

“Microhabitat Effects on the Growth Rates of Odonata Larvae”

BIOS 569: Practicum in Aquatic Ecology

Patrick C. Foy, Jr.
1646 W. Turtle Creek Dr.
South Bend, IN 46637

UNDERC Advisors-
M. Sean Dunlap
Dr. Ronald A. Hellenthal
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ABSTRACT:

Current research suggests that the vast number and diversity of dragonfly species is attributable to niche development in various microhabitats. This experiment explores the growth rates of two species of dragonfly larvae, Ladona julia and Cordulia shurtleffi, in microhabitats with different substrates. Four larvae of the same species were isolated in one of 28 random field cages that contained a substrate of either leaves or mud for an experimental growth period of 40 days. Final and initial head widths of each larva were measured, and then extrapolated to calculate dry masses. From this data, growth rates were calculated for each species in their respective microhabitat. Statistical analysis nullifies any correlation between mud/leaf substrate and growth rate for Cordulia. However, there is some suggestion that Ladona grow more quickly in mud than in leaves. This implies Ladona larvae may prefer to inhabit niches with mud rather than those with leaves. This data is not conclusive and requires further observation and research.

INTRODUCTION:

Dragonflies (Odonata: Anisoptera) are found throughout North American and global wetlands in abundance. While primarily a tropical organism, many species of odonata have evolved to life in cooler, drier climates. As evidenced by Corbet and Walker (1975), thousands of species of odonata inhabit the most northern latitudes of the United States and Canada. This striking diversity of species is particularly noticeable in the northern portions of Minnesota, Wisconsin, and the western part of the Upper Peninsula of Michigan. This geographic region is characterized by tens of thousands of lakes, large wetland areas, and countless streams. In addition, the region populated by humans at a low density and remains relatively environment-friendly. As such, dragonflies prosper throughout the region and exist in surprising special diversity. Hilsenhoff (1998) has identified hundreds of odonates that inhabit the region, while Dunlap (personal communication) has identified over 40 species of anisoptera on the 7500 acre University of Notre Dame Environmental Research Center (UNDERC).

While the abundance of odonate species is unmistakable, many questions surround the reasons for such variety of species. Current hypotheses for this dilemma often involve the idea of microhabitat and niche development for particular species. These theories predict that particular types of species have evolved to gain an advantage for living within very particular environmental conditions. Most of these theories apply to the life history of dragonfly larvae. These predatory aquatic insects live on the bottom of lakes, wetlands, and streams before emerging into their more recognizable adult forms.

Pierce (1998) demonstrated that the genus Ladona avoids colonization of habitats with fish when possible. Dunlap and Buescher (personal communication) have shown dragonflies of different genus (Ladona, Leucorrhinia, Epitheca, and Cordulia) prefer distinct artificial microhabitats in laboratory experiments. This research suggests biological and behavioral differences may result between species prefer one habitat in place of another. Claus-Walker (1997), and Wellborne and Robinson (1987) have suggested that larvae activity and feeding behavior changes considerable when living in a habitat with predatory fish. Johansson (1992) has attempted to categorize different species of dragonfly based on their microhabitat-specific behavior.

This research points to and supports the idea that microhabitats contribute to the wealth of dragonfly species. Previous research has focused primarily on the effects that microhabitats have on larvae predatory movement, defensive movement, food consumption, and habitat colonization. Little attention has focused on how microhabitat selection affects the growth and development of larvae. This investigation seeks to correlate the rate of growth for specific dragonfly larvae with various microhabitats. This investigation specifically focuses on the possible effects that various substrates have on the growth of Ladona julia and Cordulia shurtleffi. The research attempts to replicate microhabitats found at the shoreline of lakes surround by deciduous forest and those without riparian cover. A substrate of leaves will simulate a forest-surrounded habitat, while mud substrate will simulate habitats without leaf cover.

Materials and Methods:

University of Notre Dame Environmental Research Center:

Because growth rate can be influenced by a variety of factors (food supply, presence of predators, oxygen concentration, pollutant levels, etc.), this experiment must attempt to create artificial habitats that mimic the natural environment as possible. As such, UNDERC appears to be an ideal center to conduct a field experiment that minimizes unnatural disruptions. Its 7500 acres provide adequate isolation from outside human interference. Because the lakes of UNDERC are not accessible to the public, they provide as close to a natural setting as can be found in ecological studies. This setting allows for the most accurate creation of natural microhabitats as is synthetically possible, and therefore can allow the researcher to best understand what factors affect larval growth.

Procedure:

To examine microhabitat effects on odonate larval growth, this investigation explores the maturation of dragonfly species Ladona julia and Cordulia shurtleffi in field cages with differing bottom-layer substrates. The experiment consists in three distinct methodological segments. First, environmentally sensitive field cages were constructed, and then positioned in the desired location. Second, appropriate numbers of larvae were captured, measured, and placed in these cages. Finally, after a period of time, larvae were removed from the cages, identified, and then measured. Data was analyzed to see what, if any, effect variable microhabitats had on growth of the respective odonate species.

Construction of workable cages proved to be one of the most difficult challenges of the investigation. These cages allowed the larvae to be recaptured, and had to prevent larvae from escaping. Cages also needed to protect larvae from predation by fish and other odonates. At the same time, cages needed to allow for environmental conditions to be kept similar to those found in natural settings. Factors that needed to be considered included the amount and type of smaller insects that could be prey for odonate larvae, water temperature, pH, sunlight, and water clarity. Cages also needed to withstand unusual or unlikely disruptions such as heavy raining or flooding, and unintentional interactions with large mammals, principally white-tailed deer and occasional fishermen.

To obtain an appropriate number of trials, 28 cages were constructed. The base of each cage was a circular plastic flowerpot, 50.8 cm in diameter and 7.6 cm deep. A 45.7cm high, cylindrical .64 square cm hardware cloth frame was attached to the inside of each base with wire. Standard nylon mesh window screen was attached to the inside of the frame, and held in place with wire ties. The window screen was fine enough to prevent odonate larvae from escaping, but also wide enough to allow prey entrance. The screen was caulked to the base of the cage to prevent larvae from escaping from the bottom of the cage. To create an accurate substrate replication, the base was covered with approximately a half-inch layer of sifted fine sand. This layer was coated with an inch of dense lake mud taken from Morris Lake (UNDERC). The mud was dried to form a cake along the base of the cage. The cages were then transported to the site of the experimentation. The site chosen was a relatively isolated finger of Bay Lake (UNDERC). Cages were installed along the shoreline of Bay Lake in 30.4-38.1cm of

water and held in place by stakes anchored to the lakebed. The tops of the cylindrical cages surfaced above the water level. This prevented larvae from climbing up the cage and escaping out the top. A hardware cloth cover was attached to the cages to prevent intrusion from deer, frogs, and other potential disrupters. Mud and leaf substrates were chosen to be the variable microhabitats for the investigation. These correspond to shorelines that have or do not have deciduous tree canopies. While Bay Lake is tree-lined, the hardware cloth covers protected against leaves and other debris from entering the mud substrate cages. To prepare the other variable substrate, leaves were collected from the shoreline of Bay Lake and soaked. An approximately two inch deep layer of leaves was placed on top of the mud layer in 14 of the cages to replicate tree-lined, leaf substrate microhabitat. The 28 cages alternated between leaf and mud substrates to control for variability because of the location along the shoreline. The cages were allowed to settle for a week before odonates were introduced. This allowed prey to enter the cage as well as standardize water chemistry between cages. (Figure 1).

This investigation tested microhabitat growth effects for two dragonfly species, Ladona julia and Cordulia shurtleffi. Each cage required four larvae of the same species for statistical analysis. This value represented the natural population density of the site. 56 larvae of each species were needed to perform the experiment. Dragonfly larvae were collected from Bay Lake. Larvae were identified using Hilsenhoff (1998), separated, and chosen for experimentation. Small, first instar larvae were preferred over more mature larvae in both species. This allowed for maximal growth during the test period. Final instars were always discarded from possible experimentation because of possible

emergence into adult dragonfly. The head width of selected larvae was measured and recorded. Head width measurements directly correspond to a dry mass value for living odonate larvae (Dunlap, unpublished data). Groups of four larvae were chosen at random, and their estimated masses were averaged. These groups were then introduced into randomly chosen cages on June 10, 1999. A breakdown of the 28 cages included: 7 L. julia/mud, 7 L. julia /leaves, 7 C. shurtleffi/mud, 7 C. shurtleffi/leaves.

The cages were then left undisturbed for a period of nearly six weeks. On July 20, 1999, the cages were removed from Bay Lake and larvae were located. These specimens were removed from their substrates and placed in ethanol. Head widths were measured and averaged for each cage of larvae. The larvae were then dried in an oven, and then weighed. This data analyzed comparing growth rates between specimen in differing microhabitats. T-tests were used to determine whether error was attributable to chance.

Results:

The first relevant data collected following the larvae recovery concern the number of larvae recovered. In 26 of the 28 cages, at least one odonate larva was recovered following the 6-week quiescent period. In eleven cages, all four of the original larvae were recovered. In all cases, the inside of cages contained ample amounts of midges and other potential prey. The recovery percentage of each variable did vary. The initial larvae were recovered 92.9% and 82.1% of time in L. julia/mud cages and C.

shurtleffi/leaves cages respectively. On the other hand, recovery percentages were much lower for L. julia /leaves cages (50.0%) and C. shurtleffi /mud (64.3%). See Figure 2.

To investigate potential differences in growth rates, calculations were made using the instantaneous daily growth formula suggested in Hauer and Lamberti (1996). The average estimated dry weight of L. julia before trial was .0112 g and .00905 g for C. shurtleffi. This calculation required the difference between final and initial dry weight values (Figure 3). Because dry weights could not be calculated before the experimental time period, initial dry weights were estimated using a head width-dry weight extrapolation developed by Dunlap (unpublished data). The growth rates were calculated over a 40-day period corresponding to the length of time the larvae remained in the field. The growth rates showed some deviation between L. julia in mud and those in leaves. The calculated values for growth rate was -.2629 for L. julia /mud and -.2572 for L. julia /leaves. Growth rates for C. shurtleffi in the differing microhabitats remained much more consistent than those of L. julia. These values were -.2574 and -.2573 for C. shurtleffi in mud and leaves respectively.

Finally t-tests were applied to see if differences in growth rates between species could be attributed to their different microhabitats or whether these differences were a result of chance. These calculations were made using the statistics program SSPS 7.5. In comparing L. julia, t-tests revealed that the differences in growth rates between individuals in different microhabitats could possibly be by chance ($t=-1.83$, $df=5$, $p=0.123$). Unsurprisingly, the growth rates in C. shurtleffi could also be attributed to chance ($t=-0.274$, $df=5$, $p=.795$).

Discussion:

In analyzing the statistical data for this investigation, some results need to be further highlighted and accounted for. The first questions raised by the data regards the number of dragonfly larvae recovered. 72.3% of the original 112 larvae were recovered in their cages after the 40-day experimental period. This figure represents a remarkable increase in larvae recaptures from previous UNDERC experiments. It is most likely attributable to improvements made in field cage construction from years past. Placement of the nylon window screen inside the hardware cloth frame, the caulk seal at the bottom of the cage, and the drying of the mud substrate before introduction of larvae improved the quality the experiment. Despite these improvements, 31 larvae could not be accounted for after the experimental period. In two separate cages, none of the original larvae were recovered. This suggests that some of the cages may have been defective. Original larvae may have escaped through holes in the mesh, or predators may have entered into the field cage. Another cause for this relatively high rate of disappearance might be attributable to cannibalism. Dragonfly larvae are known to prey on smaller members of their own species. While an effort was made to use only larvae of relatively equal sizes, it is possible that an individual larva may have grown more rapidly than the others in its cage. Such circumstances may have lead to “dominant” larva that cannibalized the “runt” larvae.

More important than the total number of larvae recovered is the distribution of these recoveries. Most striking is the difference between the L. julia recovered in mud substrate cages and those with a leaf-based substrate. While only 50.0% of L. julia were recovered from leaf cages, 92.8% of the L. julia in mud cages were recovered. This data may suggest that environmental pressures were more substantial in leaf-substrate microhabitats. L. julia in these cages may have been more active to search for possible escapes from the field cage, or were more likely to practice cannibalism. Larvae in the mud substrate microhabitat may have had an easier time capturing prey, and thus were less mobile. This data indicates that L. julia might have a preference for a mud-based microhabitat over a leaf-based habitat. Further experimentation on microhabitat effects on mobility and colonization of L. julia would be needed to reach a more definitive conclusion. C. shurtleffi were recovered in more similar totals (18 in mud, 23 in leaves). Any conclusions based on this data would be highly subjective and open to considerably more criticism.

Analysis of the growth rate data shows that all controls showed some sign of growth during the 40-day experimental period. Once again, the most noteworthy data comes in the comparison of L. julia in different microhabitat substrates. The average larvae in mud grew in dry mass a total of .004186 grams during the 40-day period. The average L. julia in leaves added a dry mass total of only .000284 grams during the same amount of time in similar conditions. Predicted growth rate calculations show a more similar rate of development. When the growth rates of L. julia are compared in a t-test, the statistical probability of relatedness is calculated to be .123. This value lies above the

.05 value needed to prove that differences in growth rate are not attributable to chance. As such, this investigation cannot prove that L. julia grow more rapidly in mud-based microhabitats than they do in leaf-based microhabitats. However, this data should not exclude this hypothesis either. Recovery values coupled with growth data indicate that L. julia may grow more rapidly mud microhabitats than in leaves. This hypothesis would better supported if more conclusive evidence of microhabitats effecting growth rates could be established. This potential problem may be resolved if more larvae were recovered from the field cages or the period of growth was extended beyond 40 days.

Growth rates calculated for C. shurtleffi indicate that leaf or mud microhabitats have no effect on the development of larvae for this species. Predicted growth rates were calculated to be -0.2573 and -0.2574 for larvae in leaves and mud respectively. This conclusion leads one to ask what, if any, microhabitat factors favor C. shurtleffi. Perhaps other substrates such as sand or macrophytes are preferential to larval growth. Other potential areas of niche development might be water depth, water clarity, oxygen levels, pH, or predator population. This investigation cannot make the determination if any of these factors lead to specialization for C. shurtleffi.

If the suggestion that L. julia prefer mud-based microhabitats to those with leaf-based substrates is accepted, this difference must be accounted for. L. julia may have evolutionary adaptations to favor life in the mud. These larvae should be equipped to burrow or hide themselves from prey and predators in the mud. Camouflage should correspond to a mud-based habitat. The hairs on larval L. julia may aid the organisms in blending into its preferred microhabitat. L. julia would also be more likely to prey using

a “wait and ambush” technique than a “stalk and attack” technique. Research and observation regarding these considerations should be made before the suggestion is accepted.

Figure 1: Diagram of Field Cage

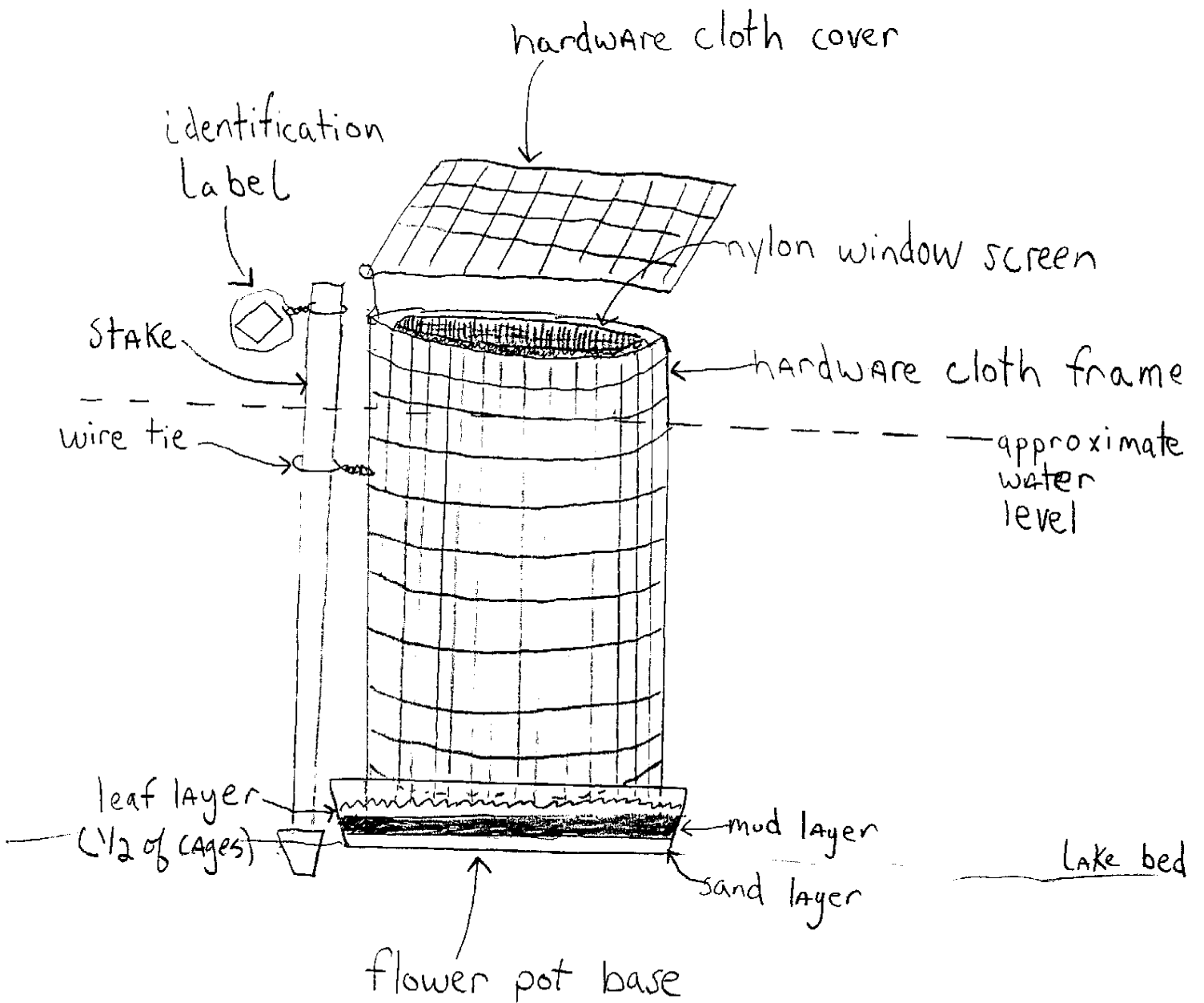


Figure 2: Larvae Recovered from Field Cages

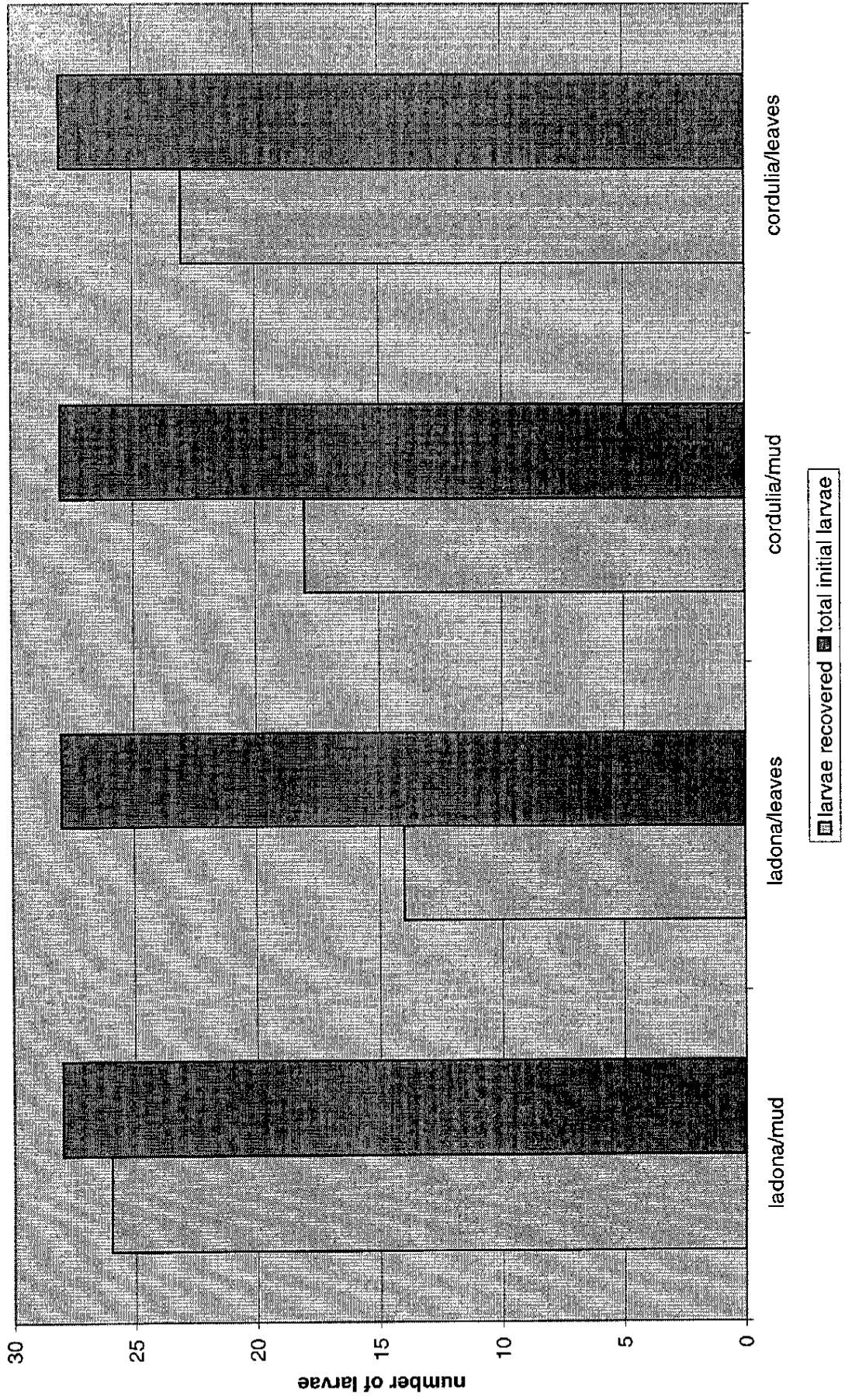


Figure 3: Final and Initial Mean Weights

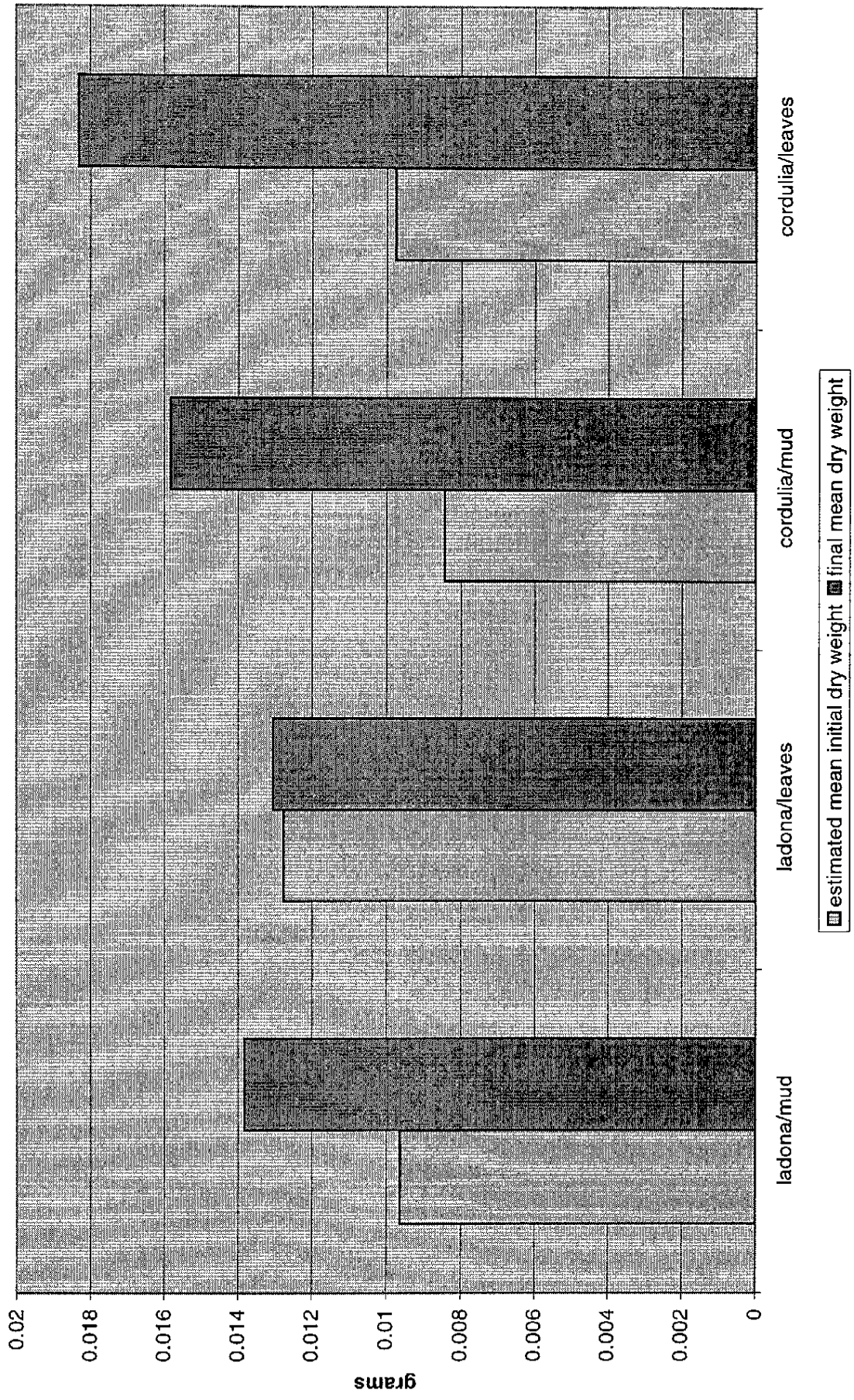


Table 1: Mean Weights and Growth Rates

| Field Cage | Mean Initial Estimated Dry Weight (grams) | Mean Final Dry Weight (grams) | Estimated Growth Rate |
|-----------------|---|-------------------------------|-----------------------|
| Ladona/Mud | 0.00964194 | 0.013827381 | -0.2629 |
| Ladona/Leaves | 0.01277285 | 0.013057143 | -0.2527 |
| Cordulia/Mud | 0.008435714 | 0.015827381 | -0.2574 |
| Cordulia/Leaves | 0.009755019 | 0.018314286 | -0.2573 |

Table 2: T-tests and P Values

| T-Tests | t value | degrees of freedom | Probability Values |
|-------------------|---------|--------------------|--------------------|
| Ladona Julia | -1.83 | 5 | 0.157 |
| Cordulia shuleffi | -0.274 | 5 | 0.989 |

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As always, "GO IRISH!"

Literature Cited:

Claus-Walker, Debra B. and Frank Johansson. 1997. Fish Predation, Cannibalism, and Larval Development in the Dragonfly *Epiheca cynosura*. *Can. Jour. Zoology*. 75:687-696.

Hauer, F.R. and G.A. Lamberti. 1996. *Methods in Stream Ecology*. Academic Press: San Diego, CA. 674.

Hilsenhoff, W.L. 1995. *The Aquatic Insects of Wisconsin*. UW Press: Madison, WI.

Johansson, Frank. 1992. Predator Life Style and Prey Mobility. *Hydrobiologie*. 126:163-173.

Pierce, C.L. 1988. Predator Avoidance, Microhabitat Shift, and Risk Sensitive Foraging in Larval Dragonflies. *Oecologia*. 77:81-90.

Walker, E.M. and P.S. Corbet. 1975 *The Odonata of Canada and Alaska*. Vol.3: Part 2, The Anisoptera- three families. Univ. of Toronto Press: Toronto, Ont. P 1-308.

Wellborne, G.A. and J.V. Robinson. 1987. Microhabitat Selection as an Anti-predator Strategy in the Aquatic Insect *Pachydiplax longipennis burmeister*. *Oecologia*. 71:185-189.