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FIELD EXPERIMENTAL APPROACH TO DETECT URBAN IMPACT ON *ERPETOGOMPHUS DESIGNATUS* HAGEN *IN* SELYS LARVAE (ANISOPTERA: GOMPHIDAE)

M. MAHATO* and J.H. KENNEDY
Department of Biological Sciences, University of North Texas, Denton, TX 76203, United States

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This study attempted to design and conduct an in situ field experiment using *E. designatus* larvae collected from the reference site and then exposed at 4 potentially impacted urban sites; all in the sub-watersheds of the city of Denton, Texas, USA. Before placing them in the urban site enclosures, head width, total width, wing pad length and wet weight were measured. The surviving larvae were retrieved after 6 weeks and all parameters were measured again in order to assess the difference between the reference and urban sites. No survival was observed at 2 urbanized sites in both spring and summer, and at another urbanized site in spring. The differences in survival of the larvae may be influenced by the differences in hydrology and water quality, especially during the summer experimental period. In the spring, a statistically significantly higher growth rate ($p < 0.05$) occurred at one of the urban sites compared to the reference site. The difference in growth rate may have been influenced by less fluctuation and higher minimum water temperature at the urban site. Although the experiment was only partially successful, it did indicate that the local common odon. taxa found at the reference site could be used for field biomonitoring experiments to assess water quality of urban sites. If fully successful, this type of in situ field experiment may indicate actual impacts rather than attempting to apply conclusions based on either laboratory microcosm or mesocosm-based toxicity tests.

INTRODUCTION

Macroinvertebrate species are widely used for ecotoxicological assessments of sites suspected of being impacted by environmental contamination. Over 50 types of macroinvertebrate species are routinely used for ecotoxicological assessments (BUIKEMA & VOSHELL, 1993). In the 1970s, researchers began to evaluate

* **Current address**: URS Corporation, Dallas, TX 75234, United States; – Mmahato@gmail.com

biological effects of synthetic as well as natural chemicals through laboratory experiments, also known as toxicity tests (BUIKEMA et al., 1982). In ecotoxicology, biologists experimentally expose test organisms to contaminated water or sediments in the laboratory to create dose-response curves for individual chemical toxicants. Laboratory derived single species toxicity tests are an efficient and economical way to assess environmental contamination. However, concerns have been expressed when applying the laboratory based criteria to *in situ* situations (CAIRNS, 1983; KIMBALL & LEVIN, 1985).

LaPOINT & PERRY (1989) state that requirements for ecosystem-level testing have developed because we have not fully understood the implications of potential damage to resources without having evaluations of the predicted impacts under field conditions. BUIKEMA & VOSHELL (1993) suggested that well replicated controlled field experiments may provide important insights into the structure and function of ecological systems. Since laboratory bioassays have the potential to be limited, mesocosms using experimental manipulations have been developed in recent years (GRANEY et al., 1994). In general, mesocosms are outdoor model aquatic systems which allow contaminant manipulation while still being subjected to natural conditions. Although mesocosms are generally more complex than traditional laboratory toxicity tests, they may serve as a bridge between laboratory toxicity tests and field bioassessments. However, mesocosms are still much less complex than the natural environment. Hence, conclusions based on mesocosm studies should only be applied to the real world with caution (CROSSLAND, 1994).

Experiments conducted in field conditions are the only way in which the actual impact of water quality on the aquatic ecosystems may be assessed. However, it is difficult to design and conduct single-species impact experiments in natural systems, especially if the system in question is lotic. It is even more challenging to conduct experiments in intermittent streams where flooding and dryness are common. Recent attempts have been made to conduct field toxicological evaluation of impacted aquatic systems (COURTNEY & CLEMENTS, 2000 & 2002; CLEMENTS et al., 2002; BURTON et al., 2005; PETTIGROVE & HOFFMANN, 2005). In those experiments, toxicity was assessed using benthic macroinvertebrate communities rather than a single species. Generally, single species field test designs have yet to be widely developed. As in microcosm and mesocosm evaluations, a single species test design requires a reference site for comparisons. The majority of urban streams of the southern United States are intermittent, yet if they are effluent dominated, they may be considered as permanent streams. However, minimally impacted reference sites with a similar hydrological regime are rarely found for comparison. Selection of the appropriate test organism can also be a challenging task.

Depending upon the objectives of the assessment, several factors should be considered when selecting a test organism. Sensitive taxa should be selected if the objective is to assess survival rate. To test chronic effects, test organism should be able to survive within the impacted environment long enough to observe endpoint in question. Organisms should therefore be selected when considering the accepted polluted tolerance limits and should preferably show a gradation of sensitivity to prevailing conditions (CAIRNS & PRATT, 1989). The use of indigenous macroinvertebrates from a reference site that has a similar hydrologic regime is advantageous because the organisms are already acclimated to a somewhat similar physical environment. Since minimally impacted reference sites with similar hydrological regimes are rarely available, very sensitive test organisms may not be available to be used for acute impact tests. In the absence of very sensitive taxa, relatively common taxa from a reference site may be used for chronic effect testing at impacted sites.

Dragonflies are predatory benthic organisms and play an important role in aquatic ecosystems. They are large in size compared to many other macroinvertebrate insects and could be a useful test organism for toxicological evaluation. Dragonflies are considered to be relatively tolerant to pollution, but very little field or laboratory data are available regarding impact evaluation for this group of organisms. In Europe, some attempts have been made to use dragonflies as bioindicative species of the environment's status (DAVID, 1998). Currently, *Erythemis simplicicollis* (Libellulidae) are being cultured for toxicological tests and are being exposed to different concentrations of heavy metals (cadmium and copper) in laboratory testing at the University of South Alabama (GERTZ et al., 2004). These researchers have found that the dragonfly larvae possess a higher tolerance of cadmium and copper than do many other commonly used aquatic test species.

An in situ field experiment study was conducted by using *Erpetogomphus designatus* larvae to observe any adverse effect on its survival and growth at urban sites. *E. designatus* is a common species found in North America (NEEDHAM et al., 2000), in the Trinity River watershed (ABBOTT, 1999), and also in the selected reference site. This species is moderately tolerant to organic pollution (USEPA, 1999). The USEPA (1999) has assigned a value 4 on a scale of 1 (very sensitive) to 10 (very tolerant to organic pollution) for this genus. However, for Texas a tolerance value of 1 is assigned for this genus by DAVIS (1997). *E. designatus* may therefore represent a good test organism to study the effect of urban water quality on mortality and growth rate.

METHODS

SITE SELECTION – This study was conducted in sub-watersheds of the City of Denton, Texas. The City of Denton is located in the North Central Texas area, approximately 50 kilometers north of the City of Dallas. The evaluated sub-watersheds are relatively small, and are all within the larger Trinity River watershed. Sites were selected at Cooper, Pecan and Hickory Creeks, and a reference site was established at Clear Creek (Fig. 1). The Cooper Creek watershed is the smallest of the selected watersheds and lies in the northern part of the city draining approximately 27 square kilometers, including some of the urban sections of the City of Denton. The greatest volume of water in this watershed originates from residential areas. The Hickory Creek watershed is the largest of the selected watersheds draining approximately 140 square kilometers to the west and northwest of the city. The drainage area for this watershed is predominately rural; however, the stream receives discharge from the City of Krum Waste Water Treatment Plant (WWTP) and also includes discharges from portions of I-35, a golf course, and commercial properties. The Pecan Creek watershed drains an area of 59 square kilometers and includes the most urbanized section of the city. The drainage area includes residences, commercial and light industry, as well as portions of I-35, Texas Woman's University, and a golf course. This watershed also receives discharge from the Pecan Creek Water Reclamation Plant. Field experiments were carried out in spring and summer 2003. A pilot study was carried out in early spring (March 2, 2003 to April 20, 2003) at a reference site and a site in Pecan Creek downstream from the Pecan Creek Waste Water Reclamation Plant.

ENCLOSURE DESIGN – Experiments were conducted using 15 cm diameter, 13 cm tall PVC cylinders stacked between two steel racks (0.6×0.9 M) (Fig.2). It was assumed that these would provide enough space for one single early instar larva to move and to capture prey colonized in the enclosures. Eight holes of 2.54 cm diameter were drilled in middle of each cylinder to allow water circulation. Even though enclosures were completely submerged, water circulation is necessary to reduce any stress on the specimens due to available gas exchange per unit volume (PETERSEN et al.,

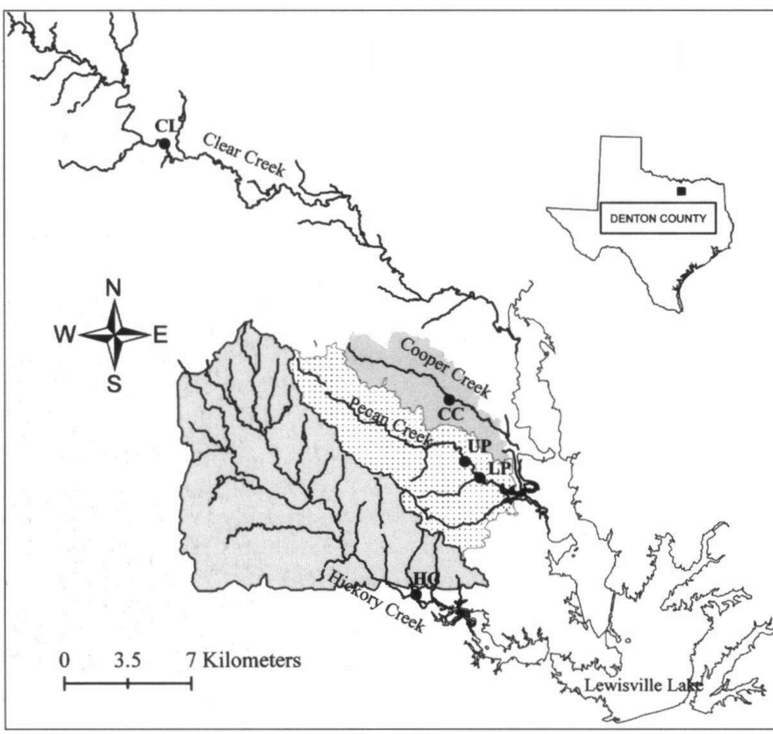


Fig. 1. Map of the city of Denton sub-watershed, Denton co., Texas, showing stream tributaries and experimental locations. – [CL = Clear Creek; – CC = Cooper Creek; – HC = Hickory Creek; – LP = Lower Pecan Creek; – UP = Upper Pecan Creek]

1999). Each hole of the cylinder was covered with 500 micron-mesh nylon outer screening to allow colonization of invertebrates to serve as prey for the dragonfly larvae and to prevent invasions from larger predators. The bottom and top of the cylinders were covered with clear plastic bucket lids for light exchange. The top lids also had a few holes with 500 micron screens. Due to excessive sedimentation in the spring (high rainfall period), the top of the cylinders were covered by lids with no screens in the summer. A 6 mm mesh plastic net was placed inside of the top and bottom part of the metal rack to block large debris. In each enclosure, 1.5 cm of sand substrate and dried detritus material were added to provide substrate for benthic invertebrate colonization.

FIELD EXPERIMENT – At each site, one set of racks containing 15 replicated enclosures was deployed and anchored for invertebrate colonization. After two weeks of deployment, one *Erpetogomphus designatus* larva was added to each enclosure. Larvae were collected from the reference site with a dip net a few days in advance of transfer. After collection, dragonfly larvae were brought into the laboratory for initial measurements. Total length, wing pad length, head width, and wet weight were measured for each collected larva. Out of the all collected larvae, smaller (head width <0.2 cm) and larger (head width >0.4 cm) specimens were discarded and were returned back into the reference site. Similar sized specimens, based on head width, were proportionally distributed in each site enclosure

Fig. 2. *Erpetogomphus designatus* larvae field enclosure experimental design: (a) enclosure assembly; – (b) enclosure with larvae in the water at Lower Pecan Creek site; – (c) dismanteling of the enclosure for larvae retrieval; – (d) retrieving larvae from the enclosure; – (e) retrieved larvae placed in separate container for measurements; – (f) *E. designatus* exuviae; – (g) emerged adult *E. designatus* from the laboratory rearing tank.

for this experimental study. Each measured larvae was kept separate from other larvae. At each site, enclosures were opened and larvae and a label were placed in each cylinder. Enclosures were checked weekly and the screen was cleaned with a soft brush to maintain water circulation. After six weeks, all enclosures were retrieved from the water, and dragonfly larvae were removed if found alive and brought to the laboratory for measurement. The timeframe was based on recommendation by GRICE (1984) who stated that an enclosure for impact assessment research with more than two interacting trophic levels can be maintained for periods up to six or more weeks. Larvae were also reared in the laboratory for species verification. Emerged adults and exuviae were verified by Dr John Abbott (University of Texas at Austin, TX).

During the experimental period, water chemistry data (dissolved oxygen and temperature) were collected at half hour intervals by in situ hydrolabs maintained at each site by the City of Denton under the Watershed Protection Program. Even though instruments were regularly checked, some data from the Pecan Creek sites were lost due to an unexpected flood event which resulted in an instrument malfunction.

DATA ANALYSIS – *E. designatus* larvae survival and mortality data during each experimental period were tabulated for site and seasonal comparisons. Even though proportionally similar-sized specimens were used in each enclosure, there was high variation in mean sizes because of un-proportional mortality between sites. Consequently, actual growth measurement data for each individual was converted into percent change to normalize the measurement parameters for statistical comparisons. The actual differences in growth and percent change data were plotted into residual plot to detect heterogeneity. If the plot indicated heterogeneity, then percent change data were log transformed for the statistical analysis. Analysis of variance (ANOVA) was conducted to observe mean percent difference of the measured parameters between sites. Normality and homogeneity of variance parametric assumptions were tested before conducting ANOVA. Data sets that did not meet parametric assumptions were compared using the Kruskal-Wallis nonparametric method to observe differences among sites and are noted in the summary table. Multiple comparisons were performed to test for post-hoc pairwise comparisons among seasons within the site. Measurements recorded from dragonflies collected during the winter, spring, and summer of 2003 and the spring of 2004 were all combined for correlation and regression analyses. Stepwise multiple regressions were calculated with head width as the dependent variable and total length, wing pad length, and wet weight independent variables as the associated predictors. A regression model and 95% confidence belt values for the regression line based on head width and total length of all dragonfly larvae collected from the reference site were calculated and this model was used to plot initial and final growth data from all evaluated sites. All statistical analyses were performed with the PC version of the Statistical Analysis System (SAS version 9.1). Tabulated minimum and maximum values per week for temperature and dissolved oxygen water quality parameters at each site were compared by using ANOVA. If data sets were normal but unequal in variance, a variance adjusted probability value was used for comparison. A non-parametric Kruskal-Wallis test was performed if any data set was not normal.

RESULTS

The differences in *Erpetogomphus designatus* mortality rate between the reference and each urban site during all seasons is presented in Table I. All dragonfly larvae died at the Upper Pecan Creek and Cooper Creek sites during both the spring and summer experimental periods. At the Hickory Creek site, 100% and 60% dragonfly larvae mortality were observed in the spring and summer, respectively. Except in the summer, mortality at the Lower Pecan Creek site was relatively higher than Clear Creek (reference site) and lower than other urban sites.

Table I

Mean percent changes (± SD) in measured growth rate parameters for *Erpetogomphus designatus* larvae. – [HW = head width; – TL = total length; – WP = wing pad length; WW = wet weight; – NC = experiment not conducted; np = probability based on Kruskal-Wallis non-parametric test, – * = Data log normal]

Seasons	Measured Parameters	Clear Creek (CL)	Cooper Creek (CC) (CC)	Hickory Creek (HC)	Upper Pecan Creek (UP)	Lower Pecan Creek (LP)	ANOVA (p)
Early Spring	HW	51.87±30.01	–	–	–	46.42±17.36	0.848*
	TL	30.23±13.49	–	–	–	33.49±11.38	0.579
	WP	50.37±59.28	–	–	–	103.97±112.44	0.193*
	WW	149.92±113.76	–	–	–	221.33±119.01	0.191
	Mortality (%)	10	NC	NC	NC	22	–
Spring	HW	21.31±12.82	–	–	–	57.32±33.14	0.003*
	TL	48.08±26.01	–	–	–	72.83±19.91	0.046
	WP	93.29±71.60	–	–	–	234.97±143.08	0.012*
	WW	61.97±44.89	–	–	–	185.02±135.39	0.007*
	Mortality (%)	40	100	100	100	47	–
Summer	HW	18.52±16.78	–	13.73±12.98	–	6.56±7.84	0.07*
	TL	21.30±12.78	–	8.15±10.39	–	9.68±11.58	0.063(np)
	WP	61.70±44.80	–	40.37±50.29	–	35.13±32.35	0.287(np)
	WW	47.43±39.09	–	32.41±37.73	–	20.17±25.03	0.115*
	Mortality (%)	27	100	45	100	20	–

In the summer, Lower Pecan Creek had 20% mortality and Clear Creek has 27% mortality of dragonfly larvae. In an average of the three seasons, Clear Creek has 29% and Lower Pecan Creek has 35% mortality, respectively. In the summer, the water flow rate at Clear Creek was reduced to zero (Fig. 3).

Average percent changes in head width, total body weight, wing pad length, and wet weight for surviving larvae during each season and at each experimental site are presented in Table I. There were no statistical differences in measured growth parameters between surviving larvae retrieved from the reference site and from the Lower Pecan Creek sites in early spring and between the reference site, Hickory Creek, and Lower Pecan Creek sites in the summer. Although no statistical difference was found, the growth rate in the summer was relatively higher at the reference site than at the Hickory Creek and Lower Pecan Creek sites. Statistically significant differences (ANOVA, $p < 0.05$) were found in mean percent difference for all four measured parameters in the spring between the reference and urban sites. In the spring, statistically higher growth ($p < 0.05$) was observed at the impacted site than at the reference site.

Because of instrument malfunction, most of the water quality data from Lower Pecan Creek and some data from Upper Pecan Creek were lost during this experimental period, but temperature data recorded for the same experimental period in 2002 and 2004 indicated relatively lower temperature fluctuation at the Lower

Pecan Creek site than at the reference site (Fig.4). Statistical comparisons based on minimum and maximum recorded values for each site and seasonal differences within the site between two experimental periods are presented in Table II. Due to insufficient data, Lower Pecan Creek was not included in this statistical analysis. During the spring experimental period, statistically significant differences ($p < 0.05$) in minimum (except temperature) and maximum recorded temperature and dissolved oxygen values were found between sites. Minimum dissolved oxygen readings were significantly higher ($p < 0.001$) at the reference site than at the other sites. During the summer, significant differences ($p < 0.05$) in temperature readings were found between sites. Among evaluated sites, minimum temperature at Hickory Creek and Upper Pecan Creek were significantly higher than the reference and Cooper Creek sites.

Seasonal differences in growth rate within the sites were also statistically compared and are presented in Table III. Percent differences for all measured parameters were statistically significantly different (ANOVA, $p < 0.05$) between seasons at both the reference and the Lower Pecan Creek sites, except for the mean percent change in wing pad measurements, which was compared by a non-parametric method. Relatively higher growth rates were observed in the spring as compared to the summer at both sites. Since no specimens survived in spring and only a few survived in the summer from the Hickory Creek site, no statistical seasonal comparisons were possible for this site. However, as in other sites, relatively low

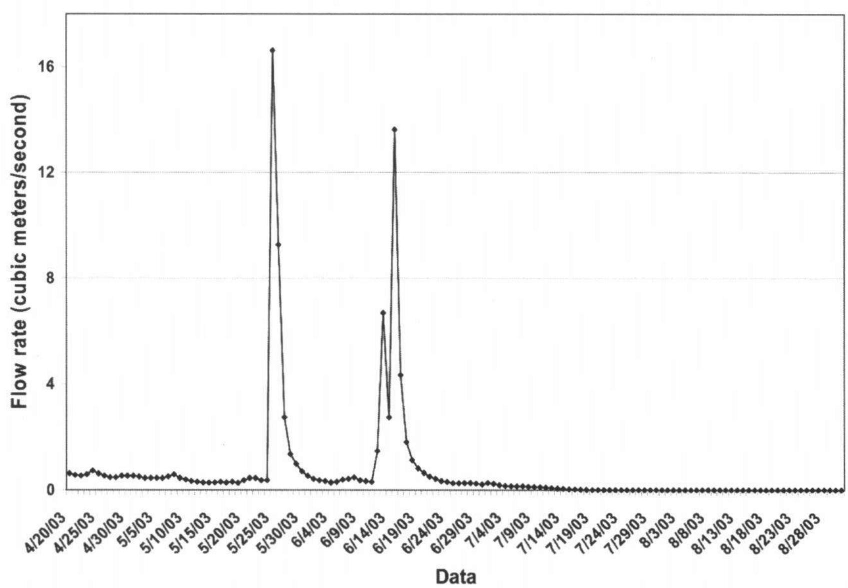


Fig. 3. Clear Creek flow rate recorded near Sanger, Denton co., Texas. – [Source: United States Geolgical Survey]

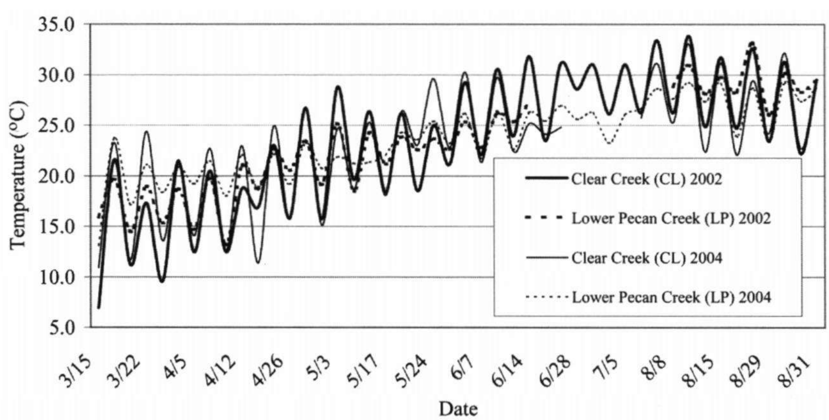


Fig. 4. Minimum and maximum temperature readings recorded per week by the in situ placed instrument at experimental sites.

percent differences in measured growth rate parameters were observed in the summer. Since the experiment was carried out in early spring, spring, and in the summer, multiple comparisons indicated higher percent difference either in early spring or in spring rather than in the summer (Tab. III).

As expected, Pearson correlation coefficients performed among all four measured parameters (head width, total length, wing pad length, and wet weight) indicated a highly statistically significant correlation coefficient ($p < 0.0001$) (Tab. IV). Although all four parameters were statistically correlated to each other,

Table II

Statistical comparison of water quality parameters summarized as minimum and maximum values recorded per week during spring and summer experimental periods. From Lower Pecan Creek site one week value in spring and two week values in summer were obtained and were not included in the statistical analysis. – [np = probability based on non-parametric tests; – * = Data log normal]

		Clear Creek (CL)	Cooper Creek (CC)	Hickory Creek (HC)	Upper Pecan Creek (UP)	Lower Pecan Creek (LP)	ANOVA p
				Temperature (°C)			
Spring	Min	20.5	20.38	21.11	20.68	23.79	0.32
	Max	29.45	26.28	28.25	24.68	26.37	0.0001*
Summer	Min	24.37	23.98	28.05	25.26	19.87	0.0001
	Max	31.64	30.67	32.69	27.39	28.99	0.01 (np)
				Dissolved Oxygen (mg/L)			
Spring	Min	4.23	1.1	1.33	1.99	3.98	0.0001*
	Max	7.73	6.33	7.5	5.77	7.18	0.02*
Summer	Min	1.02	1.13	0.8	0.4	4.64	0.82*
	Max	5.5	5.17	5.87	3.74	5.75	0.42(np)

Table III

Mean percent changes (± SD) in measured growth rate parameters for *Erpetogomphus designatus* larvae in different seasons. – [HW = head width; – TL = total length; WP = wing pad length; – WW = wet weight; – np = probability based on Kruskal-Wallis non-parametric test; – * = Data log normal]

Site		Early Spring	Spring	Summer	ANOVA (p)	Multiple Comparisons
Clear Creek	HW	46.14±20.01	21.31±12.82	18.52±16.78	0.025*	ES>SP=SU
(CL)	TL	30.23±13.49	48.08±26.01	21.30±12.78	0.011	SP>EP=SU
	WP	50.37±59.28	93.29±71.60	61.70±44.80	0.452(np)	SP=SU=EP
	WW	149.92±113.76	61.97±44.89	47.43±39.09	0.013*	ES>SP=SU
Lower Pecan						
Creek	HW	46.42±17.36	57.32±33.14	6.56±7.84	0.0001*	SP=EP>SU
(LP)	TL	31.69±11.71	72.83±19.91	9.68±11.58	0.0001	SP>EP>SU
	WP	95.24±106.99	234.97±143.08	35.13±32.35	0.0001*	SP>EP>SU
	WW	205.07±119.41	185.02±135.39	20.17±25.03	0.0001*	ES=SP>SU

head width and total length correlation had the highest correlation coefficient (r = 0.949). Multiple regressions also generated the highest coefficient of determination ($R^2 = 0.9$) value for these two variables. Multiple regressions eliminated the wet weight variable from the model and produced an R^2 of 0.918. Since total length explained 90% of the variation for head width, a regression line and 95% confidence belts for the regression line were generated based on these two variables to assess any deviation of larvae growth patterns at the urban sites.

Initial and final growth measurements based on head width and total length of dragonfly larvae retrieved from experimental enclosures during three experimental periods are plotted on a derived regression line and its confidence belt from 358 specimens collected from the reference site are presented in Figure 5. In the early spring and summer, growth rates were within the confidence belt. However, in the spring for both the reference and the Lower Pecan Creek sites, most of the growth coordinate values were outside of the upper confidence belt. This may indicate relatively higher total length in relation to head width among enclosure specimens in the spring.

DISCUSSION

Higher mortality of separately caged individual *Erpetogomphus designatus* larvae exposed to urban sites than at the reference site may indicate that differences in water flow and water quality may have caused an adverse effect on this common larva found at the reference site. The in situ field experiment showed 100% mortality of this species at the Cooper Creek and Upper Pecan Creek sites in both spring and summer and 100% mortality at Hickory Creek in the spring. Spring mortality in these three sites may not be accurately assessed since relatively high mortality at the reference and Lower Pecan Creek sites was observed, possibly due to experimental error. However, during the summer retrieval period, at both sites, enclosures were dark in color, which may indicate an anaerobic condition. At Hickory Creek, 60% mortality during the summer experimental period may be related to hydrology of the site. Hickory and Lower Pecan Creeks were the only two sites which had continuous water flow during entire summer experimental period. The survival of this species at Lower Pecan Creek was similar to the reference site, which indicates that water flow may be an important factor for the survival of this species. Although no differences in mortality were found between the reference and the Lower Pecan Creek sites, optimum conditions are important to successfully complete the life cycle. To successfully complete the life cycle, reproductive success is vital. Since dragonflies have both an aquatic and terrestrial life stage, suitable habitat characteristics are vital. At Lower Pecan Creek, there may be suitable aquatic conditions for this species to survive, but an unfavorable environment to complete reproductive cycle. SOUTHWOOD (1977) published a review paper on habitat as a template for ecological strategies, which suggested that in the process of adaptation to habitat, organisms' own dimensions of space and time are essential.

Relatively high mortality in the spring than in the summer may have been related to high sedimentation effects in the experimental enclosures at both the reference and the Lower Pecan Creek sites. Frequent rainfall in the spring and the presence of a 500 micron net covering the enclosures might have allowed higher sedimentation rates in some of the enclosures, which probably adversely affected dragonfly larvae. Although an experimental error was encountered in the spring, 100% dragonfly larvae mortality at the Cooper and Upper Pecan Creek sites in both the spring and summer may be related to differences in hydrologic

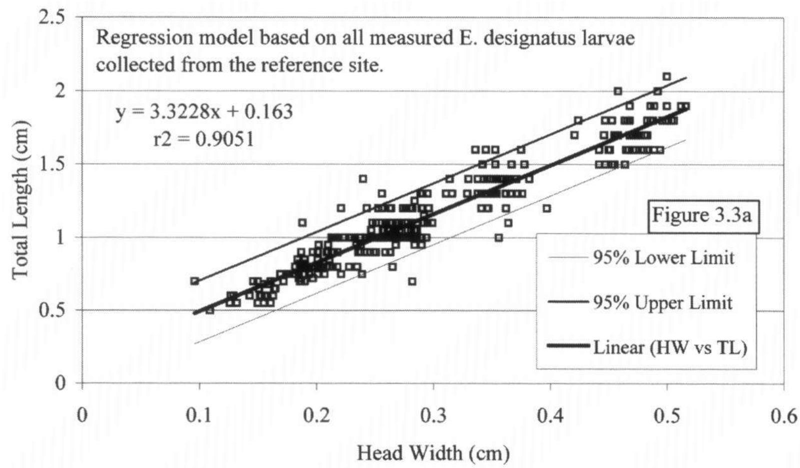
Table IV

Pearson Correlation Coefficient (r) and Multiple Regression analysis results among measured growth rate parameters for *Erpetogomphus designatus* larvae. – [HW = head width; – TL = total length; – WP = wing pad length; – WW = wet weight]

	Pearson Correlation coefficient with probability in parenthesis			
	TL	WP	WW	--
HW	0.949 (0.0001)	0.919 (0.0001)	0.734 (0.0001)	--
TL	--	0.91 (0.0001)	0.726 (0.0001)	--
WP	--	--	0.69 (0.0001)	--

	Multiple Regression Analysis (HW=)			
Intercept	TL	WP	WW	R^2
0.4833	0.1747	0.2199	0.0851	0.92
0.0393	0.1869	0.2285	--	0.918
-0.0145	0.2701	--	--	0.9

regime and water quality. Due to cessation of water flow during the summer experimental period at the reference, Cooper, and Upper Pecan Creek sites, a significant change in water quality parameters (temperature and dissolved oxygen) was observed. Also, higher mortality of larvae at the reference site (27%) than at the Lower Pecan Creek (20%) in the summer could be due to changes in hydrology and water quality during later part of the summer experimental period. In the summer, water flow was significantly reduced at the reference site and dis-



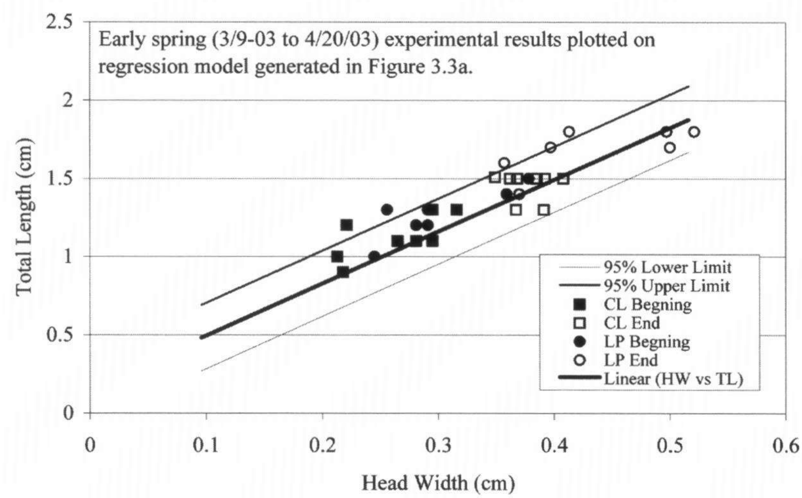
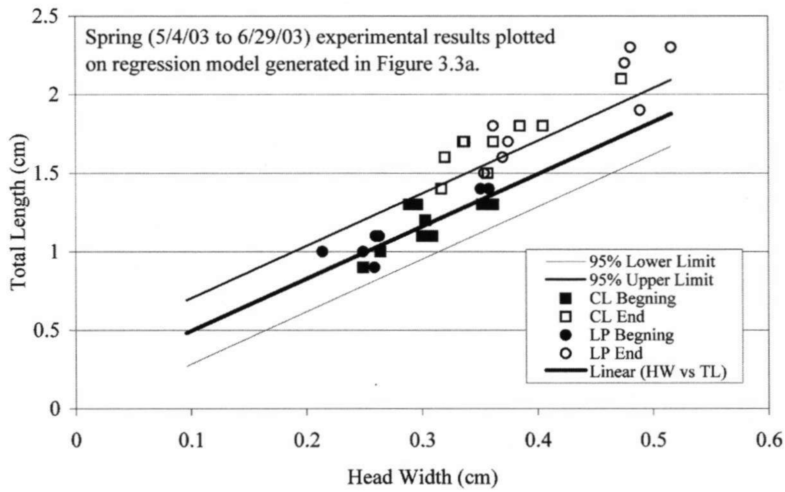


Fig. 5. Comparison of final head width and total length observed during three experimental periods, with regression line and 95% confidence belts generated from initial measurements of 358 *E. designatus* larvae collected from reference site. – [CL = Clear Creek; – HC = Hickory Creek; – LP = Lower Pecan Creek]

solved oxygen was very low during the later part of the experimental period. At the Lower Pecan Creek site, water flow remained constant due to effluent discharge from the Pecan Creek water reclamation plant. This species prefers clear streams, riffle habitat with moderate current, and they associate themselves with detritus materials (ABBOTT, 1999).

Generally, aquatic insects that have a terrestrial adult life grow faster in the spring and are influenced by the gradual increase in water temperature (SWEENEY, 1984). Experimental results indicate similar growth patterns. Even though there



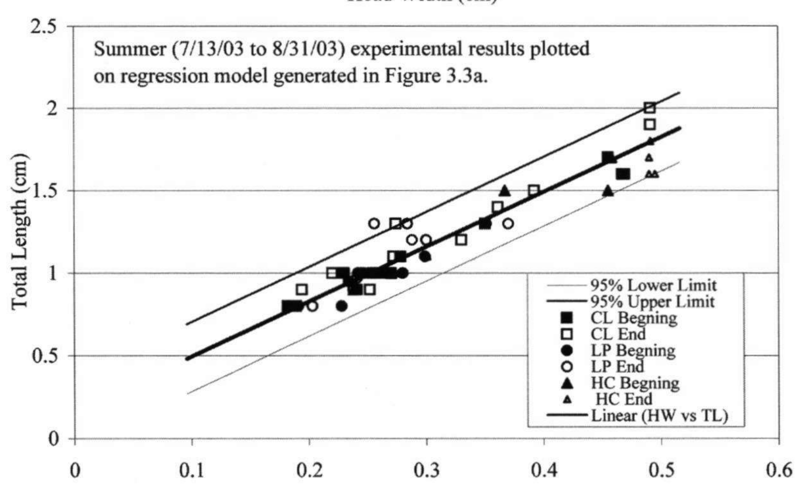


Fig. 5. Continued

were statistical differences between the early spring (March, 2 to April 20) and spring (May 3 to June 28) data, these differences may not be significant because of a very small gap between these two experimental periods. In the spring, dragonfly larvae grew significantly faster at the Lower Pecan Creek site than at the reference site. A high volume of reclaimed water discharged from the Pecan Creek Water Reclamation Plant at relatively constant temperature could have influenced these results at the Lower Pecan Creek site. In the spring, 66% and 63% of the survived larval head width and total length coordinates, respectively, at the reference and Lower Pecan Creek sites were outside of the upper 95% confidence belt of the regression line. The only possible explanation for these results is that dragonfly larvae were caged individually and in the absence of other competitors or predators, the larvae have un-proportional increase in size as compared to the natural reference conditions.

Although the wet weight of the dragonfly larvae was not well correlated with the three other measured parameters, higher percent differences between the early spring and spring experimental events at the Lower Pecan Creek site compared to the reference site may indicate presence of a higher number of prey items available for food. The benthic invertebrate colonization experiments indicate more efficient colonization of benthic macroinvertebrates at the Lower Pecan Creek site than at the reference site. The presence of high numbers of colonized prey items in the enclosure may have contributed to these results.

In summary, these dragonfly larvae field experiments demonstrated different mortality patterns between reference and urban sites. It appears that the water flow was a major factor for the mortality of *E. designatus* larvae during the summer experimental period. During the summer, the cessation of water flow had significantly altered the diel variation in water temperature, which might have caused dissolved oxygen deficiency. *E. designatus* is a common species found throughout the Trinity River watershed. Although all studied watersheds are unique, this species was expected to survive at each site during the spring experimental period. In the spring, each creek maintains constant water flow. Despite sedimentation problems, more than 50% of this organism survived from the reference and Lower Pecan Creek sites. Since no survival was observed at Cooper Creek and the Upper Pecan Creek sites, differences in growth patterns were not determined for those sites. At other sites, growth seems to be affected by water temperature. This experiment shows that the local common dragonfly taxa could be used for field biomonitoring experiments to assess water quality. However, a detailed understanding of the biology of this organism is important to accurately interpret of the experimental results. This type of *in situ* experiment indicates actual impact rather than applying conclusions that are based on either laboratory microcosm or mesocosm toxicity tests.

There are some limitations for this experimental design due to intermittent environmental conditions. In the summer, water flow is significantly reduced and changes the water quality of the streams. During this period, natural stress can play a major role in the survival of test organisms and hence summer may not be a suitable time to conduct this experiment. Other limitations are that enclosures are placed on the stream bed and due to sediment bed movement during periods of high flow rates, enclosures can become buried under the sediment. It is therefore very important to select suitable locations where there is no or minimal impact of sedimentation. Another alternative would be to suspend enclosures in the water column. Additional hazards to the tests exist, such as debris generated in the watershed which can bury or damage the experimental enclosures. Other hazards include large fluctuations in the water level due to floods that can submerge the experimental enclosures in several meters of water for extended periods. However, with proper experimental design and adequate installation considerations, the experimental approach can be an effective biomonitoring technique during the spring.

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