Effects of food availability on larval development and inter-instar predation among larvae of *Libellula lydia* and *Libellula luctuosa* (Odonata: Anisoptera)

SCOTT A. WISSINGER[1]

Department of Biological Sciences, Purdue University, West Lafayette, IN, U.S.A. 47907

Received January 27, 1987

WISSINGER, S. A. 1988. Effects of food availability on larval development and inter-instar predation among larvae of *Libellula lydia* and *Libellula luctuosa* (Odonata: Anisoptera). Can. J. Zool. **66**: 543–549.

I conducted two types of laboratory experiments with larvae of the dragonflies *Libellula lydia* and *L. luctuosa*. In one experiment I varied the number of *Daphnia magna* fed daily to these larvae to determine the effect of food availability on growth and survivorship. The time spent in each instar decreased dramatically with increased food availability, but the number of molts did not vary and the size at each molt was only slightly affected. Mortality was low in all but the lowest feeding treatment, despite 2- to 5-fold differences in instar duration. These results suggest that the number and size of instars are determinate in these species, and that starvation is an unlikely cause of larval mortality in nature. In a second experiment I used naturally co-occurring size combinations of *L. lydia* and *L. luctuosa* to determine how inter-odonate predation varies as a function of larval size difference. For both intra- and inter-specific combinations, (*i*) little or no predation occurred between larvae similar in size, (*ii*) some predation always occurred when larvae differed by more than two instars, and (*iii*) the number of larvae consumed increased dramatically as a function of instar difference. The proportional difference between the labial (gape) width of the larger instar and the head width of the smaller instar was a good estimator of inter-odonate predation rates across all instar and species combinations. Together these results suggest that the effects of inter-odonate competition and predation can be disentangled in the field by manipulating the instar structure of experimental populations.

WISSINGER, S. A. 1988. Effects of food availability on larval development and inter-instar predation among larvae of *Libellula lydia* and *Libellula luctuosa* (Odonata: Anisoptera). Can. J. Zool. **66** : 543–549.

Des larves de *Libellula lydia* et de *L. luctuosa* ont servi au cours de deux types d'expérience. Dans l'une, les larves recevaient chaque jour des nombres différents de *Daphnia magna*, dans le but de déterminer les effets de la disponibilité de la nourriture sur la croissance et la survie. La disponibilité de la nourriture a eu pour effet de diminuer considérablement la durée de chaque stade larvaire, mais n'a pas affecté le nombre de mues et la taille des larves à chaque mue variait très peu. La mortalité s'est avérée faible dans tous les cas, sauf lorsqu'il y avait peu de nourriture disponible, et pourtant la durée des stades larvaires variait par un facteur de 2 à 5. Ces résultats indiquent que la taille et le nombre des stades larvaires sont prédéterminés chez ces espèces et que le jeûne est une cause peu probable de mortalité larvaire en nature. Dans une seconde expérience, des larves de *L. lydia* et de *L. luctuosa* ont été mises ensemble en combinaisons de tailles imitant les combinaisons naturelles, afin de déterminer comment la prédation interspécifique varie en fonction des différences de taille. Pour les deux types de combinaisons, intraspécifiques et interspécifiques, (*i*) il y a peu de prédation entre larves de tailles semblabes, (*ii*) il se produit toujours un peu de prédation lorsque les larves diffèrent par plus de deux stades et (*iii*) le nombre de larves consommées augmente considérablement en fonction des différences de stades. Le rapport entre la largeur du labium de la larve la plus grande et la largeur de la tête de la larve la plus petite constitue un bon indice des taux de prédation inter-odonates, chez tous les stades et pour toutes les combinaisons de tailles. En résumé, les résultats indiquent que les effets de la compétition inter-odonates et ceux de la prédation peuvent être distingués en nature par la manipulation de la structure des populations expérimentales.

[Traduit par la revue]

Introduction

Intraspecific size differences within populations can complicate interspecific interactions. This is especially true for predators with generalized diets because the same species can interact as competitors and as predators and prey. Such mixed competition–predation interactions appear to be most important when growth is indeterminate or involves numerous developmental stages (Wilbur 1980; Werner and Gilliam 1984; Werner 1986).

Larval development in dragonflies (Odonata) entails 8–15 instars that differ in size by three to four orders of magnitude. Larvae similar in size are potential competitors, whereas larvae disparate in size can interact as predators and prey (Benke 1978; Thompson 1978*a*; Merrill and Johnson 1984). The potential for complex interactions between competitive and predatory effects has obscured the interpretation of manipulative field experiments designed to quantify interspecific interactions (Benke 1978; Benke *et al.* 1982; Johnson *et al.* 1985).

Libellula lydia and *Libellula luctuosa* (Odonata: Anisoptera) are morphologically and ecologically similar species of dragonflies that frequently co-occur in shallow, lentic habitats throughout eastern North America. These species breed throughout most of the summer, so that many different sizes of larvae co-occur at any time, and interspecific interactions are likely to be complex (Wissinger 1986). In this paper I present the results of laboratory experiments with these species that address (*i*) how larval development and survivorship vary with food availability, and (*ii*) how inter-odonate predation rates change as a function of size differences among larvae. The results of these experiments suggest a design for field experiments that should disentangle the effects of competitive and predatory interactions in these and other size-structured populations.

Methods

Both experiments were conducted at 17 ± 2°C under a 12 h light : 12 h dark photoperiod. Larvae were collected from a small pond in Tippecanoe County, Indiana (Wissinger 1986). The results of all experiments were analyzed using ANOVA, and individual means compared using the Student–Newman–Keuls multiple-range test (Nie *et al.* 1975). The robustness of ANOVA to slight departures from normality and homoscedasticity warrants the minimal use of transforma-

---

[1] Present address: Departments of Biology and Environmental Science, Allegheny College, Meadville, PA, U.S.A. 16335.

Printed in Canada / Imprimé au Canada

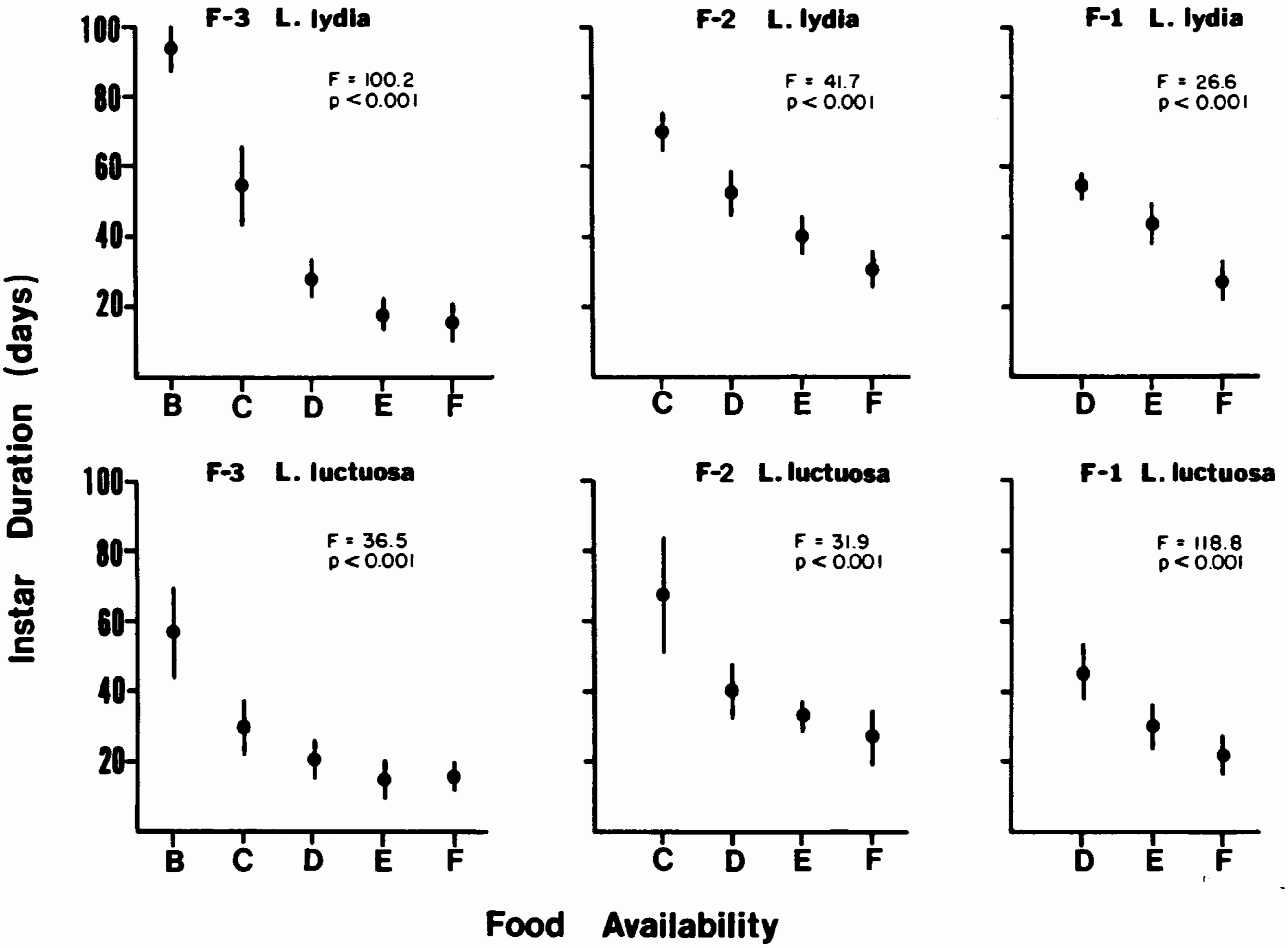

FIG. 1. The effect of food availability on instar duration. Values are expressed as mean number of days ($n = 6$). Vertical bars represent ±1 SD. F-1 to F-3, stage of larval development; B−F, feeding treatments (see Table 1).

TABLE 1. Number of *Daphnia magna* provided per day to determine the effects of food availability on the larval development and survivorship of *L. lydia* and *L. luctuosa*

	Feeding treatment						
Instar	A	B	C	D	E	F	Prey size
F-3	1	3	6	10	20	30	Small[a]
F-2	3	6	10	20	30	50	Small[a]
F-1	3	6	10	15	20	30	Large[b]
F	6	10	15	20	30	50	Large[b]

NOTE: Each treatment was replicated seven times.
[a] Body length 1.08 ± 0.04 mm.
[b] Body length 2.70 ± 0.08 mm.

tions (Scheffé 1959; see also Morin 1983). In one case where variances were markedly heteroscedastic, I used log (n + 1) transformed data.

Food availability experiment

This experiment was conducted in spring to avoid the effects that an overwintering diapause might have on development (Wissinger 1986). I collected F-4 larvae of both species, i.e., larvae that were four molts smaller than the final larval instar (F), and fed them *Daphnia magna* at constant rates until they molted to the F-3 instar. Larvae used in the experiment all entered the F-3 instar within 3 days of each other. Each larva was isolated in a small glass jar containing 100 mL of filtered pond water.

To standardize prey size, *D. magna* were passed through a series of increasingly fine sieves (1.8, 0.9, and 0.6 mm mesh sizes). F-3 and F-2 larvae were fed small *D. magna* retained by the 0.6-mm sieve (1.08 ± 0.036 mm body length), whereas F-1 and F instars were fed larger sizes that passed through the 1.8-mm sieve but were retained by the 0.9-mm sieve (2.7 ± .08 mm body length). Six feeding treatments (A−F) were used for each instar (Table 1). The maximum feeding treatment at each instar was based on the total number of prey eaten in 24 h under *ad libitum* conditions. The number of dead and live *D. magna* in each jar were counted, removed, and replaced with an appropriate number of new individuals daily for 130 days. I recorded the number of days required to complete each instar, the number of molts, and the head width after molting.

Predation experiment

To determine how relative size influenced inter-odonate predation, I combined larvae of different sizes in glass bowls (surface area 227 cm^2). The bottom of each "predation arena" was covered with 0.5 cm of silica sand and filled to a depth of 12 cm with filtered pond water. I standardized hunger levels of larvae by starving them for 48 h before experimentation. Larvae were never re-used in different trials.

Two sets of predation experiments were conducted using instar combinations that corresponded to those observed in natural populations during fall and spring. In the fall experiments I varied the size of the potential predator (F to F-6 *L. lydia* and F-1 to F-6 *L. luctuosa*) that I combined with 20 small (F-6) larvae. Five replicates of each intra- and inter-specific instar combination (130 in total) were each randomly assigned to one of four trials run during consecutive 48-h periods. At the end of each trial I counted the number of small larvae

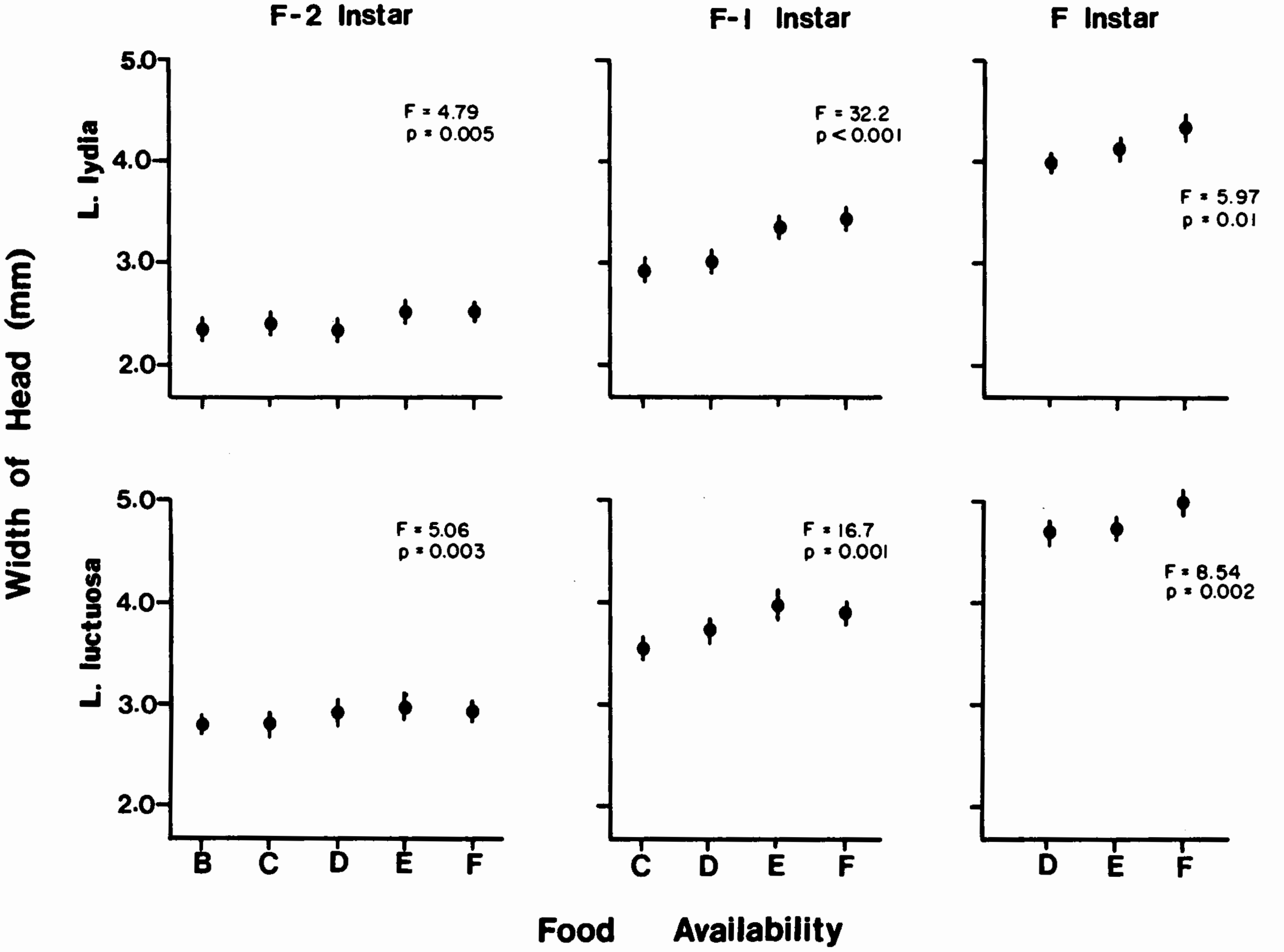

FIG. 2. The effects of food availability on the size of larvae following each molt. Values are expressed as mean head width ($n = 6$). Vertical bars represent ±1 SD. B–F, feeding treatments (see Table 1).

remaining and examined them for any evidence of appendage loss or other physical damage.

The spring experiments differed in that I varied the size of the 10 potential prey (F to F-4 *L. lydia*, and F-1 to F-4 *L. luctuosa*) that were combined with predators (either F-1 *L. luctuosa* or F *L. lydia*). Five replicates of each intra- and inter-specific instar combination (90 in total) were randomly assigned to three consecutive 48-h trials.

To compare the results of the different experiments and determine how inter-odonate predation rates varied as a function of size *per se* (rather than of instar combination), I calculated the proportional difference in the labium width (L) of the predator and the head width (H) of the prey for each treatment combination, i.e., $(L - H)/L$. In libellulid odonates I found that head width was a more repeatable measure of body size than was total length, but a less accurate measure of gape size than was labium width (Wissinger 1986).

Results

Effects of feeding rate on growth and survivorship

In both *L. lydia* and *L. luctuosa*, the duration of each instar decreased significantly with increased food availability (Fig. 1). Larvae at the lowest food levels (treatment A) did not molt during the 130-day period, those in intermediate treatments (B and C) completed one to two instars, and those in the three highest feeding treatments (D, E, and F) attained the final instar by the end of the experiment. In treatments D, E, and F, the total time required to develop from the F-3 to the F instar decreased significantly ($p < 0.001$) with increased food availability (*L. lydia*, 128.5, 106.4, and 79.6 days; *L. luctuosa*, 107.7, 83.0, and 67.7 days).

Food availability also affected the size of larvae at each molt. Although the faster growing larvae in the high food treatments were generally larger when they molted, these differences were small compared with inter-instar differences in size (Fig. 2). There was no indication that the number of instar categories varied among feeding treatments.

All larvae in treatment A died before molting. There was no mortality in the other treatments.

Inter-instar predation rates

The number of larvae consumed in the predation experiments increased as a function of instar difference (Figs. 3 and 4). The following patterns were observed with both the spring and fall instar combinations. (*i*) Little or no predation occurred among larvae that differed by one or zero instars. (*ii*) At least one larva was eaten in all interspecific and conspecific treatments in which larvae differed by two or more instars. (*iii*) Among these treatments, the number of larvae consumed increased dramatically as a function of instar difference.

Larvae in treatments with little or no predation were not missing appendages and showed no other evidence of physical

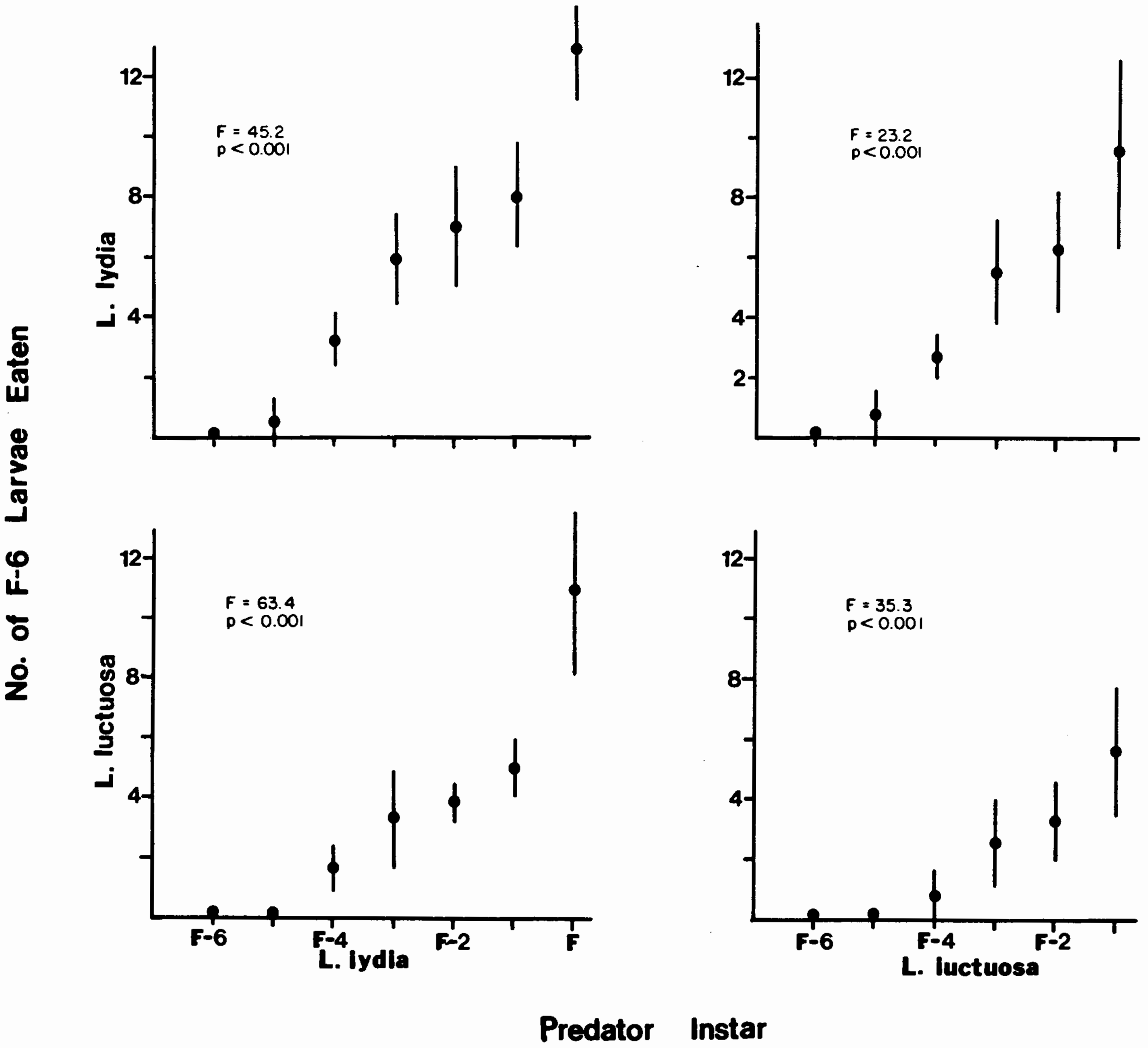

FIG. 3. Survivorship of larvae in the inter-odonate predation experiment, using fall instar combinations. ANOVA results based on (log $n + 1$) transformed data. Values are expressed as means ($n = 5$). Vertical bars represent ±1 SD.

damage. My observations during the experiments suggested that larvae of similar size rarely attacked each other.

In both experiments, *L. lydia* cannibalism was consistently higher than *L. lydia* predation on *L. luctuosa* for a given instar difference. The opposite was true for *L. luctuosa* cannibalism compared with predation on *L. lydia*. However, *L. luctuosa* is slightly larger than *L. lydia* at each instar, and when all treatments were plotted as a function of size rather than instar difference, the effects of species combination on predation rates were less obvious (Fig. 5).

Discussion

Prey availability and development response

The most obvious response of *L. lydia* and *L. luctuosa* to increasing prey availability is a decrease in instar duration (Fig. 1). Similar changes in instar duration are observed in response to food availability in other anisopteran (Hassan 1976) and zygopteran odonates (Lawton *et al.* 1980; Baker 1982). Both in my study and in previous studies, survivorship is high across a wide range of food availabilities. Even when feeding rates are insufficient for the completion of one instar, larvae can live more than 100 days (Hassan 1976; Lawton *et al.* 1980; Baker 1982; this study). Because such consistently low feeding rates are unlikely in the field, starvation should be rare in nature (Lawton *et al.* (1980) and references therein).

As in a previous growth experiment with Zygoptera (Lawton *et al.* 1980), faster developing larvae in this study molt at a slightly, but significantly, larger size than slower developing larvae (Fig. 2). However, in this study these size differences are small compared with the change in size between molts, and is similar to the within-instar variability observed in natural populations. Both the fastest and slowest growing larvae in

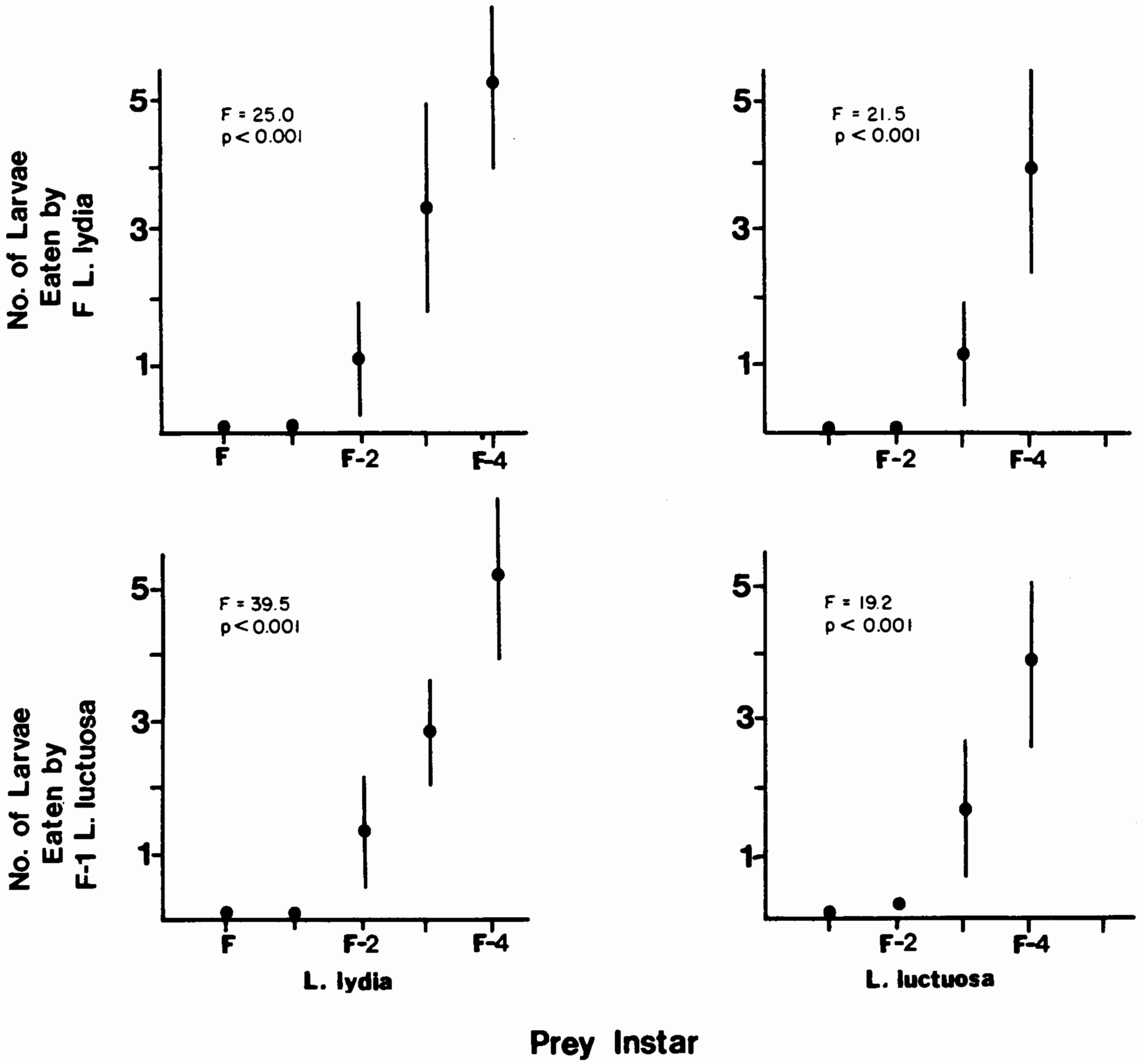

FIG. 4. Survivorship of larvae in the inter-odonate predation experiment, using spring instar combinations. Values are expressed as mean ± SD ($n = 5$).

these experiments are easily assigned to instar categories inferred from field data (Wissinger 1986).

Despite the effects of food availability on instar duration, I did not observe any change in the number of molts. This determinacy in the later instars of *L. lydia* and *L. luctuosa* may be representative of all instars. Among years in the same pond, and among different ponds in this geographic area, I found no evidence of variation in the number of instar categories for these species (S. A. Wissinger, unpublished data). This differs from the instar variability reported within and between populations of some zygopteran odonates (Sawchyn 1971; Ingram 1976; Ingram and Jenner 1976*a*, 1976*b*), and for aquatic insects in general (Sweeney 1984).

Size-specific rates of inter-odonate predation

Although the importance of interspecific predation and cannibalism among odonate larvae has received considerable recent attention (Merrill and Johnson 1984), little is actually known about how predation rates vary among different size combinations of larvae. The results of the laboratory experiments reported here demonstrate that predatory interactions between *L. lydia* and *L. luctuosa* are highly size-dependent (Figs. 3, 4). The proportional size difference between the gape of the larger instar and the head width of the smaller instar is a good predictor of predation rate across a variety of conspecific and interspecific instar combinations (Fig. 5).

My results are generally consistent with those of a recent study involving Zygoptera, in which smaller larvae were also attacked more frequently than larger larvae (Baker and Dixon 1986). Although some larvae were killed in Baker and Dixon's (1986) study, most attacks resulted in nonmortal wounds to regenerative appendages (e.g., legs and caudal lamellae). Zygopteran larvae actively defend feeding perches (Crowley 1979; Baker 1980, 1981, 1982, 1983; Rowe 1980), and such aggressive behavior may be more related to interference competition, and less directly to inter-odonate predation (but see Fischer 1961).

In contrast, aggression between *L. lydia* and *L. luctuosa* is

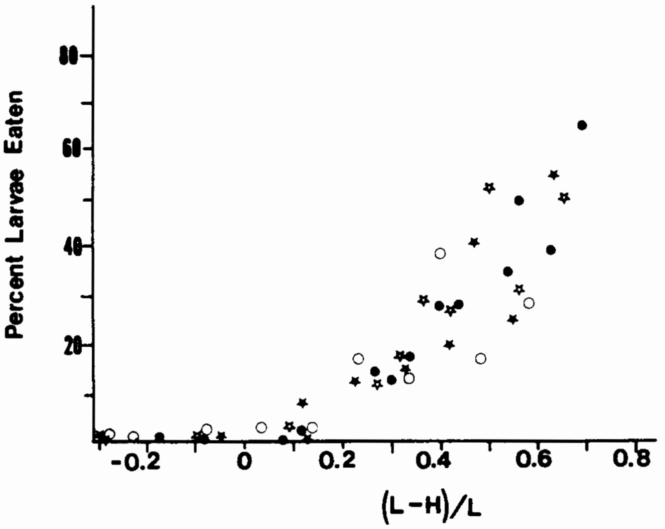

FIG. 5. The relationships between inter-odonate predation rate and the proportional size differences among larvae. Data are for both fall (Fig. 3) and spring (Fig. 4) instar combinations. Solid circles, *L. lydia* cannibalism; open circles, *L. luctuosa* cannibalism; solid stars, *L. lydia* predation on *L. luctuosa*; open stars, *L. luctuosa* predation on *L. lydia*. *L*, labial width of the predator, and *H*, head width of the prey.

clearly a trophic interaction. The absence of appendage loss or other physical damage in treatment groups containing larvae similar in size suggests that low predation rates are not a function of unsuccessful attacks, but are due to lower attack rates. Thus, it appears that there is a behavioral mechanism that mediates aggressive encounters between larvae similar in size.

Whether the predation rates I observe in the laboratory are realized in nature will depend on a variety of factors, such as the degree of habitat segregation among size classes (Macan 1964; Lawton 1970; Wissinger 1986) and (or) the defense mechanisms employed by smaller larvae (see review by Peckarsky 1984). Although encounter rates in my experiments probably exceed those in natural populations, my results clearly imply that when larvae do encounter each other, the likelihood of predation will increase as a function of their size differences.

Separating competitive and predatory interactions in field experiments

The size-structured nature of odonate populations (and those of other predatory insects) creates the potential for mixed competitive and predatory interactions that can obscure the interpretation of experiments designed to quantify the intensity of interspecific interactions (Benke 1978; Benke *et al.* 1982; Johnson *et al.* 1985). The results presented in this paper imply that the effects of these interactions should be extricable for some species by manipulating the instar structure of experimental populations. The effects of competition can be isolated from those of predation by using only larvae that are similar in size. Determining the effects of food limitation on growth rate in competition treatments should not be complicated by variability in instar size or the number of molts during development. Competition treatments can serve as survivorship controls for predation treatments that contain disparate size combinations of larvae. Although the larger larvae in predation treatments may have an asymmetric competitive effect on smaller larvae (Benke 1978; Thompson 1978*b*), starvation should not confound the effects of inter-odonate predation. Experiments that involve such size-class manipulations can provide considerable insight into the types of interactions that influence coexistence in size-structured populations (Werner 1986; Wilbur 1984).

Acknowledgements

Comments by R. D. Howard, P. M. Waser, and two anonymous reviewers greatly improved an earlier version of this manuscript. This research was supported by a Purdue Research Foundation Fellowship, a Sigma Xi grant-in-aid, and a National Science Foundation research initiation and support grant, SER 77-06731, to Purdue University.

BAKER, R. L. 1980. Use of space in relation to feeding areas by zygopteran nymphs in captivity. Can. J. Zool. **58**: 1060–1065.

——— 1981. Behavioural interactions of feeding areas by nymphs of *Coenagrion resolutum* (Coenagrionidae: Odonata). Oecologia, **49**: 353–358.

——— 1982. Effects of food abundance on growth, survival, and use of space by nymphs of *Coenagrion resolutum* (Zygoptera) Oikos, **35**: 47–51.

——— 1983. Spacing behaviour by larval *Ischnura cervula* Selys: effects of hunger, previous interactions, and familiarity with an area (Zygoptera: Coenagrionidae). Odonatologica, **12**: 201–207.

BAKER, R. L., and DIXON, S. M. 1986. Wounding as an index of aggressive interactions in larval Zygoptera (Odonata). Can. J. Zool. **64**: 893–897.

BENKE, A. C. 1978. Interactions among coexisting predators: a field experiment with dragonfly larvae. J. Anim. Ecol. **47**: 335–350.

BENKE, A. C., CROWLEY, P. H., and JOHNSON, D. M. 1982. Interactions among coexisting larval Odonata: an *in situ* experiment using small enclosures. Hydrobiologia, **94**: 121–130.

CROWLEY, P. H. 1979. Behavior of zygopteran nymphs in a simulated weed bed. Odonatologica, **8**: 91–101.

FISCHER, Z. 1961. Cannibalism among the larvae of the dragonfly *Lestes nympha* Selys. Ekol. Pol. Ser. B, **7**: 33–39.

HASSAN, A. T. 1976. The effect of food on larval development of *Palpopleura lucia* (Drury) (Anisoptera: Libellulidae). Odonatologica, **1**: 27–33.

INGRAM, B. R. 1976. Life histories of three species of Lestidae in North Carolina, United States (Zygoptera). Odonatologica, **5**: 231–244.

INGRAM, B. R., and JENNER, C. E. 1976*a*. Influence of photoperiod and temperature on developmental time and number of molts in nymphs of two species of Odonata. Can. J. Zool. **54**: 2033–2045.

——— 1976*b*. Life histories of *Enallagma hageni* and *E. aspersum* (Hagen) (Zygoptera: Coenagrionidae). Odonatologica, **5**: 331–345.

JOHNSON, D. M., CROWLEY, P. H., BOHANAN, R. E., WATSON, C. N., and MARTIN, T. H. 1985. Competition among larval dragonflies: a field enclosure experiment. Ecology, **66**: 119–128.

LAWTON, J. H. 1970. A population study on larvae of the damselfly *Pyrrhosoma nymphula*. Hydrobiologia, **36**: 33–52.

LAWTON, J. H., THOMPSON, B. A., and THOMPSON, D. J. 1980. The effects of prey density on survival and growth of damselfly larvae. Ecol. Entomol. **5**: 39–51.

MACAN, T. T. 1964. The Odonata of a moorland fish pond. Int. Rev. Gesamten Hydrobiol. **49**: 325–360.

MERRILL, R. J., and JOHNSON, D. M. 1984. Dietary niche overlap and mutual predation among coexisting larval Anisoptera. Odonatologica, **13**: 387–406.

MORIN, P. J. 1983. Preation, competition, and the composition of larval anuran guilds. Ecol. Monogr. **53**: 119–138.

NIE, N. H., HULL, C. H., JENKINS, J. G., STEINBRENNER, K., and BENT, D. H. 1975. Statistical package for the social sciences. McGraw-Hill Co., New York.

PECKARSKY, B. L. 1984. Predator–prey interactions among aquatic insects. *In* The ecology of aquatic insects. *Edited by* V. H. Resh and D. M. Rosenberg. Frederick A. Praeger Inc., New York. pp. 196–254.

Rowe, R. J. 1980. Territorial behavior of a larval dragonfly, *Xanthocnemis zealandica* (McLachlan) (Zygoptera: Coenagrionidae). Odonatologica, 9: 285–292.

Sawchyn, W. W. 1971. Environmental controls in the seasonal succession and synchronization of development in some boreal species of damselflies (Odonata: Zygoptera). Ph.D. dissertation, University of Saskatchewan, Saskatoon.

Scheffé, H. 1959. The analysis of variance. John Wiley and Sons Inc., New York.

Sweeney, B. 1984. Factors influencing life-history patterns of aquatic insects. *In* The ecology of aquatic insects. *Edited by* V. H. Resh and D. M. Rosenberg. Frederick A. Praeger Inc., New York. pp. 56–100.

Thompson, D. J. 1978*a*. The natural prey of larvae of the dragonfly *Ischnura elegans* (Odonata). Freshwater Biol. 8: 377–384.

——— 1978*b*. Prey size selection by larvae of the damselfly, *Ishnura elegans*. J. Anim. Ecol. 47: 769–785.

Werner, E. E. 1986. Species interactions in freshwater fish communities. *In* Community ecology. *Edited by* J. M. Diamond and T. M. Case. Harper and Row, Publishers, New York. pp. 344–358.

Werner, E. E., and Gilliam, J. F. 1984. The ontogenetic niche and species interactions in size-structured populations. Annu. Rev. Ecol. Syst. 15: 393–425.

Wilbur, H. M. 1980. Complex life cycles. Annu. Rev. Ecol. Syst. 11: 67–93.

——— 1984. Complex life cycles and community organization in amphibians. *In* A new ecology: novel approaches to interactive systems. *Edited by* P. W. Price, C. W. Slobodchikoff, and W. S. Goud. John Wiley and Sons Inc., New York. pp. 196–224.

Wissinger, S. A. 1986. Comparative life histories and larval population interactions in a diverse assemblage of dragonflies (Odonata: Anisoptera). Ph.D. dissertation, Purdue University, West Lafayette, IN.