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Continuous rearing of the limnephilid caddisfly, *Clistoronia magnifica* (BANKS)

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Abstract

Seven successive generations of *Clistoronia magnifica* (BANKS) were reared in laboratory cultures in 46 months at 15.6°C. Rearing techniques are described and the biology of the species, as observed in the laboratory, is discussed and compared with field data.

No obvious deterioration of vigour was noted; pupal and adult weights have remained constant or increased slightly up to the fifth generation. Laboratory-reared specimens were heavier than field material, but the laboratory colony produced fewer eggs per female.

Introduction

In my study of the distribution and biology of Oregon Trichoptera (ANDERSON, 1976a), considerable effort was expended on laboratory rearing to associate immature and adult stages. The rearing programme was gradually expanded to screen for species that were amenable to laboratory culture and could then be used as models for basic studies of growth, development and feeding responses. The desirability of growth and trophic relations studies that are based on the entire larval interval, rather than on short-term feeding trials or gut analysis, is obvious. *Pseudostenophylax edwardsi* (BANKS) was initially considered a candidate species because it had been reared for more than a generation (ANDERSON, 1974). However, later data indicate that reared adults were undersized, and developmental times in the laboratory were extremely variable. Other limnephilid genera that I have reared with mixed success are *Psychoglypha*, *Hesperophylax*, *Hydatophylax* and *Limnephilus*. Compared with these genera, *Clistoronia magnifica* (BANKS) has been remarkably easy to culture; seven generations have now been reared in 46 months. A brief description of rearing methods and the use of enchytraeid worms as a dietary supplement is given in ANDERSON (1976b). The present paper summarizes the results of the laboratory rearing and describes the biology of the species as observed under these conditions.

Field biology

Clistoronia is a genus of five species, confined to North America and largely occurring in mountainous regions of the west (WIGGINS, 1977). *C. magnifica* occurs

from British Columbia to Oregon. Most of the Oregon records are from the Cascade Mountains, from ponds or lakes above 1000 m; there is one exceptional record from Astoria, at sea level on the Pacific Coast (ANDERSON, 1976a).

WINTERBOURN (1971) conducted life history studies at Marion Lake, British Columbia (elevation approximately 300 m). He described *C. magnifica* as an early-season univoltine species whose larvae inhabit submerged marginal vegetation and open sediments. Adults emerge from early May to late June but females are not reproductively mature at that time; adults live through the summer and return to the lake during August and September to mate and lay eggs. Oviposition occurs on submerged plants or logs. Larval development through the five instars occurs from August to January, with all larvae being in the final instar from January to April.

No complete life cycle data are available for the Oregon population. In Lost Lake, Linn Co. (elevation 1200 m), the population consisted of 34% mature larvae, 60% prepupae and 6% pupae in early May, when water temperature was 11°C and about two weeks after the ice had melted. The laboratory culture was initiated from egg masses collected from nearby lakes in late July and August 1973. The globular, gelatinous masses (25-30 mm dia.) were collected by a scuba diver on the lake bottom at 3 to 5 m depth.

Rearing methods

The routine rearing of larvae was in aerated shallow pans of tap water with sand substrate at 15.6°C and long days (16 h light: 8 h dark). Pans were washed and water replaced once or twice a week to remove faeces and excess decomposing food. Overcrowding resulted in retarded development and cannibalism, so large groups were subcultured, especially for the final instar. About 50 larvae could be reared to maturity in a 25 x 40 x 5 cm pan.

Material provided for food and case-building included *Alnus* leaves, conifer needles, wheat grains and, occasionally, green grass. The leaves, conditioned in water for 1-2 weeks to allow leaching and microbial colonization, provided food and substrate for the larvae. Water-soaked needles were used primarily for case-construction. The dry wheat grains were a necessary supplement to the detrital food for normal growth. They were provided in small amounts (20-40 kernels at one time) to avoid excessive decomposition and consequent deoxygenation.

A supplement of enchytraeid worms increased the growth rate (ANDERSON, 1976b), so most rearings in the third to sixth laboratory generations included the worm supplement. The most common cause of mortality was due to toxic products and/or deoxygenation from decomposing foods, especially worms. This could be reduced by avoiding excess feeding, frequent cleaning of the pans, or rearing in a 'drippery', a series of trays with a variable water exchange (ANDERSON, 1973).

When mature larvae had sealed off their cases, they were placed in small wire cages partially submerged in the drippery. At emergence the adults were transferred to a 0.16 m^3 screen oviposition cage. The cage was streaked with dilute honey for

food and contained a pan of water with an exposed rock or stick to enable the adults to reach the water.

Adult behaviour

At 15.6 °C, the adults are long-lived, with several individuals surviving from 4-6 weeks. Though they are strong fliers, much of the daytime is spent resting on the sides or corners of the cage. They do not seem to select concealed sites, such as folds in paper towels. A trait that facilitates handling of *C. magnifica* is that escaped adults tend to reappear on or near the cage by the next day, presumably in response to pheromone attraction. Resting adults show little response to jarring of the cage, or to moving objects; they can be easily captured with the fingers or forceps. From the lack of activity during daylight hours, I infer that adults are nocturnal. However, the absence of a twilight period in the laboratory may result in atypical behaviour.

Males and females are similar in size, weight, and emergence time. Generally more females than males were obtained. Mating occurred within 1-2 days after emergence. Pairs remained in copulation for several hours during the day. Both males and females were observed to mate more than once.

Females emerge with undeveloped ovaries and require a preoviposition period of about two weeks. WINTERBOURN's (1971) observations suggest that females have a reproductive diapause over the summer. However under long-day conditions there was no evidence of diapause during the seven laboratory generations.

Egg masses occur on substrates at or below the water line or sometimes loose in the pan. The only instance of oviposition that I have observed was at 0700 hrs. The greenish egg mass, 6 mm dia., was extruded from the body before the female crawled completely under the water enveloped in an air bubble. She was submerged for 5 min. while attaching the mass to a stick; she then walked rapidly up the stick, shook her wings without attempting to fly and assumed a resting postion on the cage. Females also can rest on the water without being trapped in the surface film. This behaviour suggests that egg masses could be deposited directly into the water and then sink to the bottom. In the field, egg masses also occur both attached and loose. WINTERBOURN (1971) indicates that they occur on plants or other submerged substrates, whereas the material used to start my cultures was collected by a scuba diver from the bottom of the lake.

In laboratory cages, some small masses were deposited on damp paper and on the floor of the cage, well away from the water. These masses would expand and develop normally if placed in water but the eggs desiccated if left where deposited.

After contact with water, the gelatinous mass expands to a large sphere with the eggs arranged in lines. The mass that I observed being deposited required 8 hours to achieve full size. Though field-collected masses may contain over 300 eggs, those deposited in the laboratory contained only 50-200 eggs. Females deposit more than

one egg mass but the later ones are usually quite small and may contain 50-100% non-viable eggs.

Eggs and larval development

Eggs, incubated at 15.6°C, hatched within 2-2½ weeks. The larvae remained within the matrix for a few days, moving around slowly within the dense medium. Case-making begins within a few hours after the larvae have left the egg mass. Larvae continued to emerge from a single egg mass for over a week. Whether this irregular development is typical of field situations is not known.

The initial case, made of bits of debris, is a crude tube that is gradually made stronger and tidier in successive days. After completion of the initial case, the young larvae frequently floated at the surface. This planktonic drifting could perhaps be a dispersal mechanism.

Conifer needles were the predominant material for cases of early and mid-instar larvae, though some incorporated a component of sand, perhaps because of a shortage of the preferred material. In instar IV there was frequently a significant component of sand, but after the moult to instar V the cases were rebuilt using conifer needles. In the latter part of the final instar all larvae were in sand-grain cases. WIGGINS (1977) describes the case of mature *C. magnifica* larvae as composed of small pieces of wood arranged irregularly to form a cylinder with little curvature or taper. He indicated that a new case of fine rock fragments was constructed before pupation.

Five larval instars can readily be distinguished by head capsule measurements (Table I). Head colour darkens with successive instars; the change is particularly apparent between instar IV and V. In the latter, the head is dark brown to black with some lighter areas, especially in the fronto-clypeal area (WIGGINS, 1977).

Laboratory rates of development at 15.6°C for instars I-IV were comparable with field rates. WINTERBOURN (1971) stated that the first four instars could be completed in 10 weeks and most of the second laboratory generation were in instar IV by the ninth week (Fig. 1). Rate of development is dependent on both temperature and food quality, as will be discussed further in later sections. Even under

Table 1. Head-capsule measurements of larval instars of *Clistoronia magnifica* (Second laboratory generation).

Instar	N	Mean (mm)	Range (mm)
I	6	0.38	0.37—0.40
II	5	0.52	0.48—0.55
III	10	0.86	0.82—0.89
IV	7	1.42	1.34—1.47
V	8	2.06	1.94—2.20

constant rearing conditions, the duration of the final instar is greater than that of the other instars combined. In the field, the final instar is further prolonged because it is the overwintering stage.

The data for Fig. 1 are derived from a cohort of larvae emerging within 3-5 days. The first three instars occurred as relatively discrete units but thereafter there was considerable overlap of instars. The duration of the fourth and fifth stadia was quite variable between individuals, which results in an extended period of pupation and of adult emergence. Environmental cues in the field, such as changing photoperiod and temperature, may result in more synchronous emergence than in the laboratory. Data for the total emergence are not available because, due to space limitations, the rearing of late individuals of one generation was terminated when the next generation was beginning to be reared.

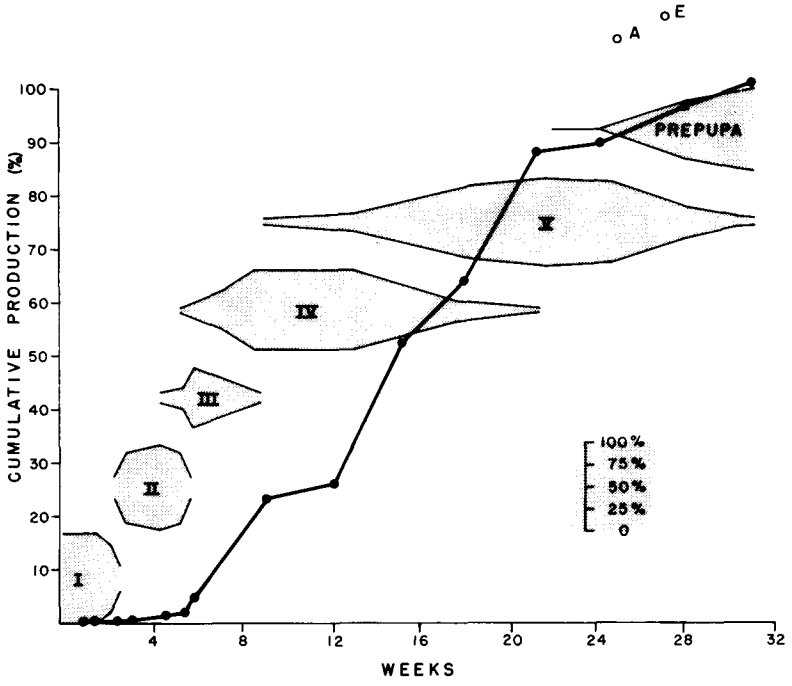

Fig. 1. Cumulative production of *Clistoronia magnifica* second laboratory generation compared with population structure (expressed as percent in each instar). Reared at 15.6°C. Occurrence of first adults and eggs is indicated by A and E.

Prepupal-pupal stages

At the end of the feeding period the final-instar larva attaches its sand case to a substrate and seals off both ends with a silk grating. The prepupal stage was completed in about one week at 15.6°C. The larval exuviae are packed in the back of the case but frequently several of the sclerites were pushed out of the case. This behaviour is atypical of most limnephilids which have a grating sufficiently fine to retain the sclerites.

As is pointed out by WIGGINS (1977), the term prepupa as used above includes both the interval of the resting larva and the period of larval-pupal apolysis. Thus this one week includes both the prepupal and pharate pupal stages. However, for comparing the performance of various treatments (e.g. effects of temperature), I do not distinguish between these two events. The most convenient markers are the closing of the case, and the casting of the exuviae when the individual is recognizable as a typical pupa. The timing of the latter can be observed without injury to the individual either by noting when sclerites are pushed out of the case, or by removing a small 'window' to observe the body form. The sexes can be distinguished by the maxillary palpi: 3 segments in males and 5 in females.

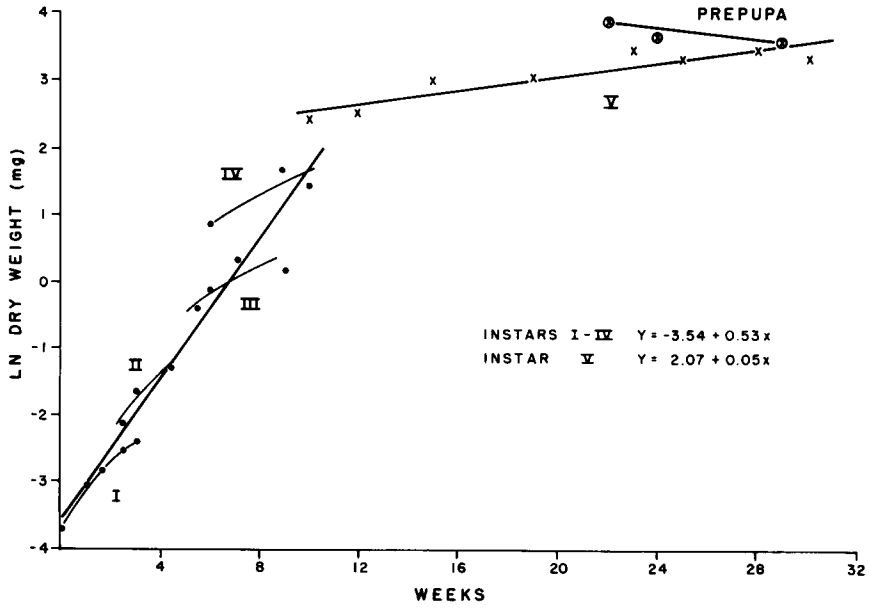

Fig. 2. Growth rates of larval instars of second laboratory generation of *Clistoronia magnifica* reared at 15.6° C.

The major advantages of using newly-moulted pupae for dry-weight comparisons are: (1) timing is predictable as one week after the case is sealed; (2) as a non-feeding stage, variability due to amount of gut content is not a factor; (3) the sexes are recognizable; (4) compared with adults, the pupae are non-mobile and the potential mortality of the three week pupal period is avoided.

Adult emergence in the laboratory occurred during the night. The pharate adult cut through the end of the case using the pupal mandibles, swam to the surface and crawled up the wire cage, where it then cast the pupal exuvia. Though most of the adults obtained under standard rearing procedures were good specimens, there were always some inferior individuals. Malformations ranged from slightly crumpled wings, through complete failure to cast the pupal exuviae, or death as pharate adults. The factors responsible for these abnormalities require further investigation, though low dissolved oxygen reduces the number of perfect adults, and dietary deficiencies may also be a factor (ANDERSON, 1976b). However, good adult emergence is not just related to larval growth. Within groups reared under uniform conditions there was no significant difference in weights of pharate adults, those with crumpled wings, or perfect specimens.

Larval growth

Larval weight of individuals of the second laboratory generation is plotted against time to illustrate within-instar and between-instar growth patterns (Fig. 2). On a log scale, growth rates can be fitted to two distinct linear regressions: instars I-IV with an instantaneous growth rate of 7.6% per day, and instar V of 0.7% per day. R^2 values are 0.92 and 0.85, respectively. For all instars there is a tendency for rapid growth early in the stage and slower growth before moulting. The values given are somewhat lower than for later generations because experience showed that this culture was somewhat crowded. Also in later generations the cultures were terminated before the slower larvae matured, whereas this series was followed for 31 weeks.

Though the instantaneous growth rate of early instars is an order of magnitude greater than that of the final instar, the latter stage is when the majority of growth and feeding occurs. This is illustrated by calculating production for the second laboratory generation, using RICKER's (1958) method:

Production = Growth rate X Mean biomass

Cumulative production is compared with the population structure in Fig. 1. This culture was sampled frequently for instar composition, but the first count of total numbers was at week 6. To arrive at an initial population, mortality for the first six weeks was assumed to be 40%. On this basis, the cumulative production was less than 5% of the total production when 80% of the population had completed instar II. If the actual mortality for the first six weeks was doubled (undoubtedly an overestimate in this culture) the contribution of the first two instars is still less than 10%. Thus it is evident that even though the early instars are abundant and have a

Table 2. Duration of Larval stage and pupal weights of *Clistoronia magnifica* reared at various temperature regimes and 16 Hours light.

Temperature (°C)	Lab generation	No.	Time to prepupa (weeks) Mean	Range	Pupal dry wt. (mg) (Mean ± 95% C.I.)
15	4	26	18	16—20	37.90 ± 2.38
15	5	23	18	15—23	39.14 ± 1.23
15 (to 10 at 15 wks)	5	6	18	17—19	40.16 ± 2.65
15 (to 10 at 15 wks)	5	11	33	24—51	39.67 ± 2.43
10	4	8	46	43—52	42.39 ± 5.14
10 (to 15 at 40 wks)	4	5	42	41—44	42.95 ± 3.65
20	5	14	22	20—24	35.09 ± 2.61
"Accelerated Field"*	5	21	24	23—26	41.38 ± 1.82

*Temperature and photoperiod: 10 wks -- 20°, 14,5-13.5 hrs; 1 wk -- from 20° to 5°, 12 hrs; 7 wks -- 5°, 10 hrs; 3 wks -- 10°, 14.5 hrs; remainder -- 15°, 15 hrs.

high instantaneous growth rate, their biomass is so small that they are unimportant in production estimates. This calculation is relevant to field situations for many invertebrates where there is a problem in accurately sampling early-instar larvae. Over 60% of the production of this population of *C. magnifica* occurred between weeks 12 and 21, when the age structure was dominated by instar IV and early-to mid-instar V.

Effects of temperature

During the fourth and fifth laboratory generations, a series of experiments were conducted to determine the temperature range tolerated by *C. magnifica* larvae, and the effects of temperature on rate of development and mature weight (Table 2). Experiments were initiated with first-instar larvae at 10° and 20°C in addition to the standard 15.6° (given as 15°). They were reared to pupae, oven-dried at 60°C, cooled in a desiccator and weighed.

As expected, larvae developed very slowly at 10°, but mortality was low and the weight of each instar was similar to that of larvae reared at 15°. Some final-instar larvae weighed 70 mg, the heaviest encountered in any of my cultures. At week 40, when larvae had not pupated in twice the time required at 15°, it seemed possible that a temperature increase was necessary to induce pupation. Five larva were transferred to 15° and the first of these sealed off its case the following week (Table 2). Thus the higher temperature accelerated development, but shortly thereafter the larvae at 10° also began to seal their cases. Though pupae from the 10° series had the highest mean weights, the 95% confidence intervals overlap with those of the 15° series.

Final-instar larvae transferred from 15° to 10° at week 15 (when the first sealed cases occurred in the stock culture) exhibited a split pupation period (Table 2). Six of these were prepupae by week 19, in synchrony with the 15° series, whereas another 11 were delayed to 24-51 weeks. Apparently those that were physiologically near maturity pupated on schedule, whereas the others were delayed almost as much as continuous rearing at 10°. Rearing at 20° throughout the life cycle was much poorer than at lower temperatures. Early-instar larvae developed rapidly and were of average weight. Mortality was high in instar V and the duration of this stadium was increased. Individuals that survived to pupation were significantly smaller than those reared at lower temperatures. The difference in response between the early instars and the final instar can be explained in terms of the field life cycle. The early instars are present during late summer, when surface water temperature may exceed 25°, whereas the mature larvae are the overwintering stage.

The 'accelerated field' series were reared with a temperature cycle similar to that experienced under field conditions, but compressed by one third to 24 weeks from the approximate nine months that larvae require for a univoltine cycle. The early instar larvae were reared at 20°, and then temperature was reduced through steps to

5°, and increased again up to 15°. This temperature regime resulted in pupae that were comparable in weight to those reared at either continuous 10° or 15°.

Comparison of generations

Weights of post-larval stages of the successive generations are compared with those of field-collected *C. magnifica* in Fig. 3. Using weight as a criterion, it is apparent that the laboratory cultures are not inferior to a field population. In most comparisons the former are significantly heavier. Though there is a tendency for highest weights in the fourth and fifth generation, the within-generation differences are not

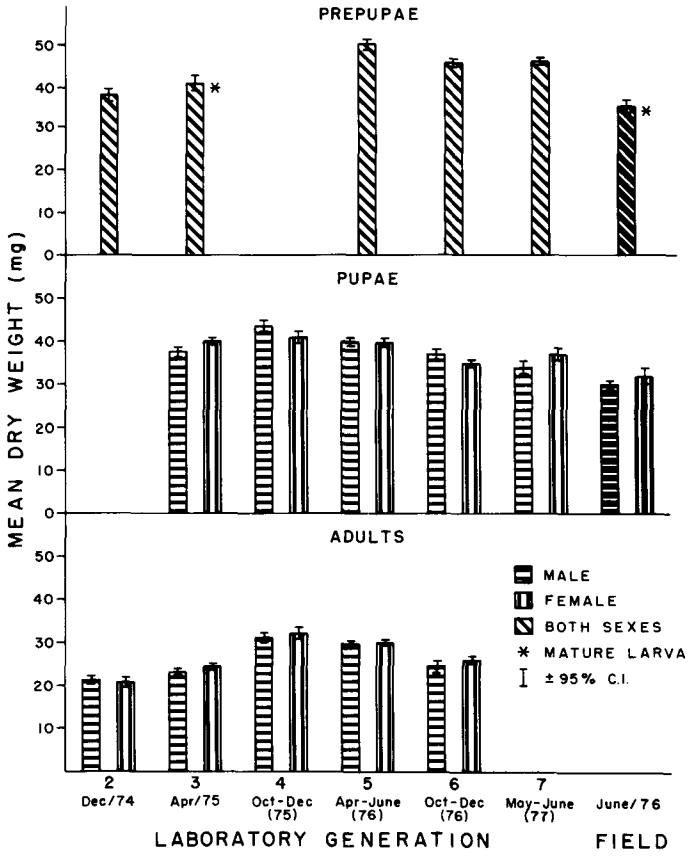

Fig. 3. Comparison of weights of post-larval stages of six generations of reared *Clistoronia magnifica* with field-collected material.

significant. I conclude from the data in Fig. 3. that current procedures are suitable to rear *C. magnifica* of a predictable size.

Though it has required 46 months to produce seven generations in the laboratory, it seems likely that two generations per year could be achieved. The trial and error involved in developing procedures and diets has resulted in some delays in the cycle. Table 3 is a generalized scheme of the timing and expected growth patterns that would result in a generation within six months. The range in weights are the extremes observed for cultures fed on suitable diets. Larvae at the low end of the range would either die or require a longer time than the durations listed for each stadium. Small prepupae or pupae would also have low viability and, if they matured, would produce adults of low vigour and fecundity. As is apparent from the range of developmental times in Fig. 1, there should be no problem in developing cultures with overlapping generations in order to have all stages available on a continuous basis.

A laboratory culture is exposed to intense selection pressures. The *C. magnifica* culture was initiated from a few egg masses, and succeeding generations have been started from less than 10 pairs. The current stock is highly inbred as no wild stock have been added in 7 generations. Despite the inbreeding, there has been no apparent decline in the vigour, or increase in abnormal adults. Though there is always some unexplained mortality in each generation, I have seen no obvious manifestations of diseases.

As *C. magnifica* has proven to be amenable to laboratory culture, it opens the

Table 3. Generalized growth and development pattern of *Clistoronia magnifica* to produce two generations per year. Based on seven generations reared at 15.6°C.

Stage	Dry Weight (mg)		Duration (weeks)
	Mean	Range	
Egg	0.02	—	2.5
Instar I, non-fed	0.02	—	
I, late	0.06	0.04—0.10	2
II, early	0.1	0.07—0.22	
II, late	0.3	0.15—0.55	2
III, early	0.7	0.45—1.2	
III, late	1.5	0.6—2.7	2
IV, early	3.5	2—6	
IV, late	7.5	4—13	2.5
V, early	11	9—12	
V, late	48	30—65	9
Prepupa, early	47	28—55	1
Pupa, early	38	27—47	3
Adult, early	26	17—40	—
Adult (preoviposition)	—	—	2

door to several exciting directions of research in ecology, behaviour and physiology. However, the extrapolation of laboratory data to field situations must be approached with caution. The merits and limitations of research based on laboratory colonies are aptly phrased by COLE (1966) in discussing mass culture techniques for body lice: '... it must be realized that an individual from a laboratory colony is no longer the same ecologically or perhaps even physically. Its reactions and responses to stimuli, (e.g. insecticides), may be more or less different than they would be in the wild state. This principle applies to all species. Still, dependable studies are possible with laboratory colonies; indeed, they are vitally necessary to modern research.'

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Discussion

MACKAY: What was the photoperiod used during rearing? Could the absence of ovarian diapause be caused by the long photoperiod?
ANDERSON: I did no experimental work on effects of photoperiods on induction of diapause. For continuous rearing I selected a photoperiod of 16 hours light and 8

hours dark. This photoperiod was selected because it is well known from work with terrestrial insects (aphids, leafhoppers, etc) that long days are conducive to continuous culturing.

MORSE: You have eliminated about 4 months preoviposition time ordinarily experienced in field populations. What do you suppose is the function of this period in natural populations?

ANDERSON: I do not know why a species from a permanent water habitat should apparently have an ovarial diapause. It could be a mechanism to avoid predation on the egg stage during the summer months. Notice that the limited field data suggest that the preoviposition period is quite variable; eggs were collected as early as July in Oregon, but not until August or September in British Columbia.

CRICHTON: You report on the small number of deformed adults in the cultured specimens, but have you any information on the number occurring in the field?

ANDERSON: No.

WINTERBOURN: In addition to differences in larval growth and adult life span, several other life history features differed in Anderson's and my studies (WINTERBOURN 1971). In Marion Lake, British Columbia, most egg masses appeared to be attached to the undersurfaces of lily pads (*Nuphar*), whereas in Oregon eggs were found on the lake bed. Marion Lake larvae also possessed cases built from plant fragements throughout the final instar; inorganic materials were not available. This provides further evidence of a high degree of ecological flexibility in this species.