riffles and high velocity runs. Average QHEI for the Paint Creek watershed was 63.8 (n = 4 sites; SD ± 5.3 QHEI). Conductivity and sulfate levels averaged 178 µS (n = 4 sites; SD ± 47 µS µS) and 47.8 ppm (n = 4 sites; SD ± 27.7 ppm) respectively, and were lower than sites maintaining *C. callainus* ($\overline{X}$ conductivity = 687.0 µS; n = 39 sites; SD ± 261.0 µS; $\overline{X}$ sulfate = 269.0 ppm; n = 39 sites; SD ± 362.0 ppm) outside the basin. *Orconectes cristavarius* ($\overline{X}$ CPUE = 11 crayfish/h; n = 87 sites; SD ± 15 crayfish/h) was the dominant crayfish occurring in the Paint Creek watershed, and co-occurred with *C. theepiensis* ($\overline{X}$ CPUE = 11 crayfish/h; n = 4 sites; SD ± 15 crayfish/h) at two of four sites. Average total crayfish CPUE was 24 crayfish/h (n = 4 sites; SD ± 15 crayfish/h).

Nine Creeks were sampled in the Mud Creek watershed (Sites 11-16, 26-28; Fig. 2 & 3; Table 1). Gradient in the Mud Creek watershed promotes riffles and fast moving runs. Consequently, sand loses its dominance over large substrate classes, resulting in increased presence of slab boulders and boulders. Anthropogenic impacts are present throughout the watershed; all sites sampled were experiencing extensive bank erosion, sedimentation including concretion, and siltation. Total average QHEI across the overall watershed, was 58.9 QHEI (n = 9 sites; SD ± 12.4 QHEI). As with Georges and Paint Creek watersheds, average conductivity and sulfate levels ($\overline{X}$ conductivity = 552 µS; n = 9 sites; SD ± 256 µS; $\overline{X}$ sulfate = 166.3 ppm; n = 9 sites; SD ± 123.8 ppm) were lower than average levels at *C. callainus* sites ($\overline{X}$ conductivity = 689.0 µS; n = 21 sites; SD ± 254.0 µS; $\overline{X}$ sulfate = 249.0 ppm; n = 21 sites; SD ± 151.2 ppm). *Orconectes cristavarius* was abundant throughout the watershed ($\overline{X}$ CPUE = 11 crayfish/h; n = 9 9 sites; SD ± 15 crayfish/h), and present at all sites sampled. *Cambarus theepiensis* also occurred within the watershed ($\overline{X}$ CPUE = 11 crayfish/h; n = 9 sites; SD ± 15 crayfish/h), though not at densities observed for *O. cristavarius.* Average total crayfish CPUE was 38 crayfish/h (n = 9 sites; SD ± 37 crayfish/h), the highest value observed in the study.

Five sites were sampled in the Johns Creek watershed, all of which possessed extensive amounts of sand as substrate (Sites 30-34; Fig. 2; Table 1). Slab boulders, boulders, and large cobbles were rare at sample reaches. Riffles and runs were present at all sites sampled. Anthropogenic impacts were limited to moderate bank erosion and isolated riparian corridor

Figure 3: Prater Creek, Floyd County Kentucky. Prater Creek was typical of streams in the Mud Creek sub-watershed of the Lower Levisa Fork

development and elimination. Average QHEI was 62.5 (n = 5 sites; SD ± 15.6 QHEI), and the highest documented in the Lower Levisa Fork watershed. Average conductivity and sulfate levels ($\overline{X}$ conductivity = 178.0 µS; n = 5 sites; SD ± 47.0 µS; $\overline{X}$ sulfate = 47.8 ppm; n = 5 sites; SD ± 27.7 ppm) were lower than average levels at *C. callainus* sites ($\overline{X}$ conductivity = 687.0 µS;n = 39 sites; SD ± 261.0 µS; $\overline{X}$ sulfate = 269.0 ppm; n = 39 sites; SD ± 362.0 ppm). *Orconectes cristavarius*, *C. theepiensis*, and a formerly undocumented *C. hatfieldi* population, compose the Johns Creek watershed crayfish community. As with the previously discussed watershed (Mud Creek), *O. cristavarius* is prevalent throughout the watershed ($\overline{X}$ CPUE = 11 crayfish/h; n = 5 sites; SD ± 15 crayfish/h). *Cambarus hatfieldi* appears to be limited to Johns Creek. Overall crayfish CPUE for all sites sampled in the Johns Creek watershed was 12 crayfish/h (n = 5 sites; SD ± 7.5 crayfish/h).

Nine sites were sampled in the Beaver Creek watershed (Sites 17-25; Fig. 2; table 1). Beaver Creek is the most mountainous of the five Lower Levisa Fork watersheds, and subsequently had the highest overall gradient of all Lower Levisa Fork watersheds sampled. Instream habitat consisted of riffles and runs with slab boulders, boulders, and isolated boulders. Anthropogenic impacts were prevalent, including but not limited to: bank erosion, riparian corridor development and destruction, point source nutrient inputs, and stream channelization. Average QHEI was 56.3 (n = 9 sites; SD ± 10.5 QHEI). Beaver Creek's average conductivity and sulfate levels ($\overline{X}$ conductivity = 640.0 µS; n = 9 sites; SD ± 198 µS; $\overline{X}$ sulfate = 184.5 ppm; n = 9 sites; SD ± 97.6 ppm) were the highest observed in the Lower Levisa Fork basin, and lower than levels observed at *C. callainus* sites ($\overline{X}$ conductivity = 687.0 µS; n = 39 sites; SD ± 261.0 µS; $\overline{X}$ sulfate = 269.0 ppm; n = 39 sites; SD ± 362.0 ppm). Beaver Creek watershed's crayfish community was typical for the Lower Levisa Fork watershed, composed of both *O. cristavarius* ($\overline{X}$ CPUE = 19 crayfish/h; n = 9 sites; SD ± 17 crayfish/h) and *C. theepiensis* ($\overline{X}$ CPUE = 4 crayfish/h; n = 9 sites; SD ± 5 crayfish/h), of which *O. cristavarius* is dominant. Overall average crayfish CPUE was 23 crayfish/h (n = 9 sites; SD ± 22 crayfish/h).

Tug Fork – Sixty five sites in 29 streams were sampled in the Tug Fork basin (Fig. 4; Table 2). Streams sampled with historic populations in Kentucky included Blackberry Creek (Taylor and Schuster 2004; Thoma 2010; Thoma et al. 2014), Knox Creek (Thoma 2010; Thoma et al. 2014), and Peter Creek (Taylor and Schuster 2004). Virginia's only known Tug Fork *C. callainus* populations occurred in Knox Creek (Thoma 2009; Thoma 2010; Thoma et al. 2014), which were sampled with this effort. Sampled streams in West Virginia's portion of the Tug Fork with previously recorded *C. callainus* populations included both Dry Fork (Loughman 2013; Loughman and Welsh 2013; Thoma et al. 2014) and the Tug Fork mainstem (Loughman 2013; Loughman and Welsh 2013; Thoma et al. 2014). Twenty four additional streams were sampled, resulting in discovery of four previously unknown populations. In total, 31.0% of streams sampled in the Tug Fork basin supported *C. callainus* populations.

Cambarus callainus was first recorded within the Pigeon/Laurel/Rockcastle Creek system with this effort (Sites 13-20, Fig. #4; Table #2). Populations were discovered at two locations on Laurel Creek, both of which experienced several anthropogenic impacts including bank erosion, elimination of riparian corridor vegetation, as well as disturbances associated with human residence. Mean QHEI for Laurel Creek was 68.8 (n = 2; SD ± 6.8). Stream substrates were similar between sites, consisting of gravel, sand, cobbles, and occasional slab boulders.

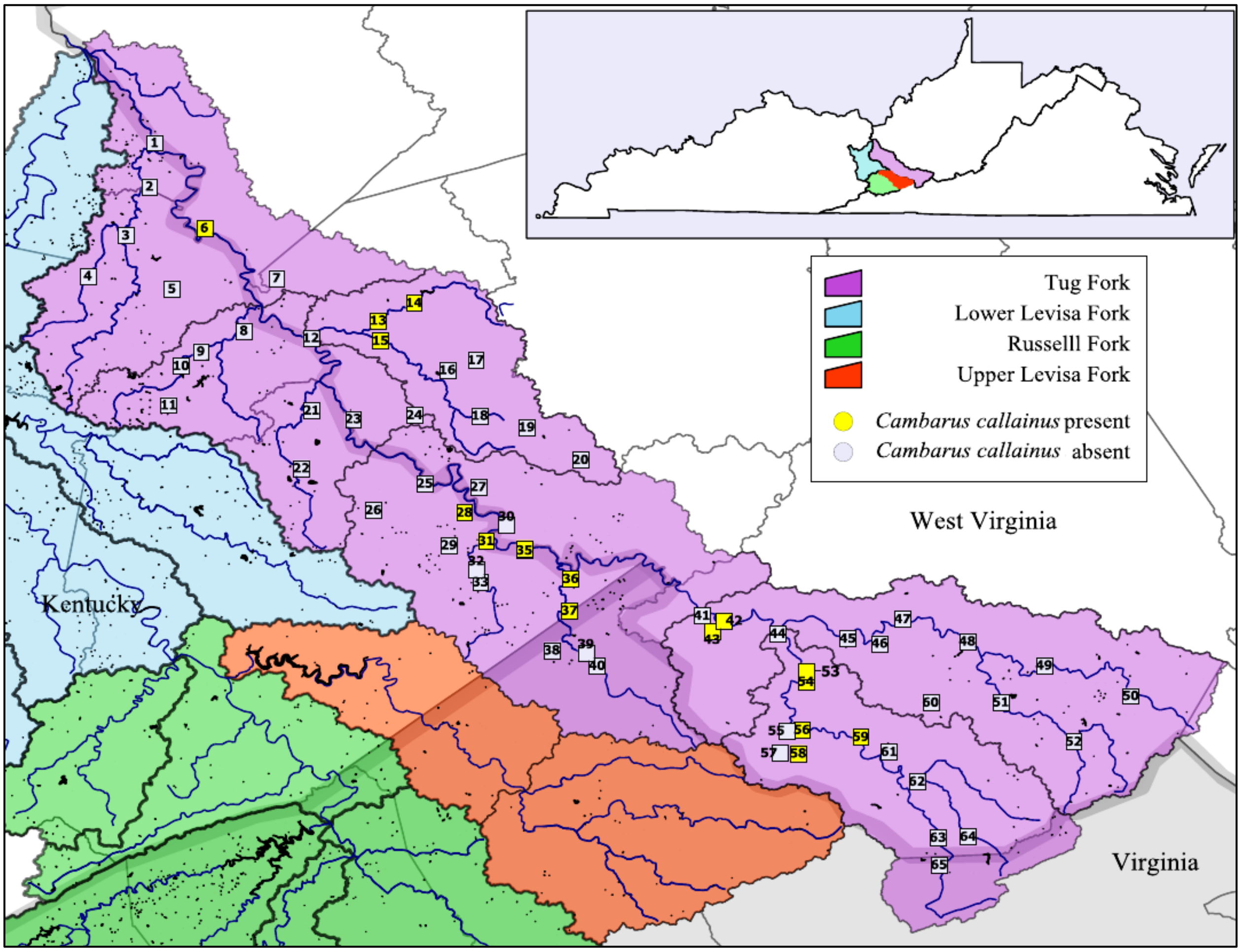


Figure 4: Tug Fork 2015 *Cambarus callainus* survey sites

Moderate sedimentation was present at both sites; the majority of boulders maintained open interstitial spaces. Mean conductivity and sulfate levels were 570 µS (n = 2 sites; SD ± 108.2 µS and 194.2 ppm (n = 2; SD ± 22.8 ppm), and lower than mean conductivity ($\overline{X}$ conductivity = 727 µS; n = 16 sites; SD ± 262.4 µS) or sulfate ($\overline{X}$ sulfate = 193.7 ppm; n = 16 sites; S ± 139.2 ppm) levels at Tug Fork sites maintaining *C. callainus.* Pigeon Creek's single *C. callainus* site (Site 15; Fig. 4; Table 2) was heavily anthropogenically impacted, with extensive bank erosion and conversion of riparian corridors to neighborhoods. Stream substrates consisted of fines, silt, sand, and occasional boulders. Pigeon Creek's QHEI score was 69. Both Pigeon Creek's conductivity (1,046 µS) and sulfate levels (225.9 ppm) were among the highest recorded at all sites across all basins. Both Laurel Creek and Pigeon Creek's crayfish fauna consisted of *C. callainus* ($\bar{X}$ CPUE = 1.0 crayfish/h; n = 3 sites; SD ± 0 crayfish/h), *C. hatfieldi* ($\bar{X}$ CPUE = 4.3 crayfish/h; n = 3 sites; SD ± 2.1 crayfish/h), and *O. cristavarius* ($\bar{X}$ CPUE = 5.7 crayfish/h; n = 3 sites; SD ± 2.5 crayfish/h). Of the three species, *O. cristavarius* was most prevalent, followed by *C. hatfieldi* and *C. callainus*. Mean crayfish CPUE for Laurel Creek/Pigeon Creek was 11 crayfish/h (n = 2 sites; SD ± 2.6). At all three sites harboring *C. callainus*, only a single animal was collected (Table 2).

Cambarus callainus was collected previously at Peter Creek (Taylor and Schuster 2004) and Blackberry Creek (Thoma 2010), and collected again in both streams with this effort. Both streams, morphologically, were among the smallest streams maintaining the species across its range. Both streams course through residential neighborhoods and experience impacts attributed to anthropogenic development. Moderate sedimentation and siltation issues were present in both streams; though neither stream experienced concretion. QHEI scores for Peter and Blackberry Creeks were 62.5 and 77.5 QHEI, respectively. Both streams had conductivity and sulfate levels (Blackberry Creek = 1,327 µS, 128.8 ppm; Peter Creek = 917 µS, 362.3 ppm) higher than average levels associated with *C. callainus* site presence in the Tug Fork basin. *Cambarus callainus* (CPUE = 1 crayfish/h for both streams) and *O. cristavarius* (Peters Creek CPUE = 1 crayfish/h; Blackberry Creek CPUE = 9 crayfish/h) were collected from both streams; *C. hatfieldi* was only taken in Blackberry Creek (CPUE = 3 crayfish/h).

Knox Creek *C. callainus* populations in Virginia and Kentucky were among the most robust populations observed in the Tug Fork basin with this effort. Two downstream Knox Creek sites (Sites 36 and 37; Fig. 4-5; Table 2) maintained *C. callainus,* one of which (Site 37) supported one of the largest populations observed in the 2015 survey. Both Knox Creek sites possessed fast runs and riffles with abundant slabs, boulder clusters, and boulders. Riparian corridors were intact, and composed of secondary mesophytic forest. Stream benthos at both sites was clear of excessive sedimentation, and lacked concretion and excessive bedload sediments. Average Knox Creek QHEI was 79.5 (n= 2 sites; SD ± 8.5 QHEI). Conductivity and sulfate levels ($\bar{X}$ conductivity = 541.0 µS; n = 2 sites; SD ± 9.9 µS; $\bar{X}$ sulfate = 180.1 ppm; n = 2 sites; SD ± 1.5 ppm) were lower than average Tug Fork levels at sites lacking *C. callainus*. Knox Creek's crayfish community consisted of *C. callainus* ($\bar{X}$ CPUE = 3.0 crayfish/h; n = 2 sites; SD ± 2.8 crayfish/h), *C. hatfieldi* ($\bar{X}$ CPUE = 0.5 crayfish/h; n = 2 sites; SD ± 0.7 crayfish/h), and *O. cristavarius* ($\bar{X}$ CPUE = 5.7 crayfish/h; n = 3 sites; SD ± 2.5 crayfish/h), of which *O. cristavarius*

Table 2: Site information for 2015 *Cambarus callainus* Tug Fork watershed collection locales. Site numbers correspond to sites depicted in Figure 4.

Site #	Stream Name	County	State	Elevation	QHEI Score	Conductivity	Sulfate	*Crayfish CPUE*	*O. cristavarius CPUE*	*C. callainus CPUE*	*C. hatfieldi CPUE*
1	Tug Fk.	Wayne	WV	700	50.0	922.0	241.2	*0*	0	0	*0*
2	Rockcastle Cr.	Martin	KY	619	56.5	413.0	131.7	*34*	30	0	0
3	Rockcastle Cr.	Martin	KY	613	52.5	479.0	135.2	*3*	3	0	0
4	Rockcastle Cr.	Martin	KY	701	54.5	240.0	86.6	*34*	6	0	15
5	Blacklog Fk.	Martin	KY	723	57.0	147.0	38.0	*11*	11	0	0
6	Tug Fk.	Wayne	WV	530	69.0	935.0	237.0	*4*	2	2	0
7	Marrowbone Cr.	Wayne	WV	622	58.5	645.0	160.0	*0*	0	0	0
8	Wolf Cr.	Martin	KY	640	44.0	802.0	308.5	*1*	1	0	0
9	Wolf Cr.	Martin	KY	614	64.0	735.0	247.0	*13*	11	0	0
10	Wolf Cr.	Martin	KY	653	58.5	708.0	248.7	*4*	4	0	0
11	Meathouse Cr.	Martin	KY	723	71.0	560.0	202.0	*26*	11	0	15
12	Pigeon Cr.	Mingo	WV	657	48.5	871.0	224.6	*0*	0	0	0
13	Laurel Cr.	Mingo	WV	625	69.5	570.0	178.1	*9*	6	1	2
14	Laurel Cr.	Mingo	WV	635	68.0	417.0	210.3	*10*	3	1	6
15	Pigeon Cr.	Mingo	WV		69.0	1046.0	2255.8	*14*	8	1	5
16	Pigeon Cr.	MIngo	WV	729	64.0	992.0	107.6	*7*	0	0	7
17	Pigeon Cr.	Mingo	WV	816	65.0	1101.0	192.6	*12*	0	0	12
18	Rockhouse Fk.	Mingo	WV	817	70.5	844.0	244.6	*12*	3	0	9
19	Rockhouse Fk.	Mingo	WV	1042	71.0	639.0	163.8	*14*	10	0	4
20	Pigeon Cr.	Mingo	WV	1222	66.5	1327.0	189.6	*4*	3	0	1
21	Big Cr.	Pike	KY	610	71.0	582.0	128.7	*5*	3	0	2
22	Big Cr.	Pike	KY	724	79.0	573.0	118.0	*8*	5	0	3
23	Tug Fk.	Mingo	WV	644	67.5	873.0	249.5	*11*	11	0	0
24	Buffalo Cr.	Mingo	WV	661	78.5	430.0	93.1	*0*	0	0	0
25	Tug Fk.	Mingo	WV	651	77.5	835.0		*14*	12	0	2
26	Pond Cr.	Pike	KY	747	73.0	762.0	233.2	*9*	8	0	1
27	Mate Cr.	Mingo	WV	752	70.9	814.0	282.1	*11*	5	0	0
28	Blackberry Cr.	Pike	KY	710	77.5	1327.0	628.0	*13*	9	1	3
29	Blackberry Cr.	Pike	KY	813	75.5	1463.0	737.5	*30*	20	0	10
30	Grapvine Cr.	Mingo	WV	707	65.5	1667.0	991.6	*9*	6	0	3
31	Peter Cr.	Pike	KY	729	62.5	917.0	362.2	*2*	1	1	0
32	Peter Cr.	Pike	KY	840	53.0	745.0	258.0	*29*	28	0	1

33	Left Fk. Peter Cr.	Pike	KY	836	60.0	749.0	245.0	*2*	1	0	1
34	Tug Fk.	Pike	KY	757	61.5	759.0	178.0	*29*	7	1	21
35	Poplar Cr.	Pike	KY	741	63.0	593.0	252.0	*45*	10	0	35
36	Knox Cr.	Pike	KY	818	85.5	534.0	179.1	*5*	0	5	0
37	Knox Cr.	Pike	KY	858	73.5	548.0	181.2	*14*	12	1	1
38	Paw Paw Cr.	Buchannan	VA	959	77.5	540.0	180.7	*42*	29	0	13
39	Knox Cr.	Buchannan	VA	948	73.5	277.0	116.3	*32*	17	0	15
40	Knox Cr.	Buchannan	VA	986	68.0	310.0	72.2	*31*	31	0	0
41	Panther Cr.	McDowell	WV	955	63.0	403.0	38.8	*18*	10	0	8
42	Tug Fk.	McDowell	WV	895	75.0	702.0	112.3	*15*	8	5	2
43	Panther Cr.	McDowell	WV	992	73.0	392.0	55.2	*12*	6	1	5
44	Dry Fk.	McDowell	WV	995	64.0	729.0	106.9	*13*	10	0	3
45	Tug Fk.	McDowell	WV	1060	64.0	594.0	104.6	*6*	1	0	5
46	Spice Cr.	McDowell	WV	1102	48.0	569.0	102.0	*10*	8	0	0
47	Tug Fk.	McDowell	WV	1099	60.5	660.0	137.5	*6*	0	0	6
48	Tug Fk.	McDowell	WV	1254	54.5	583.0	112.6	*6*	0	0	6
49	Elkhorn Cr.	McDowell	WV	1406	58.5	535.0	84.4	*0*	0	0	0
50	Elkhorn Cr.	McDowell	WV	1857	48.0	433.0	72.7	*10*	5	0	5
51	Tug Fk.	McDowell	WV	1368	56.0	865.0	160.8	*3*	1	0	2
52	South Fk. Tug R.	McDowell	WV	1570	58.0	363.0	74.6	*7*	0	0	7
53	Dry Fk.	McDowell	WV	1116	67.5	392.0	33.5	*4*	3	1	0
54	Dry Fk.	McDowell	WV	1036	69.0	719.0	160.4	*6*	2	1	3
55	Bradshaw Cr.	McDowell	WV	1374	58.0	312.7	47.6	*9*	2	0	7
56	Dry Fk.	McDowell	WV	1197	66.0	688.0	129.6	*6*	1	1	4
57	Bradshaw Cr.	McDowell	WV	1375	90.0	165.4	11.5	*20*	3	0	17
58	Dry Fk.	McDowell	WV	1378	88.5	703.0	102.8	*3*	0	3	0
59	Dry Fk.	McDowell	WV	1378	72.5	998.0	125.6	*6*	3	1	2
60	Little Indian Cr.	McDowell	WV	1475	46.5	462.0	79.5	*2*	1	0	1
61	Barenske Cr.	McDowell	WV	1371	60.0	1045.0	129.2	*6*	0	0	6
62	Jacobs Fk.	McDowell	WV	1438	68.0	795.0	234.6	*14*	6	0	8
63	Dry Fk.	McDowell	WV	1601	67.0	751.0	244.0	*13*	3	0	10
64	Jacobs Fk.	McDowell	WV	1747	67.5	868.0	365.0	*11*	0	0	11
65	Dry Fk.	Tazewell	VA	1731	70.5	723.0	19.3.0	*2*	1	0	1

was the most prolific species. Average crayfish CPUE was 9.0 crayfish/h (n = 3 sites; SD ± 5.7 crayfish/h).

Cambarus callainus was first reported occurring in Dry Fork by Loughman (2013). Results indicate that *C. callainus* populations in Dry Fork (Sites 54, 56, 58 & 59; Fig. 4; Table 2) represent the largest contiguous population occurring in West Virginia. All Dry Fork sites

Figure 5: Knox Creek, Pike County Kentucky. *Cambarus callainus* was locally abundant in this large riffle complex.

maintained fast moving riffles and high velocity runs coursing over substrates consisting of slab boulders, boulder clusters, large cobbles, and isolated boulders. Riparian corridors ranged from secondary mesophytic forest to small residential neighborhoods. Average Dry Fork QHEI was 72.7 (n = 5 sites; SD ± 9.2 QHEI). Conductivity and sulfate levels in Dry Fork averaged 700 µS ($\overline{X}$ conductivity = 700.0 µS; n = 5 sites; SD ± 214.6 µS) and 110.4 ppm ($\overline{X}$ sulfate = 110.4 ppm; n = 5 sites; SD ± 47.6 ppm) respectively, and were lower than average Tug Fork conductivity and sulfate levels ($\overline{X}$ conductivity = 727.9 µS; n = 16 sites; SD ± 262.4 µS; $\overline{X}$ sulfate = 193.7 ppm; n = 16 sites; SD ± 139.2 ppm). Dry Fork's crayfish community was typical of the Tug Fork watershed, and composed of *C. callainus* ($\overline{X}$ CPUE = 1.4 crayfish/h; n = 5 sites; SD ± 0.9 crayfish/h), *C. hatfieldi* ($\overline{X}$ CPUE = 1.8 crayfish/h; n = 5 sites; SD ± 1.8 crayfish/h), and *O. cristavarius* ($\overline{X}$ CPUE = 1.8 crayfish/h; n = 5 sites; SD ± 1.3 crayfish/h). *Cambarus callainus* CPUE values were similar to those observed by Loughman (2014) in 2009 and 2011. Mean Crayfish CPUE was 1.1 crayfish/h (n = 5 sites; SD ± 1.3 crayfish/h).

Six Tug Fork mainstem sites (Sites 1, 6, 23, 25, 34, 42) were sampled downstream of the Tug Fork/Dry Fork confluence, where *C. callainus* had been reported historically (Fig. 4; Table 2; Loughman 2013; Loughman and Welsh 2013; Loughman 2014; Thoma et al. 2014). Downstream of the Tug Fork/Dry Fork confluence, the Tug Fork mainstem's morphology and ecology is more indicative of a moderate to large size river, and no longer displays characteristics associated with small to moderate sized wadeable streams like those observed in the Tug Fork River upstream of the Dry Fork/Tug Fork confluence (Sites 45-48, 51). The furthest site downriver which *C. callainus* was observed in the Tug Fork—with this, or any effort—was Site 6 (Fig. 4; Table 2), where the substrate consisted of sand with a single boulder cluster. The three lower Tug Fork River sites maintaining *C. callainus* all occurred in areas with moderate to high anthropogenic impacts, characterized by bank erosion, riparian corridor manipulation, and residential development.

Overall average Tug Fork River mainstem site QHEI was 66.8 (n = 6 sites; SD = 10.0), conductivity was 837.7 µS (n = 6 sites; SD = 92.1 µS), and sulfate levels were 203.6 pm (n = 6 sites; 58.4 ppm) respectively. Average lower Tug Fork QHEI at sites maintaining the species was 68.5 QHEI (n = 3 sites; SD ± 6.8 QHEI), higher than average lower Tug Fork QHEI at sites lacking *C. callainus* ($\bar{X}$ QHEI = 65.0; n = 3 sites; SD ± 13.9 QHEI). All Tug Fork River sites maintaining *C. callainus* had at least one boulder cluster composed of two to five slab boulders. Sites lacking *C. callainus* possessed monotypic sandy substrates or experienced high levels of substrate concretion. Both average conductivity and sulfate levels ($\bar{X}$ conductivity = 798.7 µS; n = 3 sites; SD ± 121.5 µS; $\bar{X}$ sulfate = 175.8 ppm; n = 3 sites; SD ± 62.4 ppm) at Tug Fork *C. callainus* sites were higher than average levels at sites maintaining the species across the Tug Fork basin ($\bar{X}$ conductivity = 727.9 µS; n = 16 sites; SD ± 262.4 µS; $\bar{X}$ sulfate = 193.7 ppm; n = 16 sites; SD ± 139.2 ppm), though lower compared to Tug Fork River mainstem sites lacking the species ($\bar{X}$ conductivity = 876.7 µS; n = 3 sites; SD ± 43.6 µS; $\bar{X}$ sulfate = 245.4 ppm; n = 3 sites; SD ± 5.9 ppm).

Cambarus callainus was not captured en-masse in the Tug Fork River, with an average CPUE of 2.7 crayfish/h (2.7 crayfish/h; n = 3 sites; SD ± 2.1 crayfish/h) at Lower Tug Fork sites maintaining the specie. The farthest upstream Tug Fork River site (site 42) maintaining the species exhibited the highest Tug Fork *C. callainus* CPUE; the farthest downstream site (site 6) produced two animals including a malformed individual. Both *C. hatfieldi* (7.7 crayfish/h; n = 3 sites; SD ± 11.6 crayfish/h) and *O. cristavarius* (5.7 crayfish/h; n = 3 sites; SD ± 3.2 crayfish/h) also occurred in the Tug Fork of which *C. hatfieldi* was the dominant species. Average crayfish CPUE at Tug Fork sites with *C. callainus* was 15.3 crayfish/h (n = 3 sites; SD ± 13.5 crayfish/h).

Four Tug Fork mainstem sites were sampled upstream of the Dry Fork/Tug Fork confluence. *Cambarus callainus* has not been noted in the Tug Fork River headwaters upstream of the Dry Fork/Tug Fork confluence historically, and this effort also failed to find the species there (Sites 45-48, 51). Habitat conditions in this section of the Tug Fork are similar to Dry Fork and the two streams are separated by a single mountain range. Mean upper Tug Fork conductivity ($\bar{X}$ conductivity = 675.5 μS; n = 4 sites; SD ± 130.8 μS) and sulfate levels ($\bar{X}$ sulfate = 128.9 ppm; n = 4 sites; SD ± 25.5 ppm) were lower than average levels where *C. callainus* was present within the basin ($\bar{X}$ conductivity = 727.9 μS; n = 16 sites; SD ± 262.4 μS; $\bar{X}$ sulfate = 193.7 ppm; n = 16 sites; SD ± 139.2 ppm). Stream width and depth were lower in the upper Tug Fork compared to lower Tug Fork mainstem sites where *C. callainus* was present.

Cambarus callainus was not collected or observed at 69% (n = 49 sites lacking *C. callainus*) of sites sampled with this survey in the Tug Fork watershed. Site quality ranged from poor (Sites 8, 50, and 60; Fig. 4) to high (Sites 22, 24, 57; Fig. 4). Several smaller tributaries sampled (Sites 4, 50, and 64; Fig. 4) also lacked *C. callainus* populations. Common to all of these sites was increased sedimentation, concretion, and elevated bedload sediments. Average QHEI for Tug Fork tributaries lacking *C. callainus* was 63.8 (n = 49 sites; SD ± 10.0 QHEI). Among sites lacking *C. callainus*, other crayfish species were present at 91.7 % of sites, with both *O. cristivariaus* (6.8 crayfish/h; n = 49 sites; SD ± 8.3 crayfish/h) and *C. hatfieldi* (5.2 crayfish/h; n = 49 sites; SD ± 6.6 crayfish/h) maintaining relatively high densities compared to sites harboring *C. callainus* (4.4 *O. cristavarius*/h; n = 16 sites; SD ± 3.6 *O. cristavarius*/h; 3.4 *C. hatfieldi*/h; n = 16 sites; SD ± 6.6 *C. hatfieldi*/h). Both average conductivity and sulfate levels were lower ($\bar{X}$ conductivity = 681.6 μS; n = 49 sites; SD ± 303.1 μS; $\bar{X}$ sulfate = 187.9 ppm; n = 49 sites; SD ± 165.0 ppm) at sites lacking *C. callainus* compared to sites harboring *C. callainus* ($\bar{X}$ conductivity = 727.9 μS; n = 16 sites; SD ± 262.4 μS; $\bar{X}$ sulfate = 193.7 ppm; n = 16 sites; SD ± 139.2 ppm) in the Tug Fork basin.

ULF - Russell Fork – Thirty nine sites in 20 streams were sampled in the Russell Fork basin (Fig. 6; Table 3). Streams sampled in Kentucky with historic locations included Shelby Creek

(Taylor and Shuster 2004; Thoma 2009; Thoma et al. 2014), Long Fork (Thoma 2009; Thoma et al. 2014), Elkhorn Creek (Thoma 2009; Thoma et al. 2014), and the Russell Fork mainstem (Thoma 2009; Thoma et al. 2014). A previously undocumented population was discovered in Robinson Creek, a tributary of Shelby Creek, in Pike County Kentucky. Virginia historic populations sampled included Cranes Nest River, McClure River, Open Fork, Pound River, Prater Creek, and Russell Fork (Thoma 2009; Thoma et al. 2014). Previously undocumented populations were recorded in Virginia in Caney Creek, Frying Pan Creek, and Indian Creek. *Cambarus callainus* was collected in 51.2% of streams surveyed in the ULF-Russell Fork watershed.

For purposes of this study, the Shelby/Robinson/Long Fork (Sites 1, 4-7; Fig. 6; Table 3) and Elkhorn Creek systems (Site 10; Fig. 6: Table 3), are included in ULF-Russell Fork basin. The morphology of both of these stream systems is more similar to those of the ULF-Russell Fork watershed than those associated with the Lower Levisa Fork system. Specifically, both stream's gradient is moderate to high, and both stream's substrates are composed of bedrock glides, or streambeds maintaining gravels, cobbles, and slab boulders. Lower Levisa Fork streams lacked gradient levels observed in both the Shelby/Robinson/Long Fork/Elkhorn Creek system and ULF-Russell Fork watersheds.

Cambarus callainus populations in the Shelby Creek/Robinson Creek/Long Fork system (Sites 1, 4-7; Fig. 6; Table 3) exhibited the highest densities observed in Kentucky portions of the Upper Levisa Fork basin. Shelby Creek, Robinson Creek, and Long Forks average QHEI was 68.3 (n = 2 sites; SD ± 6.7 QHEI), 74, and 67.8 (n = 2; SD ± 1.8 QHEI) respectively. All streams course through residential neighborhoods or areas of human habitation, and experience impacts including riparian corridor elimination, bank erosion, and nutrient inputs. Substrate composition for all sites included slab boulders, boulder clusters, and anthropogenic substrates (concrete slabs and blocks). Interstitial spaces were mostly free of sediment; all sites lacked concretion. Average conductivity and sulfate values for all three streams ($\overline{X}$ conductivity = 849.0 µS; n = 5 sites; SD ± 102 µS; $\overline{X}$ sulfate = 322.9 ppm; n = 5 sites; SD ± 70.0 ppm) was higher than sites maintaining *C. callainus* in the ULF-Russell Fork watershed ($\overline{X}$ conductivity = 689.0 µS; n = 21 sites; SD ± 254.0 µS; $\overline{X}$ sulfate = 249.0 ppm; n = 21 sites; SD ± 151.2 ppm).

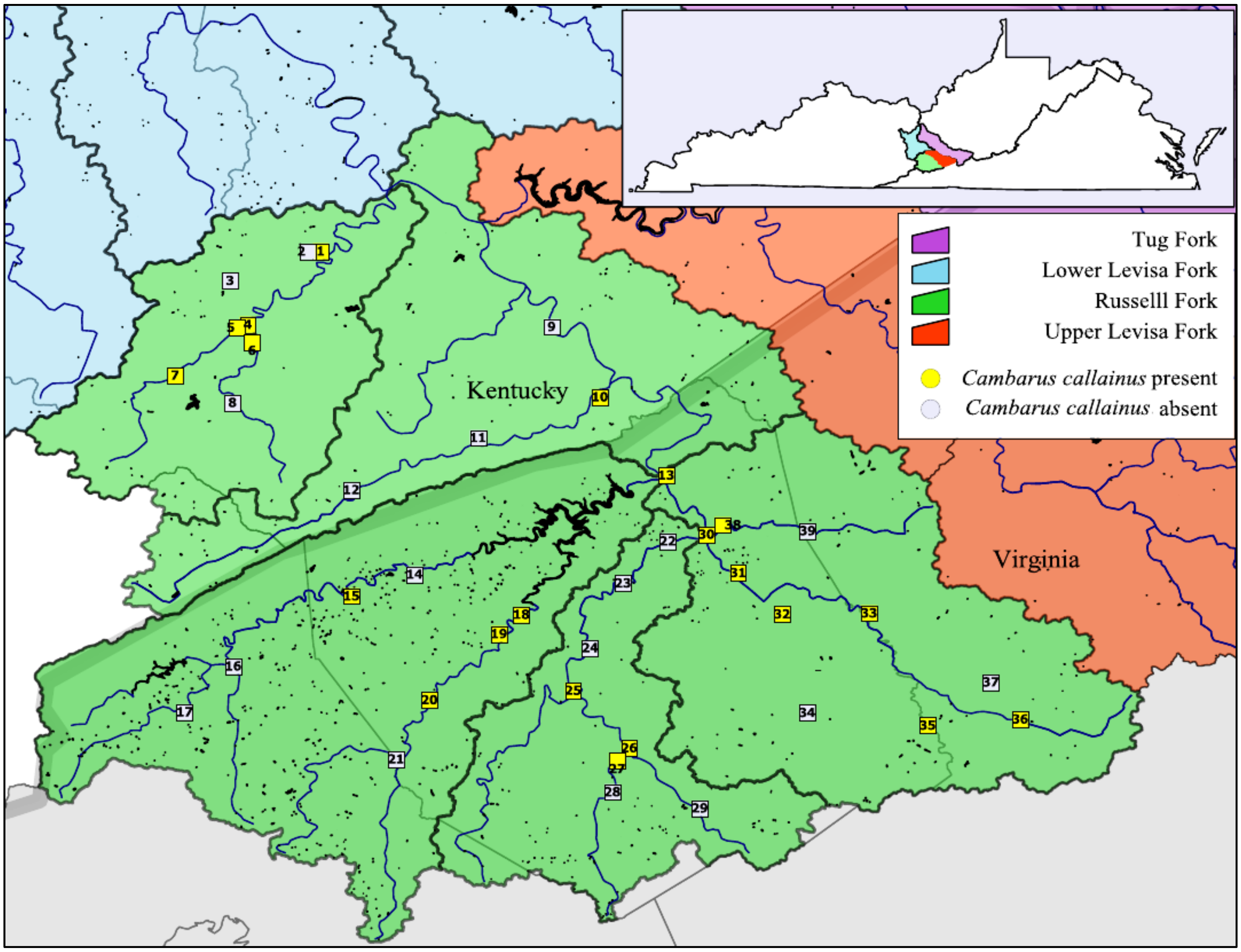


Figure 6: ULF-Russell Fork 2015 *Cambarus callainus* survey sites

Cambarus callainus was the dominant *Cambarus* species in Long Fork ($\bar{X}$ CPUE = 2.5 crayfish/h; n = 2 sites; SD ± 0.7 crayfish/h) and Shelby Creek ($\bar{X}$ CPUE = 1.5 crayfish/h; n = 2 sites; SD ± 0.5 crayfish/h); *C. theepiensis* was more prevalent in Robinson Creek than *C. callainus* (CPUE = 1 crayfish/h). *Orconectes cristavarius* was the dominant crayfish in the Shelby Creek/Robinson Fork/Long Fork system.

Cambarus callainus was first documented by Thoma (2010) in Elkhorn Creek, Pike County, Kentucky, and again with this effort. Within Elkhorn Creek *C. callainus* were procured near the Elkhorn Creek/Russel Fork confluence. Elkhorn Creek's QHEI, conductivity, and sulfate levels were 75 QHEI, 695 µS, and 221.4 ppm at the single *C. callainus* location. The single animal collected was taken under a large slab boulder in a high velocity riffle. Extensive bedrock glides dominate downstream Elkhorn Creek reaches, and could represent barriers to upstream expansion given the complete lack of slab boulders, boulder clusters, and other

Table 3: Site information for 2015 *Cambarus callainus* ULF-Russell Fork watershed collection locales. Site numbers correspond to sites depicted in Figure 6.

Site #	Stream Name	County	State	Elevation	QHEI Score	Conductivity	Sulfate	Crayfish CPUE	*O. cristavarius* CPUE	*C. callainus* CPUE	*C. theepiensis* CPUE
1	Robinson Cr.	Pike	KY	729	74.0	923.0	337.7	*46*	42	1	3
2	Robinson Cr.	Pike	KY	752	64.0	1185.0	564.7	*24*	18	0	6
3	Little Robinson Cr.	Pike	KY	953	71.0	1011.0	437.2	*16*	9	0	7
4	Shelby Cr.	Pike	KY	804	73.0	723.0	251.0	*7*	5	2	0
5	Long Fk.	Pike	KY	869	66.5	798.0	295.0	*40*	37	3	0
6	Shelby Cr.	Pike	KY	829	63.5	978.0	412.0	*40*	37	1	2
7	Long Fk.	PIke	KY	945	69.0	821.0	247.0	*38*	33	2	3
8	Shelby Cr.	Pike	KY	967	61.5	1135.0	442.7	*64*	63	0	1
9	Russell Fk.	Pike	KY	717	81.0	790.0	242.5	*5*	5	0	0
10	Elkhorn Cr.	Pike	KY	812	72.5	695.0	221.4	*7*	5	1	1
11	Elkhorn Cr.	Dickenson	VA	1079	72.0	932.0	312.0	*22*	20	0	2
12	Elkhorn Cr.	Pike	KY	1250	59.5	1023.0	340.7	*40*	39	0	1
13	Russell Fk.	Dickenson	VA	1216	81.0	511.0	121.7	*4*	2	1	1
14	Georges Fk.	Dickenson	VA	1489	77.0	1266.0	539.5	*15*	7	0	8
15	Pound River	Dickenson	VA	1476	85.0	1224.0	550.0	*3*	0	3	0
16	Pound River	Wise	VA	1563	51.5	969.0	450.5	*13*	13	0	0
17	South Fk. of the Pound R.	Wise	VA	1602	73.5	1555.0	952.0	*17*	11	0	6
18	Cranes Nest R.	Dickenson	VA	1515	90.5	837.0	431.0	*3*	0	3	0
19	Cranes Nest R.	Dickenson	VA	1457	89.5	870.0	428.0	*5*	0	2	3
20	Cranes Nest R.	Dickenson	VA	1533	73.0	986.0	408.5	*5*	1	3	1
21	Cranes Nest R.	Wise	VA	1562	61.0	336.0	81.7	*14*	14	0	0
22	McClure River	Dickenson	VA	1273	88.5	517.0	148.0	*1*	1	0	0
23	McClure River	Dickenson	VA	1344	85.5	416.0	27.5	*0*	0	0	0
24	McClure River	Dickenson	VA	1475	59.0	507.0	101.5	*1*	1	0	0
25	Caney Cr.	Dickenson	VA	1452	71.5	354.0	64.5	*3*	1	2	0
26	McClure River	Dickenson	VA	1526	74.5	534.0	142.2	*8*	3	3	2
27	Open Fk.	Dickenson	VA	1610	74.0	508.0	132.7	*6*	4	1	1
28	Middle Fk.	Dickenson	VA	1627	78.5	443.0	147.7	*14*	0	0	14
29	Roaring Fk.	Dickenson	VA	1694	83.0	653.0	116.2	*15*	0	0	15
30	Prater Cr.	Dickenson	VA	1277	79.0	454.0	429.0	*5*	2	3	0
31	Russel Fk.	Dickenson	VA	1270	60.0	468.0	117.0	*2*	0	2	0
32	Fryingpan Cr.	Dickenson	VA	1316	73.5	622.0	198.0	*13*	8	4	1
33	Russell Fk.	Dickenson	VA	1350	79.5	444.0	126.7	*4*	3	1	0
34	Fryingpan Cr.	Dickenson	VA	1442	77.0	716.0	138.0	*16*	0	0	16

35	Indian Cr.	Buchannan	VA	1489	62.0	558.0	177.0	*14*	5	2	7
36	Russell Fk.	Buchannan	VA	1559	70.5	206.0	24.0	*17*	10	1	6
37	Hurricane Cr.	Buchannan	VA	1558	71.5	168.0	19.0	*17*	4	0	13
38	Russell Prater Cr.	Dickenson	VA	1293	70.5	955.0	116.5	*5*	2	3	0
39	Russell Prater Cr.	Buchannan	VA	1458	67.5	1003.0	449.2	*44*	43	0	1

instream habitat frequently utilized by *C. callainus* as refuge. Upstream Elkhorn Creek sites did not support *C. callainus* populations (Sites 11-12; Fig. 6; Table 3), though instream habitat was similar to downstream reaches that maintained the species. Both *C. theepiensis* ($\bar{X}$ CPUE = 2 crayfish/h; n = 2 sites; SD ± 1 crayfish/h) and *O. cristavarius* ($\bar{X}$ CPUE = 30 crayfish/h; n = 2 sites; SD ± 13 crayfish/h) were present in upstream reaches.

Both the Pound and Cranes Nest Rivers and their tributaries drain into the 1,143 acre Flannagan Reservoir, and as such, are treated together in this section. Populations were reported previously in both rivers (Thoma 2009; Thoma et al. 2014). *Cambarus callainus* was collected in the Pound River mainstem at Site 15. Here the Pound River consists of a series of high velocity riffles and runs with abundant slab boulders, boulders, and boulder clusters. Stream substrates experienced moderate sedimentation, including concretion, in low velocity situations. In higher velocity situations interstitial spaces were open. *Cambarus callainus* was taken under large slab boulders at both the head of fast moving runs and mid-riffle. Zero crayfish species co-occurred with *C. callainus* at Site 15. Upstream Pound River sites (Sites 16-17; Fig. 6; Table 3) did not produce *C. callainus*, and were heavily impacted by development ($\bar{X}$ QHEI = 62.5; n = 2 sites; SD ± 15.6 QHEI).

Cambarus callainus was collected at three sites on Cranes Nest River (Sites 18-20; Fig. 6; Table 3). Site characteristics for all Cranes Nest River sites were similar, with riparian zones composed of secondary growth mesophytic forests, stream substrates consisting of abundant slab boulders, boulder clusters, isolated boulders, and course woody debris, with moderate to little sedimentation. Concretion of stream substrates was absent at all *C. callainus* Cranes Nest River sites. Mean QHEI was 84.3 (n = 3 sites; SD ± 9.8 QHEI). Average conductivity ($\bar{X}$ conductivity = 898 µS; n = 3 sites; SD ± 78.3 µS) and sulfate levels ($\bar{X}$ sulfate = 422.5 ppm; n = 3 sites; SD ± 12.2 ppm) were higher than average levels at ULF-Russell Fork sites maintaining the species ($\bar{X}$ conductivity = 689.0 µS; n = 21 sites; SD ± 254.0 µS; $\bar{X}$ sulfate = 249.0 ppm; n = 21 sites; SD ± 151.2 ppm). *Cambarus callainus* was the dominant cambarid species ($\bar{X}$ CPUE = 3 crayfish/h; n

= 3 sites; SD ± 1 crayfish/h) in the Cranes Nest River; average *C. theepienisis* CPUE at Cranes Nest River sites was 1 crayfish/h (n = 3 sites; SD ± 2 crayfish/h). *Orconectes cristavarius* was only present at a single *C. callainus* site (CPUE = 1 crayfish/h) in the Cranes Nest River. Overall average crayfish CPUE was 4.3 crayfish/h (n = 3 sites; SD ± 1.2 crayfish/h). With the exception of the Russell Fork mainstem, Cranes Nest River *C. callainus* populations were the densest populations observed in Virginia during 2015.

Thoma (2009) was the first to report *C. callainus* from the McClure River in Virginia. Virginia Department of Transportation reported *C. callainus* in 2014 from Open Fork. In the current effort, *C. callainus* was collected from a single site on the McClure (Site 25; Fig. 6; Table 3) as single site on Open Fork (Site 27; Fig 6; Table 3) , as well as Caney Creek (Site 25; Fig. 6; Table 3) which represented a new distribution record for the species. McClure River sites downstream of the Caney Creek/McClure River confluence and upstream of the Open Fork/McClure River confluence did not produce *C. callainus*. Average QHEI at sites maintaining *C. callainus* in the McClure River watershed was 73.3 QHEI (n = 3 sites; SD ± 1.6 QHEI). Stream substrates were composed of moderate and fast moving runs and riffles with sand substrates, isolated slab boulders, and boulder clusters. Sedimentation was low to moderate at all sites; substrates were not experiencing concretion. Average conductivity and sulfate levels were 465 µS (n = 3 sites; SD ± 97.3 µS) and 113.2 ppm (n = 3 sites; SD ± 42.4 ppm) respectively. McClure River sites that lacked *C. callainus* were composed of sand bottomed, slow to moderate velocity runs, with higher average QHEI scores ($\bar{X}$ QHEI = 77.7; n = 3 sites; SD ± 16.3 QHEI), and substrates composed of silt and sand with large cobbles, course woody debris, as well as isolated boulders and slabs. Only *O. cristavarius* ($\bar{X}$ CPUE = 0.7 crayfish/h; n = 3 sites; SD ± 0.6 were captured in upstream portions of the McClure River watershed. Total crayfish CPUE for all McClure River sites was 3.2 crayfish/h (n = 6 sites; SD ± 3.2 crayfish/h).

Cambarus callainus was collected in Prater Creek at Haysi, Virginia, in the vicinity of the stream's confluence with the Russell Fork (Site 38; Fig. 6; Table 3). Shultz and Reid were the first to collect *C. callainus* at this location in 1937 (Loughman 2014). Collections by Thoma (2009) in 2008 were the most recent confirming the species presence in Prater Creek prior to this effort. Prater Creek experiences several anthropogenically mediated impacts, including: elimination of riparian corridors, bank erosion, and possible chemical inputs associated with road runoff. Instream habitat consisted of abundant slab boulders and isolated boulders, cobbles, and sand. Sedimentation was limited, with the majority of slab boulders maintaining open interstitial spaces. QHEI, conductivity, and sulfate levels were 79 QHEI, 454 µS and 429 ppm at the site harboring *C. callainus*, and 67.5 QHEI, 1,003 µS and 449.3 ppm at the upstream site lacking the species. *Cambarus callainus* was the only *Cambarus* species when present (CPUE = 3 crayfish/h); *C. theepiensis* was present at the upstream Prater Creek site (CPUE = 1 crayfish/h). *Orconectes cristaivarius* was markedly more abundant in the upstream site lacking *C. callainus* ($\overline{X}$ CPUE = 43 crayfish/h) compared to the downstream site (CPUE = 2 crayfish/h) that maintained the species. Average crayfish CPUE for both Prater Creek sites was 25 crayfish/h crayfish/h (n = 2 sites; SD ± 28 crayfish/h).

Cambarus callainus was consistently collected within the Russell Fork mainstem from the confluence of the Pound River upstream to the river's headwaters (Sites 13, 31, 33, 36; Fig. 6-7; Table 3). Thoma (2009) observed similar densities in Virginia in 2008, also recording the species in sections of the Russell Fork in Kentucky (Thoma 2009). In the current survey, only one Russell Fork mainstem site was surveyed downstream of the confluence of the Russell Fork with the Pound River (Site 13 and 9; Fig. 6; Table 3) and zero *C. callainus* were collected at that location. Future survey efforts should focus on this section of the Russell Fork mainstem to determine the downstream extent of *C. callainus* in the Russell Fork. Russell Fork mainstem sites (Sites 9, 13, 31, 33, 36; Table 3) shared similar morphology, and herein are treated collectively. QHEI scores were high at most sites ($\overline{X}$ QHEI = 70.1; n = 5 sites; SD ± 8.0 QHEI), reflecting the river's relatively sediment free and heterogeneous substrate. Slab boulders, boulder clusters, isolated boulders, and large cobbles were present at Russell Fork sites. Both fast moving runs and riffles were prevalent throughout the Russell Fork and present at all collection locales.

All *C. callainus* collected at Russell Fork sites were taken under slab boulders or boulders in riffles and runs. Habitat in slack water environments was sampled at all sites, and produced zero *C. callainus*. Average conductivity and sulfate levels were lower ($\overline{X}$ conductivity = 407 µS; n = 4 sites; SD ± 137 µS; $\overline{X}$ sulfate = 97.4 ppm; n = 4 sites; SD ± 49.1 ppm) than average levels at sites maintaining *C. callainus* within the ULF-Russel Fork watershed ($\overline{X}$ conductivity = 689.0 µS; n = 21 sites; SD ± 254.0 µS; $\overline{X}$ sulfate = 249.0 ppm; n = 21 sites; SD ± 151.2 ppm). *Cambarus callainus* was captured in low numbers at all sites ($\overline{X}$ CPUE = 1.0 crayfish/h; n = 4 sites; SD ± 1.0 crayfish/h); *C. theepiensis* was only collected at 1 (25%) Russell

Figure 7: Russell Fork, Dickenson County Virginia. *Cambarus callainus* was observed at several Russell Fork mainstem sites in Virginia upstream of the Pound River/Russell Fork mainstem with site conditions depicted in picture.

Fork site. *Orconectes cristavarius* ($\overline{X}$ CPUE = 3.8 crayfish/h; n = 4 sites; SD ± 4.3 crayfish/h) was collected most frequently with *C. callainus*. Average Russell Fork crayfish CPUE was 6.8 crayfish/h (n = 4 sites; SD ± 6.9 crayfish/h). Russell Fork *C. callainus* population densities were similar to those observed by Thoma (2009); at present, *C. callainus* has the largest contiguous populations across the species range in the Russell Fork River.

Cambarus callainus were discovered in Frying Pan (Site 32; Fig. 6; Table 3) and Indian (Site 35; Fig. 6; Table 3) Creeks for the first time with this survey. Both streams' morphologies were similar, consisting of riffles and fast moving runs with sand substrates, isolated slab boulders, and boulder clusters. Indian Creek's substrate experienced more sedimentation than Frying Pan Creek, and subsequently experienced a higher degree of concretition than the latter stream. Both streams' riparian corridors consisted of secondary mesophytic forests. QHEI, conductivity, and sulfate levels were 73.5 QHEI, 622 µS, and 198 ppm for Frying Pan Creek and 62 QHEI, 558 µS, and 198 ppm for Indian Creek. *Cambarus callainus* was taken at both sites

(Frying Pan Creek CPUE = 4 crayfish/h; Indian Creek CPUE = 2 crayfish/h) under isolated slab boulders in fast moving, sand bottomed runs. *Cambarus theepiensis* (Frying Pan Creek CPUE = 1 crayfish/h; Indian Creek CPUE = 7 crayfish/h) and *O. cristavarius* (Frying Pan Creek CPUE = 8 crayfish/h; Indian Creek CPUE = 5 crayfish/h) occurred syntopically with *C. callainus* in both streams, and were collected under the same slab boulders as *C. callainus*.

With the exception of downstream McClure River sites (Sites 22-24; Fig. 6; Table 3), the majority of ULF-Russell Fork tributary sites southeast of the Pine Mountain Divide maintained *C. callainus*. The sites lacking *C. callainus* (Sites 16-17, 28-29, 34, 37, 39; Fig. 6; Table 3) in the ULF-Russell Fork basin were the farthest upstream sites sampled in the ULF-Russell Fork basin and are treated collectively herein given their similar site morphology and “headwater” characteristics. All upstream sites had stream widths ranging from 5-15 m and were characterized by short riffles (3-5 m) followed by expansive slow to moderate (15-50m) runs. The substrates of these streams were dominated by cobbles and gravels with occasional boulders, and in the absence of cobble and gravel, substrates were dominated by sand. Unlike the ULF-Russell Fork sites maintaining *C. callainus*, large riffles and fast moving runs coursing over boulder fields with slab boulders and boulder clusters were not present in the farthest upstream sampled sites in the ULF-Russel Fork basin. Average QHEI was the same at upstream sites lacking *C. callainus* ($\overline{X}$ QHEI = 71.0; n = 7 sites; SD ± 13.1 QHEI), compared to sites maintaining the species ($\overline{X}$ QHEI = 71.0; n = 21 sites; SD ± 14.5 QHEI). Both average conductivity ($\overline{X}$ conductivity = 643 µS; n = 7 sites; SD ± 316 µS) and sulfate levels ($\overline{X}$ sulfate = 218.4 ppm; n = 7 sites; SD ± 172.0 ppm) were lower at ULF-Russell Fork headwater sites lacking *C. callainus* compared to average values for sites maintaining the species ($\overline{X}$ conductivity = 689.0 µS; n = 21 sites; SD ± 254.0 µS; $\overline{X}$ sulfate = 249.0 ppm; n = 21 sites; SD ± 151.2 ppm). Both average *C. theepiensis* CPUE ($\overline{X}$ CPUE = 4 crayfish/h; n = 7 sites; SD ± 7 crayfish/h) and *O. cristavarius* CPUE ($\overline{X}$ CPUE = 11 crayfish/h; n = 7 sites; SD ± 15 crayfish/h) were higher at ULF-Russell Fork headwater sites compared to sites maintaining the species ($\overline{X}$ *C. theepiensis* CPUE = 1.0 crayfish/h; n = 21 sites; SD ± 1.0).

Upper Levisa Fork - Levisa Fork – Fifteen sites were sampled in seven streams across the ULF-Levisa Fork basin (Fig. 8; Table 3). *Cambarus callainus* was collected most recently by Thoma (2009) in the watershed in Dismal Creek. The current sites sampled with this effort

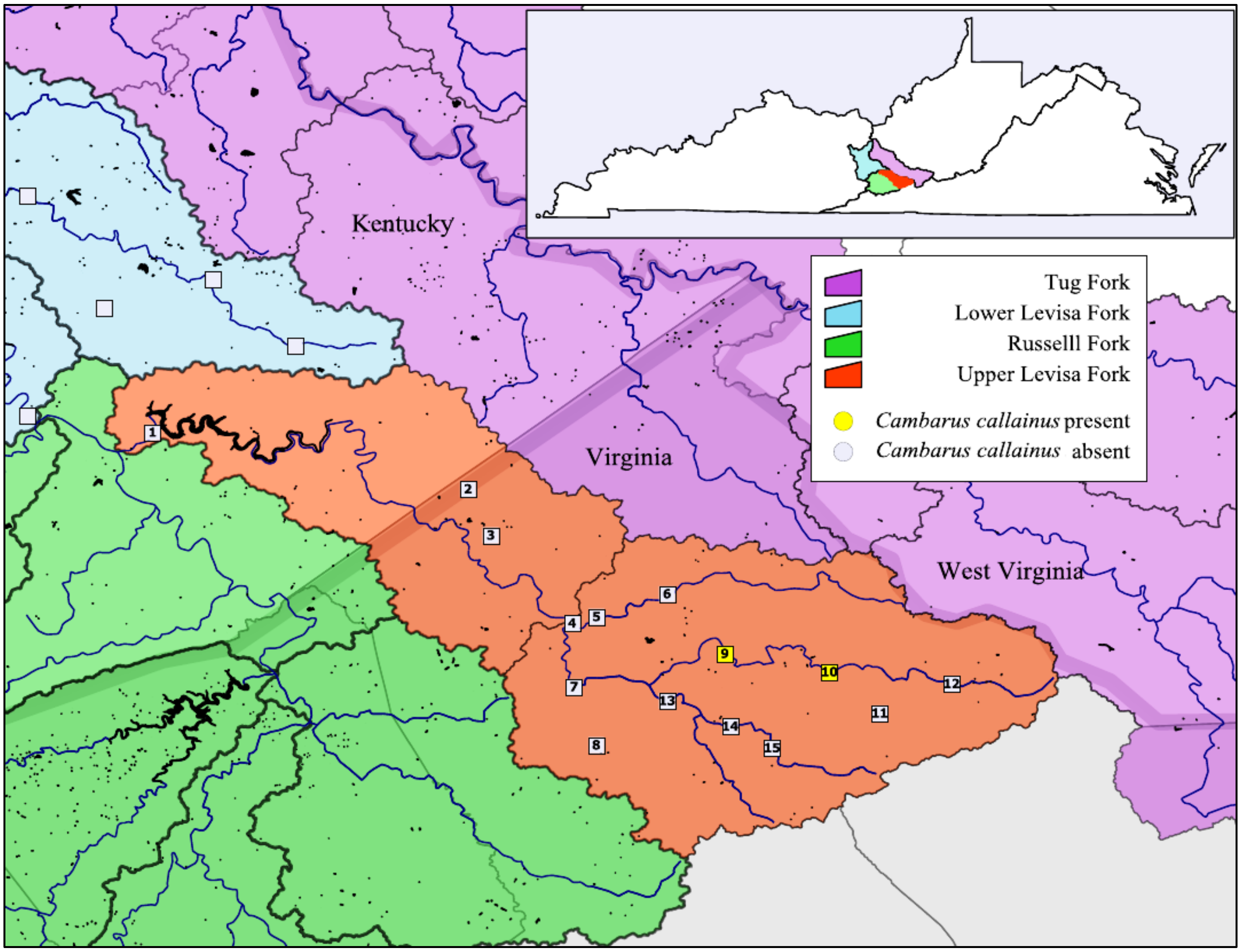


Figure 8: ULF-Levisa Fork 2015 *Cambarus callainus* survey sites

included three sites on Dismal Creek, and six sites on the Levisa Fork mainstem; five of which occurred upstream of Fishtrap Reservoir, with one occurring in Fishtrap reservoir's outflow (Fig. 8; Table 3). Big Prater, Home, Rocklick, and Slate Creeks were also sampled (Fig. 8; Table 3). Thoma (2010) sampled these streams in 2010 and did not procure *C. callainus*.

Of the aforementioned streams, Dismal Creek was the only stream in which *C. callainus* was collected during the current study, with animals captured at two (Sites 9-10; Fig. 8; Table 3) of three sites sampled. Dismal Creek's mean QHEI was 73.0 (n = 2; SD ± 9.9 QHEI). Both *C. callainus* site's substrates were composed of large slab boulders, boulder clusters, and course woody debris snags. Siltation was present in eddies and on boulder surfaces in low flow situations. Sedimentation was limited to these same environments, with limited evidence of concretion. High velocity habitats lacked concretion, and maintained open interstitial spaces. All *C. callainus* observed at both Dismal Creek sites were collected under large slab boulders at the

Table 4: Site information for 2015 *Cambarus callainus* Upper Levisa Fork watershed collection locales. Site numbers correspond to sites depicted in Figure 8.

Site #	Stream Name	County	State	Elevation	QHEI Score	Conductivity	Sulfate	Crayfish CPUE	*O. cristavarius* CPUE	*C. callainus* CPUE	*C. theepiensis* CPUE
1	Levisa Fk. - Fishtrap Res.	Pike	KY	714	60	744	167.2	0	0	0	0
2	Rocklick Cr.	Buchannon	VA	1068	78.5	279	60.8	24	13	0	11
3	Home Cr.	Buchannon	VA	945	79	552	205.3	17	2	0	15
4	Levisa Fk.	Dickenson	VA	1036	65	671	218.0	40	20	0	0
5	Slate Cr.	Buchannon	VA	1152	73	419	115.3	7	6	0	1
6	Slate Cr.	Buchannon	VA	1233	63.5	346	84.8	22	14	0	8
7	Levisa Fk.	Buchannon	VA	1120	72	434	93.8	1	0	0	1
8	Big Prater Cr.	Buchannon	VA	1381	62.5	254	64.5	36	1	0	35
9	Dismal Cr.	Buchannon	VA	1323	80	312	74.0	7	1	5	1
10	Dismal Cr.	Buchannon	VA	1592	66	357	66.5	7	0	5	2
11	Laurel Fk.	Buchannon	VA	1756	71	352	84.0	16	13	0	3
12	Dismal Cr.	Buchannon	VA	1833	85.5	374	101.8	20	3	0	17
13	Levisa Fk.	Buchannon	VA	1226	78.5	485	90.3	1	1	0	0
14	Levisa Fk.	Buchannon	VA	1401	72.5	404	97.0	10	5	0	5
15	Levisa Fk.	Buchannon	VA	1560	72.5	305	46.0	16	10	0	6

heads of riffles or mid-run. Zero animals were collected under small boulders, rounded boulders or other sampled rock classes. Mean conductivity and sulfate levels ($\bar{X}$ conductivity = 335 µS; n = 2 sites; SD ± 31.8 µS; $\bar{X}$ sulfate = 70.3 ppm; n = 2 sites; SD ± 5.3 ppm) were lower than sites lacking the species in the ULF-Levisa Fork ($\bar{X}$ conductivity = 432.0 µS; n = 10 sites; SD ± 147.0 µS; $\bar{X}$ sulfate ± 109.9 ppm; n = 13 sites; SD ± 53.9 ppm). Dismal Creek maintains a diverse crayfish assemblage, composed of *C. callainus*, *C. theepiensis*, and *O. cristavarius*. *Cambarus callainus* appears to be the dominant *Cambarus* species, with a mean CPUE of 5.0 crayfish/h (n = 2 sites; SD ± 0 crayfish/h) compared to *C. theepiensis'* mean CPUE of 1.5 crayfish/h (n = 2 sites; SD ± 0.7 crayfish/h). *Orconectes cristavarius* was not prevalent in Dismal Creek sample reaches ($\bar{X}$ CPUE = 0.5 crayfish/h; n = 2 sites; SD ± 0.7 crayfish/h), and subordinate to *C. callainus* in both high velocity runs and riffles. Mean Crayfish CPUE for Dismal Creek was 7.0 crayfish/h (n = 2 sites; SD ± 0 crayfish/h).

Zero *C. callainus* were captured or observed from the five Levisa Fork mainstem sites sampled upstream of Fishtrap Reservoir (Sites 4, 7, 13-15; Fig. 8; Table 4). Mean Levisa Fork QHEI score was 72.1 (n = 5 sites; SD ± 9.9 QHEI). All Levisa Fork sites sampled possessed riffles and fast moving runs coursing over substrates maintaining slab boulders, boulders, and course woody debris snags. All Levisa Fork sites maintained some degree of sedimentation and concretion, specifically concretion of slab boulders in riffles and runs. Both conductivity and sulfate levels ($\overline{X}$ conductivity = 460 µS; n = 5 sites; SD ± 135 µS; $\overline{X}$ sulfate = 109 ppm; n = 5 sites; SD ± 64.4 ppm) were higher than sites maintaining *C. callainus* ($\overline{X}$ conductivity = 335 µS; n = 2 sites; SD ± 31.8 µS; $\overline{X}$ sulfate = 70.3 ppm; n = 2 sites; SD ± 5.3 ppm) within the basin. *Cambarus theepiensis* ($\overline{X}$ CPUE = 2.4 crayfish/h; n = 5 sites; SD = 2.9 crayfish/h) and *O. cristavarius* ($\overline{X}$ CPUE = 9.6 crayfish/h; n = 5 sites; SD ± 8.2 crayfish/h) make up Levisa Fork's crayfish community of which *O. cristavarius* is more prevalent. Overall crayfish CPUE was 9.6 crayfish/h (n = 5 sites; SD ± 8.6 crayfish/h) for the Levisa Fork mainstem in the ULF-Levisa Fork.

A single site was sampled downstream of Fishtrap Reservoir (Site 1; Fig. 8; Table 4) in the Levisa Fork mainstem, located immediately downstream of Fishtrap Reservoir Dam. Here the Levisa Fork is channelized, and consists of a large moderate to low velocity run. Stream substrates were composed of silt, sand, small boulders and cobbles; QHEI score was 60. Conductivity (744 µS) and sulfate (167.2 ppm) levels were higher compared to sites maintaining *C. callainus* in the ULF-Levisa Fork ($\overline{X}$ conductivity = 335 µS; n = 2 sites; SD ± 31.8 µS; $\overline{X}$ sulfate = 70.3 ppm; n = 2 sites; SD ± 5.3 ppm). Notably, the only crayfish collected was *Orconectes juvenilis*, a species not native to the Levisa Fork, though native to the state of Kentucky in the Lower Kentucky River system (Taylor and Schuster 2004). This was the first time introduced *O. juvenilis* populations were recorded in the ULF-Levisa watershed. Two form I males were collected from under substrate debris in large eddies.

ULF-Levisa Fork tributaries (Sites 2-3, 5-8, 11; Fig. 8; Table 4) lacking *C. callainus* had similar physical habitat and physiochemical characters, and are treated as a pooled group. Mean tributary QHEI was 71.3 (n = 6; SD ± 7.1 QHEI). All tributaries possessed riffles and runs, with substrates composed of large cobbles, boulders, and slab boulders. Sedimentation issues were present in all ULF-Levisa Fork tributaries, ranging from moderate sedimentation (Sites 7-8; Fig. 8; Table 4) to complete concretion of the stream benthos (Site 2-3; Fig. 8; Table 4). In all streams, slab boulders were observed lacking interstitial spaces, and were concreted into the stream benthos. Both conductivity and sulfate levels ($\overline{X}$ conductivity = 367 µS; n = 6 sites; SD ± 108 µS; $\overline{X}$ sulfate = 102.5 ppm; n = 6 sites; SD ± 54.0 ppm) were higher than ULF-Levisa Fork streams harboring *C. callainus* ($\overline{X}$ conductivity = 335 µS; n = 2 sites; SD ± 31.8 µS; $\overline{X}$ sulfate = 70.3 ppm; n = 2 sites; SD ± 5.3 ppm). Crayfish were present in all ULF-Levisa Fork tributaries. *Cambarus theepiensis* ($\overline{X}$ CPUE = 12.0 crayfish/h; n = 6 sites; SD ± 12.0 crayfish/h) was dominant over *O. cristavarius* ($\overline{X}$ CPUE = 8.2 crayfish/h; n = 6 sites; SD ± 5.9 crayfish/h) in all tributaries. Overall average crayfish CPUE was 20 crayfish/h (n = 6 sites; SD ± 9.7 CPUE).

Comparison of sites with and without C. callainus - Sites with *C. callainus* (n = 39 sites; 25.2% of sites sampled) physical habitats differed compared to those lacking the species (n = 116 sites; 75.8% sites sampled). Slab boulders, boulder clusters, and isolated boulders, as well as fast moving runs and riffles, were present at all *C. callainus* sites. Furthermore, sites with *C. callainus* did not possess substrate concretion, and maintained open interstitial spaces. Zero *C. callainus* were observed at sites where slab boulders and boulders were concreted into the substrate. Several streams lacking *C. callainus* possessed slab boulders and isolated boulders; many, like those in downstream sub-watersheds in the Lower Levisa Fork lacked these habitats. A statistically significant difference was present between average QHEI scores between sites with and without *C. callainus* presence (U = 1,357.5; p = 0.0004; sig. < 0.05; 2 tailed); average QHEI was higher at sites with *C. callainus* present (QHEI = 71.4; n = 39 sites; SD ± 11.6 QHEI) compared to sites where the species was absent (QHEI = 65.0; n = 113 sites; SD ± 11.0 QHEI). Statistically significant differences were not present between sites with/without *C. callainus* for any physiochemical covariates.

Cambarus callainus site presence may have an effect on syntopic crayfish abundance, or vice versa. *Orconectes cristavarius* was present at a slightly higher percentage of *C. callainus* sites (n = 31 sites; 17.8% of sites sampled), compared to sites where *C. callainus* was absent (n = 99 sites; 17.2% of sites sampled). Average *O. cristavarius* CPUE values were highest at sites lacking *C. callainus* ($\overline{X}$ CPUE = 11 crayfish/h; n = 116 sites; SD = 16; Fig. 9) compared to those with the species ($\overline{X}$ CPUE = 7 crayfish/h; n = 39 sites; SD ± 11 crayfish/h); there was not a significant difference in *O. cristavarius* CPUE between sites with/without *C. callainus* (α = 0.05; $t_{(153)}$ = 1.6; p = 0.11). Average *C. theepiensis* CPUE was significantly higher (α = 0.05; $t_{(153)}$ =

2.1; p = 0.03) at sites lacking *C. callainus* ($\bar{X}$ CPUE = 1 crayfish/h; n = 39 sites; SD ± 2 crayfish/h) compared to those with species present ($\bar{X}$ CPUE = 3 crayfish/h; n = 119 sites; SD ± 6 crayfish/h; Fig.9). *Cambarus theepiensis* was present at a slightly higher percentage of sites lacking *C. callainus* (n = 116 sites; 62.2 %), compared to those with the species (n = 26 sites; 66.7%). Similar results were observed between *C. hatfieldi* and *C. callainus* ($\bar{X}$ CPUE *C. callainus* present = 3 crayfish/h; n = 119 sites; SD ± 6 crayfish/h; $\bar{X}$ CPUE *C. callainus* absent = 3 crayfish/h; n = 119 sites; SD ± 6 crayfish/h; Fig. 11). Overall average crayfish CPUE was significantly higher (α = 0.05; $t_{(153)}$ = 1.9; p = 0.05) at sites lacking *C. callainus* ($\bar{X}$ CPUE = 17 crayfish/h; n = 119 sites; SD ± 17 crayfish/h) compared to sites maintaining the species (Fig. 12).

Model selection and statistical analysis – *Cambarus callainus* was present at 39 of 153 sampling sites, including a total of 81 individuals captured per 1,530 seine hauls. For sampling basins, *C. callainus* was found at 2 of 15 sites in Levisa Fork of the upper Levisa Fork (n = 10 *C. callainus* captured), 21 of 39 sites in Russell Fork of the upper Levisa Fork (n = 44 *C. callainus* captured), and 16 of 65 sites in Tug Fork (27 *C. callainus* captured). . *Cambarus callainus* was not found in the lower Levisa Fork. Additionally, *O. cristavarius, C. hatfieldi,* and *C. theepiensis* were present at 128 (n = 1598 *O. cristavarius* captured), 54 (n = 373 *C. theepiensis* captured), and 57 (n = 354 *C. hatfieldi* captured) of the 153 sampling sites, respectively. For sampling basins, *O. cristavarius, C. hatfieldi,* and *C. theepiensis* were present at 31, 6, and 16 of 33 sites in lower Levisa Fork (n = 621, 37, and 101, respectively); 12, 1, and 12 of 15 sites in Levisa Fork of upper Levisa Fork (n = 89, 20, and 105, respectively); 33, 1, and 24 of 39 sites in Russell Fork of upper Levisa Fork (n = 475, 4, and 121, respectively); and 52, 46, and 5 of 65 sites in Tug Fork (n = 413, 312, and 27, respectively). *Cambarus callainus* was sympatric with *O. cristavarius, C. hatfieldi,* and *C. theepiensis* at 32, 11, and 14 sites, respectively.

Logistic regression model results - The Goodness of Fit (GOF) statistic supported a good fit of the global model to the data (Chi-Sq = 8.62, df = 8, P = 0.38), and an ROC curve (AUC = 0.84) supported high predictive accuracy for the selected model. Pearson correlation coefficients supported near collinearity between conductivity and salinity (r = 0.98), conductivity and TDS (r = 0.99) and salinity and TDS (r = 0.98), so we retained conductivity as a covariate, but did not use salinity and TDS as model covariates. We did not model %DO as a covariate because it had

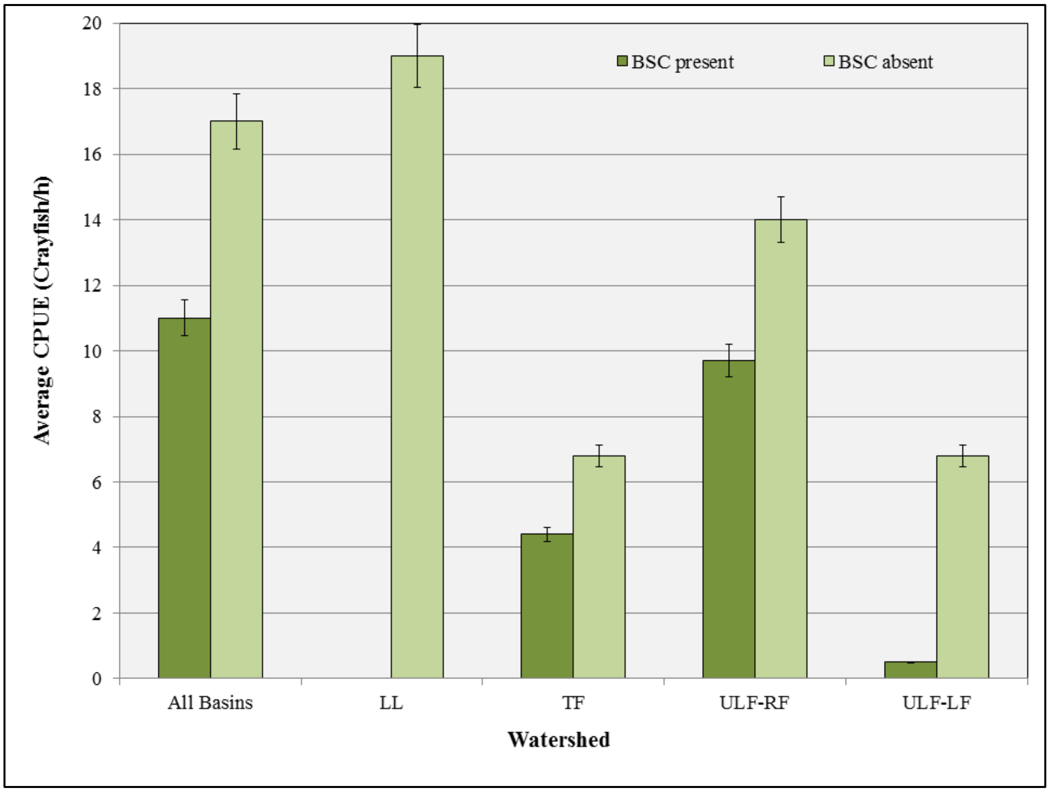


Figure 9: Mean overall *O. cristavarius* CPUE at sites where *C. callainus* was present or absent

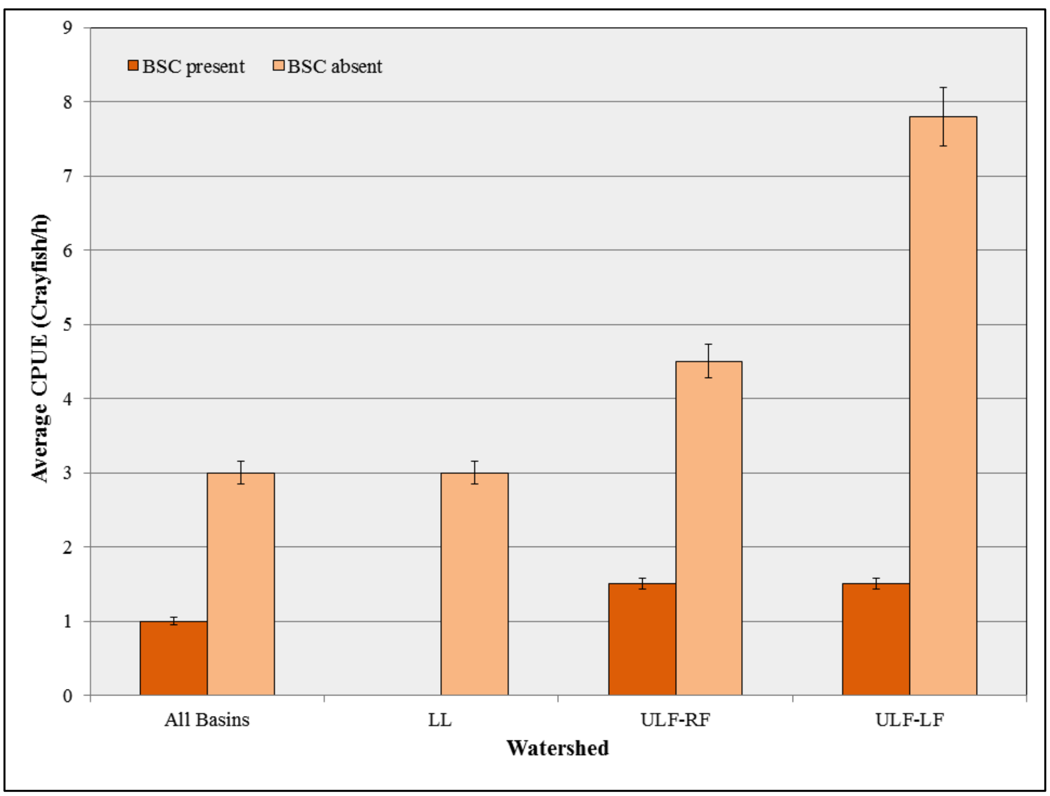


Figure 10: Mean overall *C. theepiensis* CPUE at sites where *C. callainus* was present or absent

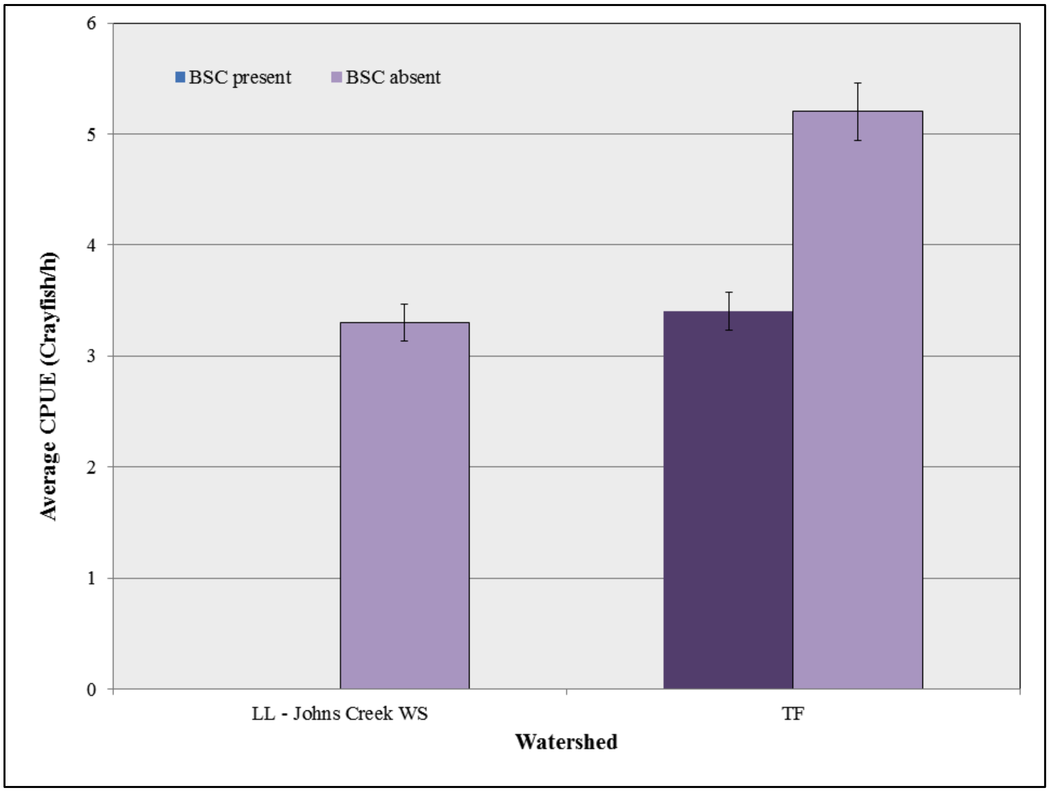


Figure 11: Mean overall *C. hatfieldi* CPUE at sites where *C. callainus* was present or absent

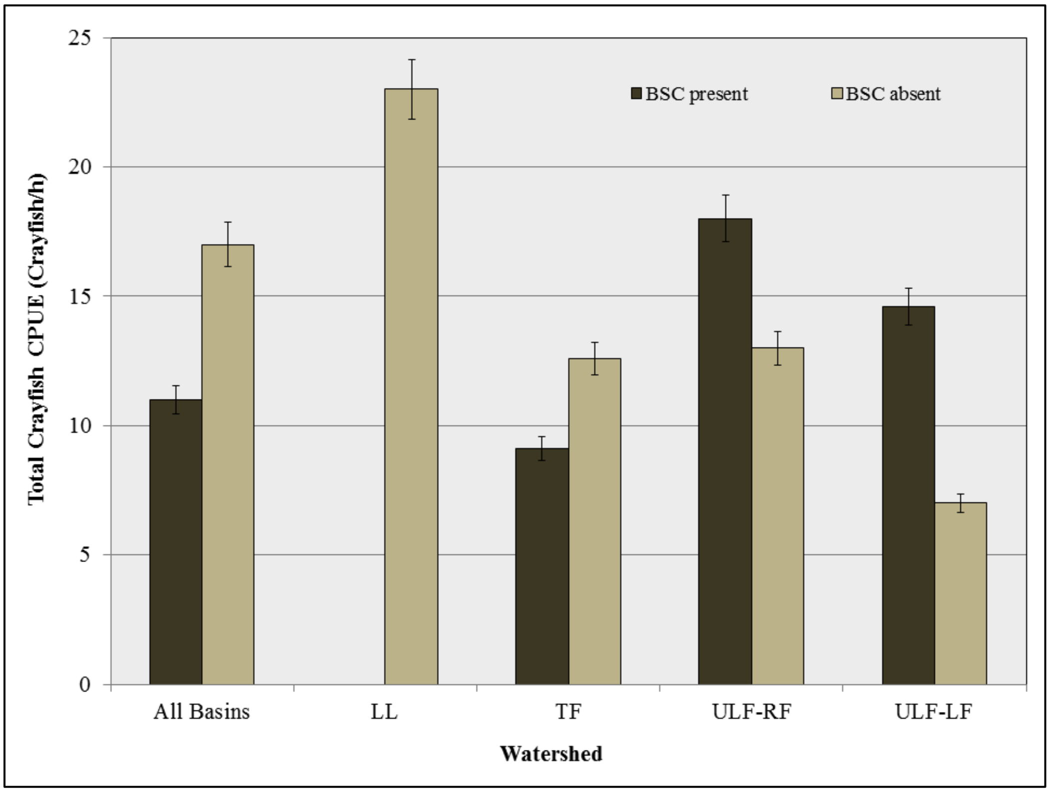


Figure 12: Mean overall crayfish CPUE at sites where *C. callainus* was present or absent

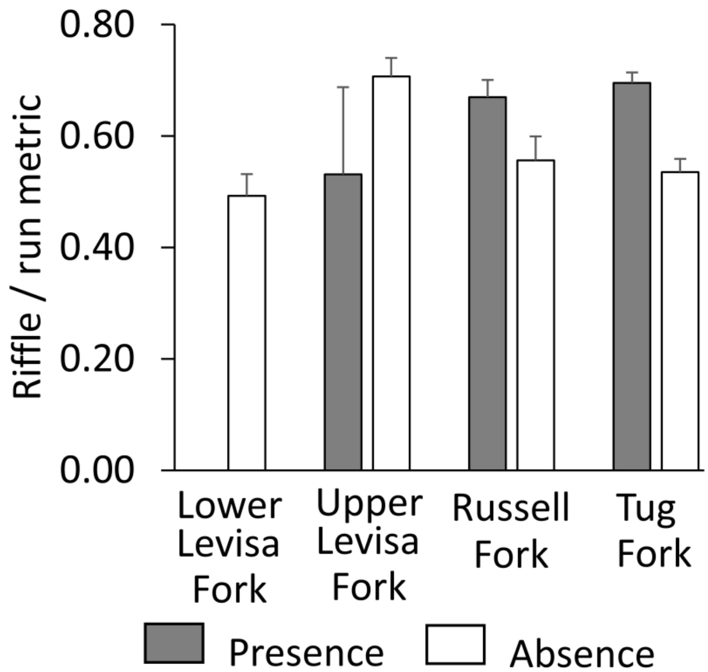


Figure 13: Mean ± SE of riffle/run metric scores by basin for sites with presence and absence of *C. callainus*.

little among-site variation (mean = 95.4, SD = 2.7).For the logistic regression analysis, data supported a single model: Basin + riffle/run metric (ΔAIC_c = 0.0, wt = 0.57; Table 5). Based on the logistic regression model, a positive beta value for the riffle/run metric was consistent with a higher mean value of that covariate at sites with species presence (Fig. 13). Mean values of covariates for sites with presence and absence of *C. callainus* aided interpretation of model results. Sites in Russell Fork and Tug Fork where *C. callainus* was present had higher mean ± SE values for the riffle/run metric than sites where the species was absent (Table 6; Table 7). However, *C. callainus* was present at only two of 15 sites in Levisa Fork of the upper Levisa Fork basin, and the mean riffle/run score was lower at these two sites than sites with species absence. Of the four basins, the lower Levisa Fork basin had the lowest mean riffle/run scores, and *C. callainus* was not found in the lower Levisa Fork basin.

Linear regression model results - Based on linear regression analysis, the CPUE data supported a single model: Basin + pool/current metric (ΔAIC_c = 0.0, wt = 0.60, Table 8). For this model, the beta estimate was positive for the pool/current metric covariate. Relationships for the

Table 5. Model selection statistics from logistic regression models of *Cambarus callainus* presence/absence data, including Akaike Information Criteria corrected for small sample size (AIC_c), the distances among models (ΔAIC_c), and AIC_c model weights (wt).

Model	AICc	ΔAICc	wt
Basin + riffle/run metric	138.7	0.0	0.57
Basin + QHEI	142.2	3.4	0.10
Basin + substrate metric	142.8	4.0	0.08
Basin + QHEI + SO_4	143.3	4.6	0.06
Basin + QHEI + elevation	143.4	4.6	0.06
Basin + QHEI + temp	143.4	4.7	0.05
Basin + QHEI + Cond	144.1	5.4	0.04
Basin + pool/current metric	145.0	6.2	0.03
Basin + instream cover metric	148.2	9.4	0.01
Basin + Riparian zone metric	149.5	10.7	0.00
Basin + gradient metric	150.3	11.6	0.00
Basin + water temperature	150.5	11.8	0.00
Basin + SO_4	151.1	12.4	0.00
Basin + channel morphology metric	151.4	12.6	0.00
Basin + *C. theepiensis* presence/absence	151.8	13.0	0.00
Basin + conductivity	151.8	13.0	0.00
Basin + elevation	151.8	13.1	0.00
Basin + *C. hatfieldi* presence/absence	151.9	13.2	0.00
Basin + O. *cristavarius* presence/absence	151.9	13.2	0.00
QHEI	160.0	21.2	0.00
QHEI + Cond	160.4	21.7	0.00
QHEI + temp	160.9	22.1	0.00
QHEI + elevation	161.4	22.7	0.00
QHEI + SO_4	161.8	23.1	0.00
Riffle/run metric	162.6	23.8	0.00
Substrate metric	164.2	25.5	0.00
Pool/current metric	165.5	26.8	0.00
Global model	165.9	27.2	0.00
Instream cover metric	170.5	31.8	0.00
Riparian zone metric	171.9	33.2	0.00
Elevation	174.8	36.1	0.00
Channel morphology metric	175.6	36.9	0.00
Gradient metric	175.9	37.1	0.00
Water temperature	176.4	37.7	0.00
Intercept	176.9	38.2	0.00
Conductivity	177.4	38.7	0.00
C. hatfieldi presence/absence	177.9	39.2	0.00
SO_4	178.5	39.8	0.00
C. theepiensis presence/absence	178.9	40.2	0.00
O. *cristavarius* presence/absence	178.9	40.2	0.00

Table 6. Mean values, standard errors (SE), and ranges of physical habitat and water quality variables from three basins (lower Levisa Fork, upper Levisa Fork, and Tug Fork) and sites with presence and absence of *Cambarus callainus*.

	All sites			*C. callainus* present			*C. callainus* absent		
Variable	mean	SE	Range	mean	SE	Range	mean	SE	Range
Lower Levisa Fork									
Conductivity (*u* S/cm)	477.7	45.5	87.0–949.0	*	*	*	477.7	45.5	87.0–949.0
Dissolved oxygen (%)	96.8	0.35	92.9–102.8	*	*	*	96.8	0.35	92.9–102.8
Elevation (m)	209.7	4.6	171.3–314.6	*	*	*	209.7	4.6	171.3–314.6
pH	8.0	0.05	7.6–8.8	*	*	*	8.0	0.05	7.6–8.8
QHEI	59.9	2.3	32.0–81.5	*	*	*	59.9	2.3	32.0–81.5
Salinity	0.23	0.0	0.04–0.47	*	*	*	0.23	0.02	0.04–0.47
SO_4	139.8	17.8	10.9–369.3	*	*	*	139.8	17.8	10.9–369.3
Total dissolved solids	310.4	29.6	57.6–617.0	*	*	*	310.4	29.6	57.6–617.0
Water temperature (°C)	22.1	0.30	18.5–25.4	*	*	*	22.1	0.30	18.5–25.4
Upper Levisa Fork - Levisa Fork									
Conductivity (*u* S/cm)	419.2	36.4	254.0–744.0	334.5	22.5	312.0–357.0	432.2	40.9	254.0–744.0
Dissolved oxygen (%)	94.3	0.28	92.2–96.4	94.6	1.85	92.7–96.4	94.2	0.24	92.2–95.8
Elevation (m)	393.0	24.1	217.6–558.7	444.2	41.0	403.3–485.2	385.1	26.9	217.6–558.7
pH	8.4	0.05	8.0–8.7	8.5	0.0	8.5–8.5	8.4	0.05	8.1–8.7
QHEI	72.0	1.9	60.0–85.5	73.0	7.0	66.0–80.0	71.8	2.1	60.0–85.5
Salinity	0.20	0.02	0.12–0.36	0.16	0.01	0.15–0.17	0.21	0.02	0.12–0.36
SO_4	104.6	13.4	46.0–218.0	70.3	3.8	66.5–74.0	109.9	14.9	46.0–218.0
Total dissolved solids	272.5	23.7	165.0–484.0	217.5	14.5	203.0–232.0	281.0	26.6	165.0–484.0
Water temperature (°C)	22.3	0.56	19.5–26.3	20.5	0.97	19.5–21.5	22.6	0.60	19.5–26.3
Upper Levisa Fork - Russell Fork									
Conductivity (*u* S/cm)	746.2	48.0	168.0–1555.0	689.0	55.4	206.0–1224.0	806.3	78.6	168.0–1555.0
Dissolved oxygen (%)	95.7	0.36	90.4–101.6	95.9	0.47	91.6–101.6	95.5	0.55	90.4–100.8
Elevation (m)	382.9	15.2	194.2–516.3	382.1	19.6	222.2–490.7	383.6	23.8	194.2–516.3
pH	8.2	0.02	8.0–8.5	8.2	0.03	8.0–8.5	8.3	0.03	8.0–8.5
QHEI	71.8	1.7	42.5–90.5	74.4	1.9	60.0–90.5	69.2	2.7	42.5–88.5
Salinity	0.36	0.02	0.08–0.79	0.34	0.03	0.10–0.61	0.39	0.04	0.08–0.79
SO_4	266.3	29.8	19.0–952.0	233.2	31.4	24.0–550.0	301.0	51.2	19.0–952.0
Total dissolved solids	485.4	31.2	109.0–1011.0	448.5	36.1	134.0–796.0	524.2	51.1	109.0–1011.0
Water temperature (°C)	22.5	0.38	17.1–27.3	22.7	0.43	19.3–27.3	22.4	0.63	17.1–26.2
Tug River									
Conductivity (*u* S/cm)	692.9	36.0	147.0–1667.0	727.9	65.6	392.0–1327.0	681.6	42.9	147.0–1667.0
Dissolved oxygen (%)	94.7	0.40	76.1–99.5	94.4	0.78	84.0–98.1	94.9	0.47	76.1–99.5
Elevation (m)	293.1	12.6	161.5–566.0	272.0	20.4	161.5–420.0	299.9	15.3	185.9–566.0
pH	8.2	0.04	6.3–9.0	8.4	0.06	8.1–9.0	8.2	0.05	6.3–9.0
QHEI	65.7	1.2	44.0–90.0	71.7	1.8	61.5–88.5	63.8	1.4	44.0–90.0
Salinity	0.35	0.02	0.07–0.85	0.37	0.04	0.19–0.66	0.34	0.02	0.07–0.85
SO_4	195.3	19.9	11.5–991.7	193.7	34.8	33.5–628.0	195.9	24.0	11.5–991.7
Total dissolved solids	449.6	23.4	96.0–1083.0	473.3	42.6	255.0–863.0	442.1	27.9	96.0–1083.0
Water temperature (°C)	21.2	0.23	15.9–25.4	22.0	0.34	19.0–24.1	20.9	0.27	15.9–25.4

Table 7. Mean values, standard errors (SE), and ranges of scaled (0–1) metric scores of the Qualitative Habitat Evaluation Index from three basins (lower Levisa Fork, upper Levisa Fork, and Tug Fork), and sites with presence and absence of *Cambarus callainus*

	All sites			*C. callainus* present			*C. callainus* absent		
Variable	mean	SE	Range	mean	SE	Range	mean	SE	Range
Lower Levisa Fork									
Channel morphology metric	0.59	0.02	0.25–0.8	*	*	*	0.59	0.02	0.25–0.8
Instream cover metric	0.66	0.03	0.30–0.95	*	*	*	0.66	0.03	0.30–0.95
Pool/current metric	0.50	0.02	0.17–0.75	*	*	*	0.50	0.02	0.17–0.75
Riffle/run metric	0.49	0.04	0.0–0.94	*	*	*	0.49	0.04	0.0–0.94
Riparian zone metric	0.47	0.02	0.20–0.75	*	*	*	0.47	0.02	0.20–0.75
Stream gradient metric	0.64	0.02	0.20–0.80	*	*	*	0.64	0.02	0.20–0.80
Substrate metric	0.68	0.05	0.0–1.0	*	*	*	0.68	0.05	0.0–1.0
Upper Levisa Fork - Levisa Fork									
Channel morphology metric	0.69	0.03	0.40–0.88	0.69	0.11	0.58–0.80	0.69	0.03	0.40–0.88
Instream cover metric	0.72	0.04	0.25–0.95	0.75	0.05	0.70–0.80	0.71	0.05	0.25–0.95
Pool/current metric	0.62	0.04	0.25–0.83	0.75	0.08	0.67–0.83	0.60	0.05	0.25–0.83
Riffle/run metric	0.68	0.04	0.38–0.94	0.53	0.16	0.38–0.69	0.71	0.03	0.50–0.94
Riparian zone metric	0.57	0.03	0.40–0.75	0.65	0.10	0.55–0.75	0.55	0.03	0.40–0.75
Stream gradient metric	0.75	0.02	0.70–0.90	0.70	0.00	0.70–0.70	0.75	0.02	0.70–0.90
Substrate metric	0.89	0.04	0.38–1.0	0.88	0.03	0.85–0.9	0.89	0.05	0.38–1.0
Upper Levisa Fork - Russell Fork									
Channel morphology metric	0.67	0.02	0.15–1.0	0.65	0.03	0.15–0.95	0.70	0.03	0.48–1.0
Instream cover metric	0.76	0.02	0.40–1.0	0.79	0.03	0.55–1.0	0.74	0.04	0.40–1.0
Pool/current metric	0.63	0.03	0.25–1.0	0.67	0.03	0.50–1.0	0.58	0.05	0.25–1.0
Riffle/run metric	0.61	0.03	0.0–0.88	0.67	0.03	0.38–0.88	0.56	0.04	0.0–0.81
Riparian zone metric	0.57	0.02	0.25–0.80	0.56	0.04	0.25–0.80	0.58	0.03	0.25–0.80
Stream gradient metric	0.72	0.02	0.30–1.0	0.73	0.03	0.30–1.0	0.72	0.02	0.50–0.90
Substrate metric	0.86	0.02	0.25–1.0	0.89	0.03	0.55–1.0	0.84	0.04	0.25–1.0
Tug Fork									
Channel morphology metric	0.60	0.01	0.35–0.85	0.67	0.02	0.50–0.85	0.58	0.01	0.35–0.78
Instream cover metric	0.71	0.02	0.35–1.0	0.76	0.04	0.35–1.0	0.70	0.02	0.40–1.0
Pool/current metric	0.57	0.02	0.25–1.0	0.64	0.04	0.33–1.0	0.55	0.02	0.25–1.0
Riffle/run metric	0.57	0.02	0.0–0.88	0.70	0.02	0.63–0.88	0.54	0.02	0.0–0.81
Riparian zone metric	0.49	0.02	0.20–0.80	0.56	0.03	0.40–0.80	0.47	0.02	0.20–0.80
Stream gradient metric	0.69	0.01	0.50–0.9	0.67	0.01	0.60–0.70	0.69	0.01	0.50–0.90
Substrate metric	0.80	0.02	0.20–1.0	0.90	0.02	0.80–1.0	0.76	0.03	0.20–1.0

Table 8. Model selection statistics from linear regression models of *Cambarus callainus* CPUE data, including Akaike Information Criteria corrected for small sample size (AIC_c), the distances among models (ΔAIC_c), and AIC_c model weights (wt).

Model	AICc	ΔAICc	wt
Basin + pool/current metric	-259.4	0.0	0.60
Basin + QHEI	-255.5	3.9	0.09
Basin + QHEI + SO_4	-254.1	5.2	0.04
Basin + QHEI + Cond	-253.5	5.9	0.03
Basin + QHEI + temp	-253.4	6.0	0.03
Basin + QHEI + elevation	-253.3	6.0	0.03
Basin + riffle/run metric	-253.1	6.3	0.03
Basin + *C. theepiensis* CPUE	-253.1	6.3	0.03
Pool/current metric	-252.8	6.5	0.02
Basin + instream cover metric	-252.3	7.0	0.02
QHEI + elevation	-252.1	7.3	0.02
QHEI	-251.6	7.7	0.01
Basin + Riparian zone metric	-250.7	8.6	0.01
Basin + substrate metric	-250.1	9.2	0.01
QHEI + Cond	-249.7	9.6	0.00
QHEI + temp	-249.5	9.8	0.00
QHEI + SO_4	-249.5	9.8	0.00
Basin + channel morphology metric	-249.4	10.0	0.00
Basin + O. *cristavarius CPUE*	-249.2	10.2	0.00
Global model	-249.0	10.3	0.00
Basin + *C. hatfieldi* CPUE	-248.9	10.4	0.00
Basin + SO_4	-247.8	11.6	0.00
Basin + conductivity	-247.2	12.1	0.00
Basin + elevation	-247.1	12.2	0.00
Basin + water temperature	-247.0	12.4	0.00
Riffle/run metric	-244.6	14.8	0.00
Basin + gradient metric	-244.4	14.9	0.00
Instream cover metric	-243.8	15.6	0.00
Riparian zone metric	-242.8	16.6	0.00
Substrate metric	-242.2	17.2	0.00
Elevation	-241.6	17.8	0.00
Channel morphology metric	-240.6	18.8	0.00
C. hatfieldi CPUE	-237.7	21.6	0.00
O. *cristavarius* CPUE	-237.2	22.1	0.00
C. theepiensis CPUE	-237.0	22.3	0.00
Intercept	-236.1	23.2	0.00
Conductivity	-234.4	24.9	0.00
Water temperature	-234.4	25.0	0.00
SO_4	-234.1	25.2	0.00
Gradient metric	-232.4	26.9	0.00

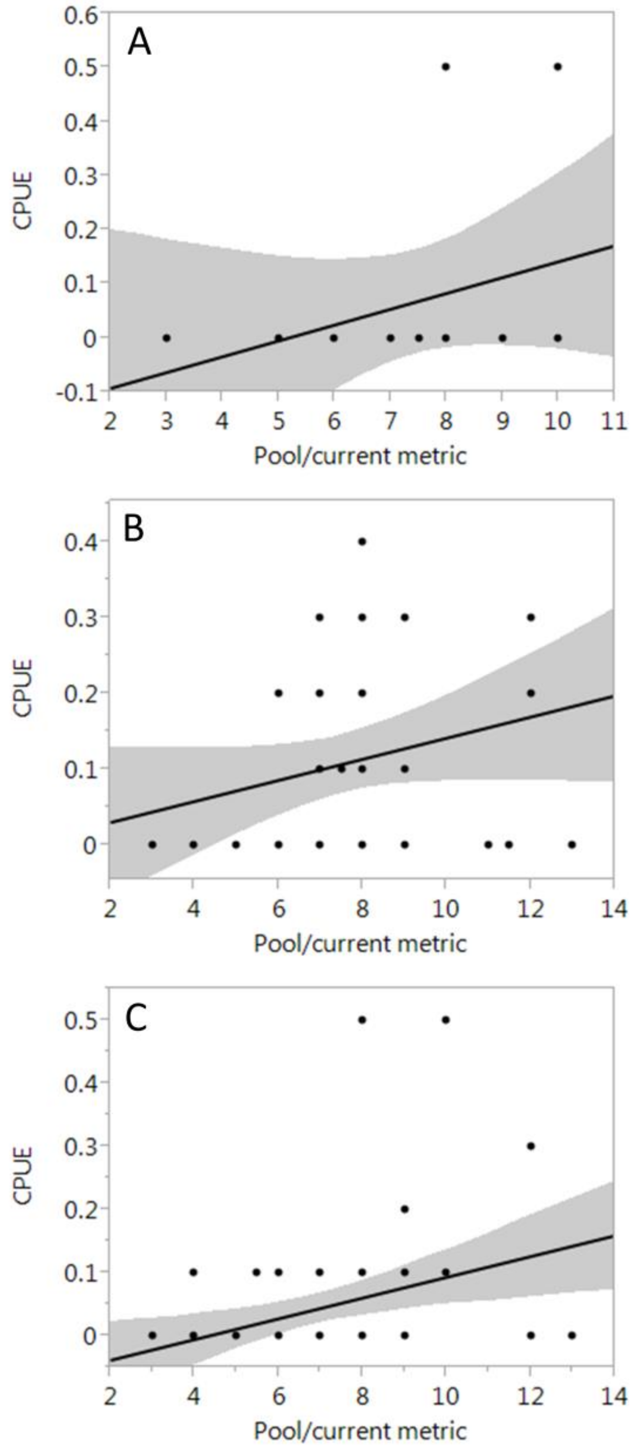


Figure 14. Relationships of the pool/current metric score and *C. callainus* CPUE for (A) the Levisa Fork basin of the upper Levisa Fork, (B) the Russell Fork basin of the upper Levisa Fork, and (C) the Tug Fork basin.

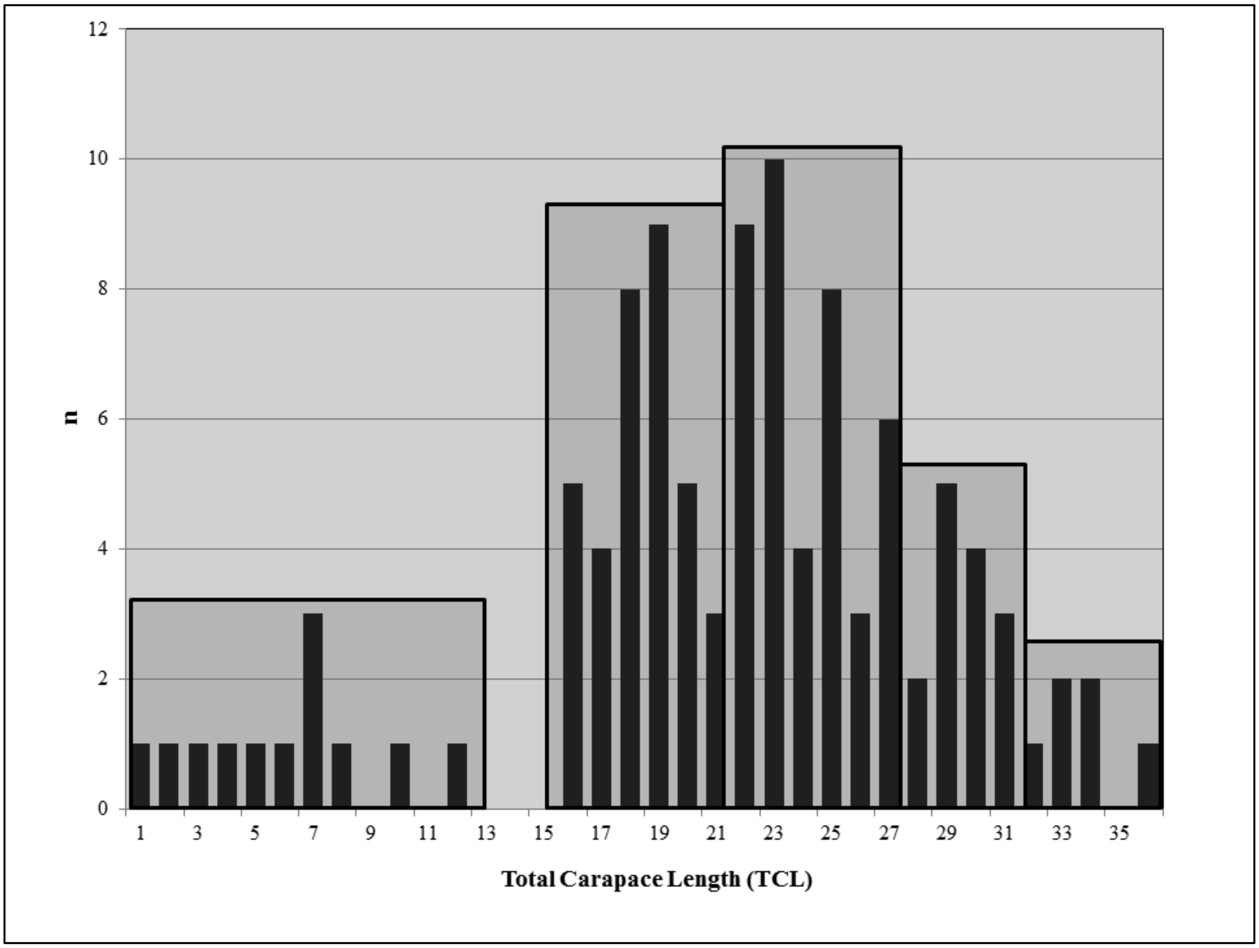


Figure 15: *Cambarus callainus* size histogram. Shaded boxes indicate possible size cohort.

pool/current metric vs. CPUE were similar for the Levisa Fork and Russell Fork basins of the upper Levisa Fork drainage and the Tug Fork basin (Fig. 14).

Cambarus callainus *life history - 2015* – One hundred six *C. callainus* (11 I♂; 40 II♂; 55 ♀) were captured in 23 streams in the ULF-Russell Fork, ULF-Levisa Fork, and Tug Fork Rivers. Individual site CPUE values are presented in Tables 2-4 for each respective basin. It is important to note that additional collecting was performed in select creeks following each site's 10 seine hauls in order to collect additional animals and subsequent life history information; these values do not match values depicted in Tables 2-4, which are reflective of the number of *C. callainus* captured during each site's respective 10 seine hauls used for standardized CPUE determination.

Captured *C. callainus* exoskeletons in June and July exhibited varying degrees of encrustation and biological fouling. Given carapace encrustation/fouling level inconsistencies between sites, determination of a possible mass molt within the greater populations was impossible for the spring/early summer months. Animals collected in Prater Creek at Haysi, Dickenson County, Virginia, on 10, September 2015 all exhibited clean exoskeletons devoid of encrustation or biological fouling. This single population had undergone a mass molt within the weeks prior to early September. It is not known if other populations experience a similar molt *en masse*.

Thoma et al. (2014) reported on the life history of all animals used in their analysis to describe the species, and indicated the majority of males collected in September, October, and November were all Form I, lending support to the possibility that a mass molt does occur within *C. callainus* in late summer during which, males molt from Form II to Form I. Further evidence for this occurring is the ratio for Form II males to Form I males for 2015 collections, which was skewed 3.6:1 in favor of form II males. Collections for the current study took place in late June and early July. Zero ovigerous females were collected, which corresponds to observed egg extrusion dates presented by Thoma (2009; 2010). Fifty two percent of adult females collected in late June and early July exhibited active glare glands. Sex ratio between males and females was almost equal at 1.0:1.1. All demographics were most frequently encountered under large slab boulders and boulders in fast moving runs and riffles with open interstitial spaces. Collections in 2015 determined five possible size cohorts (Cohort #1 = 15.0 mm-26.0 mm; Cohort #2 = 16.0 mm- 21.0 mm; Cohort #3 = 22.0 mm-27.0 mm; Cohort #4 = 28.0 mm-31.0mm; Cohort #5 = 32.0 mm -36.0 mm) were present within both populations when data was pooled for all *C. callainus* sites (Fig. 15). Average TCL was 36.0 mm (n = 106; SD ± 7.3 mm). Form I males were the largest sex, with an average TCL of 42.9 mm (n = 11; SD ± 4.6 mm). Female ($\bar{X}$ = 36.3; n = 55; SD ± 7.6 mm) and form II male ($\bar{X}$ = 33.6; n = 40; SD ± 6.2 mm) average TCL was similar, and close to that of the overall population.

DISCUSSION

Thoma (2009; 2010) was the first to perform dedicated surveys for *C. callainus* across its range in Kentucky and Virginia, though Taylor and Schuester (2004) were the first to critically review the species distribution within the state. Loughman and Welsh (2013) were the first to document *C. callainus* presence within West Virginia. Thoma's (2009) ULF-Russell Fork results were corroborated with this effort. All recent ULF-Russell Fork streams surveyed by Thoma (2009; 2010) surveyed in the current effort maintained *C. callainus* populations. Populations were recorded in the ULF-Russell Fork for the first time in Caney Creek, Frying Pan Creek, and Indian Creek, as well as within all major tributaries to either the Russell Fork or McClure Rivers. Thoma's (2009) ULF-Levisa Fork results determined *C. callainus* was limited to the Levisa Fork mainstem and Dismal Creek. Dismal Creek was the only stream in which *C. callainus* was observed in the ULF-Levisa Fork watershed during 2015. Efforts to find *C. callainus* in the Levisa Fork mainstem resulted in zero *C. callainus* captures. Both Thoma (2009; 2010) and Loughman and Welsh (2013) surveyed the Tug Fork; Thoma (2009; 2010) focusing on Kentucky and Virginia populations, Loughman and Welsh (2013) focusing on West Virginia. All recent recordings by both previous efforts were extant in 2015. *Cambarus callainus* was reported for the first time at five sites in West Virginia, most notably from the greater Pigeon Creek and Panther Creek watersheds. Furthermore, *C. callainus* was collected from the Tug Fork mainstem near Crum, Wayne County, West Virginia, the farthest downstream the species has been collected in the Tug Fork drainage.

Taylor and Schuster (2004) were the first to record *C. callainus* from the Lower Levisa Fork, reporting the species from the Levisa Fork mainstem in Auxier, Floyd County, Kentucky. Thoma (2010) collected the species from the Levisa Fork at Auxier again in 2009, and at an additional, previously undocumented, Levisa Fork location in Pikeville, Pike County. Concerted efforts undertaken with the current effort across the Lower Levisa Fork basin were unable to recover any *C. callainus* at both historic locations, and at additional semi-random sites. Zero *C. callainus* were collected from the Levisa Fork mainstem including both the Upper Levisa Fork and Lower Fork watersheds. Concretion rates were high at all Levisa Fork sites. Overall crayfish CPUE values were markedly low within the Levisa Fork mainstem for *Cambarus* spp., specifically.

Gradient within the Lower Levisa Fork watershed decreases in the Georges Creek, lower Mud Creek, and Johns Creek watersheds, resulting in increased sand and decreased boulders in stream benthos throughout these watersheds. These conditions likely are at the extreme of *C. callainus*' preferred habitat, and could potentially preclude *C. callainus* from these sub-watersheds naturally. However, the Levisa Fork mainstem does maintain several riffles with slab boulders and boulder clusters, well within the known ecological theater of *C. callainus,* and likely historically represented the core *C. callainus* population within the Lower Levisa Fork. As stated, *C. callainus* was not observed in the Levisa Fork mainstem in the Lower Levisa Fork watershed in 2015.

Modeling and linear regression analyses results indicated that physical habitat quality has a higher impact on *C. callainus* site presence than physiochemical attributes. Conductivity, pH, sulfate, and dissolved oxygen all were relatively equal at sites both possessing and lacking *C. callainus*. Regarding physical habitat specifically, the only selected logistic regression model was the additive model Basin+Riffle/Run, indicating that riffle run quality was not equal across all watersheds. Riffle Run subscores are resultant of (1.) riffle depth, (2.) run depth, (3.) substrate stability and rock size classes, and (4.) substrate embeddedness. Loughman (2014), as well as Thoma (2009; 2010) proposed that *C. callainus* populations are dependent on the presence of large slab boulders with open interstitial spaces that are not concreted into the stream benthos.

Model presence selection results from this study support this hypothesis, indicating that stable, non-embedded boulders and slabs are associated with *C. callainus* occupancy of stream reaches within the ULF-Levisa Fork, ULF-Russell Fork, and Tug Fork River watersheds. Also supported by the model was the impact of sedimentation; stream reaches with elevated sedimentation rates lacked *C. callainus*, indicating that stream sedimentation likely represents the most pressing threat for *C. callainus* populations. Modeling results were further supported by field observations. Slab boulders, riffles and fast moving runs were present at all sites in which *C. callainus* was observed in this study; zero animals were observed in concreted, heavily sedimented stream reaches sampled with this effort.

Cambarus callainus presence or absence appears to have an effect on both the composition and density of syntopic crayfish species. *Orconectes cristavarius* was the most prolific crayfish species in all watersheds. In the absence of *C. callainus*, at sites that supported crayfish populations, *O. cristavarius* density was higher at sites lacking C. callainus compared to sites maintaining *C. callainus*. The same result was observed with *C. theepiensis* in the Lower Levisa, ULF-Levisa Fork, and Russell Fork watersheds, as well as *C. hatfieldi* in the Tug Fork watershed. *Orconectes cristavarius* density actually increased as physical habitat quality decreased. Why this occurs is unknown, but possible explanations include increased nutrient inputs associated with stream degradation leading to higher forage availability, lack of competition with larger *Cambarus* species precluded by environmental degradation, or a synergistic interaction of both explanations.

Cambarus theepiensis and *C. hatfieldi* exhibit high overlap in habitat preference for *C. callainus'* preferred refugia (large slab boulders and boulders), and in the absence of *C. callainus*, occupy these habitats at higher densities than those observed when *C. callainus* is present. Loughman (2014) proposed that sympatric crayfish species could exclude *C. callainus* from reinvasion of sites where extirpation occurred, via competitive inhibition. Results of the current study support the hypothesis of a competitive exclusion effect existing within the Greater Big Sandy basins' crayfish assemblage. How habitat degradation leads to the loss of *C. callainus*' competitive ability in this ecological continuum is not known, and is an area of needed research if *C. callainus* conservation is going to be efficient and effective.

Work Cited

Burskey, J. L. & T. P. Simon, 2010. Reach- and watershed-scale associations of crayfish within an area of varying agricultural impact in west-central Indiana. Southeastern Naturalist 9 (Special Issue 3): 199–216.

Gazendam, E., B. Gharabaghi, F. C. Jones & H. Whiteley, 2011. Evaluation of the Qualitative Habitat Evaluation Index as a planning and design tool for restoration of rural Ontario waterways. Canadian Water Resources Journal 36: 149–158.

Jezerinac, R.F., G.W. Stocker, and D.C. Tarter. 1995. The Crayfishes (Decapoda:Cambaridae) of West Virginia. Bulletin of the Ohio Biological Survey, New Series 10:1–193.

Loughman, Z. J. 2013. Rediscovery of *Cambarus veteranus* (Big Sandy Crayfish) in West Virginia with a discussion of future conservation needs within the state. Report submitted to the West Virginia Division of Natural Resources and the United States Fish and Wildlife Service Elkins West Virginia Field Office. Pp. 17.

Loughman, Z. J. 2014. Biological Status Review of *Cambarus veteranus* for U. S. Fish and Wildlife Service. Review prepared for United States Fish and Wildlife Service. 49 pp.

Loughman, Z. J., S. A. Welsh, J. W. Fetzner, and R. F. Thoma. 2015. Conservation of Imperiled Crayfish, Cambarus veteranus (Decapoda: Reptantia: Cambaridae). Journal of Crustacean Biology. DOI: 10.1163/1937240X-00002383. 1-11.

Ohio Environmental Protection Agency, 2006, Methods for assessing habitat in flowing waters—using the Qualitative Habitat Evaluation Index (QHEI), Division of Surface Water, Ecological Assessment Section, Groveport, OH. http://www.epa.state.oh.us /portals/ 35/ documents/ qheimanualjune2006. pdf

Rankin, E. T., 1995. Habitat indices in water resource quality assessments. Pp. 181–208. In Davis, W. S. & T. P. Simon (eds), Biological Assessment and Criteria. Lewis Publishers, Boca Raton, FL: 415.

Taylor, C. A. and G. A. Schuster. 2004. Crayfishes of Kentucky. Illinois Natural History Survey Bulletin 28; 219 pp.

Thoma, R. F. 2009. The conservation status of *Cambarus* (*Puncticambarus*) *veteranus*, Big Sandy Crayfish; *Cambarus* (*Jugicambarus*) *jezerinaci*; Spiny Scale Crayfish; and *Cambarus* (*Cambarus*) sp. A; BlueRidge Crayfish. Final project report submitted to the Virginia Department of Game and Inland Fisheries. Pp. iv + 20.

Thoma, R. F. 2010. The conservation status of *Cambarus* (*Puncticambarus*) *veteranus*; Big Sandy Crayfish and *Cambarus* (*Jugicambarus*) *parvoculus*; Mountain Midget Crayfish in Kentucky. Final project report submitted to the Kentucky Department of Fish and Wildlife Resources. Pp. iii + 9.

Thoma, R. F., Z. J. Loughman, and J. Fetzner. 2014. A new species of crayfish (Decapoda: Cambaridae) from the Big Sandy River basin in Kentucky, Virginia, and West Virginia, USA. Zootaxa 3900(4); 541–554. DOI: dx. doi. Org / 10.11646 /zootaxa. 3900.4.5

Welsh, S. A. and Z. J Loughman. 2014. Physical habitat and water quality correlates of crayfish distributions in a mined watershed. Hydrobiologia 745: 85-96. DOI: 10.1007/s10750-014-2095-y