

**ONTOGENETIC DIFFERENCES IN INTRASPECIFIC  
AGGRESSION OF DAMSELFLY LARVAE:  
*ISCHNURA POSITA* (HAGEN)  
(ZYGOPTERA: COENAGRIONIDAE)**

L.R. SHAFFER and J.V. ROBINSON

Department of Biology, Box 19498, University of Texas at Arlington,  
Arlington, TX 76019, United States

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Differences in behaviour between ultimate and penultimate instar larvae of *I. posita* were examined using caudal lamella loss as an assay. Equal densities of each instar were separately assembled in 20 L aquaria using 39 vertical dowels as an artificial weed bed. After 24 h there were 5 deaths in the ultimate instar assemblages and 1 death in the penultimate assemblages. One hundred sixty one ultimate instar larvae (72%) suffered some loss of lamellae while only 54 penultimate larvae (24%) were so affected ( $P < 0.001$ ). Comparison of number of lamellae lost yielded similar results: 254 lost among ultimates, 65 among penultimates ( $P < 0.001$ ). The hypothesis that lamella loss follows a binomial distribution could not be rejected. This pattern suggests that lamellae are lost singly and that no individual has a higher probability of loss than another. Video recorded trials of paired, same instar larvae suggest that differences in lamellae loss result from differential use of agonistic behaviour patterns rather than differences in encounter rates due to locomotion patterns.

**INTRODUCTION**

Larval zygopterans are aggressive toward conspecifics (FISHER, 1960; ROWE, 1980). Interactions between individuals can be complex and involve a wide range of aggressive behaviour (BAKER, 1980, 1981; ROWE, 1980, 1985; COHN, 1989; SHAFFER, 1990). The behaviour patterns used in an interaction are influenced by the relative size of the individuals involved (McPEEK & CROWLEY, 1987). In both laboratory and field experiments, small *Ischnura verticalis* Say larvae are wounded more frequently than large larvae (BAKER & DIXON, 1986).

In addition to the relative size effect, ROWE (1985) indicates that behavioural

changes may accompany changes in ontogenetic stage. Behavioural differences between early and late instar larvae of *Xanthocnemis zealandica* McLachlan are described in detail by ROWE (1985) but not quantified. The outcome of an encounter between larvae, therefore, may be due in part to relative size and in part to behavioral characteristics associated with the contestants' ontogenetic stage.

One way to examine the behaviour of larval zygopterans involves placing the larvae in an arena, staging an encounter, and evaluating the subsequent maneuvers of the subjects. There are potential problems with this approach: The staged encounter may be unrealistic or so limiting as to not accurately represent field conditions (e.g., researchers controlling the orientation of the larvae at the onset of the experiment), the choice of patterns quantified is highly subjective, and the criteria used to evaluate the "winner" or "loser" of an encounter may be subjective.

BAKER & DIXON (1986) propose using caudal lamellae loss as an index of aggressive interactions between zygopteran larvae. This method is independent of many of the subjective components of staged contests. We evaluate possible behavioural differences that may accompany the ontogeny of *Ischnura posita* using lamellae removal as an assay. In our experiment, equal densities of ultimate and penultimate (F-1) instar larvae were placed in comparable chambers and the resulting damage and mortality after 24h determined. We assigned levels of aggression to final and F-1 instar larvae in these assemblages based on lamella removal. Since a single larva may be missing 0, 1, 2 or 3 lamellae, the interpretation of this assay was made more complicated. However, data concerning the number of lamellae lost per individual are useful and can be analysed to establish whether attacks that lead to lamellae loss are unexpectedly concentrated on a small subset of the damselfly population. Larvae in paired symmetric contests were video recorded to determine if locomotion and the use of certain recurring behaviour patterns could be associated with the results of the assemblage assay.

## METHODS

### Assemblage experiment

Thirty-nine 6 mm diameter wooden dowels were affixed to a 23 x 23 cm sheet of plexiglass 5 mm thick. The dowels were arranged in a hexagonal fashion, each 3.5 cm from all adjacent dowels. The plexiglass sheet was placed at the bottom of a 20 L aquarium with the dowels projecting upwards 30 cm, extending approximately 2 cm above the surface. Cleaned, coarse sand was poured into the chamber to cover the plexiglass sheet and the surrounding gap between the plexiglass sheet and the aquarium walls, thus preventing larvae from taking refuge under the stalk arrangement. The experimental chambers were aerated using an airstone placed in one corner. Low air pressure was used to minimize currents generated by rising bubbles. The entire chamber was wrapped with opaque paper to prevent activity outside the chamber affecting larval behaviour.

Head widths of field collected larvae were measured and any missing or regenerating caudal lamellae noted. Instar determinations were made from frequency distributions of head widths (BENKE & BENKE, 1975). Of the larvae determined to be in the final or F-1 instars, only those with 3 non-regenerating lamellae were used. Final instar larvae nearing ecdysis were not used. Larvae were fed, ad libitum, a mixture of zooplankton for at least 15 h before each trial.

32 larvae were added to each chamber in an approximately uniform pattern at the water surface. 24 h after initiation of a trial the stalk arrangement was lifted from the chamber and the surviving larvae removed. Larvae that remained in the chamber, as well as partially consumed ones, were removed using a fine mesh net. Seven 24 h trials were performed with each of the two instars. Wilcoxon 2 sample tests were used to determine if mortality and the number of individuals affected by lamellae loss were significantly different between instar assemblages.

For each of the two instar treatments, a binomial distribution was used to determine the expected number of larvae having 0, 1, 2 and 3 lamellae at the termination of an experiment. Parameters for this distribution were derived intrinsically for each treatment by approximating the probability that a lamella would be lost. This was done by calculating the relative frequency of lamellae lost for each instar in all seven trials. The actual distribution of larvae having 0, 1, 2 or 3 lamellae was compared to these theoretical distributions using a G test for goodness of fit. The degrees of freedom used to determine the critical values were adjusted appropriately for the intrinsic nature of the hypothesis (SOKAL & ROHLF, 1981). A lack of fit between these distributions would indicate that some individuals experienced an inordinate amount of lamellae loss or that once a lamella was lost an individual was less likely to lose another.

### Contest between two larvae

All trials were video recorded and took place in a plexiglass chamber 5 cm x 2 cm x 5 cm deep. Two dowels 3 mm in diameter were cemented to the bottom of the chamber along the center of the long axis so that the distance between them was the same as the distance from either dowel to the side wall of the chamber. The dowels extended above the surface of the water when the chambers were filled. A translucent plastic cylinder 1 cm in diameter was placed over each dowel and then a larva was placed on each dowel. This allowed the larva to recover from being handled and acclimate to the chamber without seeing the other larva. After a 30 min acclimation period the plastic cylinders were lifted out of the chamber and video recording begun. Five pairs of final instar larvae and five pairs of F-1 instar larvae were recorded for 2 h.

Initiation and termination times of crawling and swimming bouts were used to determine the total time an individual spent moving. In each trial, the number of times individuals engaged in one of four behaviour patterns was noted. The behaviour patterns quantified were labial strikes, a forward thrusting of the labium toward the opponent; slash, a rapid lateral movement of the abdomen directed toward the opponent; abdomen wagging, a broad, slow, lateral wagging of the abdomen; and S-bend, a curving of the abdomen in one direction at segments 2-3 and in the opposite direction near segment 8. These correspond respectively to damselfly behaviour patterns defined as "labial strike" (BAKER, 1981); "slash" (BAKER, 1981; ROWE, 1985) "rigid abdomen wave" (BAKER, 1981) and "SCS" (ROWE, 1985); and "S-bend" (ROWE, 1985).

## RESULTS

In the seven trials with final instar larvae a total of 5 individuals were killed but only one F-1 instar died. This difference was not statistically significant ( $U=22.5$ ,  $P>0.05$ ). Among the final instar larvae, 161 individuals (72%) lost one

or more caudal lamellae, whereas only 54 (24%) F-1 instar larvae did, a highly significant difference ( $U=49$ ,  $P<0.001$ ).

Final and F-1 instar treatments can be similarly shown to be different by analyzing the assay using number of lamellae removed as an index of aggression rather than number of damaged larvae. Final instar larvae lost 254 lamellae during these trials, while only 65 were lost by F-1 instar larvae ( $U=49$ ,  $P<0.001$ ).

The probability that a lamella was removed from a surviving final instar larva was 0.39 (i.e., number of missing lamellae / 3 x the number of survivors); the corresponding probability for F-1 larvae was 0.10. These probabilities were used to estimate the expected distribution of surviving larvae having 0, 1, 2 or 3 lamellae (Fig. 1). Goodness of fit analyses indicated that the hypothesis that lamella loss follows a binomial distribution could not be rejected ( $G=3.65$ ,  $P>0.05$  for final instars;  $G=5.22$ ,  $P>0.05$  for F-1 instars).

Video analysis indicated that the duration of larval locomotion was nearly identical for both instars (Fig. 2). Final instar larvae wagged their abdomens and struck each other more with their labia than F-1 instars which displayed slashes and S-bends more frequently (Fig. 3).

## DISCUSSION

Final instar larvae lost caudal lamellae nearly 3 times as often as their F-1

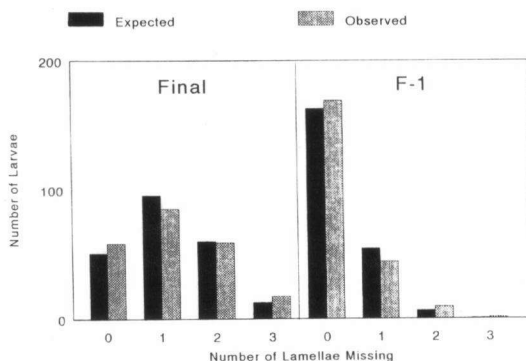


Fig. 1. Distribution of expected and observed lamella loss among final and F-1 instar larvae. Expected frequencies are based on a binomial distribution and the intrinsic hypotheses that the probability of lamella loss = 0.39 for the final instar and 0.10 for the F-1 instar.

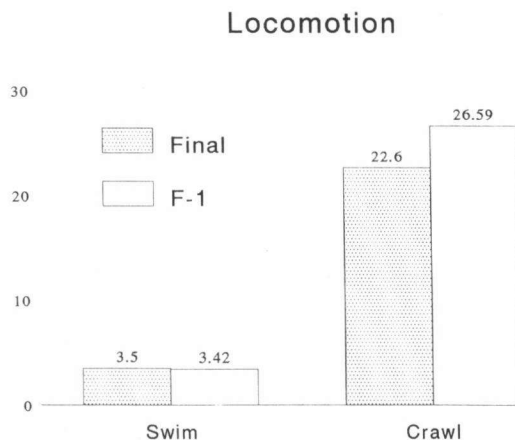


Fig. 2. Percentage of the two hour trial in which larvae engaged in locomotion.

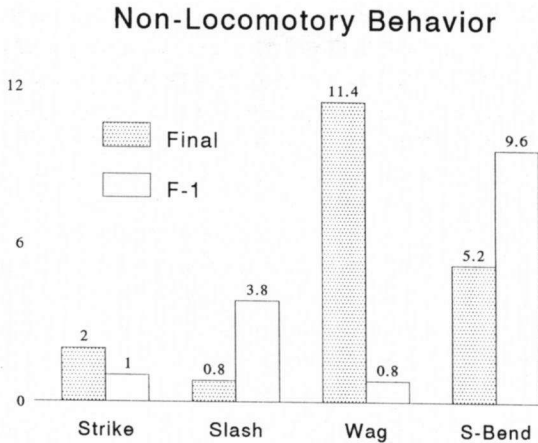


Fig. 3. Mean number per trial of occurrences of the four non-locomotory behaviour patterns.

instar counterparts in symmetric contests within experimental chambers. More final instar larvae were killed in these assemblages as well, but not significantly more, probably due to the overall rarity of killing in the experiment. The experimental results indicate that a difference in the aggressive tendencies occurred between the two instars and support BAKER & DIXON's (1986) conclusion that the damage observed in damselfly larvae may be caused by other damselfly larvae.

BAKER & DIXON (1986) reported a higher frequency of lamella loss in earlier than late instars of *I. verticalis*, both in nature and the laboratory. This may have been because younger larvae were at a size disadvantage in aggregations which included larger larvae. However, penultimate instar larvae of *I. posita* lose lamellae less frequently in nature than their final instar counterparts (ROBINSON, et al., 1991). Interpretation of such lamella loss in field collections is problematic because: (1) the duration of instars varies; — (2) the milieu of organisms that remove lamellae is uncontrolled and — (3) the amount of damage during collection cannot be precisely determined. However, our laboratory results are consistent with results reported from field collections of *I. posita* and the opposite of those reported for *I. verticalis*.

We can not reject the hypothesis that lamella loss per individual followed a binomial distribution. This implies that the probability of an individual losing a lamella is independent of the number of lamellae that it possesses. There are 2 potential reasons for suspecting that this hypothesis may be erroneous. First, a single strike might result in the loss of multiple lamellae and, second, some individuals may be significantly more subject to attack than others. The former reason undoubtedly occurs; however it provides limited biological insight into the dynamics of intraspecific interactions in *I. posita*. Despite biasing our test of this hypothesis toward rejection because of such simultaneous lamella losses we must still accept the null criterion. This suggests that inequality among larvae in their ability to avoid lamella removal contributed little to our results.

A possible reason why damage was higher in final instar assemblages was

that encounter rates were more frequent because final instar larvae were more active than instar F-1 larvae. If this were true, no difference in aggressiveness would be needed to explain lamella removal patterns. However, the locomotory activity of the two instars was nearly identical. This suggests the importance of other behaviour patterns in explaining differences in caudal lamellae loss.

Video recorded behaviour suggests trends in the behaviour of final and F-1 instar larvae that correspond to the assemblage results. Thus, strikes were twice as frequent among final instar larvae. Another method of displacing an enemy, slash, also involves physical contact. However, a slashing larva neither has the potential to kill its opponent nor does it incur a high risk of being killed. Slash was used more by the F-1 larvae. Abdomen wagging and S-bend occur at a distance from the opponent and involve no physical contact. Abdomen wagging is associated with the final instar and S-bend with the F-1 instar. Using the assemblage results we conclude that abdomen wagging is invoked in more dangerous encounters than S-bending. Indeed it may be precursor to predatory behaviour (COHN, 1988). Perhaps abdomen bending reflects the increased respiratory demands associated with "excitement" as suggested by Baker (*in* JOHNSON, 1991).

Considerable evidence is accumulating that foraging behaviour changes ontogenetically in vertebrates which undergo continuous growth (WERNER, 1988). Ontogenetic behavioural differences of individuals may be more abrupt in animals exhibiting stepwise development than in those with continuous development. Aquatic insect larvae exhibit stepwise growth at each molt. Larvae of *I. posita* increase in size approximately 26% between the F-1 and final instar (SHAFFER & ROBINSON, 1989). In this study we found ontogenetic trends in the development of behavioural patterns not associated with foraging and demonstrated that, in addition to the morphological changes that occur between these two instars, there were marked changes in aggression. Such changes in behaviour may be important when considering the life history and size structure of populations of aquatic insects.

#### REFERENCES

- BAKER, R.L., 1980. Use of space in relation to feeding areas by zygopteran nymphs in captivity. *Can. J. Zool.* 58: 1060-1065.
- BAKER, R.L., 1981. Behavioral interactions and use of feeding areas by nymphs of *Coenagrion resolutum* (Coenagrionidae: Odonata). *Oecologia* 49: 353-358.
- BAKER, R.L. & S.M. DIXON, 1986. Wounding as an index of aggressive interactions in larval Zygoptera (Odonata). *Can. J. Zool.* 64: 893-897.
- BENKE, A.C. & S.S. BENKE, 1975. Comparative dynamics and life histories of coexisting dragonfly species. *Ecology* 56: 302-317.
- COHN, S.L., 1988. *Damsels in distress: feeding, predator avoidance, and competition in the damselfly larva Ischnura verticalis (Say)*. M. Sci. thesis, Univ. Kentucky, Lexington.
- FISHER, Z., 1960. Cannibalism among the larvae of the dragonfly *Lestes nympha* Selys. *Ekol. pol.* (B) 7: 33-39.

- JOHNSON, D.M., 1991. Behavioral ecology of larval dragonflies and damselflies. *Trends Ecol. Evol.* 6: 18-13.
- McPEEK, M.A., & P.H. CROWLEY, 1987. The effects of density and relative size on the aggressive behavior, movement and feeding of damselfly larvae (Odonata: Coenagrionidae). *Anim. Behav.* 35: 1051-1061.
- ROBINSON, J.V., L.R. SHAFFER, D.D. HAGEMEIERS & N.J. SMATRESK, 1991. The ecological role of caudal lamellae loss in the larval damselfly, *Ischnura posita* (Hagen) (Odonata: Zygoptera). *Oecologia* 7: 1-7.
- ROWE, R.J., 1980. Territorial behaviour of a larval dragonfly *Xanthocnemis zealandica* (McLachlan) (Zygoptera: Coenagrionidae). *Odonatologica* 9: 285-292.
- ROWE, R.J., 1985. Intraspecific interactions of New Zealand damselfly larvae I. *Xanthocnemis zealandica*, *Ischnura aurora*, and *Austrolestes colenisonis* (Zygoptera: Coenagrionidae: Lestidae). *N. Z. J. Zool.* 12: 1-15.
- SHAFFER, L.R., 1990. *A multivariate analysis of the behavioral response of Ischnura posita (Hagen) (Odonata: Zygoptera) larvae in encounters with trophically dissimilar macroinvertebrates.* M. Sci. thesis, Univ. Texas, Arlington.
- SHAFFER, L.R. & J.V. ROBINSON, 1989. Sex ratio and sexual dimorphism in late instar larvae of *Ischnura posita* (Hagen) (Zygoptera: Coenagrionidae). *Notul. odonatol.* 3: 40-41.
- SOKAL, R.R. & F.J. ROHLF, 1981. *Biometry* (2nd ed.). Freeman, San Francisco.
- WERNER, E.E., 1988. Size, scaling, and the evolution of complex life cycles. In: B. Ebenman & L. Persson, [Eds], *Size structured populations*, pp. 60-81, Springer, Berlin.