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The laboratory rearing of stoneflies.

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THE LABORATORY REARING OF STONEFLIES

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INTRODUCTION

Little is known of the biology of Plecoptera. The rearing of stoneflies, with which this thesis is concerned, is still in a highly exploratory stage with most of the earlier techniques being unsatisfactory. Most specimens reared have been nearly mature naiads brought through to adulthood to associate the naiads with adults of the species for taxonomic purposes. Among the few species whose life cycles have been studied, there is considerable variation in the number of instars found, not only between species (12-33), but also within species. The food and temperature requirements of a few European species have been reported, but little has been done anywhere else.

Rearing stoneflies in the laboratory provides advantages over field rearing, such as decreasing the number of lengthy field trips and thus allowing relatively more time for observation and study, and reducing the danger of vandalism and accidental loss. Laboratory rearing should also prove expedient in permitting better control and evaluation of individual environmental factors and likewise possibly permitting the production of a greater number of generations in a given period of time. Thus, although the laboratory does not provide a completely normal habitat and in some insects produces morphological and developmental aberrations, its potential advantages are such that its use should be

explored further for stonefly biological studies.

Stoneflies are commonly used as stream pollution indicators. Much basic life cycle and ecological information is needed to exploit the potential of stoneflies for this purpose.

LITERATURE REVIEW

Little research has been reported on rearing Plecoptera through their life cycles. The following is a summary of the techniques and results of both laboratory and field life-cycle studies as well as rearing techniques utilized for taxonomic work.

Schoenemund (1912) reported keeping naiads of three species of Perla in aquaria in the laboratory. The naiads were transported to the laboratory in jars, each containing one or two specimens. The jars were one-eighth full of fresh stream water which was changed daily in transit. It was found that rapidly circulating tap water could be utilized for laboratory rearing. Tiny naiads were fed infusoria and larger specimens were given Asellus aquaticus or Ephemeroptera naiads.

Smith (1913) attempted to rear Paragnetina immarginata Say using running tap water in a "roof aquarium" and an artificial pond. She was unable to keep the naiads alive for more than two months and thus failed to rear a complete life cycle. Her cages were constructed of galvanized screen which probably had a toxic effect on the naiads (page 32).

Wu (1923) reared Nemoura vallicularia Wu naiads in the stream from which they were collected. Ten first-instar naiads were confined individually within shell vials by silk bolting cloth held by a rubber ring. The specimens in their

cages were then placed in an enamel tray which was submerged in the stream. During nine months of naiad development, Wu identified 22 stadia by the recovery of exuviae. He stated that "Each ecdysis marked the beginning of an instar and the heads of the naiads, collected immediately after the molts, were measured...with an ocular micrometer." The actual head measurements were compared with computed head widths based on the rate of increase in head width between two stadia, 0.95, and found to agree closely for each stadium.

Three questions must be raised in relation to Wu's paper. Firstly, he has not clearly indicated whether the measurements were taken from his ten confined naiads or from others which were collected from the stream after each molt of his caged specimens. The latter appears more likely because it hardly seems possible that all ten of Wu's specimens would molt on the same date. Data presented here and those of Brink (1949) and Khoo(1964) support this contention. Secondly, it hardly seems possible that all specimens studied in a certain instar would all have exactly the same head width. Thirdly, the figure 0.95 given for the growth rate of head width would show an almost two-fold increase in head width at each molt, which is clearly an impossibility. On examining his data for the computed head width, one finds that .095 is the figure which should have been given.

Samal (1923) reared Perla burmeisteriana Claassen [abdominalis Burm.] in a laboratory aquarium and found 22 stadia during the three and one-half to four years of development of this species. Naiads were fed small Odonata, Ephemeroptera and Tubifex worms. Samal found frequent cannibalism among his specimens. Data are presented on body length and antennal and cercal segments for each instar.

Schoenemund (1925) found approximately 33 instars for females of Perla cephalotes Curtis during its three year life cycle. A single female was reared through the second and third year and the data combined with other rearing data for the first year. Since the male naiad was considerably smaller, Schoenemund concluded that it would have fewer molts.

Frison (1929) used small tins containing moist leaves to rear mature naiads for taxonomic purposes. He also attempted to hatch the eggs of three species of Plecoptera in tubes covered at both ends with linen cloth. In three months, naiads of Taeniopteryx nivalis Fitch had hatched and died. Quite unexpectedly, the eggs of Allocapnia vivipara Claassen did not hatch until a year later. This was the first reported instance of such a long egg stage in Plecoptera. Frison did not isolate the specimens to study growth patterns.

Helson (1934) reared naiads of Stenoperla prasina Newman to adulthood for taxonomic purposes. The naiads were

transported to the laboratory in jars containing moist moss and ferns which were rinsed with fresh stream water each day during transit. Water-tight rearing cages constructed with glass fronts and gauze tops were maintained at room temperature in the laboratory. Sand and stones were placed on the bottom of the cages and the water was aerated continually. The stoneflies were fed mayfly naiads.

Helson (1935) reared the first instar of Stenoperla prasina Newman from eggs at a laboratory temperature which averaged 13.6 degrees Centigrade. The eggs were kept in a covered glass capsule, the bottom of which was covered by sand. A water weed (Elodea) provided oxygen and was replaced when unhealthy. Helson presented data on the first instar which hatched from eggs in 91-94 days. Dyar's law[1] and Przibram's rule[2] were used to identify second instar specimens collected in the field. Data were also presented on these second instar naiads.

Frison (1935) described a second method used to rear large naiads to adulthood. Cages, 4½" by 4½" by 12" having

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[1]Dyar's law states that parts of the insect body grow by geometrical progression. There are many exceptions to this rule.

[2]Przibram's rule states that an insect's weight is doubled during each instar and at each molt all linear dimensions are increased by a factor of 1.26. This assumes that during each instar every cell in the body divides once and grows to its original size. Although the ratio holds true for some insects, the doubling of cells in the body was found not to be the usual case.

removable tops held by screws, were constructed of copper strips and fine wire copper mesh. A 2' by 6' wooden raft, anchored in the stream, had 16 holes, 4 3/4" by 4 3/4", to receive the cages, which were supported by two brackets in order to immerse the cages to three quarters of their depth.

Miller (1939) reared the first four instars of Pteronarcys proteus Newman in the laboratory. Eggs obtained from adults which were reared from mature naiads in pillow cages[3] were placed in Syracuse watch glasses containing lake water. Three hundred days passed before the eggs hatched. Miller found, however, that eggs of P. proteus could be hatched in less than ten months by manipulating water temperature. After 5½ months of development, egg hatching could be induced by a short exposure to winter temperatures followed by normal spring temperatures. Miller measured body length and head width of naiads in their first two instars and determined the duration of the first three stadia at several temperatures.

Holdsworth (1941, 1941a), using statistical methods on head-width measurements of 79 naiads of Pteronarcys proteus Newman collected during a twelve month period, found that males had 12 instars and females 13. When the collecting

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[3] The pillow cage, devised by J. G. Needham as reported by Morgan (1930), is constructed from a single square of woven wire cloth formed into a cylinder with the edges folded together. The ends are then flattened and folded. These cages may be partially or completely submerged in water.

date for each specimen is examined one finds that the naiad collection was poorly distributed throughout the year. For nine months an average of only three naiads a month was collected with no naiads present for three of these months. Secondly, head-width measurements fail to fall into distinct groups since the variations between instars are no greater than the gaps in measurements within some instars. Thus, additional data, preferably from rearing, are needed to ascertain the number of instars for this species.

Hynes (1941), conducting taxonomic studies of the plecopterous naiads of Britain, successfully reared all but one (24) species through some of the latter instars to adulthood in the laboratory. Several species inhabiting "still water" were reared in dishes of standing water which remained throughout the year at a nearly constant temperature of 14 degrees Centigrade. The remaining species, collected from running water habitats, were reared in an artificial stream. Enamel pie plates containing naiads were placed on a series of six sloping shelves so arranged that the lower end of each lay over the middle of the one below it with tap water originating from tubing set above the top shelf dripping from plate to plate. Each plate held food for the naiads and stones for their support and emergence. Mosquito netting or bolting silk sewn onto wire frames fitted over each plate confining the naiads.

Mature naiads of the one British species which failed to live under laboratory conditions were reared to adulthood in the field. Cages for this purpose were constructed from non-corroding metal boxes with two sides of metal gauze and were partially submerged in the stream from which the naiads were collected.

Hynes obtained eggs from 14 British species of Plecoptera. Eggs from 9 species were successfully hatched. Each egg mass was enclosed in a small tube covered on both ends with bolting cloth and kept in the plates with the naiads in the artificial stream. Hynes later found that the eggs hatched as well in shallow standing water in glass containers at laboratory temperature (14 degrees Centigrade).

Harden (1942) utilized jars approximately 20 centimeters in diameter containing 5 to 25 centimeters of water to rear mature naiads to adulthood. The jars were supplied with air forced through alundum bacteriological filters at two pounds pressure. A wire screen cylinder, covered at the top by cheese cloth, was placed around each jar, providing a place for emergence and confinement of adults. To maintain a low temperature for early spring species, the jars were set in running tap water at approximately 10 degrees Centigrade. Harden reared one nearly mature Pteronarcys naiad to adulthood in four months and a second in nine months.

Harden and Mickel (1952) associated naiads of 5 species of stoneflies with adults using essentially the techniques

of Harden (1942). Wide-mouth quart jars were used for rearing cages rather than the larger jars used previously. Naiads were transported to the laboratory in quart jars having screen tops. During warm weather these jars were provided with fresh stream water every hour.

Khoo (1964) successfully reared five specimens of Capnia bifrons Newman through their complete life cycle in the laboratory. The naiads were kept in a cold room with both temperature and light controlled to simulate stream conditions. The very tiny specimens were reared in "small vessels", then transferred to petri dishes and later to enamel pie dishes as they increased in size. The cages were checked every two or three days for exuviae. Khoo verified the findings of Hynes (1941)and Brink (1949), that most eggs of C. bifrons hatch within 24 hours after being deposited, although he found that some hatched as much as 48 hours later. He found this species to have a diapause in the early stages of its one-year cycle. The number of instars determined varied: 14 in two males, 15 in another male and a female, and 16 for another female. Khoo also found that photoperiod affects growth rate.

Perhaps the variations in number of instars of C. bifrons found by Khoo could be attributed to his sole use of the exuviae for instar identification. Tiny exuviae could easily be overlooked in the rearing chamber and naiads of some species consume their exuviae. Had measurements of head width or

some other method been used to supplement the exuvial data, the instar information presented would have been more dependable.

HABITAT

The Nemoura naiads utilized in this study were obtained from a small stream flowing from Johnson Spring on Wendell Road in Warwick, Massachusetts. Located in a mixed hardwood forest dominated by maple, black birch and oak trees, the stream originates with water issuing through an iron pipe from the concrete enclosed spring. Being primarily spring-fed, the 40 foot long stream has characteristics not consistent with larger neighboring streams. Temperature fluctuations are moderate owing to its proximity to the spring. Moreover, the volume of water issuing from the spring does not fluctuate as much as in near-by streams from drought to spring flood. The slope of the stream remains fairly constant for about 10 feet, at an angle of approximately 30 degrees, except for two abrupt drops of about 6 inches. The majority of the Nemoura naiads are found in this 10 foot portion of the stream, which is 6 inches wide and one inch deep, although obscured by a heavy cover of leaves in the fall. The sides of the stream are lined with leaves throughout the year and the bottom consists of coarse sand with half-buried leaf packs and a few stones three inches or larger in diameter (Fig. 1,2).

The following section, approximately 30 feet in length, connecting the Nemoura habitat with Grace Brook, consists mostly of seepage through the ground litter. The seepage

barrier effectively isolates the immature insects of the stream from those of Grace Brook, thereby preventing carnivorous plecopterous naiads from entering the small stream. The small size of the stream may account for the fact that carnivorous plecopterous adults have avoided the site for oviposition, leaving the herbivorous species to flourish abundantly virtually free from predation.

METHODS AND MATERIALS

Collecting and rearing

The collecting and rearing materials and techniques utilized were essentially those of J. F. Hanson for field rearing.

Two types of sieves were necessary for collecting naiads of Plecoptera. The more important of these was constructed by attaching a disk of 150 mesh stainless steel screen[4] to the top of a coffee can from which the bottom had been removed (Fig.4). The sieve was fine enough to retain not only the smallest naiads but also plecopterous eggs. It was also used to collect large naiads except in rapidly flowing water where, because the mesh restricted water flow, a backwash was created which swept many specimens aside and away from the sieve. To overcome this problem, another type of sieve was used (Fig. 3), a strainer with a much coarser mesh (approximately 12). Removal of its detachable handle allowed the sieve to be used in recesses difficult of access.

Where water flow was adequate, the sieve was held directly downstream from a point where the bottom was disturbed and the current carried the dislodged specimens

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[4]The 150 mesh stainless steel screen is made of .0026" diameter wire at 150 x 150 strands per square inch and is manufactured by the Cambridge Wire Cloth Company of Cambridge, Maryland.

and debris into the sieve. Where flow was sluggish, the water was stirred and the sieve was passed quickly through the roily water to collect the dislodged naiads and debris. The contents of the sieve were then washed into a 12 quart enamel pail to be sorted later in the laboratory.

During the return trip to the laboratory, oxygen tension was a critical factor in maintaining the naiads' healthy condition. Since oxygen capacity of cold water is greater than that of warm water and oxygen consumption of both specimens and debris is less, no special precautions were needed during cold weather. When specimens and debris were transported in warm weather, one of two methods of maintaining oxygen tension were utilized. One lowered the water temperature thereby increasing its oxygen capacity and coincidentally lessening the oxygen demand of the specimens and debris, whereas the other provided as much oxygen as possible to the warm water. Lowering the water temperature while in transit was accomplished by placing a frozen can of commercially available coolant into the pail containing the naiads. To furnish adequate oxygen in water that was not cooled, a light portable vibrator-type aerator (Bait-air-ator, Fig. 5) powered by either the car battery through the cigarette lighter receptacle, or four D-type dry cells, was used.

In the laboratory the naiads and debris were provided with aeration in the pail which was placed in a cold water

bath. Within 36 hours after collecting, specimens and debris were placed in petri dishes and the larger specimens removed by tweezers and pipette after being located with the naked eye. A stereomicroscope was then used to locate the smaller specimens which were likewise removed. The naiads were placed by size and species groups directly into jars of cool stream water which were in turn placed in a cool water bath. Whenever a relatively large number of specimens was assembled in one jar, aeration was provided to supply as much oxygen as possible. Within a day these specimens were placed in rearing cages either individually for instar studies, or in groups for the water and food preference phases of the life-cycle study.

Two types of rearing cages were used in both the laboratory and the field. The first, used for all sizes of specimens, was a wide-mouth glass jar three inches high and one and one half inches in diameter with a capacity of 4 ounces (Fig. 6). A disk of 150 mesh stainless steel screen[5]

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[5]The 150 mesh stainless steel screen greatly facilitates the handling of the cages. If the partly filled cages are quickly inverted, the extremely fine mesh allows a film of water to form on the screen by adhesion and capillarity. This film, held by surface tension, excludes additional air and although a small amount of water is lost while the weight of the water stabilizes with the partial vacuum created, the film remains intact, preventing further escape of water. Even if the cage is inadvertently placed on its side no water is lost unless the film is broken on some part of the screen or evaporates, thus releasing the partial vacuum. When the cages are placed in water with the screen end down, the barrier of the surface tension is lost and water circulates freely in and out of the cage, still maintaining the air pocket.

was secured over the mouth of the jar by a bakelite screw-type cover from which the top had been removed except for a narrow rim (Fig. 6a). The second type of cage, frequently used for minute specimens, was made from a 2 inch length of glass tubing of approximately one quarter inch bore with a disk of 150 mesh stainless steel secured over one end by a ring of letter-type sealing wax. The other end was closed with a cork stopper (Fig. 8). Both types of cages were only partially filled with water with a pocket of air retained sufficiently large to float them. The large cages were filled while upright and then inverted after the covers were screwed tight. The smaller cages were lowered upright into a dish of water until the water reached the desired level at which time the stoppers were inserted.

A stereomicroscope was used to observe the general health and development of the caged specimens and their food supply. For this purpose the small cages were submerged in a dish of water to eliminate the optical distortion otherwise caused by the curvature of the glass tube. The specimens in the large cages were observed through the open top. Before opening the large cages, it was usually necessary to shake the specimens from the screen. For more detailed observations the specimens were removed from their cages to Syracuse watch glasses or petri dishes. At all times when living naiads were illuminated for stereomicroscopic viewing, a jar of water was placed between the specimen

and the light as a heat-absorption barrier.

The large cages were prepared for emergence of adults by pressing a moistened strip of thin white paper (2" x 2½") firmly against the inside surface of the jar (Fig. 7). When the cage was in the screen-down position the lower end of the paper was in the water and the top end in the air chamber, thus providing a firm footing for the mature naiads to leave the water for their final molt.

An effort was made to prevent contamination of rearing equipment and to provide circulating, well-aerated water. To avoid contamination, the "non-reacting" cages containing specimens were placed in glass aquaria of from one to five gallon capacity filled with water from a stream in which a variety of Plecoptera were found. Fresh water from this source was brought to the laboratory every other week in a five gallon polyethelene container to replace the water in each aquarium. Aeration was maintained in each aquarium by an aquarium stone through which air was forced. Air bubbles released from the stones also provided circulation which in turn helped to maintain the temperature of the water in the aquaria in close approximation to that of the surrounding water bath.

The flowing water bath for temperature control of rearing aquaria was maintained in a 4' x 6' cypress wood aquarium (Fig. 9) partitioned into fourteen chambers. Chlorinated tap water entering at a controlled rate flowed

through each successive chamber in serpentine fashion to an exit at the far end, a distance of approximately 32 feet. An immersion-type refrigeration unit, capable of lowering the temperature of the water to the freezing point if necessary, was placed in the second chamber. Temperatures were maintained at nine degrees Centigrade at the cooling unit and warmed gradually to 16 degrees Centigrade at the exit in the warm laboratory. In any given rearing aquarium the water temperature did not vary more than ±1 degree Centigrade during the course of the experiments.

Food preference

The Nemoura naiads used for food preference studies were collected from the stream originating at Johnson Spring, which is located in a mixed hardwood forest. Predominant among the trees whose leaves made up the organic matter and leaf packs of this stream were white oak (Quercus alba), red maple (Acer rubrum), black birch (Betula lenta), and hemlock (Tauga canadensis). Leaves from those deciduous species plus apple (Pyrus malus), from just outside the laboratory, were presented to the naiads. Dry powdered yeast, as a replacement for the microorganisms present on the leaves, and rat pellets (Purina), as a substitute balanced diet, were also evaluated as foods.

Two procedures were involved in determining food preference. The first was to offer the naiads a single

food and the second involved presenting the specimens with a choice of foods. The shape of tissue sections cut from the leaf identified the type of leaf. Feeding was quantitatively determined by observing the portions of leaf missing and the resultant frass pellets. Feeding observations were also made in the field during the developmental period of the naiads.

Polluted water types

Nemoura naiads were exposed to both local chlorinated tap water and brook water under several rigorous conditions in order to gain perspective on the more important health factors necessary for long-term rearing experiments with specimens of this genus.

Three containers of brook water were provided, one of galvanized steel and two glass aquaria. The water in the galvanized container and in one aquarium was allowed to stand throughout the test, a period of several months, while that in the second aquarium was changed every four weeks when the discoloration due to the organic matter present became visibly detectable.

Tap water experiments were maintained under three conditions as follows:

1. Standing for two days with aeration before specimens were introduced.
2. Cooled in the second chamber next to the refrigeration unit.

3. Warmed to 16 degrees Centigrade in last chamber of flowing water bath with the amount of chlorine presumably lessened.

With the exception of the 16 degree Centigrade test which was conducted directly in the chamber of the flowing water bath, the tap water for the tests was also contained in glass aquaria. With all examples of both brook and tap water, aeration and circulation were provided by forcing air through aquarium stones.

Nemoura naiads were placed in the large (4oz.) rearing cages and provided with portions of maple and birch leaves for food. The cages were then placed in each of the six water treatments. Each week a careful check was made to determine the health of specimens, presence or absence of cast skins (as an indication of growth) and number of naiads dead.

Species identification

The species determination of the naiads utilized in the intensive life-cycle study required that they be reared to adulthood since the literature was not adequate to identify the naiads to species. Later, it became necessary to distinguish the naiads selected from those of several other Nemoura species present in the stream during their developmental period.

Both laboratory rearing and field collecting were employed to obtain the adult specimens necessary for identi-

fication of the species used in the intensive life cycle study. In the laboratory nearly mature naiads having large wing pads were placed in the large cages and supplied with food and emergence paper. The cages were checked weekly for food supply and emergence of adults. In the field, adults were sought by sweeping, light collecting, and by examining the debris along the sides of the stream. Both the low vegetation consisting of grass, ferns and brush, and the higher tree branches, mostly hemlock, were swept with a net for a distance of about thirty feet from the stream. Light collecting was done after dusk utilizing a white sheet suspended by a rope between two trees and illuminated by a gasoline lantern hung two to three feet to one side. Automobile headlights were occasionally substituted as the light source. Adult specimens attracted by the white light to the sheet were collected from the surface with tweezers. In searching for adults among the debris along the sides of the stream, rocks were lifted and scrutinized on the bottom and sides and loose wet and dry leaves were carefully turned over for examination.

In order to distinguish the naiads of Nemoura selected for the life-cycle study from others present in the stream, members of each discernable group were collected and reared to adulthood. Comparisons of the general appearance, activity, and anatomical differences of the naiads were made, and the times of their appearance in the stream and emergence as adults noted.

Life cycle of *Nemoura washingtoni*

Naiads of the species *Nemoura washingtoni* inhabiting the stream flowing from the Johnson Spring were utilized in the life-cycle study. Tiny naiads believed to be in their first instar and successively larger specimens collected at later dates were taken to the laboratory and isolated in either of the two types of cages as described earlier. Fragments of black birch and red maple leaves, each approximately one-eighth square inch in size were provided as food. Each week the contents of each cage were poured into a petri dish and examined for exuviae, food supply and the health of the naiad. Each specimen was then placed under a stereomicroscope for detailed observation and measurements. Frequently, it was found necessary to quiet the normally active specimens by placing them in a Syracuse watch glass containing a narcotic agent, Chloretone, at a concentration of two percent in water. Using ocular micrometer units (1 unit equals 16.6 microns) and estimating to tenths of units, measurements were made of head width, body length, and wing pads when present. While measurements were being made, precautions were taken to have the specimen completely horizontal to obtain accurate measurements as well as to have the specimen sufficiently covered by the liquid to eliminate optical distortion caused by curvature of the liquid surface. The head was measured across its widest

point, the eyes. Body length measurements excluded the cerci and antennae. Length and width of both fore and hind wing pads, when present, were measured. Because of the poorly defined base of the early wing pads, the widths were measured from the side of the notum to the point of the pad farthest from the insect's mid-line (Fig. 11) and the lengths were measured from the posterior margin of the notum to the posterior most point of the wing pads. This procedure resulted in negative figures for the smallest wing-pad lengths because they did not project back to the posterior margin of the notum.

Antennal and cercal segments were counted while the specimens were still in the Chloretone. Because the cercal segments on the larger specimens are relatively smaller and thus more difficult to count, the whorls of setae, which were found on all but the last segment, were counted as segments. The specimens were then removed from the Chloretone and returned to their cages.

RESULTS AND CONCLUSIONS

Collecting and rearing

An effort was made to provide favorable conditions for the naiads throughout the entire collecting and rearing process. The naiads were maintained under what would appear from preliminary tests to be satisfactory rearing conditions with fresh brook water provided bi-weekly to prevent contamination, continuous aeration, constant circulation, temperatures controlled within narrow limits and an acceptable diet of black birch and maple leaves similar to those utilized by the naiads in nature.

Obviously, normal conditions could not be so easily maintained while the naiads were being transported to the laboratory, sorted and observed. It was inevitable then that during these activities the specimens would be subjected to stresses of unknown intensity. The procedures followed to reduce such stresses appeared to be successful. The naiads appeared to be in an active, healthy condition when sorted, indicating that the oxygen and temperature controls provided by either the coolant or aeration technique were adequate during the forty-five minute return trip to the laboratory. The naiads appeared to resume their normal activities quickly after the fifteen minute sorting period and after the thirty or more minutes needed for detailed

observation when Chloretone was usually employed. During sorting and observation periods, warmth of the room and heat from the microscope illuminator caused a significant temperature increase of the water or Chloretone mixture containing the naiad even with the jar of water present as a heat absorption barrier.

Despite the precautions taken during the collecting and rearing periods, the survival rate for the naiads was low. Only seven of the seventy specimens observed for the intensive life-cycle study lived for more than five stadia. This low survival rate could have been the result of latent effects of the transportation, sorting and observation activities.

Successful rearing temperatures ranged from 10 to 13 degrees Centigrade. Higher temperatures usually resulted in death, whereas a lower temperature of approximately 6 degrees Centigrade, caused an obvious retardation in development.

Both types of rearing cages utilized in this study were satisfactory and felt to be superior to those used by other workers in past studies. They were constructed from non-corroding, non-dissolving materials in contrast to galvanized iron or copper used in many previous cages. They could be conveniently opened and closed and could be removed from the rearing aquaria without separating the naiads from their environment, thus minimizing the stress

on the specimens. The small size of both cage types permitted isolation of individual naiads for life-cycle studies without requiring excessive laboratory space.

Food preference

Naiads of the three species of *Nemoura* inhabiting the stream flowing from Johnson Spring exhibited a definite preference for certain foods. When presented with a choice of apple (*Pyrus malus*), black birch (*Betula lental*), maple (*Acer rubrum*) and oak (*Quercus alba*) leaves gathered from dry ground litter, the naiads in the laboratory consistently selected apple and black birch leaves. Maple leaves were eaten sparingly but oak leaves were completely refused. When only oak leaves were available as food, the naiads still refused them, even to the point of starvation. On the other hand, when either maple, apple, or black birch leaves were provided, the specimens were maintained in good health.

Field observations supported the above preference findings with an interesting exception noted in the spring. The naiads first fed upon black birch leaves, exhausting the supply by mid-winter. Maple leaves were then used as food. However, in the spring, the naiads began to include oak leaves in their diet. Such leaves were at this time in a decaying state and perhaps this is a necessary condition for their use as food. This conclusion was supported in the

laboratory at a later date when approximately ten specimens were reared for a period of several months exclusively on partially decayed oak leaves.

Both yeast and rat pellet trials proved to be unsatisfactory. Given either of these foods, the specimens died after about one week. In both instances, a fungal growth appeared from the bodies of the naiads. Moreover, the rat pellets produced a fungal growth which covered the screens of the cages and in most cases completely surrounded the specimens.

An interesting observation indicates that microorganisms may form an important part of the diet. After the paper labels in the rearing jars had been there long enough to become slimy from the growth of microorganisms, some naiads ate the surface portion of the label. Clear evidence of this was supplied by missing portions of the India ink identifying numbers on the labels and by the paper frass pellets produced.

Polluted water types

Results of the polluted water tests, with numbers of specimens involved and temperatures utilized, are presented in Table 1. The six types of water are arranged according to their effect on the length of life of Nemoura naiads.

These tests were purely exploratory and not replicated. They were intended as a guide to subsequent extensive laboratory rearing work to give a rough indication as to the

TABLE 1

POLLUTED WATER TESTS WITH NEMOURA NAIADS

Type of Water	Length of Life in Laboratory		Number of Specimens Utilized	Temperature: Degrees Centigrade
	Average Days	Maximum Days		
Brook - Changed[a]	69[g]	84[g]	11	14
Brook - Unchanged[b]	31	71	17	14
Tap - Conditioned[c]	22	66	17	14
Tap - Cold[d]	25	36	9	6
Tap - Circulated[e]	14	46	10	15
Brook - Galvanized[f]	15	26	24	10

[a]Water changed approximately every 4 weeks.

[b]Water unchanged throughout the test period.

[c]Conditioned by 2 days aeration prior to introducing naiads.

[d]Located in coolest chamber containing cooling unit.

[e]Located directly in the last chamber of the wooden water bath.

[f]Water in a galvanized container.

[g]Seven naiads remained alive at the conclusion of the test.

critical or noncritical nature of the source of water and type of container needed for rearing stoneflies.

Brook water, as expected, proved superior to tap water for rearing purposes, although an unexpected factor was discovered. Because approximately half of the original naiads in brook water had died within a month, a second container of brook water was set up containing brook water which was later replaced every four weeks. Under these conditions, 7 of the 11 original naiads were alive and healthy at the end of the test period 84 days later, whereas all naiads in the unchanged brook water died within 71 days, with the average life span being only 31 days. A further verification test was run with a new group of thirteen naiads in the previously used unchanged brook water and observed for 36 days. No cast skins were recovered from the cages and all specimens were dead by the fifteenth day, with the average length of life being only 7 days. Of thirteen specimens placed in fresh brook water at the same time, 11 were healthy at the termination of the test and had been growing well, producing 13 cast skins during the 36 day period. Apparently toxicity from the metabolites of naiads and decaying vegetable matter accumulated to such an extent that Nemoura naiads could not survive. Such an obvious toxic effect was unexpected since seemingly few (17) small naiads had been present in a large quantity (1 gallon) of water for a relatively short period of time (average of

31 days each).

As a result of these tests, about three times as much brook water was provided for each naiad during the subsequent laboratory rearing work and the water was changed every two weeks in an attempt to avoid completely the toxic effects as a factor in the rearing process.

Tap water was unsatisfactory for rearing Nemoura naiads because the average length of life was less than one month presumably due to the presence of chlorine. The naiads confined in cold tap water attained the longest average life span for tap water. The low temperature of 6 degrees Centigrade may have slowed metabolism and development with a consequent retardation in susceptibility to the toxic elements in the water. The relatively small number of naiads confined to the gallon of cold tap water may also have been a factor in the apparent lessening of toxicity.

The circulated tap water, which was contained in the wooden water bath rather than in glass aquaria as were the others, showed a high degree of toxicity to the naiads. Probably, contaminants from the wood and joint cement as well as from the chlorinated tap water had a poisonous effect on the stoneflies.

Water in the galvanized container proved to be the most toxic water tested. This was expected and presumably was

due to zinc dissolving from the container. Thus, all zinc-coated items were kept from contact with the rearing water during subsequent rearing work.

Species identification

Three distinct species of Nemoura naiads were found in the stream below Johnson Spring. These three were easily distinguished by their chronology of development, their appearance and their behavior.

Nemoura (Soyedina) washingtoni Claassen, utilized in the intensive life-cycle study, appeared as tiny naiads during the last week of June. In the following spring the mature naiads had robust bodies with short legs and no gills and were dark brown in color with muscle attachment patterns on the thorax in a lighter shade of brown. This species emerged in both the laboratory and the field during the month of April. Both collected and reared adults were used for identification.

Tiny naiads of Nemoura (Ostrocerca) albidipennis Walker [serrata Claassen] appeared in the stream during the last week of August and the first week of September, two months later than N. washingtoni. Their emergence as adults in early June occurred four weeks after the conclusion of the emergence of N. washingtoni. Unlike the other species,

the continual movements of the active naiads of N. albidipennis so hampered observation that it was always necessary to use Chloretone to calm them. Like N. washingtoni, these naiads had no gills, but they were more delicate in appearance, smaller in size and had relatively longer legs. A white triangle between the three ocelli aided in the recognition of these gray-brown naiads. This species was identified from one reared male.

The naiads of Nemoura (Amphinemura) nigritta Provancher [venosa Banks] were distinguished from the aforementioned species by the presence of four groups of from six to eight gills on the cervix. The size of these naiads approximated that of N. washingtoni yet in physical appearance they more nearly resembled N. albidipennis. The mature naiads of N. nigritta were a uniform light brown. They appeared in the stream during the latter part of August and the first week of September as did N. albidipennis, although N. nigritta emerged as adults two to three weeks later, during mid-June.

It was virtually certain that only Nemoura washingtoni naiads were used for the intensive rearing since they were identifiable by anatomical and size differences throughout the year.

Life cycle of Nemoura washingtoni

Instar determination

Molting indicators. The most direct method of determining the number of instars required for the development of a species would be to observe each molt. This was not practical since an actual molt was seen only once during this study. Thus, an indirect approach which could reveal when a molt was about to occur, or had just occurred was necessary. In Plecoptera, a molt can be detected by the presence of any of the following: a cast skin, a change in head-capsule size, changes in the eye transparency thickness and other changes in the appearance of the naiad.

Unfortunately, the complete molting process was not observed. The longest observation of molting was made over a period of 7 hours before the project was abandoned late at night, unfinished. By morning, the molt was complete. Since the specimen died a few days later, it appears that it may not have been in good health at the time of molting and therefore the time required for this particular molt is suspected of being abnormally long. No reliable estimate of the usual length of the molting period is possible at this time. It would seem that if the molt period were normally many hours in length, more opportunities to observe molting would have presented themselves during the hundreds of observations made over the two-year course of this project.

The easiest way to detect an impending molt is to observe the eye transparency thickness changes reported by Hanson (1962). Under a stereoscope at high magnification the compound eye in profile is seen to be composed of two parts, the outer transparent body wall and the dark eye pigment below it. Between the body surface and the pigment is observed a clear layer the thickness of which varies. This eye transparency thickness gradually increases during each stadium and just prior to the molt increases rapidly until approximately a four-fold increase is present. For example, in naiads that are three-quarters grown, the eye transparency thickness is approximately 9 microns, increasing to 36 before the molt. In the latter stage of the increase, a separation of the newly forming head from the rigid outer shell seems to be involved. Thus, having observed a substantial increase in eye transparency thickness, one is assured that a naiad is about to molt and for the counting of instars, the specimen need not be observed again for approximately three days in the case of the young specimens to two weeks in older ones which molt less frequently.

Another criterion for determining time of molting is the appearance of new chitinous body appendages within the old exoskeleton. This is not as useful a criterion as eye transparency thickness however, because they are observable for only a few hours before the molt. New cercal and

antennal joints were easily seen because they did not coincide with the old joints. New pretarsal claws were also observed within the old claws during the pre-molt hours. The exact number of hours prior to the molt that the new appendages became visible was not determined because the eye character alone was quite adequate.

In later instars the thickening of wing pads and in the final instar their darkening, as reported by Hanson (1962), were also found to be useful indicators of an impending molt.

The use of these indicators of molting or even of eye transparency thickness alone is more reliable than depending on the finding of cast skins and less dangerous to the specimen than anesthetizing it and measuring the head capsule. Thus, the use of molting indicators saves time for the observer and avoids considerable stress to the naiads.

Evaluation of instar identification criteria. The following types of data were collected for use in determining the number of instars of Nemoura washingtoni: head width, body length, wing-pad sizes, cercal segment counts and antennal segment counts. These proved to be of differing value for the purpose of instar identification.

Head-width measurements were found to be the most reliable for the initial third of naiad development for several reasons. First of all, growth in the head capsule

is confined to a very short period immediately following each molt before the hardening of the integument is completed since no membranous joints are present to allow for later expansion. Thus, the accuracy or variability of measurements taken in any stadium after the hardening of the head capsule is a direct function of the observer and his methods. Secondly, the head-width measurements were found to be reliable criteria during these early instars because the ranges in head-width measurements did not overlap from instar to instar. In fact, the gap between successive stadia was about equal to the ranges of either stadium. Thus, young naiads can be identified to instar by head-width measurements alone. However, in the intermediate stadia, variations in head-width measurements progressively increased toward the later instars so that identification of individual naiads to stadium using this characteristic alone became increasingly insecure. By the time development had progressed to the wing-pad stadia, identification by head-width measurements alone was impossible.

The number of segments composing the cerci and antennae was also useful for identifying the instar of naiads in the first three stadia. Were it not for the frequent loss of segments, these two criteria would probably be adequate to identify all instars. Segments were lost readily, not only at molting but also in normal movement about the habitat, as is seen by specimen S80 which had lost one segment from

the left cercus during the first instar. After the first molt, both antennal and cercal segments were lost by some specimens as is shown by second instar naiads in Table 2. During the last molt recorded for specimen S92, the left cercus was lost completely, probably contributing to the death of the naiad. N. washingtoni naiads do at times regenerate lost cercal segments as is indicated by data for instars 18 and 19 of specimen S100. Eight segments were added to the left cercus to replace some of those lost at the preceding molt, whereas only two were added to the apparently normal right cercus. Hynes (1941) and Kühtreiber (1934) mention frequently observing partially regenerated cerci in another Nemoura species. Thus, it appears that the loss and inconsistent regeneration of cercal segments may be common among Nemoura naiads. Evidence of regeneration in antennal segments was not found. The antennal segment count was less reliable than the cercal segment count because of more frequent segment loss as is apparent from the variations in counts and their overlapping between instars. Therefore, data on cercal and antennal segment counts can be used only as a guide in estimating the stadium of a given naiad of N. washingtoni for other than the first three stadia.

Body length of the naiads was a poor criterion for instar determination because the measurement does not stabilize following hardening of the integument. This is related to

the telescoping properties of the abdominal segments. In fact, the body length is always shorter immediately after the hardening of the integument than later in the stadium after feeding and internal growth has occurred and is often less following a molt than it was just before the molt. Therefore, individual body length measurements are not presented in this paper although they were made on each naiad at each measuring session.

The gaps in wing-pad measurements between instars were great enough so that growth variations during each instar and certain measuring difficulties did not prohibit making accurate identifications of each of the six instars bearing wing-pads. The ranges of measurements on wing-pad size within stadia included the additional effects caused by the pattern of wing-pad change during the stadium. The overall sizes of the larger wing-pads remained constant for each individual during a stadium, but their positions changed. When viewed from above just after molting, the wing pads appeared thin, curving downward and then outward from the body. Just prior to the next molt they were swollen and projected straight out from the notum of the specimen. Therefore, wing-pad width measurements on living naiads varied within a stadium depending on the time at which they were made. Length measurements of wing pads were more consistent throughout each stadium for each individual than width. Further measuring difficulties were caused by the

ill-defined bases of the wing pads. The problem was worst for the two smallest wing-pad-bearing instars on which the wing pads were little more than lobes on the sides of the notum. Thus, measurements were read only to the nearest whole ocular micrometer unit (each equal to 16.6 microns).

From the discussion in the foregoing section, it is clear that head-capsule width is most important for identifying the early instars and that the wing-pad sizes and shape are most important in the last instars. Because there are no diagnostic wing pads in the intermediate instars and the head-width measurements overlap, it is impossible to determine the total number of instars involved in the postembryonic development of this species without rearing and keeping close watch on individual specimens over extended periods of time.

Numbering of instars. Naiads of the species Nemoura washingtoni inhabiting the stream flowing from Johnson Spring in Warwick, although not reared for their entire nine to ten months of post-embryonic development, are, on the basis of laboratory data, estimated to pass through 22 stadia.

Tiny naiads collected during the last week of June are believed to have been, for several reasons, in their first instar. First of all, no specimens were found during thorough search in the preceding week. Secondly, the

smallest naiads possessed three cercal segments as did first instar specimens studied by Samal (Perla, 1923), Hynes (Perla, Chloroperla, Taeniopteryx, Protonemura, Nemoura, 1941) and Hanson (Acroneuria, unpublished). Three cercal segments were also possessed by the first tiny specimens collected of both N. albidipennis and N. nigritta when they made their similar sudden appearances in the same stream. Although a greater number (4) of cercal segments was found for the first instar specimens of a Leuctra species which I reared from eggs as well as for first instar specimens of Stenoperla (Helson, 1934)and Leuctra (Hynes, 1941), no stonefly naiads have been reported to have fewer than 3 cercal segments. A third indication that the smallest naiads studied were first instar specimens was the presence of fat globules which were scattered throughout the bodies of the tiny naiads but were not present after the first observed molt. Miller (1939) also reported such fat globules in Pteronarcys proteus in the first instar only. Thus, there seems to be no doubt that the smallest specimens studied were first instar naiads.

Since I was unable to rear even a single specimen through all of its stadia in the laboratory, the determination of the number of instars had to be based on integration of diagnostic data from many specimens. Head capsule size measurements and cercal and antennal segment counts of the first few stadia fit so distinctly into groups that the

early instars could be identified easily from unreared field collected specimens without exception. Corroborative data were obtained from the rearing of several specimens(6) through a number of stadia (2-4) in the laboratory. When integrated, these data provide a reliable numbering of instars 1 through 8 (Table 2).

Specimen number S85, although not providing information on the earliest or the last instars, was a very important specimen for determining the total number of instars because data from this specimen bridged the gap in our knowledge between the easily distinguishable early and late instars. The head-width data of S85 closely correlated with the previously mentioned early instar data through several stadia making it clear that S85 was collected in the third stadium and died in the nineteenth which was the third wing-pad instar.

Head-width data of S100, reared through 10 instars, matched those of S85 so well that there is little doubt of the identity of the stadia particularly since the wing-pad data also matched in the two specimens. Thus, it would seem that at least these two specimens possessed exactly the same number of molts through the intermediate instars. Until more than two naiads can be reared, one can only tentatively conclude that there are typically 16 instars prior to wing-pad development.

TABLE 2

HEAD WIDTH, AND CERCAL AND ANTENNAL SEGMENT COUNTS OF INSTARS ONE TO NINETEEN FOR NEMOURA WASHINGTONI

Instar	Specimen number S80	S82	S84	S90	S89	S92	S85	S100
1	180[a] 2 3[b] 9 9[c]							
2	195 2 3 11 11	195 4 4 9 9	187 4 4 9 6					
3		207 5 5 11 8	202 5 5 11 8	210 5 5 11 11	210 5 5 11 11	210 5 5 11 11	212 5 5 10 10	
4			219 - -	225 8 8 12 12	-	-	-	
5			242 10 10 14 14	244 8 8 12 13	-	-		
6			252 10 10 16 16	263 10 10 15 15	265 10 10 15 15	260 7 7 12 12		
7				293 12 12 16 16	287 11 10 16 16	290 10 10 14 14		
8					303 0 13 14 15	305 11 11 16 16		

[a]Head-width measurements in microns.

[b]Cercal segment count (left fig. rep. left cercus right fig. rep. right cercus).

[c]Antennal segment count (left fig. rep. left antenna right fig. rep. right antenna).

TABLE 2 -- Continued

Instar	Specimen number S80	S82	S84	S90	S89	S92	S85	S100
9							327 15 15 19 18	
10							388 16 17 13 20	380* - -
11							423 17 22 17 22	423 12 16 25 26
12							466 18 19 24 19*	-
13							504 20 21 26*24*	-
14							575 23 23 -	567 19 21 31*32*
15							645 25 25 -	673 24 23 - 37
16							751 28 27 -	757 29 29 -
17							837 29 30 40*40*	835 29 29 -
18							922 31 34 -	930* 12 30 -
19							987 33 33 -	1055 20 32 -

*S85 and S100 were the longest lived and thus most valuable specimens. Therefore, when an unusually long period in chloretone had not quieted them, an approximation or no reading at all was taken.

The last six instars (Table 3) were distinguished by the presence of wing pads in both sexes. These instars presented distinct measurement gaps in data on wing-pad dimensions. Since the last three instars of S85 (17-19) presented in Table 2 possessed wing pads, the six wing-pad instars can be identified as 17 to 22.

Instar 17, the initial wing-pad instar, had very small hind-wing pads and no measurable fore-wing pads. The second wing-pad instar (18) was easily distinguished from the first by the presence of measurable fore-wing pads. The third wing-pad instar was characterized by an increase in length of the hind-wing pads so that they reached approximately to the hind margin of the notum. The fourth wing-pad instar (20) had a conspicuous increase in both length and width of the hind-wing pads. On the contrary, it was the fore-wing pads which had a distinct increase in length and width in the fifth wing-pad instar (21). A large increase in size of both wing pads distinguished the twenty-second or last naiad instar. Convoluted wings became visible within the wing pads about midway through the final stadium, being white at first but becoming pigmented black a few days prior to emergence. Also, the last abdominal segment of male naiads in the final instar had a large dorsal hump or dome which provided space for the developing supra-anal process of the adult specimen. Thus, last instar males were identified as such by both the

TABLE 3

WING-PAD SIZES AND HEAD WIDTHS OF INSTARS SEVENTEEN TO TWENTY-TWO FOR *NEMOURA WASHINGTONI*[a]

Instar	Specimen number	Fore-wing pad		Hind-wing pad		Head width	Notes
		Length	Width	Length	Width		
17	S85	-	-	-33	132	837	
17	S108	-	-	-	99	761	
18	S85	-16	83	-33	215	922	
18	S100	-83	66	-49	199	930	approx.
18	S128	16	132	-33	182	917	
18	S119	33	116	-49	199	987	
18	S118	49	-	-49	-	864	
19	S120	33	116	0	215	1027	
19	S85	49	116	0	249	987	Just molted dead.
19	S100	0	232	0	298	1055	
19	S130	49	166	0	265	981	
19	S116	116	182	-16	182	903	
20	S122	249	265	49	365	986	
20	S130	265	249	99	448	1077	
20	S134	298	232	116	415	1042	

[a]All measurements are presented in microns.

TABLE 3 -- Continued

Instar	Specimen number	Fore-wing pad		Hind-wing pad		Head Width	Notes
		Length	Width	Length	Width		
21	S121	365	348	116	614	1402	
21	S125	348	348	33	498	1112	Male
21	S129	332	332	99	464	1152	Female
22	S124	830	614	498	996	1445	Male
22	S127	996	630	498	913	1296	
22	S123	1195	879	415	1211	1591	Female
22	S137	996	664	415	747	1230	
22	S134	913	747	415	996	1200	
22	S143	830	581	448	846	1052	Late individ.
22	6 males	-	697-846	-	747-846	1195-1311	1966
22	6 females	-	697-846	-	813-1095	1361-1460	1966

modification of the last abdominal segment and the distinctive wing pads, and females by the wing pads alone. Thus, because of specimen S85 which united the data on the early instars (Table 2) and the data on the wing-pad instars (Table 3), it appears that naiads of Nemoura washingtoni pass through twenty-two stadia before reaching adulthood.

Analysis of instar data

Head-width measurements for the early stadia fit so well into distinct instar groups that statistical analysis of the data was not necessary. In the intermediate stadia the only instar-correlated data available were from two reared naiads. Identification of unreared naiads to instar in the intermediate stages was not possible because of increasing variability in head-capsule width and the absence of other criteria usable for identification purposes. Thus, although desirable, little meaningful analysis is possible with the data available. Because of the presence of instar-distinguishing wing pads on late instar naiads, additional data were collected from unreared naiads. Since variations among individuals of each instar were relatively large and few specimens were available, further analysis was needed to evaluate the groupings mentioned in the previous paragraph.

A cumulative figure consisting of the sum of the length and width of both the fore and hind wing pads was used to

gather several small differences into a single larger figure with correspondingly larger gaps allowing easier identification of particular specimens to instar. The resultant figures for the individuals in Table 3 are presented in Table 4. Between instars 19 and 20 and 21 and 22, the gaps are particularly large. In view of the small number of specimens involved, a statistical evaluation of the reliability of these data was undertaken (Tables 5 and 6). A multiple comparison test was needed to evaluate the significance of the differences between the means of the cumulative wing-pad figures. Duncan's multiple range test, modified to test group means with unequal numbers of individuals, was selected as the best for this purpose. Duncan's test is not valid unless analysis of variance yields a significant F. In this case it was found to be highly significant (Table 6). The results of Duncan's multiple range test are presented at the 95% level of significance in Table 5. Thus, instar 22 is shown to be different from 21, instar 21 is different from 20 and instar 20 is different from 19, but by Duncan's test, instars 19, 18 and 17 are not distinguishable on the basis of presented evidence. Fortunately, specimens S85 and S100 were reared through each of these three stadia and their measurements were obtained. Thus, by combining the rearing evidence for instars 17, 18 and 19 with the statistical support for

TABLE 4

CUMULATIVE WING-PAD SIZES[a] OF INSTARS 17 TO 22 FOR NEMOURA WASHINGTONI

Instar	Specimen Number	Wing-pad Size	Instar	Specimen Number	Wing-pad Size
17	S85	99	20	S122	928
17	S108	99	20	S130	1061
18	S100	133	20	S134	1061
18	S85	249	21	S125	1227
18	S128	297	21	S129	1227
18	S119	299	21	S121	1443
19	S120	364	22	S143	2705
19	S85	414	22	S137	2822
19	S116	464	22	S124	2938
19	S130	480	22	S127	3037
19	S100	530	22	S134	3071
20	S126	829	22	S123	3700

[a]The sum of the length and width measurements for both fore and hind wing pads.

TABLE 5

MEAN, RANGE, STANDARD DEVIATION AND STATISTICAL SIGNIFICANCE OF THE CUMULATIVE WING-PAD FIGURES OF INSTARS 17 TO 22 FOR NEMOURA WASHINGTONI

Instar	n	W.P. $\bar{x}$	Range	Standard Deviation	Statistical Significance
22	6	3045	995	350	
21	3	1299	216	124	
20	4	969	231	112	
19	5	448	166	63	
18	4	244	166	77	
17	2	99	0	0	

TABLE 6

ANALYSIS OF VARIANCE OF CUMULATIVE WING-PAD FIGURES FOR NEMOURA WASHINGTONI

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Instars	5	29633932	7926786	264. **
Individuals	18	541368	30180	

instars 20, 21 and 22, it is virtually a certainty that there are six wing-pad instars present in the development of Nemoura washingtoni. It remains to be seen whether greater numbers of naiads would allow unreared specimens to be identified in instars 17, 18 and 19.

The conclusion that head-widths varied too much to allow their use in instar identification for the late instars with wing pads was confirmed by testing the head-width data by Duncan's method (Tables 7 and 8). None of the means of the instars was found to be significantly different from adjacent instars to be diagnostically useful. The means for the head-width measurements for each instar, however, fall approximately at intervals of 100 microns with the exception of instar 20. Linear regression of the head-width means data was established and tested for significance to evaluate the observed interval trend in head-width (Fig. 12). Although growth rate is exponential in form, the changes in the head-width measurements are approximately linear over the final quarter of the naiad's development represented by the wing-pad instars, thus justifying the use of linear regression. The regression was found to be statistically very significant. Thus, in view of the small sample size, the deviation of instar 20 from the expected norm is not surprising. The average head-width increment for each successive instar for the six final instars was 98 microns.

TABLE 7

MEAN, RANGE, STANDARD DEVIATION AND STATISTICAL SIGNIFICANCE OF HEAD-WIDTH MEASUREMENTS OF INSTARS 17 TO 22 FOR *NEMOURA WASHINGTONI*

Instar	n	H.W. $\bar{x}$	Range	Standard Deviation	Statistical Significance
22	6	1302	539	186	
21	3	1222	290	175	
20	4	1022	95	46	
19	5	991	152	57	
18	5	924	163	43	
17	2	799	76	53	

TABLE 8

ANALYSIS OF VARIANCE OF HEAD-WIDTH MEASUREMENTS FOR *NEMOURA WASHINGTONI*

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Instars	5	578666	115733	4.65**
Individuals	19	471334	24800	

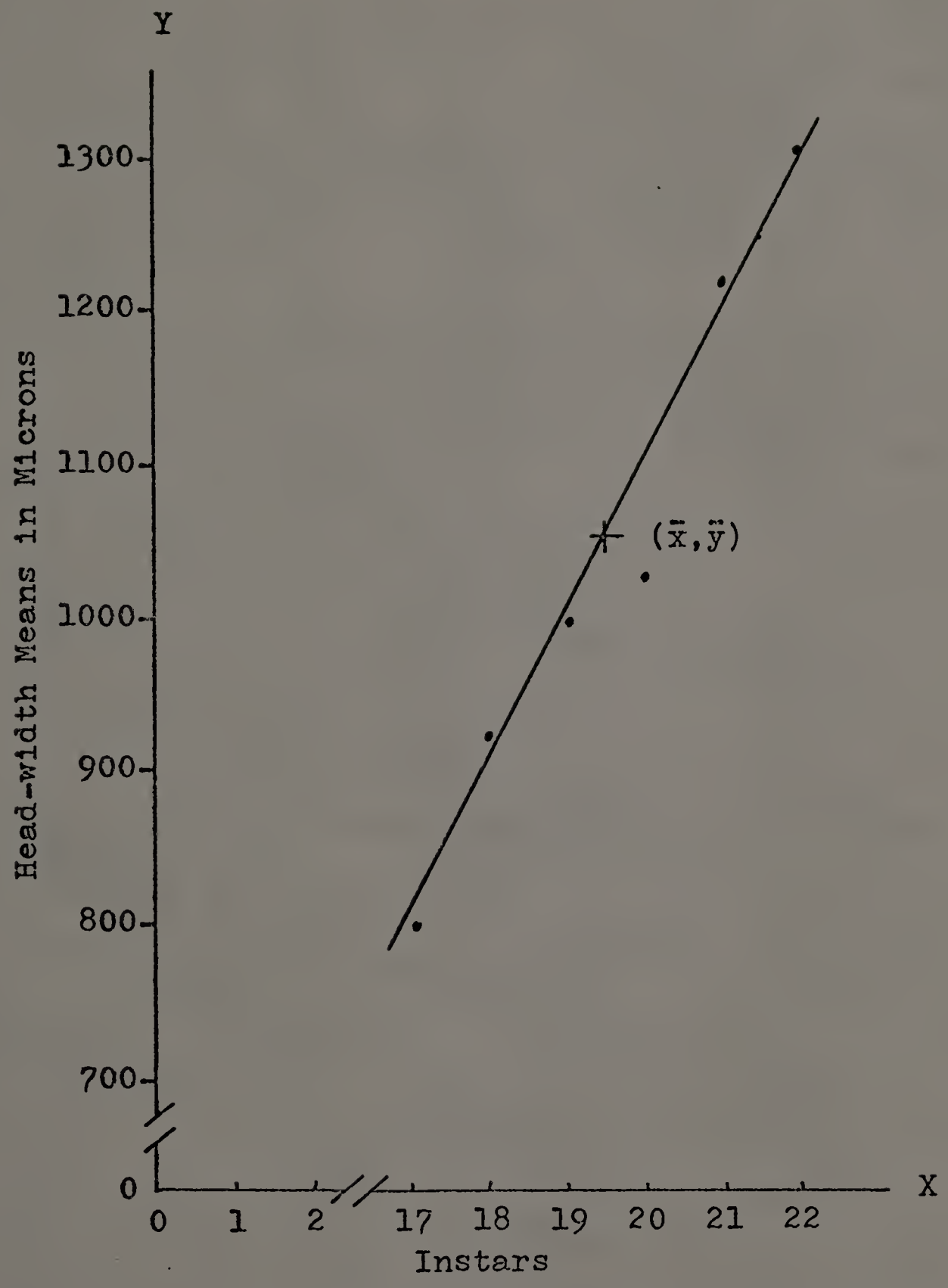

Fig. 12.--Regression of head-width means on instars for Nemoura washingtoni. 24 specimens. Y = 98X - 866 2/3. t = 9.25** d.f. = 4.

A final presentation (Fig. 13) indicates visually the relationship between head-width measurements and the cumulative wing-pad measurements. Both measurements for each specimen in Table 3 are plotted on logarithmic scales. From left to right, the graph clearly indicates the overlapping head-width ranges. Just as clearly, the fact that the ranges for the cumulative wing-pad figures do not overlap can be seen on the vertical axis of the graph. The grouping of the plotted points into 6 separate units indicating six wing-pad instars for N. washingtoni is as expected from previous analyses.

The head-width data for the last instar naiads collected in 1966 (Table 3) fell into two distinct size groups by sex with no overlap in measurements. The head-width measurements of the six males varied from 1195 to 1311 microns, and those of the six females from 1361 to 1460 microns, thus indicating the possibility that female specimens require a greater number of instars to develop. The range of the cumulative wing-pad figures (Table 5) for the last instar shows almost a two-fold increase in variability over the preceding instars. Thus, the greater size of females in the 22nd instar appears to be caused by sexual dimorphism in the last instar only.

Although 22 stadia are presently strongly indicated for the development of N. washingtoni, additional data from specimens of each sex reared through all stadia are needed.

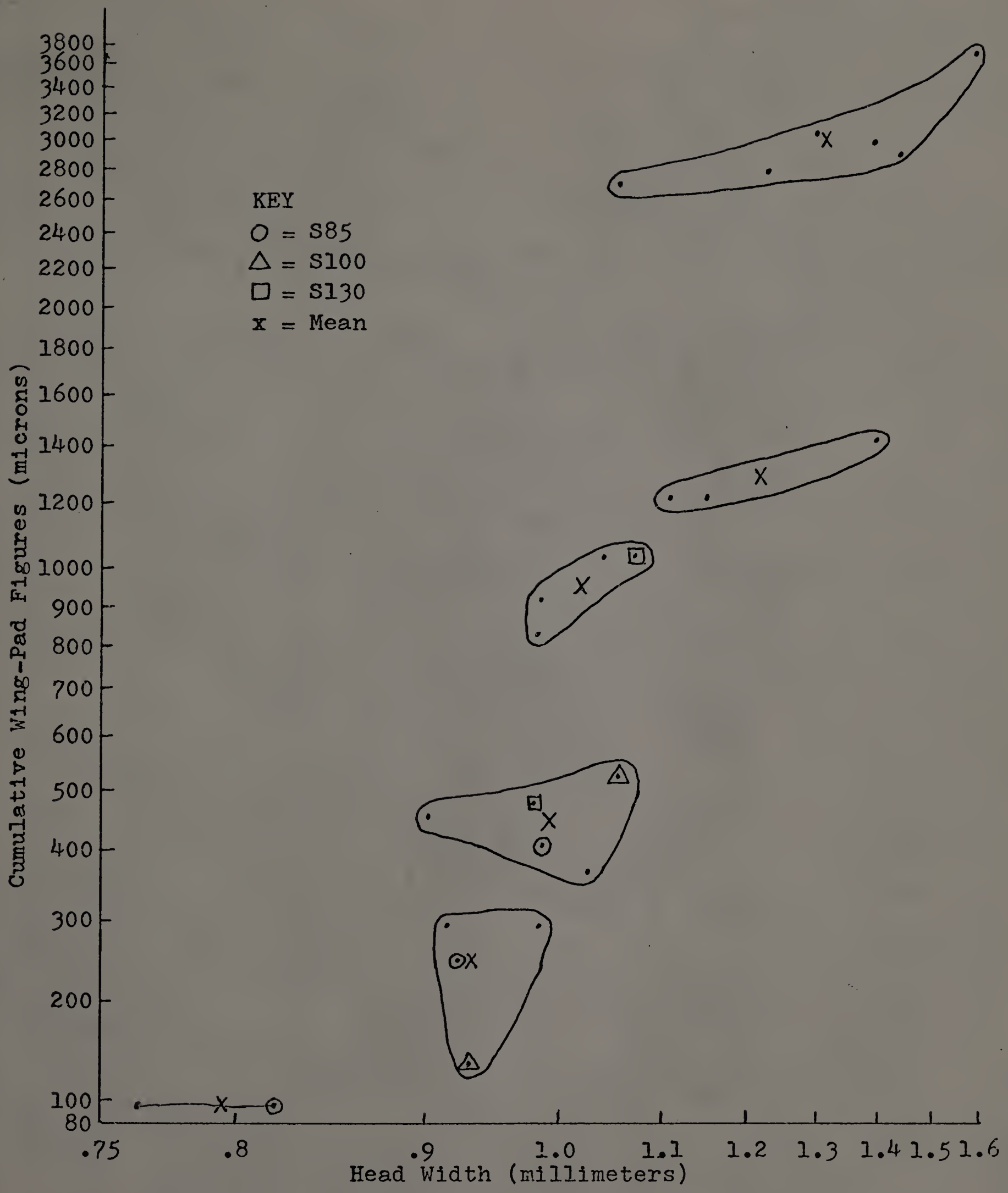

Fig. 13.--Cumulative wing-pad figures plotted against head width for individual specimens of instar 17 to 22 for Nemoura washingtoni.

Miscellaneous observations

Growth. There was remarkable uniformity in the time of hatching; most of the eggs apparently hatched within a single week. First and second instar naiads were initially found together and yet not a single tiny naiad was found seven days earlier. By the following week, only second and third instar naiads were found. This would indicate that all N. washingtoni naiads hatched during the last week of June, and that the duration of the first stadium was about one week. Laboratory rearing confirmed field observations showing that the second stadium also lasted approximately one week. Succeeding stadia were increasingly longer reaching about 30 days duration in the last few instars.

Body-length measurements for the first and last instar naiads of Nemoura washingtoni should prove useful for general size comparisons with other species of Nemoura. First-instar naiads were approximately 280 microns in length excluding the cerci and antennae. The last-instar naiads were between 5.9 and 8 millimeters in length, also excluding the cerci and antennae. The average length was 6.9 millimeters.

The average increment in head-capsule width for each molt during the 22 stadia was ten percent. Calculated head widths, using this percentage figure, were similar to the actual head widths but the uniformity indicated for

Nemoura vallicularia by Wu (1923) was not observed.

Respiration. The tracheal system, not visible throughout the first stadium, appeared in some naiads during the second and was visible in all specimens as silvery passageways by the third stadium. It would seem that gases enter the tracheal system late in the second stadium in Nemoura washingtoni as opposed to the first eleven minutes of the first stadium as reported by Helson (1935) for Stenoperla prasina. The first tracheae to become visible (i.e., emptied of fluids) were those in the legs again differing from S. prasina in which those of the head and longitudinal trunk were the first to be cleared of fluids.

Initial feeding. Numerous fat globules were present throughout the bodies of the first instar naiads. These naiads appeared not to have fed, as no solid material could be seen in the alimentary canal. By the second stadium, the globules had disappeared and brown and green particles (the color of the leaf tissues provided for food) were visible in the food canal. The fat globules probably supplied the nourishment needed during the first stadium. These observations agree with data presented by Miller (1939) on the feeding habits of Pteronarcys proteus during the first two stadia.

Coloration. Body color in the first-instar naiads was a translucent gray, with reddish-brown eyes and two tiny black lateral ocelli being the only distinct markings present. In the second stadium, a third black ocellus could be seen and food was present in the gut. In the third stadium, a very light brown general coloration of the naiad obscured the ocelli which at a later undetermined date became large enough to be again visible since they were easily seen on half-grown naiads. By the seventh instar, a darker brown (still a light brown) color was present over all of the body and there was a distinct darker transverse band across the head between the median ocellus and the fork in the epicranial suture (Fig. 10). The brown color gradually intensified in later instars and other distinct markings appeared on the naiad. By the twelfth stadium, the darkening thorax revealed lighter muscle attachment markings and the abdominal segments developed color bands with the posterior half of each segment a darker brown than the remaining portion. Successively in following stadia, further darkening of the lighter portions resulted in a loss of distinct patterns over all of the body. The coloration of mature naiads was a dark brown with the head pattern and some of the thorax muscle attachment markings still discernable. In the final stadium, the wing pads became black prior to emergence.

Emergence. Adults of N. washingtoni were observed emerging at the stream during the early morning hours in April of 1966 on the under sides of rocks and leaves within an inch or so of the water. Presumably, molting occurred during the night for newly emerged specimens were present at 7 A.M. when observations were made. Adults were found within a few inches of their emergence sites as indicated by the nearby cast skins. A few adults were found in the process of emerging. The adults obviously dispersed from the stream during the day as very few could be found near the stream at any one time, and yet large numbers of naiads must have emerged from the stream since predators to diminish the large supply of naiads were lacking. Light collecting and intensive sweeping in the area during the emergence period each yielded but a single specimen.

SUMMARY

Naiads of Nemoura washingtoni, Nemoura albidipennis and Nemoura nigritta found inhabiting the stream from Johnson Spring in Warwick, Massachusetts, were reared under controlled conditions in the laboratory. Easily handled glass cages confined the naiads individually or in groups in aquaria which were provided with fresh brook water frequently to avoid possible contaminant effects. The naiads preferred leaf tissues of apple and black birch, but also ate red maple and decaying white oak leaf tissues.

An intensive life cycle study was conducted in the laboratory on naiads of Nemoura washingtoni which were estimated to pass through 22 stadia during their 9-10 months of postembryonic development. Measurements and observations for possible instar identification were collected on head-capsule width, body length, wing-pad sizes, antennal and cercal segment counts, coloration, etc. Combined data on head-capsule width from eight naiads were used to identify the first eight instars since no single specimen lived in the laboratory through all of these stadia. Wing-pad measurements, primarily from field-grown specimens collected late in their development, were utilized to identify the six final instars. A single specimen which lived from stadium 3 through 19 provided overlap and continuity between the identified early and late instars.

Additional observations of interest included the transition from liquid to gas-filled tracheae near the end of the second stadium. This was found to occur much later than had been found for Stenoperla prasina (Helson, 1935). As found for Pteronarcys proteus by Miller (1939), the first-instar naiads did not feed but apparently subsisted on fat globules present in the body. These globules disappeared at the molt to the second instar and the naiads then began to feed on leaf tissues.

Numbering of stadia was possible without measuring head capsules or recovering cast skins but rather by observing certain molting indicators the most useful of which was eye transparency thickness. The transparent portion is present throughout the stadium, but its size increases considerably prior to each molt. A subsequent observation revealing a smaller eye transparency thickness indicates that a molt has taken place. Also useful as indicators for a few hours prior to the molt are the presence of newly formed tarsal claws and antennal and cercal segments visible within the old appendages. In the later instars, the swelling of the wing pads is indicative of an impending molt.

REFERENCES

Banks, N. 1897. Perlidae. In New North American neuropteroid insects. Amer. Ent. Soc. Trans. 24: 21-22.

Barrett, C. 1929. Rearing stoneflies. Victorian Nat. 45: 301.

Brink, P. 1949. Studies on Swedish stoneflies. Opus. Ent., Supp., Lund. 11: 1-250.

Claassen, P. W. 1923. New species of North American Plecoptera. Canad. Ent. 55: 257-263, 281-292.

_____. 1931. Plecoptera nymphs of America (north of Mexico). Thomas Say Foundation of the Entomol. Soc. Amer., Publ. 3, pp. 1-199.

_____. 1940. A catalogue of the Plecoptera of the world. Cornell Univ. Agr. Exp. Sta. Memoir 232, pp. 1-235.

Frison, T. H. 1929. Fall and winter stoneflies, or Plecoptera, of Illinois. Illinois Nat. Hist. Survey, Bul. 18: 345-409.

Hanson, J. F. 1962. Visible changes preceding molting in Plecoptera. Verh. XI int. Ent. Kongr., Wien. 3: 271.

Hanson, J. F. and J. Aubert. 1952. First supplement to the Claassen Catalogue of the Plecoptera of the World. Privately printed, pp. 1-23.

Harden, P. H. 1942. The immature stages of some Minnesota Plecoptera. Ann. Ent. Soc. Amer. 35: 318-331.

Harden, P. H., and C. E. Mickel. 1952. The stoneflies of Minnesota (Plecoptera). Tech. Bul., Univ. Minn., Minneapolis. 201: 1-82.

Helson, G. A. H. 1934. The bionomics and anatomy of Stenoperla prasina (Newman). Trans. Roy. Soc. N.Z. 64: 214-248.

_____. 1935. The hatching and early instars of Stenoperla prasina (Newman). Trans. Roy. Soc. N.Z. 65: 11-14.

Holdsworth, R. P. 1941. The life history and growth of Pteronarcys proteus Newman (Pteronarcidae: Plecoptera). Ann. Ent. Soc. Amer. 34: 495-502.

_____. 1941. Additional information and a correction concerning the growth of Pteronarcys proteus Newman (Pteronarcidae: Plecoptera). Ann. Ent. Soc. Amer. 34: 714-715.

Hynes, H. B. N. 1941. The taxonomy and ecology of the nymphs of British Plecoptera with notes on the adults and eggs. Trans. Roy. Ent. Soc. Lond. 91: 459-557.

Khoo, S. G. 1964. Studies on the Biology of Capnia bifrons (Newman) and notes on the diapause in the nymphs of this species. Verhandlungen des 3. Internationalen Symposiums uber Plecopteren, pp. 23-30.

Kühtrieber, J. 1934. Die Plekopteren fauna Nordtirols. Ber. naturw. -med. Ver. Innsbruck 44: 1-219.

Miller, A. 1939. The egg and early development of the stonefly Pteronarcys proteus Newman (Plecoptera). J. Morph. 64: 555-609.

Morgan, A. H. 1930. Field book of ponds and streams. G. P. Putnam's Sons, N. Y. pp. 1-448.

Needham, J. G., and P. W. Claassen. 1925. A monograph of the Plecoptera or stoneflies of America north of Mexico. Thomas Say Foundation of the Entomol. Soc. Amer., Publ. 2, 1-397.

Provancher, [L.] 1876. Une pluie d'insectes. Nat. Canad. 8: 125-127.

Ricker, W. E. 1952. Systematic Studies in Plecoptera. Indiana Univ. Publications, Science Series 18, pp. 1-200.

Samal, J. 1923. Etude morphologique et biologique de Perla abdominalis Burm. (Plecoptera). Ann. Biol. lacustre 12: 229-273.

Schoenemund, E. 1912. Zur Biologie und Morphologie einiger Perla Arten. Zool. Jahrb., Abt. Anat. und Ontog. Tiere 34: 1-56.

Schoenemund, E. 1925. Beiträge zur Biologie der Plecopteren-Larven, mit besonderer Berücksichtigung der Atmung. Arch. Hydrobiol. 15: 339-369.

Smith, L. 1913. The biology of Perla immarginata Say. Ann. Ent. Soc. America. 6: 203-211.

Walker, F. 1852. Perlides. In Catalogue of the specimens of neuropterous insects in the collection of the British Museum. Part I-(Phryganides-Perlides), pp. 136-192.

Wu, C. 1923. Morphology, anatomy and ethology of Nemoura. Lloyd Libr. Bul. 23, Ent. Ser., no. 3: 1-81.

EXPLANATION OF PLATES

PLATE I

Fig. 1. Habitat, upstream view

2. Habitat, downstream view

PLATE II

3. Kitchen sieve, disassembled
   a. Handle and bail
   b. Removable sieve

4. Coffee can sieve, exploded view
   a. Can with 150 mesh stainless steel screen in place
   b. Coffee can cover adapted for holding screen in place

PLATE III

5. Portable aerator powered by car battery through cigarette lighter receptacle or by 4 self-contained "D" type dry cells

PLATE IV

6. Rearing jar, disassembled view
   a. Bakelite cover
   b. 150 mesh stainless steel screen
   c. 4 ounce glass jar

7. Rearing jar assembled, with paper in place for emergence of adults

8. Small rearing tube, assembled

PLATE V

Fig. 9. Flowing water bath

PLATE VI

10. Nemoura washingtoni dorsal head color pattern

11. Outline of metathoracic wing pad (right side) illustrating measuring technique
    a. Width of wing pad
    b. Length measurement of wing pad; posterior-most point of wing pad to posterior-most margin of notum

PLATE I - HABITAT

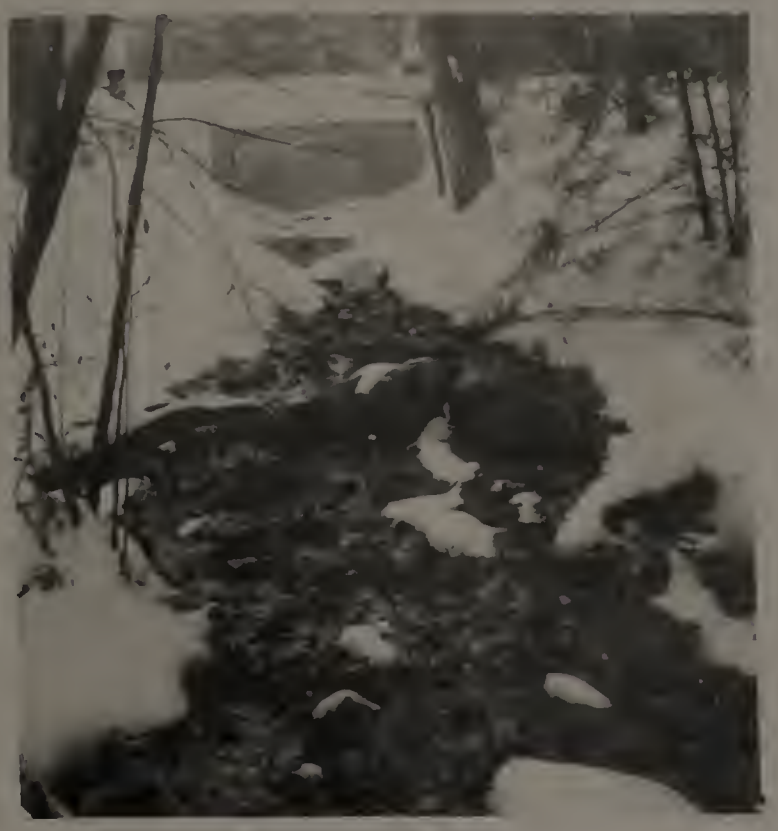

Fig. 1. Habitat - upstream view

Fig. 2. Habitat - downstream view

PLATE II - COLLECTING SIEVES

Fig. 3a

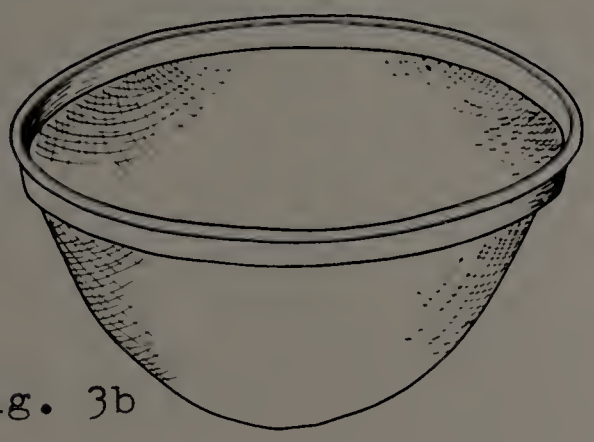

Fig. 3b

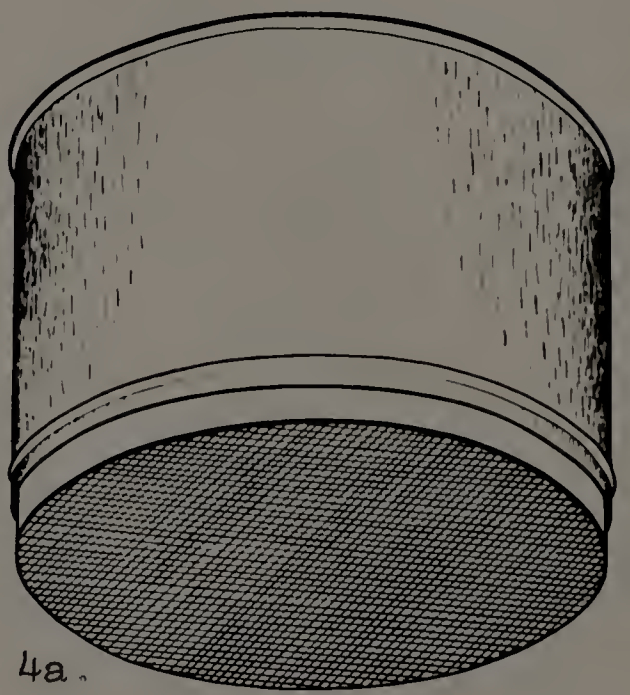

Fig. 4a.

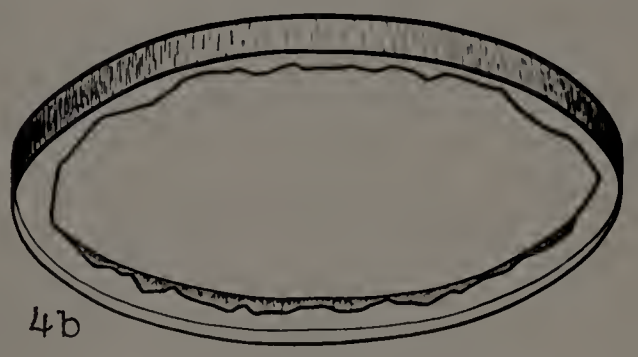

Fig. 4b

3. Kitchen sieve, disassembled
   a. Handle and bail
   b. Removable sieve

4. Coffee can sieve, exploded view
   a. Can with 150 mesh stainless steel screen in place
   b. Coffee can cover adapted for holding screen in place

PLATE III - AERATOR

Fig. 5. Portable aerator powered by car battery through cigarette lighter receptacle or by 4 self-contained "D" type dry cells

PLATE IV - REARING CAGES (ACTUAL SIZE)

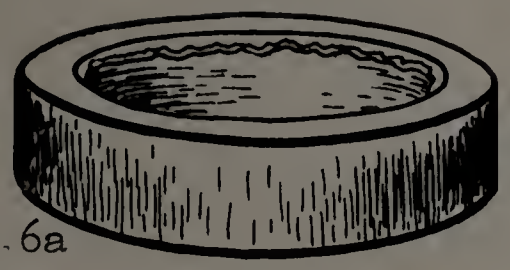

Fig. 6a

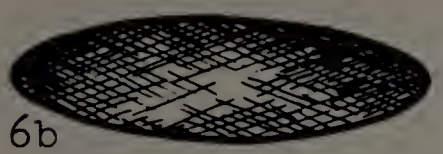

Fig. 6b

Fig. 6c

Fig. 7

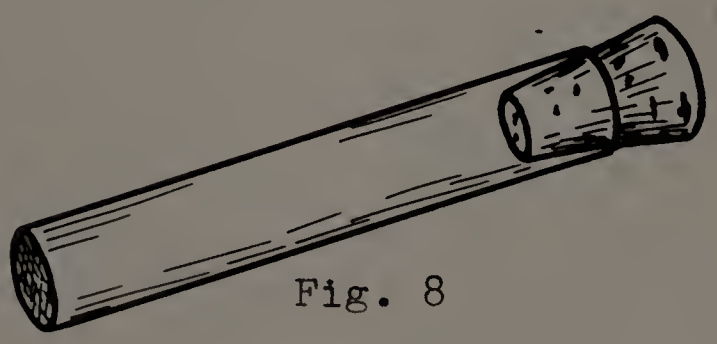

Fig. 8

6. Rearing jar, disassembled view
   a. Bakelite cover
   b. 150 mesh stainless steel screen
   c. 4 ounce glass jar

7. Rearing jar assembled, with paper in place for emergence of adults

8. Small rearing tube, assembled

PLATE V - FLOWING WATER BATH

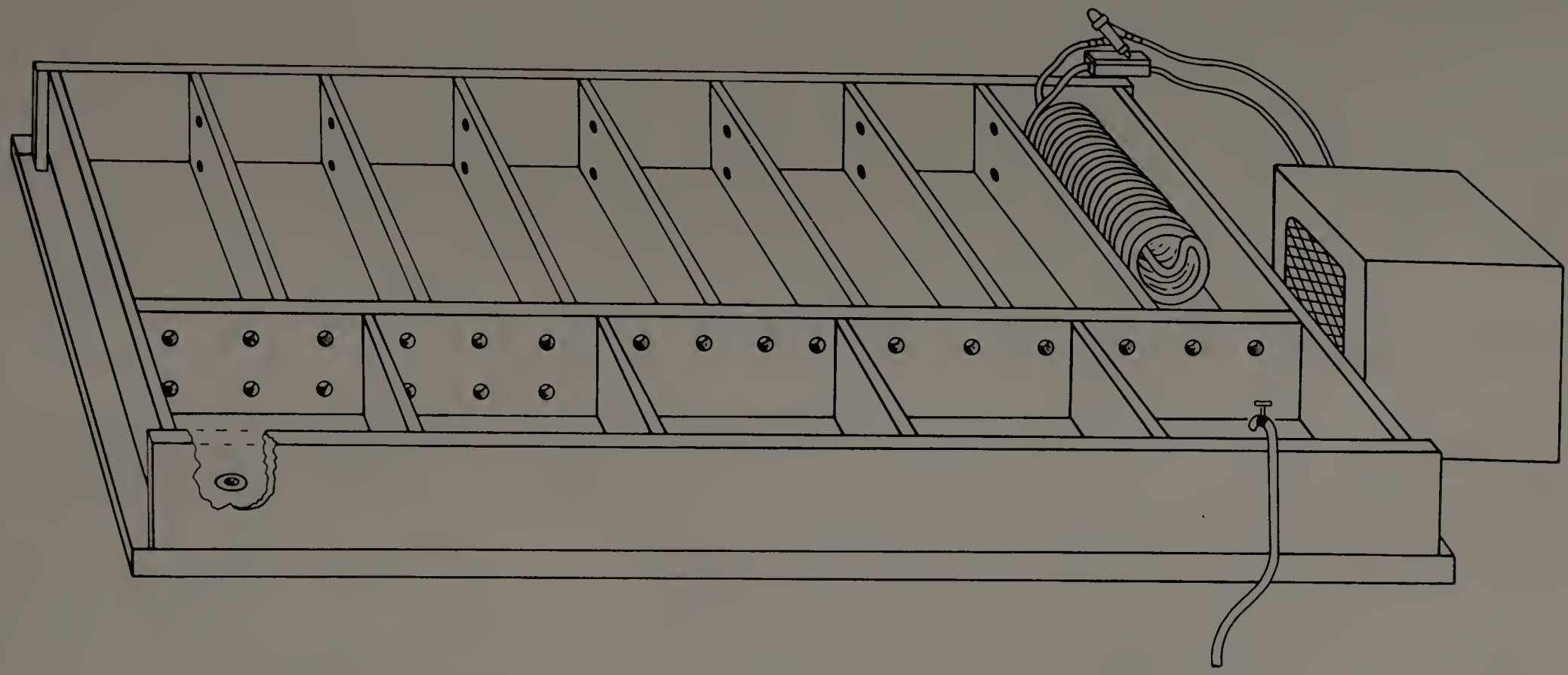

Fig. 9. Flowing water bath

PLATE VI - DIAGNOSTIC CHARACTERISTICS

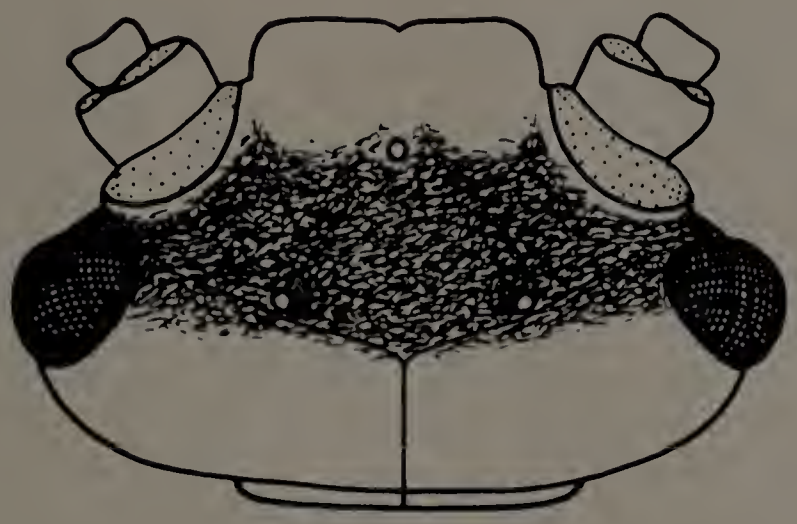

Fig. 10. Nemoura washingtoni dorsal head color pattern

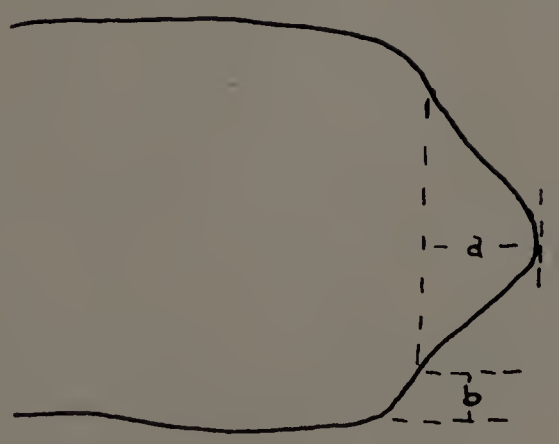

Fig. 11. Outline if metathoracic wing pad (right side) illustrating measuring technique
a. Width of wing pad
b. Length measurement of wing pad; posterior-most point of wing pad to posterior-most margin of notum