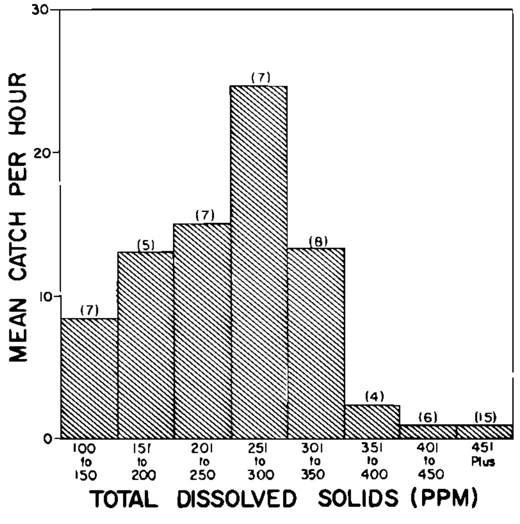

FIGURE 3.—Mean catch per hour of striped bass in relation to total dissolved solids in the San Joaquin River at Stockton, California, in 1966. Numbers of gill net drifts are in parentheses.

occurred in our study area (Chadwick, 1958; Farley, 1966; Donald E. Stevens, pers. comm.); most of it takes place downstream where the concentrations of dissolved solids are lower. Most bass migrating up into relatively high concentrations of dissolved solids must therefore drop back downstream to spawn. Under present conditions this is not a serious problem, as the run of striped bass in the San Joaquin River above Stockton is small even under ideal water conditions. However, with some proposed water development plans the entire spawning migration in the San Joaquin River could be threatened.

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Methods for Mass-Rearing *Hexagenia* Mayflies (Ephemeroptera: Ephemeridae)[1]

INTRODUCTION

Hexagenia bilineata and *Hexagenia limbata* mayfly nymphs are important food organisms for Mississippi River fishes (Hoopes, 1960) and they seem to be excellent indicators of general water quality (Fremling, 1964a). The general life histories of *Hexagenia* mayflies are well known (Needham, Traver, and Hsu, 1935; Hunt, 1953; Fremling, 1960). The burrowing nymphs construct U-shaped respiratory tubes in the muddy bottoms of lakes and rivers where they ingest mud, organic detritus, algae and bacteria. *Hexagenia* nymphs require from three months to a year to mature in the Upper Mississippi River, whereupon they rise to the surface, usually at night, cast their nymphal exuviae and emerge as subimagoes. Subimagoes rest in the shade along the river bank until the following afternoon when a final molt occurs and the imagoes emerge. Mating occurs aerially along the shoreline at dusk and the females return to the river where each alights on the surface and deposits two egg packets, each of which contains about 4,000 eggs. The eggs sift downward to the river bottom where most of them hatch in 10–12 days, if conditions are favorable. Male and female imagoes die within hours after they have mated.

Hexagenia mayflies tend to emerge *en masse*, and river residents are accustomed to nuisance

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problems caused by the insects during periods of maximum emergence. Analyses of over 500 mayfly collections along the Upper Mississippi River indicate that mass emergences of *H. bilineata* mayflies tend to occur at intervals of about 6 to 11 days from mid-June through mid-August (Fremling, 1964b).

We have studied the rhythmic emergence phenomenon for the past 8 years to determine the environmental factors which influence it. Much of our investigation has been done indoors under controlled conditions. The purpose of this paper is to present the techniques developed to maintain large laboratory populations of *Hexagenia* mayflies.

COLLECTION OF EGGS

Female imago mayflies are most easily collected from beneath lights along the water's edge. Mature females are usually more common around lights than are imago males or subimagoes of either sex. Virtually all of the mayflies collected beneath bridge lights are inseminated imago females. Apparently these have been diverted to the light as they flew upriver to lay their eggs.

If small amounts of eggs are desired, it is usually simplest to return the gravid females to the laboratory. They may be transported easily by picking them up by the wing tips and placing them in a large inflated paper bag. If egg collection must be postponed for some reason, the bag and its contained insects should be refrigerated to slow the metabolism of the insects and thus lengthen their life span.

Females are dropped, a few at a time, into a large water-filled funnel, the stem of which is fitted with a rubber tube and a pinch clamp. As they struggle on the surface of the water, the females release their eggs which sink slowly into the stem of the funnel where they may be tapped off in the desired quantities.

Imago females release their eggs most readily when they are lying right side up on the water surface with their outstretched wings adhering to the surface film. They have difficulty ovipositing unless their wings are immobilized in some manner. Thus, they also lay eggs when they are caught in spider webs, or if they are stuck to wet pavement or if they are weighted down by an accumulated mass of other mayflies.

Large numbers of eggs are most easily collected by parking an automobile along the water's edge and by placing a water-filled tub beneath the headlights. Insects which are attracted to the headlights fall into the tub where they lay their eggs. When the layer of insects becomes too thick, the insects are skimmed off to make room for more. On one occasion we parked our automobile on a little-used bridge at Winona, Minnesota, and collected 5.5 liters of eggs (*ca.* 345 million) in one hour and 40 minutes.

Eggs may also be extracted from adults reared in the laboratory. It is unlikely, however, that mating will occur under laboratory conditions because *H. bilineata* mayflies have an elaborate mating behavior pattern which involves swarming and sight recognition of the female by the male (Fremling, 1960). Unmated female imagoes have been observed to lay eggs in laboratory aquaria, but unsuccessful attempts have been made on 12 occasions to hatch *H. bilineata* eggs parthenogenically. Fertile eggs may be obtained readily, however, by artificial insemination. Eggs stripped from female imagoes or subimagoes are mixed with the macerated sexual elements of the male in the insect's own body fluids, as described by Needham (1935). If insects arise from the rearing tank in very small numbers, and if males are not immediately available, females may be kept for up to two days in paper bags in the refrigerator at 10 C.

STORAGE AND INCUBATION OF EGGS

Eggs are conveniently stored and incubated in water-filled polyethylene bags which measure $10 \times 16 \times .0015$ inches. The eggs are able to respire because polyethylene is permeable to oxygen and carbon dioxide. Up to 3 cc of eggs may be stored per bag for 120 days at 12 C with maximum viability (Flattum, 1963). After 120 days, the eggs will begin to hatch at this temperature if oxygen is available to them.

We have stored *H. bilineata* eggs for 380 days at 12 C by immersing the bags in water which contained soured milk. Because milk has a high bio-chemical-oxygen demand, the dissolved oxygen concentration of the ambient water varied from 0.1 to 0.3 ppm during the 380 days. The viability of the eggs decreased steadily with time. The normal time required for the eggs to hatch after removal from this cold anaerobic environment was increased by 4 days at 22 C.

Four liters of eggs in a small amount of water have been stored anaerobically at 12 C for 79 days in a sealed jar. The water turned black and smelled strongly of hydrogen sulfide. Viability decreased steadily with time and only 5% of the eggs were viable after 79 days. No eggs remained viable longer than 110 days.

Eggs may be easily incubated in the polyethylene bags in which they were stored. A black counter top is an ideal place for incubation because the newly-hatched nymphs are white and their movements may be discerned with the naked eye.

The rate of development of *Hexagenia* eggs is dependent upon temperature. A cold period is not necessary as it is for some other mayflies such as *Ephoron album* (Britt, 1962). *H. bilineata* eggs hatch in 12 days at 22 C and in 8 days at 32 C. If the eggs are clumped, hatching time is greatly prolonged. The eggs on the outside of the clumps hatch first because they are best oxygenated.

MAINTENANCE OF NYMPHAL POPULATIONS

Nymphs have been successfully reared in galvanized stock-watering tanks, galvanized wash tubs, various glass aquaria, and in large polyethylene bags. The containers are filled to a depth of 15 cm with rich garden soil which is prepared by spading in moderate amounts of thoroughly composted hay. Inorganic fertilizer (Armour Vertegreen 10-10-10) is mixed in at the rate of 100 gm per m^2 of soil area. We have successfully used the same soil for five years by enriching it in the preceeding manner.

Enough water is added to fill the containers. Constant aeration is provided to maintain a high level of dissolved oxygen in the water. Polyethylene bags are well suited for isolated individuals because the bags are permeable to oxygen and thus require no bubbler. We attach Kerr jar covers upside down in the mouths of polyethylene bags with strong rubber bands. The bags are then hung in slots which are cut in a shelf. A cork stopper is tacked to the center part of each Kerr cover which functions as a cover for the bag.

Nymphs ingest quantities of mud, detritus, algae, and bacteria principally from the mud surface, using their fore-legs to carry the material to their mouths. As the nymphs excavate the mud for food, their nymphal burrows constantly change positions. Nymphal feeding activities and respiratory movements cause the water to become turbid. Indeed, highly turbid water indicates a vigorous population.

A constant algae bloom is maintained by hanging 250-watt incandescent lights, with reflectors, within one foot of the water surface. The lights also maintain the tanks at temperatures which exceed room temperature. The constant burrowing activities of the nymphs make the soil nutrients available for algae growth and the algal rain provides a constant source of food.

Large, illuminated reservoir tanks are filled with tap water to which inorganic fertilizer is added (20 gm of Armour Vertegreen 10-10-10 per 50 gallons of water) to promote an algae bloom. This algae-rich water is used to replace water lost through evaporation from the rearing tanks.

Eggs may be placed directly into the rearing chamber for incubation or they may be hatched and then added. In the latter case it is advisable to place the unopened bags full of newly-hatched nymphs in the rearing tank for an hour before opening to equalize the water temperatures.

Nymphal growth is negligible at 14 C, but the rate increases with temperature. Only 79 days were required to develop newly-hatched *H. bilineata* nymphs to adults in a tank which was maintained between 24 and 27 C.

When nymphs are crowded, they exhibit differential growth rates. The larger nymphs apparently inhibit the growth of smaller nymphs. In one instance a crowded population which was initiated at about 7000 nymphs in 2.3 m^2 of substrate finally produced 479 adult insects. The adults matured at varying rates, however, the last adults emerging 327 days after the first ones. Differential growth rates among *Hexagenia* nymphs has been reported previously by Spieth (1938).

Nymphs may be collected from large tanks by scooping vigorously, back and forth through the water with a screen-bottomed bucket. This violent agitation dislodges the nymphs from their burrows and they are trapped, unharmed in the bucket. This procedure eliminates the tedious task of sifting mud to find the nymphs.

Nymphs have also been collected by removing the aerator and by scattering granulated sucrose at the rate of 500 gm per m^2. The sugar settles to the bottom where it acts as a high biochemical-oxygen-demand pollutant. When the oxygen concentration near the mud-water interface drops to 3 ppm, the nymphs leave their burrows and rest on the mud surface. When the dissolved oxygen concentration drops to 2 ppm (usually in about 24 hours) the nymphs swim to the surface of the water where they may be easily collected. They are able to swim for about two days, whereupon they become fatigued and sink to their deaths in the oxygen-deficient zone at the bottom. The bacteria and yeast which decompose the sugar add organic matter to the soil, thus enriching it for the next population of nymphs. Dissolved oxygen determinations at the mud-water interface are made by siphoning and by using water-filled polyethylene bags as samplers (Fremling, 1963).

Occasionally, a residual population of nymphs is killed so that a tank can be restocked for another experiment. Hot tap water (59 C) was added to a tank to kill the nymphs. To our surprise, however, two subimagoes emerged from the tank two days later. Sampling revealed that many nymphs were still alive. They apparently escaped the hot water by burrowing deep into the mud where they remained until the water had cooled. Between rearing experiments, we now routinely add sugar as described previously. A week later, all water is drained from the tank and the soil is stirred thoroughly as hot water is added to insure that each nymph is killed.

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The Effect of the Lamprey Larvicide, 3-trifluoromethyl-4-nitrophenol, on Selected Aquatic Invertebrates

INTRODUCTION

The chemical compound 3-trifluoromethyl-4-nitrophenol (TFM) is used to control the sea lamprey (*Petromyzon marinus*) in the upper Great Lakes. It is introduced into streams in which sea lampreys have spawned, to kill the larvae. These "treatments" are carried out at intervals shorter than the larval phase of the sea lamprey's life cycle (about