# Growth and Survival of Larvae of *Ephydra hians* Say (Diptera: Ephydridae) on Unialgal Diets

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**ABSTRACT** The algae *Nitzschia latens* Hustedt, *Ctenocladus circinnatus* Borzi, and two species of *Oscillatoria* were isolated from Mono Lake, which is near Lee Vining, CA, and used to produce cultures containing only one algal species (unialgal cultures). Survival to pupation and rates of larval growth and development were compared among larvae of *Ephydra hians* Say, which were reared on one of these algal species or on a diet prepared using the fish food Tetramin. It was found that larvae could be reared from the second larval molt to pupation when fed on a single species of algae. Larvae reared on *Nitzschia* showed significantly increased weight gain and survival compared with larvae reared on *Ctenocladus*. Growth performance was always ordered *Nitzschia*, *Oscillatoria*, then *Ctenocladus*. Larvae reared on *Nitzschia* or *Oscillatoria* developed significantly faster than those reared on Tetramin. The results indicate that single species of *Nitzschia* and *Oscillatoria* are adequate food sources for *E. hians* growth. Although larvae of this fly appear to be generalist algal herbivores, food quality varies between algal species.

**KEY WORDS** algae, diet, brine flies

Recent studies have examined the possible effects of changes in lake level at Mono Lake which is near Lee Vining, CA, on the major benthic invertebrate species in the lake, *Ephydra hians* (Herbst et al. 1988, Herbst 1990, Herbst & Bradley 1993). As a result of diversions of inflowing freshwater streams, the lake level has declined more than 40 ft over the last half-century and the salinity of the lake water has roughly doubled (Patten et al. 1987, Botkin et al. 1988). Concerns have arisen regarding the effects of changes in the quality and quantity of benthic substrate available to the flies and the effects of salinity on fly growth and survival (Patten et al. 1987, Botkin et al. 1988).

The overwhelming majority of *E. hians* larvae and pupae at Mono Lake are found in association with hard substrate on the lake bottom, principally tufa. Studies have attempted to relate the changes in lake benthic area and lake benthic substrate composition with possible changes in fly population size and productivity (Herbst 1990, Herbst & Bradley 1993).

Studies of the salinity tolerance of the larvae (Herbst 1986, Herbst et al. 1988, Herbst & Bradley 1989b) have attempted to relate changes in salinity to fly survival. Concerns have also arisen that direct effects of salinity on algal productivity, survival, or competition might affect the flies indirectly (Herbst 1986, Herbst & Bradley 1989a).

Previous studies have established that the productivity and composition of the algae in Mono Lake will change with changing salinity (Herbst 1986, Herbst & Bradley 1989a). If *E. hians* larvae survive and develop better on some species of algae rather than others, studies concentrating on the effects of lake changes on the nutritionally more valuable algal species would seem warranted. Alternatively, if all algal species or even most algal species are roughly equivalent in food value for the larvae, then future studies could concentrate largely on the gross overall effects of lake level changes on algal productivity and nutritional quality, as opposed to algal succession. Finally, if none of the algae can support larval growth in a manner equivalent to Tetramin, then a mix of algae may be necessary for fly development. This requirement should be borne in mind in future studies of fly physiology and ecology.

In the current study we address one aspect of *E. hians* biology, namely whether different algal species differ in their capacity to support fly growth and development. The capacity of larvae to grow and develop on unialgal cultures was compared with their growth on Tetramin fish food, a food source known to support larval growth and permit complete development from egg to adult (T. J. Bradley, personal observation).

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## Materials and Methods

Algae were collected in Mono Lake by hand using scuba diving techniques. Samples of mud, sand, and rock (often tufa) substrate were collected and used for isolation of unialgal cultures.

Unialgal cultures were produced using only physical techniques. Filamentous algae were separated from adhering algal contaminants by dragging filaments across filter paper. Following a period of regrowth, the isolation procedures were repeated. Generally, two isolation procedures were sufficient to produce unialgal colonies of *Oscillatoria* (a cyanobacterium) and *Ctenocladus* (a green alga). The diatom species, *Nitzschia latens* Hustedt, was isolated using a fine pipette made from a Pasteur pipette pulled in a flame. Individual cells or small colonies of the diatom were aspirated into the pipette and then transferred to a flask for culturing. One to two repetitions of this procedure produced cultures of diatoms contaminated only by a flagellated alga, probably *Dunaliella*. Ultimately, isolated unialgal cultures of *Nitzschia* were produced by extreme dilution of the cultures. A mixed culture of the two algae was agitated and then a small amount (~0.1 ml) of the culture medium was transferred to a new culture flask. This procedure was replicated, producing hundreds of subcultures. These were inspected individually; the contaminated cultures being discarded and the unialgal *Nitzschia* cultures being retained.

For all three algal species, care was taken to assure that the separate cultures represented separate isolates of the originally collected population. Therefore, the replicate cultures used in the experiments are not pseudoreplicates with regard to clonal origin. These replicate cultures were subcultured to provide algae to feed to the flies on a regular basis.

No attempts were made to sterilize the cultures chemically (e.g., with antibiotics), therefore, we cannot be certain what species of nonalgal microorganisms were present in the cultures. In no case did these nonalgal species reach an abundance that was noticeable when samples of the cultures were examined by light microscopy.

All the subcultures of algae used were examined by light microscopy to determine that they were unialgal and that the algae were all morphologically identical. Multiple samples of these algae were sent to J. P. Kociolek of the California Academy of Sciences, San Francisco, for expert identification. Four species of algae were chosen for the present experiments involving larval rearing. These were *Nitzschia latens* Hustedt, *Ctenocladus circinnatus* Borzi, and two species of *Oscillatoria* (one with a large thallus and one with a small). The *Oscillatoria* were judged to be of different species because they maintained this difference in size under identical culture conditions even when cultured in the same flask. No morphologically intermediate forms were ever observed. For the remainder of this paper, *Oscillatoria* species number 1 (Os1) is used to indicate the species with the large thallus and *Oscillatoria* species number 2 (Os2) for the smaller one.

Algae were reared in filtered Mono Lake water adjusted to a salinity of 80 g/liters (total dissolved solids per liter of water) [TDS], which had been autoclaved, cooled, and enriched with 1 mM $NaNO_3$. Algae were cultured in 250-ml Ehrlenmeyer flasks using Grow-Lux lights with a light: dark timing regime of 14 h:10 h.

Flies were collected at Mono Lake as second instars. Pans of larvae in natural sediment were maintained in the laboratory at 18°C in a 14:10 light:dark regime. Newly molted third instars were placed in petri dishes containing Mono Lake water (adjusted to a salinity of 80 g/liter TDS) and a piece of cheesecloth ≈3 by 3 cm provided as physical substrate.

Larvae in the experimental groups (i.e., fed on algae) were fed ad libitum on algae isolated from the unialgal cultures by filtering through a paper filter. The filter was then placed in the petri dish where the larvae grazed on it. Controls were fed on a medium derived from Tetramin fish food and agar. Tetramin is a commercial fish food derived from both animal and plant products and containing 45% crude protein, 5% crude fat, and 2% crude fiber. Bacto agar (Difco, Detroit, MI) was dissolved in hot distilled water to produce a 3% solution. When this cooled to 40°C, it was mixed with ground Tetramin (9 ml agar mixture to 1 g Tetramin) and placed in plastic tubing (3 mm diameter) for storage in a refrigerator at 4°C. Fresh food was prepared every 2 wk. Three millimeters of this Tetramin/agar mixture was extruded from the tubing, cut into 0.5-cm lengths, and fed to the larvae on the days that the experimental group received fresh algae. This quantity was sufficient to provide Tetramin ad libitum to the controls. Both controls and the experimental group received fresh filter paper, food, and water three times a week. All larvae were reared at 18°C, with a 14:10 light:dark regime. Ten larvae were used per dish, with seven replicates of each food treatment.

Larvae were weighed on a Cahn Electrobalance (Cahn Instruments, Cerritos, CA) on day 25 after the molt to the third instar. Survival was recorded on each day that the food was changed. Pupation occurrences were recorded daily.

Values for each of the variables measured were compared using analysis of variance (ANOVA) calculated on an IBM PC using the SYSTAT statistical package. Significant differences were observed among treatments for each variable. The Student-Newman-Keuls standardized range test was used to determine which treatments were

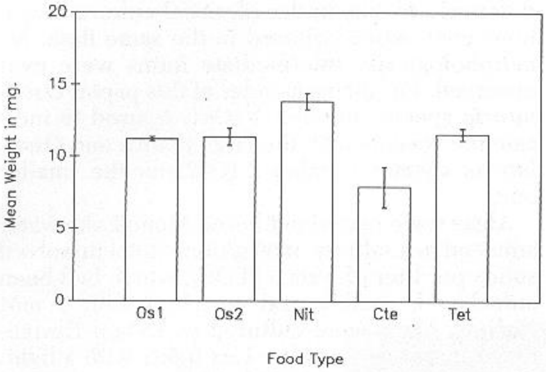


**Fig. 1.** Mean and standard error of the weight in milligrams of E. hians larvae 25 days after molting to the third instar and fed either on Os1, the *Oscillatoria* species with the large thallus; Os2, the *Oscillatoria* species with the small thallus; Nit, *Nitzschia latens;* Cte, *Ctenocladus circinnatus;* or Tet, the mixture of Tetramin and agar.

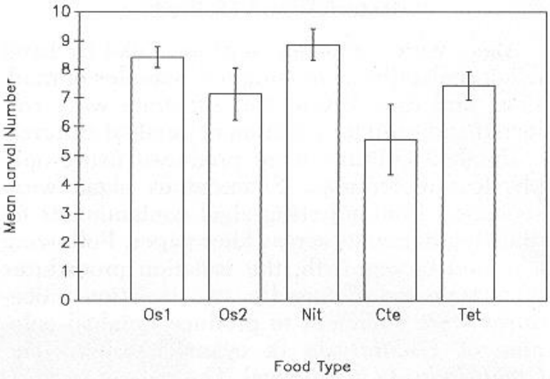


**Fig. 2.** Mean and standard error of the number of larvae per dish (out of an initial number of 10) surviving to pupation when fed either on Os1, the *Oscillatoria* species with the large thallus; Os2, the *Oscillatoria* species with the small thallus; Nit, *Nitzschia latens;* Cte, *Ctenocladus circinnatus;* or Tet, the mixture of Tetramin and agar.

significantly different from each other (Sokal & Rohlf 1969).

Unialgal cultures of *Ctenocladus* were obtained later than those of the other three algal species. Because the experiments could not be run simultaneously, a second set of controls was run with the *Ctenocladus* experimental group. It was subsequently found that the controls that were run at these two separate times did not differ in any of the characteristics measured ($P > 0.05$ using Student's $t$ test). Control values from the two experiments were therefore pooled for data analysis. Statistical analyses that were run with pooled controls did not yield conclusions different from those using the two controls as separate treatments. In all figures, $n = 7$ represents the unialgal diets and $n = 14$ the Tetramin controls.

## Results

**Larval Culture.** Fig. 1 illustrates the mean for all seven dishes in a treatment (i.e., food type) of the mean larval weight in the dish on day 25 after they had molted to the third instar. Larvae fed on *Nitzschia* had the greatest mean weight on day 25, whereas those fed on *Ctenocladus* had the lowest. Larvae fed exclusively on one of the two species of *Oscillatoria* or on Tetramin were intermediate in weight. Statistical analysis revealed that larvae reared on *Nitzschia* were significantly heavier ($P < 0.05$) than those reared on *Ctenocladus* but all other comparisons were not significantly different.

Fig. 2 illustrates the mean number of larvae surviving to pupation per dish. Each dish initially had 10 larvae. The highest survival values were obtained with larvae fed exclusively on *Nitzschia,* whereas the poorest survival was observed with larvae fed exclusively on *Ctenocladus.* Statistical analysis revealed that larvae reared on *Nitzschia* had significantly higher survival rates ($P < 0.05$) than those reared on *Ctenocladus* but all other comparisons were not statistically significant (Table 1).

The length of time required by larvae to complete the third stadium is expressed in our data in two ways. The minimum period of time from the initiation of the experiment until the first pupa appeared in a dish is shown in Fig. 3. This is equivalent to the minimum time to pupation in this dish. It can be seen that the results from the larvae reared on the two species of *Oscillatoria* and on *Nitzschia* are essentially identical. Larvae reared on *Ctenocladus* required a longer period to pupate, and those reared on Tetramin required a few days longer still. Statistical analysis revealed that the length of time to first pupation was significantly longer ($P < 0.05$) in the controls reared on Tetramin than in the larvae reared on *Nitzschia* or either species of *Oscillatoria* (Table 1). No significant differences were observed among the larvae reared on algae. No significant difference was observed between larvae reared on *Ctenocladus* and those reared on Tetramin (Table 1).

In Fig. 4, the mean period of time from the initiation of the experiment until pupation by the larvae is shown. Statistical analysis reveals no significant differences in mean time to pupation for larvae reared on algae, but a significant lengthening of the time to pupation for larvae reared on Tetramin (Table 1).

Though not always statistically significant, it should be noted that for best growth, survival, or maturation, diets were always ordered *Nitzschia* > *Oscillatoria* > *Ctenocladus.*

**Table 1. Parameters measured for each of the food treatments tested.**

| | |
|---|---|
| Weight of larvae on day 25[a] | |
| *Ctenocladus circinnatus* | a |
| *Oscillatoria* (species #1) | a, b |
| *Oscillatoria* (species #2) | a, b |
| Tetramin | a, b |
| *Nitzschia latens* | b |
| Survival of larvae to pupation[b] | |
| *Ctenocladus circinnatus* | a |
| *Oscillatoria* (species #1) | a, b |
| *Oscillatoria* (species #2) | a, b |
| Tetramin | a, b |
| *Nitzschia latens* | b |
| Time to first pupation[c] | |
| *Nitzschia latens* | a |
| *Oscillatoria* (species #1) | a |
| *Oscillatoria* (species #2) | a |
| *Ctenocladus circinnatus* | a, b |
| Tetramin | b |
| Mean time to pupation for all larvae in a dish[d] | |
| *Nitzschia latens* | a |
| *Oscillatoria* (species #1) | a |
| *Oscillatoria* (species #2) | a |
| *Ctenocladus circinnatus* | a |
| Tetramin | b |

Treatments with the same letter listed in the right-hand column are not statistically significantly different at the $P = 0.05$ level using the Student-Newman-Keuls standardized range test.

[a] Treatments in order of increasing weight.
[b] Treatments in order of increasing survival.
[c] Treatments in order of increasing time to first pupation.
[d] Treatments in order of increasing time to pupation.

## Discussion

A number of interesting and novel observations are provided by the results presented in this paper. First, the results demonstrate that species of algae fed ad libitum differ in their capacity to support the survival and growth of larvae of *E. hians*. Larvae reared on *Nitzschia latens* showed significantly ($P < 0.05$) greater weight at day 25 and improved survival compared with larvae reared on *Ctenocladus circinnatus*. These results agree with those of Herbst (1986), demonstrating that *Ctenocladus* is a poor food for *E. hians*. Our results further demonstrate, however, that Herbst's result was not caused by rearing *E. hians* on a restricted diet consisting of a single species of alga but rather to the poor quality of *Ctenocladus* as a larval food.

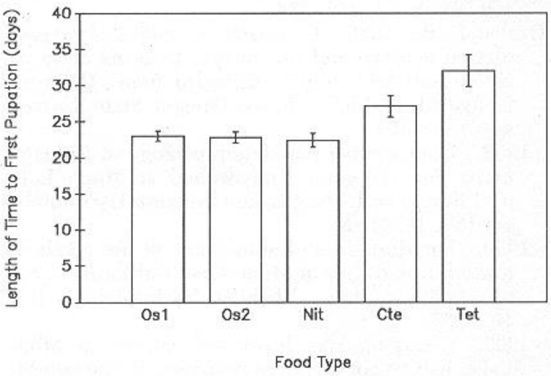


Fig. 3. Mean and standard error of the minimum development time for completion of the third stadium. Larvae were fed either Os1, the *Oscillatoria* species with the large thallus; Os2, the *Oscillatoria* species with the small thallus; Nit, *Nitzschia latens;* Cte, *Ctenocladus circinnatus;* or Tet, the mixture of Tetramin and agar.

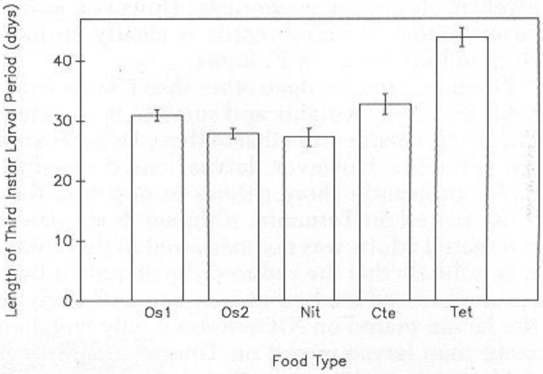


Fig. 4. Mean and standard error of the mean period of time in days in the third stadium for larvae fed either on Os1, the *Oscillatoria* species with the large thallus; Os2, the *Oscillatoria* species with the small thallus; Nit, *Nitzschia latens;* Cte, *Ctenocladus;* or Tet, the mixture of Tetramin and agar.

A second conclusion to be drawn from these data is that diets composed of unialgal species compare favorably with Tetramin as a food for *E. hians*. Tetramin is a balanced fish food containing ingredients derived from plant and animal sources as well as vitamins and minerals. While this food was designed to support fish development, it has also been shown to support multigenerational development of *E. hians* (T. J. Bradley, personal observation). It is, therefore, of some interest that single species of alga can match and, with regard to some variables, exceed Tetramin as a food for *E. hians*.

In Mono Lake, larvae of *E. hians* are found predominantly on pieces of tufa on the lake bottom. These tufa fragments are often coated with golden brown algal layers rich in diatoms. Diatoms are a large and important portion of the diet of the larvae (Herbst 1986). The results of this paper demonstrate that the diatom *N. latens*, which is quite common in the lake (Kociolek & Herbst 1992), supports growth and survival of *E. hians* as well as Tetramin. Development to pupation is actually more rapid than with Tetramin. The growth and development of larvae reared on the two species of *Oscillatoria* is indistinguishable from that of those reared on *Nitzschia*. It is not the case, therefore, that only one alga can be used as food by *E. hians* larvae, with all other algal species being inadequate. However, as discussed above, *C. circinnatus* is clearly an inferior food for larvae of *E. hians*.

Larvae reared on algae other than *Ctenocladus* exhibited body weights and survival to pupation indistinguishable from that of those larvae reared on Tetramin. However, larvae reared on algae had significantly shorter times to pupation than those reared on Tetramin. Although body size of the reared adults was not measured in this study, it is unlikely that the reduced development time resulted in reduced adult size because many of the larvae reared on *Nitzschia* not only weighed more than larvae reared on Tetramin in this experiment but also more than larvae reared on Tetramin in a previous study at the same salinity and temperature employed in the present experiment (T. J. Bradley, personal observation).

Differences in pupal or adult body size in *E. hians* have previously been observed when flies were collected on different years (Herbst 1988), in different seasons of the same year (Herbst 1988), at different sites in the same year (T. J. Bradley, personal observation), or reared at different salinities on algal food (Herbst 1992). Clearly, a number of factors including salinity, temperature, and the quantity and quality of food during the larval growth period, can affect subsequent adult body size in *E. hians*.

Other studies have shown that the ephydrid larvae of *Ephydra bruesi* Cresson and *Paracoenia bisetosa* Curran, inhabitants of thermal spring algal mats, feed on mixed types of algae (Brock et al. 1969). *Scatella stagnalis* Fallen also is a trophic generalist, able to develop to the adult stage on a variety of unialgal diets of diatoms, green, yellow-green and blue-green algae (Zack & Foote 1978). However, significant differences in nutritional suitability were also found, with the most rapid growth occurring on the diatom *Navicula pelliculosa*, and the slowest growth on the blue-green alga *Nostoc muscorum*. Other ephydrids appear to be trophic specialists, with species of *Axysta, Hyadina, Lytogaster*, and *Nostima* developing only on a diet of blue-green algae (Foote 1977, 1983), and *Parydra* only on diatoms (Doenier 1972, Doenier & Regensburg 1978).

Coexistence of ephydrids on the same habitat may depend on niche partitioning of the food resources available. In the case of *Ephydra hians*, apparently a trophic generalist, the usual absence of coexisting ephydrids in its saline lake habitats, probably caused by extreme salinity and alkalinity, may have permitted a broad exploitation of algal food resources. Superior performance on diatoms suggests that conditions favoring growth of these algae will support the optimum development of *E. hians* larvae.

It should be noted that the present study examines *E. hians* larval performance over only a portion of the life cycle, from the second larval molt to pupation. However, as a bioassay of growth, the third instar is most appropriate because it is the longest period of larval growth during which the larvae increase in mass almost 10-fold.

Our results indicate that individual algal species differ in quality as a food for *E. hians*. The growth performance parameters measured were always ordered: *Nitzschia* > *Oscillatoria* 1 = *Oscillatoria* 2 > *Ctenocladus*. It would seem that larvae of *E. hians* are not dependent on a single algal species for growth and survival.

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