

AGGREGATION BEHAVIOR IN BALTIMORE CHECKERSPOT
CATERPILLARS, *EUPHYDRYAS PHAETON*
(NYMPHALIDAE)

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ABSTRACT. The aggregation behavior of early instars, overwintering fourth instars, and late instars of *Euphydryas phaeton* Drury was examined in natural populations. Activity outside the webs increased as larvae progressed through the first three instars. Mortality at diapause in late summer was 46 to 64% per web. The mean size of overwintering larval groups was one-fifth that of mean group size at diapause, as a consequence of mortality and group subdivision and separation. Mortality of overwintering larvae was 18 to 53%. Post-diapause instars were gregarious, until just prior to pupation.

The size of a group of animals can influence the fitness of an individual for a variety of reasons, such as improved thermoregulation, feeding facilitation, and effective defensive mechanisms (Allee et al., 1949; Alexander, 1974; Wilson, 1975; Morse, 1977; Bertram, 1978; Stamp, 1980a). In contrast to the larvae of most butterflies, the caterpillars of the Baltimore checkerspot aggregate in all instars. These larvae are conspicuous with their communal webs in mid-summer and their coloration and aggregated behavior in the spring. Information on larval aggregation behavior of *E. phaeton* has been largely descriptive. My objectives were to quantify the larval aggregation tendencies of early instars, overwintering fourth instars, and late instars of *E. phaeton* and to identify factors contributing to aggregation behavior.

METHODS

I observed *E. phaeton* from 1977 through 1979 at the Conservation and Research Center of the National Zoological Park at Front Royal, Warren Co., Virginia. Turtlehead (*Chelone glabra* L.: Scrophulariaceae), the larval host plant, grew there in dense patches in wet meadows. This host plant is a clonal, perennial; thus, each plant group consisted of numerous stalks.

Eggs and Early Instars

Egg clusters were located on turtlehead, and these host plant stalks were tagged. To determine the mean number of eggs per cluster, I collected and counted the eggs of 32 and 35 new clusters in 1977 and 1978, respectively.

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Twenty-one prediapause larval aggregations were observed at 1000, 1200, 1400 and 1600 h on three warm, sunny days in July to determine the numbers of larvae outside the webs. Webs were collected after larval diapause in August 1977, 1978, and 1979; 18, 37, and 10 webs, respectively.

Overwintering Fourth Instars

Fifteen plots 1 m apart were set up in late October 1978 to determine group size of overwintering fourth instars, which leave their webs in late fall to overwinter in plant litter on the ground (Bowers, 1978). To facilitate finding larvae, each plot consisted of a 1 × 1 m sheet of clear plastic on the ground. An eighth of a bale of hay was used for each plot. The hay was similar in amount and texture to the plant litter on the ground at this site. A web tied to a stake was placed in the center of each plot. To avoid damaging the webs, the number of larvae per web was not determined. However, the mean number of larvae per web was probably similar to that of the 1978 set of dissected webs ($n = 37$ webs, $\bar{x} = 110$ larvae ± 11 S.E.). By mid-November, after the caterpillars had moved from the webs into the hay, a few spines were marked of each caterpillar within a group, using a different color of Testor's enamel paint for each aggregation on a plot to monitor exchange of individuals among the groups over the winter. Preliminary tests indicated that the paint (primary and secondary colors) did not affect larval behavior. Larvae were checked in mid-November and mid-March to determine the number of groups, size of groups, and dispersal distance from the webs.

To determine the number of larvae surviving over the winter, I set up a second experiment with 30 boxes containing caterpillars in mid-October. Each clear plastic box (13 × 25 × 8 cm) had holes in the bottom, covered with window screen for drainage. Tanglefoot was sprayed on the upper sides of the box to prevent the caterpillars from leaving it. Dried grass in the boxes was similar in texture and depth to the plant litter on the ground in the study site. Fifteen boxes each contained 100 larvae and no cover, and another 15 boxes each had 100 larvae and a screen cover. Each box was held in place on the ground by four stakes. The surviving larvae were counted in mid-March.

Post-diapause Instars

To determine the aggregation behavior of late instars, three study sites were examined weekly from mid-April through mid-July in 1979. The number of larvae within one body length of each other (0.5 to 2

cm, depending on the instar) and the number of those greater than one body length from each other were recorded.

In June 1978 and 1979 the patches of turtlehead were mapped in two study sites. In both sites 3×3 m plots were divided into a hundred 30×30 cm squares. Plant groups were located within these squares. To determine the height of the turtlehead which was available in May, plant stalks were chosen by placing a rod through a plant group until 30 stalks were partitioned, and these stalks were measured. Because there were so few healthy plant groups during this period, only three plant groups were sampled.

To examine aggregation tendencies of late instars further, caterpillars were collected in mid-April and kept in cages ($25 \times 25 \times 76$ cm) in the laboratory at 23°C , 70% RH, and 16 h of light, approximating conditions in late spring. Ten larvae were placed in each cage with pots of snapdragon (*Antirrhinum* sp.: Scrophulariaceae). Snapdragon was chosen because turtlehead was rare in the study sites from April through May; snapdragon was easy to grow; *E. phaeton* larvae feed on other Scrophulariaceae in addition to turtlehead (Tietz, 1972); and they readily ate snapdragon. The first cage contained one plant about 25 cm in height, a second cage had two such plants, and a third had four plants. Five replicates were run. Observations of the larvae began 17 h after placing them in the cages and they were monitored at half-hour intervals for 3 h. Larvae within a body length (less than 1.5 cm) of each other were considered as aggregated. After the experiment, all of the larvae pupated; thus, it is unlikely that any behaved differently during the experiment due to unapparent parasitism. Furthermore, in the course of host-parasitoid studies (Stamp, 1980b, 1981b), I have not obtained tachinid flies, which are the major parasitoids emerging in the pupal stage of *E. phaeton*. If they occur in this *E. phaeton* population, they are rare.

RESULTS

Eggs and Early Instars

The mean number of eggs per cluster was 273.8 (± 23.1 S.D.; 95% confidence limits for a mean pooled for years). Of 42 aggregations of newly-hatched larvae, 95% moved to the top of the host plant stalk before feeding on the turtlehead.

Larvae fed from late June through early August on leaves enclosed within and adjacent to their communal webs. Larval activity outside the webs varied through the day (two-way ANOVA after square root transformation of count data, $P < .001$; with interaction between days and times of day, $P < .05$; Fig. 1). In July larval activity outside the

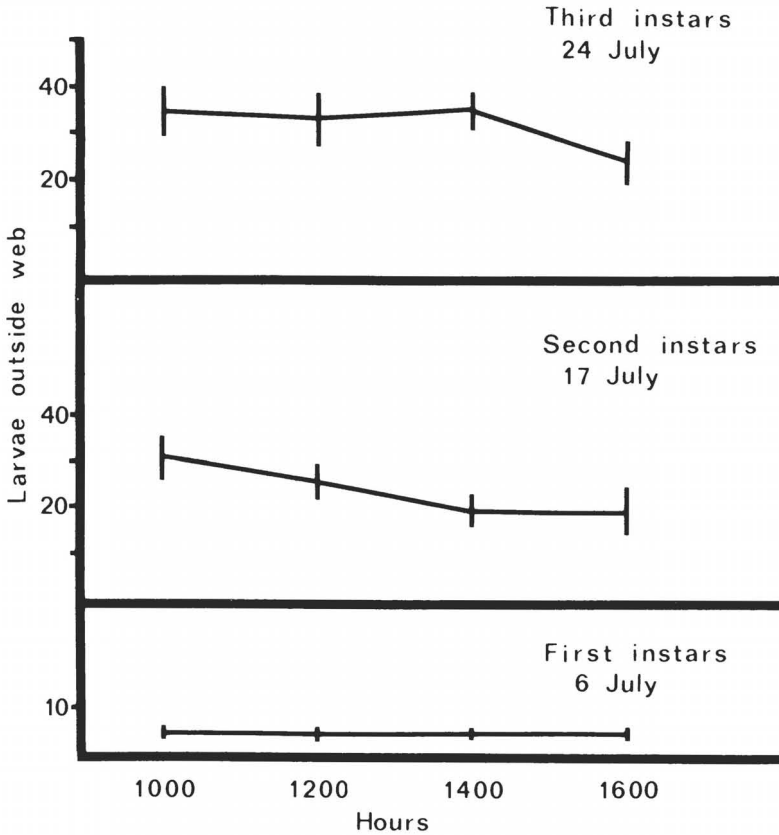


FIG. 1. Mean number of larvae outside webs ($n = 21$) with \pm one standard error.

webs increased as larvae progressed through the first three instars, with significantly fewer first instars outside the webs than during second and third instars (two-way ANOVA, $P < .001$; Newman-Keuls multiple range test [Zar, 1974]; Fig. 1). By the third instar, 86% more larvae were on the outside of the webs or feeding on adjacent leaves during the day relative to the first instars outside the webs. Larvae inside the webs, especially near the center, were quiescent or molting.

The larval aggregations collected after diapause were all in the center of their webs, usually on the upper half of plant stalks. The mean number of larvae per web at diapause was 110 to 216, with considerable variation among years (Fig. 2). Larval mortality due to predators within webs was probably small. Few potential predators were

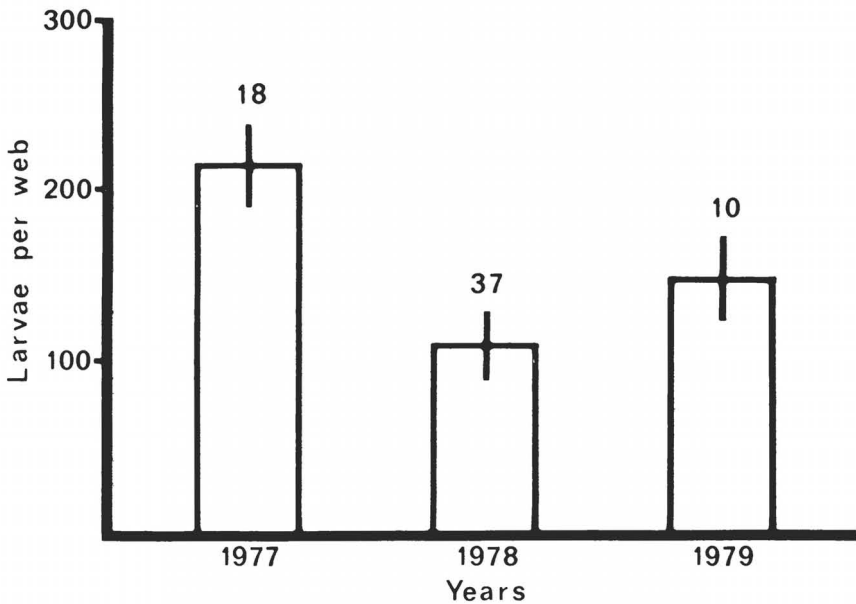


FIG. 2. Mean number of larvae per web at diapause, with \pm one standard error. The numbers of webs are indicated.

found in the 65 webs collected in 1977-79: two chrysopid larvae, two ant colonies, and 27 spiders.

Mortality of the egg and larval stages up to diapause was at least 46%, based on a mean of 274 eggs per cluster and a mean of 148 larvae (± 18 S.E.) per web at diapause for 1977-79. This is a conservative estimate of mortality at this stage for two reasons. First, some webs were composed of larvae from more than one egg cluster on a plant stalk. For example, for plant stalks with egg clusters in 1979, the mean number of clusters per stalk was 1.5 (or 411 eggs; Stamp, 1980b). Furthermore, entire egg clusters rarely disappeared (Stamp, 1981a). Based on this mean of multiple egg clusters per stalk and mean number of diapausing caterpillars per web, mortality of the egg and larval stages up to diapause was 64%. Second, larval aggregations seldom split up, even when they defoliated their host plant stalk. The caterpillars expanded their web down the plant stalk and onto adjacent leaves, remaining together by using silk trails (e.g., Bush, 1969). None of the aggregations observed in this study subdivided. Thus, mortality up to diapause was in the range of 46 to 64%.

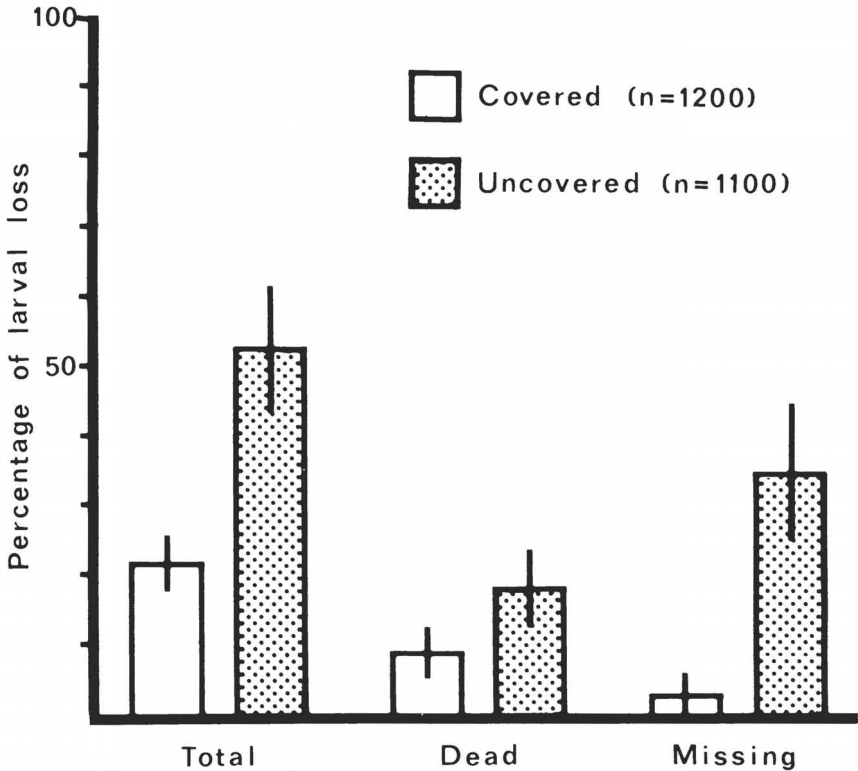


FIG. 3. Percentage of larval loss from screen-covered and uncovered boxes over the winter. Total numbers of larvae are indicated. The numbers of dead larvae per box between covered and uncovered boxes were not significantly different (Mann-Whitney U test, $P > .20$). The numbers of missing larvae between covered and uncovered boxes were significantly different (Mann-Whitney U test, $P < .002$).

Overwintering Fourth Instars

The mean size of overwintering larval aggregations was one-fifth that of mean group size at diapause, which was a consequence of group subdivision and mortality. The sizes of overwintering larval groups on plots were similar in November and March (mean of 21.4 larvae \pm 24.3 S.D. and 17.9 larvae \pm 26.9 S.D., respectively; two-sample *t* test, $P > .50$). Larvae were usually found in dry litter or on the plastic sheet 5 to 8 cm below the litter surface. In November most groups were tightly aggregated (larvae touching each other), but in March most were in loose aggregations (larvae within a body length of each other). All of the groups had moved between November and March. The larval aggregations in March were farther from their webs than in November (11.7 cm \pm 7.9 S.D. and 20.9 cm \pm 14.2 S.D.,

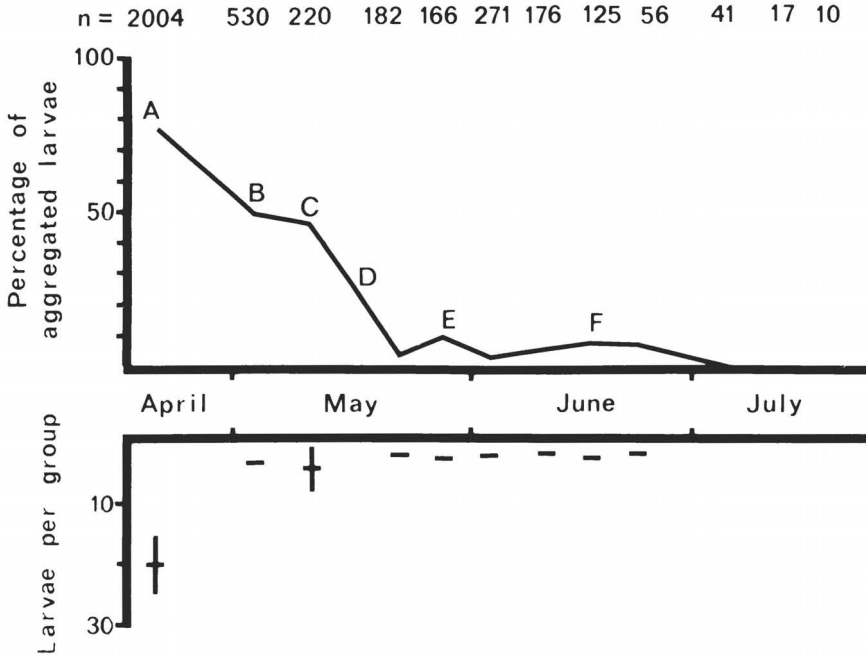


FIG. 4. Aggregation tendencies among post-diapause larvae. Top—line indicates the percentage of aggregated larvae, with total larvae shown by numbers. Factors related to aggregation patterns are: **A**. small patches of turtlehead in mid-April, **B**. no turtlehead above ground in two of three areas, **C**. no turtlehead in two areas and one-third of third area reduced to leafless, 15 cm stalks, **D**. pupation started and turtlehead beginning to reappear, **E**. turtlehead in all areas and first *E. phaeton* adults flying, and **F**. pupation ended. Bottom—mean number of larvae per group of those aggregated individuals, with \pm one standard error.

respectively; normal approximation to Mann-Whitney U test, $P < .01$). Some individuals changed groups over the winter. Over half of the larvae originally on the plots in November were missing in March. In mid-March there were 1.5 groups per plot (± 1.9 S.D.) in contrast to 2.3 groups per plot (± 1.0 S.D.) in mid-November, but this was not a significant difference (Mann-Whitney U test, $P > .05$). The missing groups had either moved off the plots (greater than 45 cm from their webs) or were dead. It appears that groups lost some individuals, but there is no evidence that entire groups died together.

Up to 18% of the caterpillars were dead and up to 35% were missing in the plastic boxes, for a total larval loss of 53%. The numbers of dead larvae per box in the covered and uncovered boxes were similar (Fig. 3); however, the numbers of missing larvae per box were greater in the uncovered boxes than in the covered boxes. Seven boxes were broken by deer and, thus, excluded from the experiment.

TABLE 1. Comparison of defoliated plant stalks and solitary caterpillars. Six completely defoliated plant groups were sampled in May, with a total of 397 stalks. The 226 larvae feeding on these stalks were classified as solitary (greater than a body length apart) or aggregated. Statistical analysis showed no correlation between number of stalks per plant group and the percentage of solitary larvae per plant group (Spearman rank correlation, $n = 6$, $P > .50$). Thus, at this time larvae occurring singly did not appear to be in response to the limited availability of the larval host plant.

	Plant group					
	1	2	3	4	5	6
Leafless plant stalks	31	33	60	82	91	100
Percentage of larvae which were solitary	26	26	12	36	47	0

Post-diapause Instars

Some 77% of the late instars were aggregated in mid-April (beginning of the post-diapause period), but only 20% prior to pupation in mid-May (Fig. 4). The mean group size of aggregated larvae was 20.5 (± 52.1 S.D.) in mid-April and 5.0 (± 7.1 S.D.) three weeks later. In both 1978 and 1979 a few small patches of turtlehead occurred in each of three study sites by mid-April. Less than 15% of the caterpillars were on turtlehead at this time. The patches of turtlehead were completely defoliated by late instars in two of three sites by mid-May in both years. Caterpillars in the two sites with defoliated turtlehead then mainly ate rosaceous shrubs about 10 m from the defoliated host plants. In the third area turtlehead was reduced by one third by mid-May. Many of the plants had few if any leaves, and the stalks were mere stubs, often with one or more caterpillars feeding on them. No correlation between the number of stalks per plant group and the percentage of single larvae on stalks per plant group was evident at this time (Table 1). Thus, the late instars were neither aggregated (that is, no negative correlation) nor dispersed (no positive correlation) in response to the limited amount of turtlehead available to them. Shortly after pupation began, the turtlehead began to recover and by mid-June the mapped areas of turtlehead very closely resembled the patches mapped the year before.

In the laboratory larval aggregation was highest when fewer plants were available (two-way ANOVA after square root transformation; for the number of plants per cage for all late instars aggregated in cages, $P < .05$; for number of plants per cage for larvae aggregated on plants, $P < .001$). The larvae fed actively, and individuals changed aggregations frequently. Half of the larvae were aggregated (mean of 4.7 aggregated larvae per cage). However, most were aggregated on the cages rather than on the plants (mean of only 1.6 aggregated larvae

on plants per cage). Of those caterpillars aggregated on the cages, 93% were at the top of the cages. Similarly, in the spring fifth and sixth instars were aggregated frequently at heights above the vegetation on dead plant stalks, even when turtlehead was readily available.

DISCUSSION

The small number of first instars on the outside of webs, relative to the numbers of second and third instars, was a consequence of how these three instars used the webs. Their abilities to defend themselves differed greatly. First instars were cryptically colored with single hairs extending from tubercles on their bodies. However, second and third instars had conspicuous reddish-brown bodies with dense, black spines projecting at 45 degree angles from tubercles in rows across their bodies. In contrast to first instars, third instars successfully knocked parasitoids away from them by head-jerking (Stamp, 1981b). Also, second and third instars of *E. phaeton* were probably toxic to some vertebrate predators, because larvae that were reared on turtlehead were unpalatable to blue jays (*Cyanocitta cristata* L.: Bowers, 1980). Thus, second and third instars of *E. phaeton* may be better protected against parasitoids and predators because of their size, spines and toxicity and consequently, less dependent on webs for protection against their enemies than first instars (Stamp, 1980b). Similar between-instar differences (in terms of body hair and response by predators) also occur in tent caterpillars (*Malacosoma americanum* [Fabricius]; Ayre & Hitchon, 1968).

The scarcity of first instar *E. phaeton* on the outside of the webs may also be a consequence of adequate food remaining within the webs for them. They enclosed the top two furled leaves of the stalk with silk and fed within the web. In contrast, second and third instars consumed leaves at a faster rate than first instars and often fed from the outside of the webs.

In addition, first instars may be more susceptible to low humidity than second and third instars. Morris & Fulton (1970) suggested that the webs of fall webworms increased microhabitat humidity, an important factor maintaining a high feeding rate and shortening the developmental period. Second and third instars may leave the web on warm days when they are most active to avoid overheating (Morris & Fulton, 1970).

The movement of groups of fourth instars between November and March may be an anti-predatory response, probably directed to invertebrate predators and insectivorous mammals. Larvae which remain aggregated in the course of such movement may enhance the

effect of aposematic coloration and unpalatability on most vertebrate predators. Maintaining a clumped distribution also reduces the chance of being discovered by predators, and being surrounded by others provides individuals with less risk of being attacked (Hamilton, 1971; Taylor, 1976, 1977).

Field observations and the aggregation tests in the laboratory suggest that late instars of *E. phaeton* were intrinsically gregarious, in contrast to a statement that they were largely solitary (Klots, 1951). They stayed together in April, sometimes even joining other aggregations and after defoliation of host plants. Solitary sixth instars collected during the pupal and adult flight periods were invariably those attacked by parasitoids earlier (98% of 95 larvae; Stamp, 1981b). Occasionally, even these parasitized caterpillars occurred in groups of two to four, either on top of leaves or between leaves bound by silk. A similar pattern was found for sawfly larvae (*Neodiprion swainei* Midd.), in that after defoliating their host plant, larvae migrated 180 m or more and continued to aggregate; whereas, diseased larvae were solitary (Smirnoff, 1960). The aggregated behavior of late instars of *E. phaeton* may be beneficial by enhancing the defensive mechanisms discussed above.

The decline in aggregation tendencies of late instars was correlated with food availability, pattern of larval mortality due to predators and parasitoids, and pupation. In areas where the host plants were totally defoliated and few other acceptable food plants were available, larvae became increasingly dispersed with time. If late instars fed primarily on other food plants, it might be advantageous for individuals to be solitary if the food they ate rendered them palatable. Bowers (1980) demonstrated that *E. phaeton* larvae were palatable to blue jays when as latter instars they fed on English plantain (*Plantago lanceolata* L.). Latter instars were reported to feed on this and a variety of other plants (Tietz, 1972). Thus, as a consequence of both the quality and quantity of available food, the advantages of aggregating in relation to predators may frequently change from one instar to the next.

These caterpillars were actively gregarious through all instars, this behavior contributing to an average group size at each larval stage. It is clear that the costs and benefits of aggregation changed as group size changed and as these animals progressed through the life cycle, a consequence of different factors operating on the larval stages.

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