

RELATIONSHIP OF OXYGEN REQUIREMENTS TO INDEX-ORGANISM CLASSIFICATION OF IMMATURE AQUATIC INSECTS

Theodore A. Olson and Myrtle E. Rueger

Hynes (1) has classed pollution of water as a biological phenomenon in that its primary effect is on living things. For this reason, biological examinations, usually combined with chemical and physical examinations, have been used for many years to assess the degree of pollution of water. In the biological analyses, immature aquatic insects have been used extensively as indicators because many possess useful characteristics such as sensitivity to changes in their environment, aquatic stages lasting for months to several years, sufficient size for ease in collection, and ability to move out of an area without the great mobility of fish.

Over the years fairly extensive lists of aquatic insects frequently encountered in water undergoing natural purification have been compiled (2) (3) (4) (5) (6). However, some disagreement exists over the exact status of many organisms, whether they are considered single species or communities of species. This may be because many associations of insects have been correlated with water conditions as determined by chemical and physical examinations taken only at the time of sampling. Data obtained by Gameson and Griffith (7) using continuous recordings of oxygen concentration show that no reliable idea of the oxygen con-

tent of a body of water can be gained from a few spot samples. Even with continuous recording of chemical and physical factors, care should be taken in associating index-organisms with the records because, as pointed out by Needham and Usinger (8), the insects may not be distributed evenly throughout the stream. Macan (9) has stated that we still have too few fundamental facts about distribution of insects in a stream to justify any scheme of classification. The same lack of fundamental facts pertains to their status as index-organisms. Oxygen is a basic need of aquatic insects, yet respiration studies have been done on relatively few species, and a list correlating their oxygen needs to their usual index-organism classification is not available.

Wigglesworth (10), Edwards (11), and Macan (9) summarize most of the work that has been done on respiration rates of insects. The majority of the publications on immature aquatic insects have been on European species. In addition, the conditions under which the experiments were done often make comparisons difficult. In most studies, multiple specimens of varying weight were used. It is now known that the respiration rate does not remain stable throughout the development of an insect; younger and less heavy specimens of a species usually use more oxygen per gram body weight than older and heavier specimens. In using multiple specimens, the average rate may be altered because one specimen often disturbs the other in the

Theodore A. Olson and Myrtle E. Rueger are, respectively, Professor of Public Health Biology and Research Fellow, School of Public Health, University of Minnesota, Minneapolis, Minn.

TABLE II.—Oxygen-Consumption Rate per Live Body Weight

Immature Insect	Oxygen Consumption ($\mu\text{l/hr/mg}$)		
	Mean	Variance	S. E. Mean
Diptera: Tipulidae: <i>Tipula</i> sp.	0.0505	0.0001	0.0029
Ephemeroptera: Baetiscidae: <i>Baetisca laurentina</i> McDunn.	0.3138	0.0069	0.0208
Ephemeroptera: Ephemeridae: <i>Ephemer</i> <i>simulans</i> Walker	0.2425	0.0065	0.0185
Ephemeroptera: Leptophlebiidae: <i>Leptophlebia</i> sp.	0.4004	0.0083	0.0228
Ephemeroptera: Potamanthidae: <i>Potamanthus myops</i> (?) (Walsch)	0.2937	0.0118	0.0272
Odonata: Calopterygidae: <i>Calopteryx</i> sp.	0.0920	0.0010	0.0069
Odonata: Calopterygidae: <i>Hetaerina</i> sp.	0.1663	0.0049	0.0193
Plecoptera: Perlidae: <i>Paragnetina media</i> (Walker)	0.1869	0.0039	0.0166
Plecoptera: Pteronarcidae: <i>Pteronarcys pictetii</i> Hagen	0.0996	0.0027	0.0078
Trichoptera: Hydrophilidae: <i>Macronemum zebrafatum</i> (Hagen)	0.2069	0.0018	0.0130
Trichoptera: Limnephilidae: <i>Pycnopsyche guttifer</i> (Walker)	0.0935	0.0032	0.0141

confined space or because of a buildup of toxic waste products. Extensive work on individual, immature, aquatic insects was done by Balke (12) on European species of the insect orders Neuroptera, Odonata, Plecoptera, and Trichoptera. Although her study was not concerned with their oxygen-consumption rates in relation to their index-organism classification and no individual figures are given, one can obtain approximate values from the graphs given in the publication.

Patton (13) describes the rate of oxygen consumption of any insect species as an elusive value that varies with many internal and external factors. And, as pointed out by Keister and Buck (14), there are various types of puzzling fluctuations in rate familiar to anyone who has measured

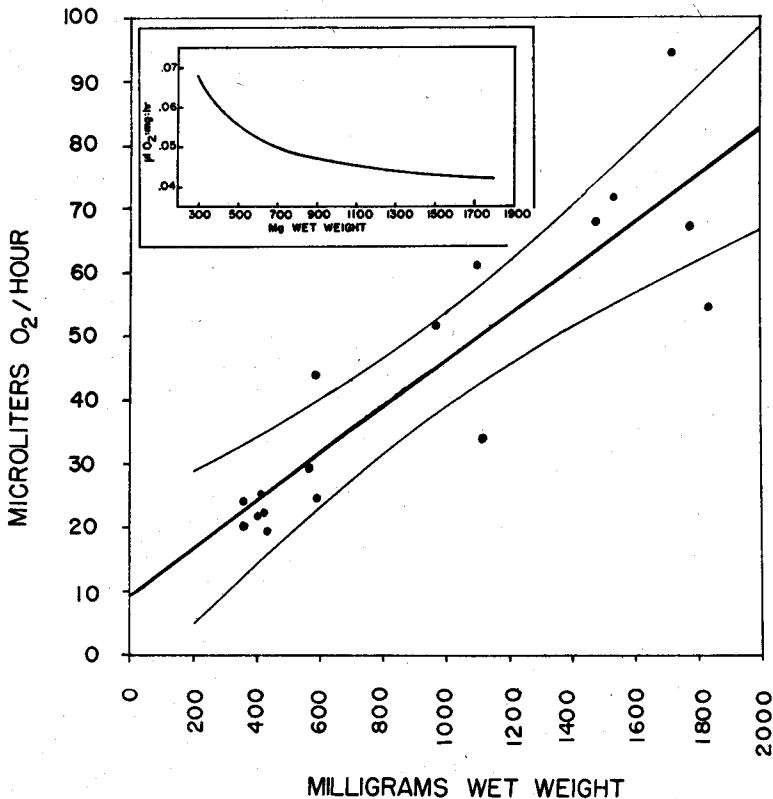


FIGURE 1.—Relationship of oxygen consumption (ordinate) to live body weight of 17 *Tipula* sp. larval specimens at 20° C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 9.23572 + 0.03683X$ (SE slope = 0.00492). Insert: Oxygen consumption rate in relation to live body weight.

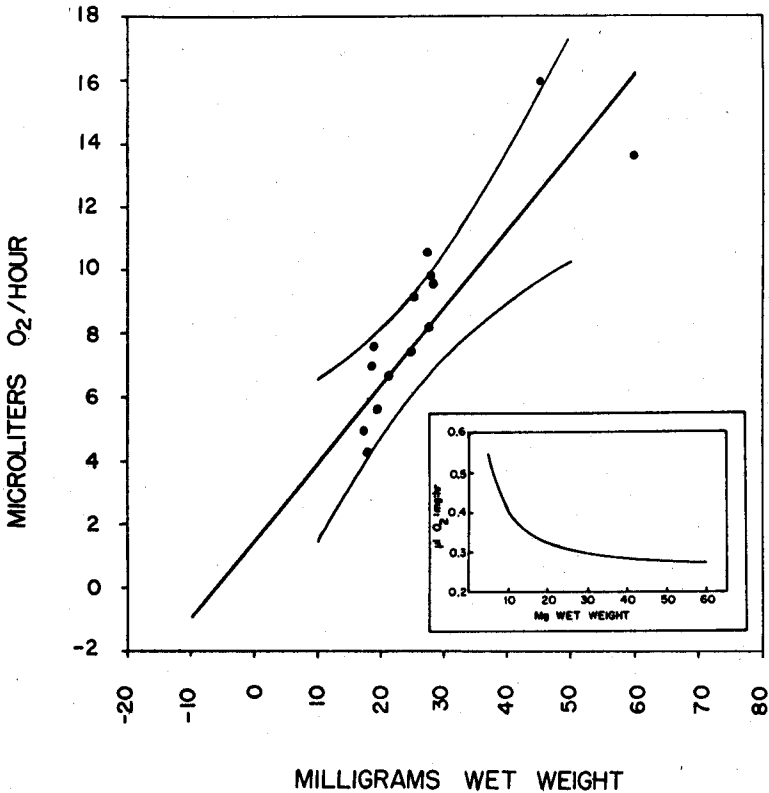


FIGURE 2.—Relationship of oxygen consumption (ordinate) to live body weight of 16 *Baetisca laurentina* naiid specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 1.48386 + 0.24510X$ (SE slope = 0.04686). Inset: Oxygen consumption rate in relation to live body weight.

respiration rates of individual insects. They believe these fluctuations suggest the possibility of relatively sudden metabolic regulation independent of any externally visible activity. Studies on standard respiration rates of insects undoubtedly will need to be patterned after human standard basal metabolism rates in which samples were obtained from all ages and these then were subjected to statistical analysis (15) (16) (17). Boothby *et al.* (15) pointed out that variability, being a universal biological phenomenon, is inherent in metabolism values. They maintained that there is not a single lowest value for basal metabolism for an individual, but a variable quantity that can be standardized but not absolutely fixed and the variability should be taken into account by proper statistical methods.

As pointed out oxygen is a basic need of aquatic insects and respiration studies have been done on very few American species. Therefore, the primary objective of this study was the determination of oxygen-consumption rates of common benthic insects in order to obtain information which may aid in the determination of their status as members of an index-organism community. Taking into account the studies of previous workers, the main emphasis of our studies has been on individual insects of varying weights and statistical analysis of the data. The results obtained at 20°C are reported in this paper.

Methods

The insects were collected from rapidly flowing rivers in Minnesota; the *Macronemum zebratum* (Hagen)

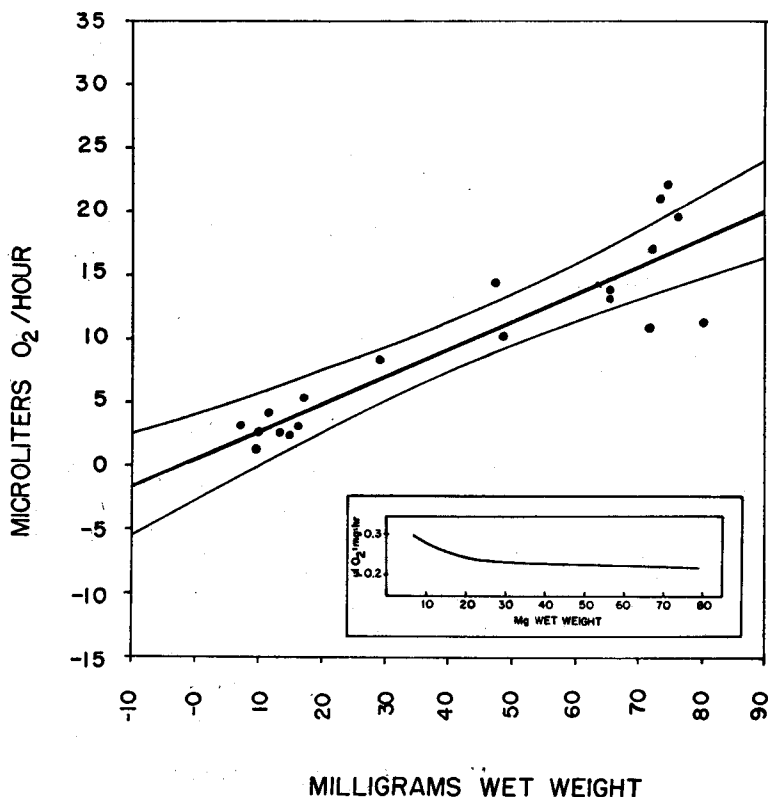


FIGURE 3.—Relationship of oxygen consumption (ordinate) to live body weight of 19 *Ephemera simulans* naiad specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 0.61023 + 0.21710X$ (SE slope = 0.02491). Inset: Oxygen consumption rate in relation to live body weight.

(Trichoptera) from the St. Croix River and the other insects from Elk River. In the laboratory the insects were kept in aerated aquariums containing water and rocks from the original or a similar habitat. Light was provided during at least part of the day. Most of the aquariums were maintained at 20°C and were equipped with bottom filters of coarse sand; the water was filtered through activated charcoal after passing through the sand.

The Warburg apparatus was used for the respiratory studies. This method has been used for many years in respiration studies and is of sufficient sensitivity to measure the oxygen consumption of single specimens of most index-organisms. The method followed the general plan as out-

lined in Umbreit *et al.* (18), using 3 ml of deionized water in 15-ml flasks with 0.2 ml of 10-percent KOH and folded filter paper in the well. A plastic screen to which the insects could cling was added to each flask, including the thermobarometer. Flasks were shaken at 88 times/min at an excursion distance of 3 cm with a 9-station circular Warburg apparatus in an unlighted, temperature-controlled room. Preventing leakage in manometer stopcocks is particularly important. Therefore, as recommended by Ludwig *et al.* (19), all old grease and lint were removed with xylol. New grease was applied longitudinally on three sides of the stopcock. The plug then was warmed gently, inserted in the barrel, twisting only sufficiently to coalesce the three zones of grease. All

stopcocks were examined for a smooth appearance, free of any air. Additionally, the reaction flasks must be strictly clean. Ludwig *et al.* (19) found that a procedure in which the flasks were scrubbed with soap, rinsed well, filled with sodium dichromate cleaning solution, warmed at 100° for 2 hr, and rinsed about 20 times using tap, distilled, and doubly distilled water was satisfactory. This procedure was used.

The insects were brought to approximately 20°C about 2 hr before the beginning of an experiment and conditioned to deionized water by progressively increasing its proportion to the aquarium water. For trichopteran larvae which live in cases, the oxygen-consumption studies were done with the larvae in their shelters. However,

the cases of *M. zebra* are destroyed to some extent when removed from the rocks to which these larvae are attached and in these instances the cases were not included in the experiments. Individual insects were allowed to equilibrate for $\frac{1}{2}$ to 1 hr before readings were begun. Readings then were taken each hour for a period of four hours. After the experiment, the insects were blotted dry and their live weight and volume determined. The respiratory rate is expressed in microliters of oxygen per hour (an average of the four-hour period), and all weights referred to are unit whole body live weights. In the measurement of the oxygen consumption, the regression of the microliters of oxygen consumption per hour against the live body weight was calculated by pro-

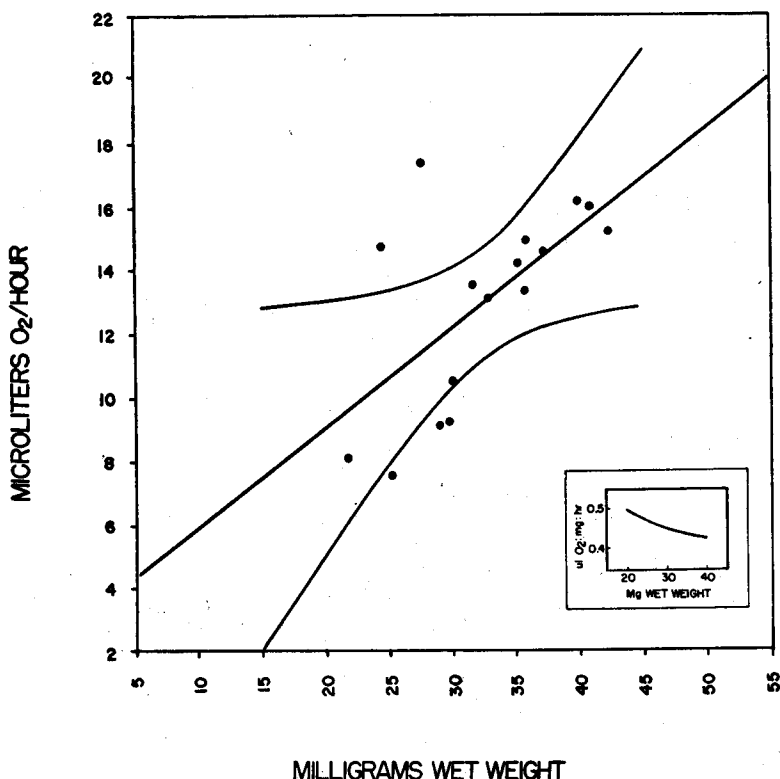


FIGURE 4.—Relationship of oxygen consumption (ordinate) to live body weight of 16 *Leptophlebia* sp. naiad specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 2.78825 + 0.31174X$ (SE slope = 0.10571). Inset: Oxygen consumption rate in relation to live body weight.

gramming all data for each species into a pooled regression analysis performed on a 1604 Control Data computer.

Results

Oxygen consumption by immature aquatic insects of the orders Diptera, Ephemeroptera, Odonata, Plecoptera, and Trichoptera have been determined with the Warburg respiratory method. The results obtained at 20°C are presented in Tables I and II and Figures 1 through 12. As has been found by previous workers (20) (21) (22) (23), smaller specimens usually had higher oxygen-consumption rates than larger specimens of the same species. Two exceptions, over the weight range tested, were *Peteronarcys pictetii* Hagen and *Paragnetina media* (Walk-

er) (Figures 8 and 9). In general, the oxygen-consumption rates agree with the index-organism classification given by Fair and Whipple (5) and Gaufin and Tarzwell (24).

When compared with the other orders, the average rates of *Tipula* (Diptera) were the lowest among those tested (Figure 12). *Tipula abdominalis* and *Tipula* sp. were found in the clean-water zone of a stream by Gaufin and Tarzwell (25); *Tipula* sp. also was found in the zone of moderate pollution. Although the average oxygen-consumption rates of the families of the Ephemeroptera varied somewhat (Figure 12), they were higher than rates of the other orders. *Leptaphlebia*, which cling to submerged plants in the stream, had a higher rate than genera (*Ephemera*

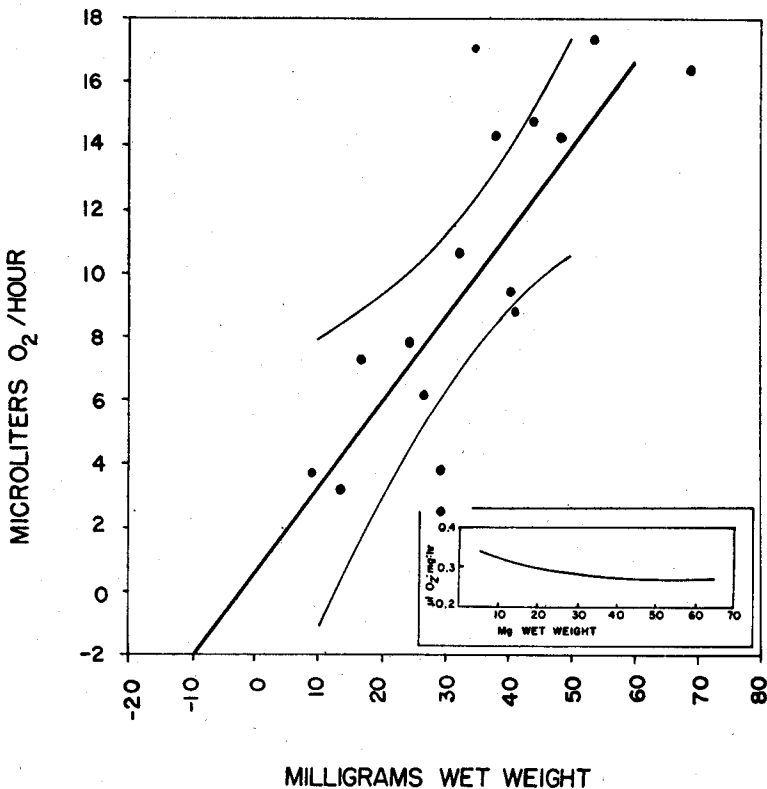


FIGURE 5.—Relationship of oxygen consumption (ordinate) to live body weight of 16 *Potamanthus myops* (?) naiad specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 0.71468 + 0.26584X$ SE slope = 0.05727. Inset: Oxygen consumption rate in relation to live body weight.

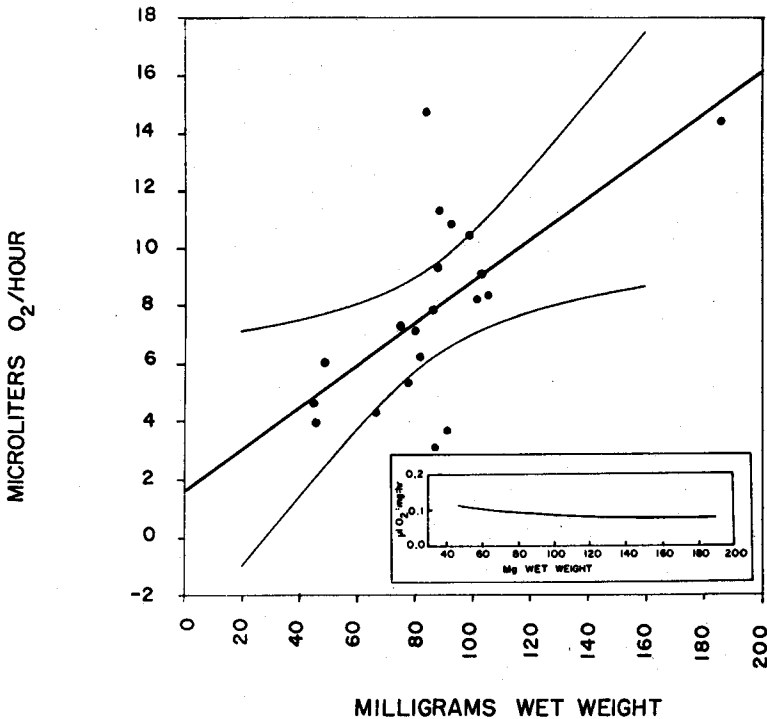


FIGURE 6.—Relationship of oxygen consumption (ordinate) to live body weight of 20 *Calopteryx* sp. naiad specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 1.60219 + 0.07197X$ (SE slope = 0.02075). Insert: Oxygen consumption rate in relation to live body weight.

and *Potamanthus*) which burrow in the sand or are found on rocks at the bottom of the stream. The high oxygen-consumption rate of the Ephemeroptera corresponds to their index-organism classification. In the Fair and Whipple tabulation (5), members of this order are classified in the clean-water zone for the most part. The data on the naiads of the suborder Zygoptera order Odonata indicate that these insects have a low oxygen-consumption rate. In the Fair and Whipple tabulation (5), the Odonata for the most part are listed in the clean-water zone. Gaufin and Tarzwell (25) found *Agrion* sp. (= *Calopteryx*) few to common in the clean-water zone and common in the moderately polluted and recovery zone; in their 1952 publication (24), the genus was listed only from the clean-water zone. *Paragnetina media*, the

smaller of the two Plecoptera tested, approached a moderate oxygen-consumption rate while *Pt. pictetii* had a low rate (Figure 12). Fair and Whipple (5) list several species of *Perla* (some species of this genus have been transferred to *Paragnetina*) as occurring in the clean-water zone. In the same tabulation (5), six families of Trichoptera are listed as having been found in the recovery zone. In our study, the oxygen-consumption rate of *Pycnopsyche guttifer* (Walker) was low and that of *M. zebratum* moderate (Figure 12). For the latter, the rate may have been somewhat higher than that found in nature because the larvae were removed from their cases.

Discussion

The *Tipula* larvae have posterior respiratory spiracles by which they are

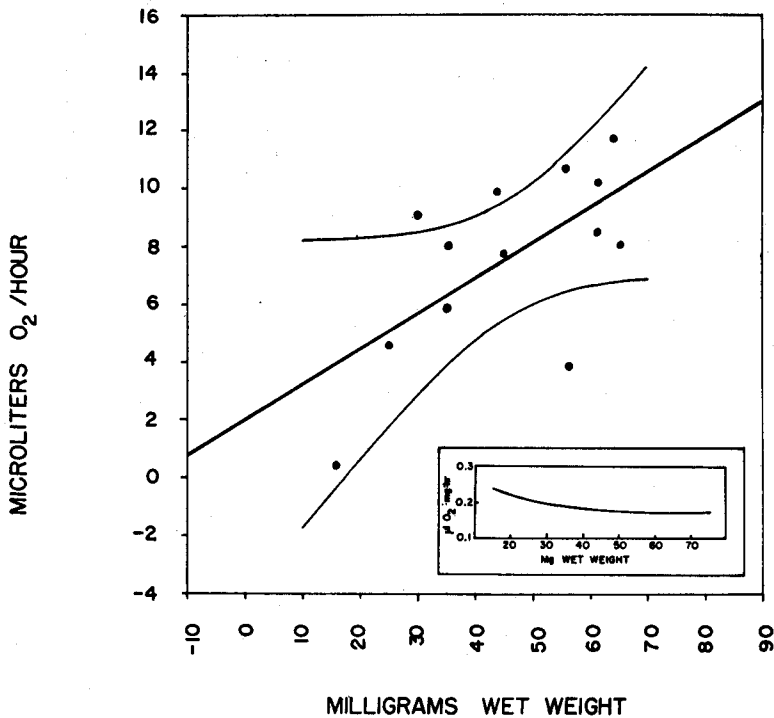


FIGURE 7.—Relationship of oxygen consumption (ordinate) to live body weight of 13 *Hetaerina* sp. naiad specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 1.97760 + 0.12173X$ (SE slope = 0.04478). Insert: Oxygen consumption rate in relation to live body weight.

able to obtain atmospheric oxygen; however, these and other Dipteran larvae with open spiracles may have to depend on dissolved oxygen when the surface tension of the water is reduced by pollution. For example, mosquito (*Culicidae*) larvae are unable to hold to the surface when the tension is reduced from the normal 70 dynes/sq cm to about 27–36 dynes (10). In the experiments reported here, the *Tipula* larvae never were observed coming to the surface of the water for oxygen. Apparently, under conditions in which the water contains sufficient oxygen for their low rate, these larvae were able to obtain the necessary amount by cutaneous respiration.

Respiration through gills is the method usually utilized by the Ephemeroptera in obtaining their oxygen. Some species can survive in highly

oxygenated water without gills, and under these conditions oxygen is obtained by cutaneous respiration (10). Fox *et al.* (26), using the Barcroft method at 10°C, obtained a range of 250–300 cu mm/hr/g dry weight for *Ephemera vulgata* and 274–486 cu mm for *Ephemera danica* when anaesthetized, and ranges of 695–826 cu mm and 720–921 cu mm, respectively, for unanaesthetized specimens. Eriksen (27), using 5 nymphs of *Ephemera simulans* Walker in flasks containing “the most suitable particle size substratum” and the Winkler method at $13.0^\circ \pm 1.4^\circ\text{C}$, obtained a range of 150–290 cu mm/hr/g dry weight in February and a range of 190–290 cu mm for May specimens.

The naiads of the Zygopteran Odonata have three lamellate caudal gills at the extremity of the body. Although these appear to have some respiratory

function, naiads can obtain oxygen by cutaneous respiration. Pennak and McColl (28) found that the mortality rate of gill-less naiads greatly exceeded that of normal specimens. In other experiments by the same authors, the range of minimum oxygen toleration by normal naiads was 0.11–0.37 (average 0.19) mg/l while for the gill-less it was 0.70–1.69 (average 1.14) mg/l. Munchberg (29), while testing 7 different species (6 genera) of Zygoptera, found that the respiratory rate decreased 16.7–83.34 percent after the removal of the caudal lamellae in all but one species, which showed an increase. However, Pattee (30) obtained higher values for naiads of the genus *Calopteryx* without gills than those with gills. Munchberg (29), using a 50-mg *Agrion puella* (L.), obtained a

value of 222.6 cu mm/hr/g with the Warburg method. A 55-mg specimen at the same temperature used 303.5 cu mm/hr/g, and 2 specimens weighing 100 mg used 244.9 cu mm/hr/g. Harnish (31), with the Warburg method at 20°C, obtained averages of 247.08 and 125.08 cu mm/hr/g using 2 *Calopteryx* naiads. The value obtained from the regression line from experiments by Balke (12) using the Warburg method with 47 specimens at 21°–23°C for *Calopteryx virgo* appears to be 8 cu mm/hr for a 60-mg specimen. This may be compared with the 5.92 cu mm/hr obtained from the regression line (Figure 6) for a 60-mg *Calopteryx* specimen. With *Agrion puella* (*pulchellum*), the value on the regression line determined by Balke (12) for a 50-mg specimen appears to

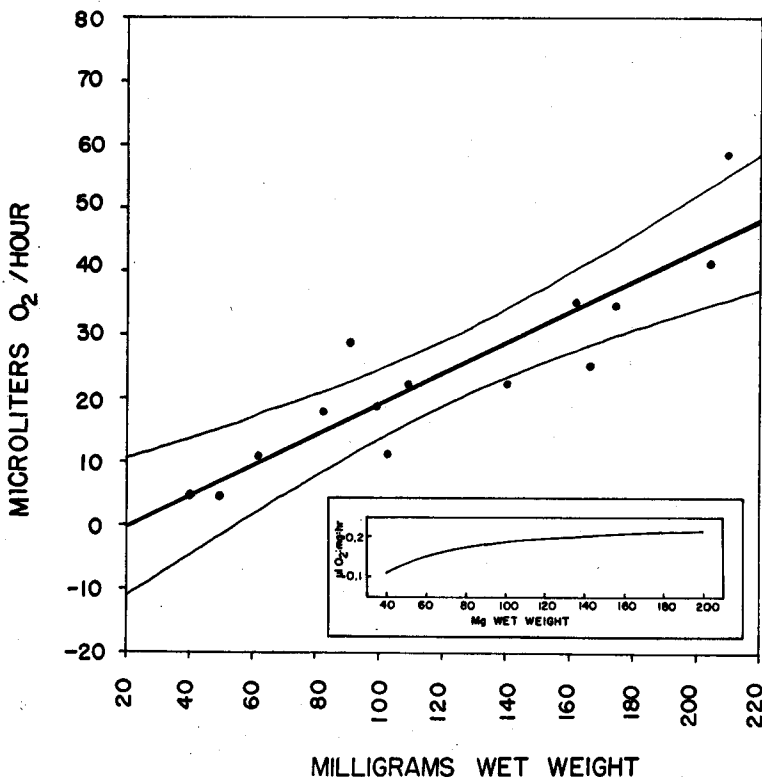


FIGURE 8.—Relationship of oxygen consumption (ordinate) to live body weight of 14 *Paragnetina media* naiad specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = -5.15670 + 0.24028X$ (SE slope = 0.03391). Inset: Oxygen consumption rate in relation to live body weight.

be about 9.2 cu mm/hr (153 cu mm/hr/g), somewhat lower than that obtained by Munchberg (12) but probably representing a truer value since it was based on 35 specimens representing a range of weights. These values were higher than were obtained in this study, but may represent species difference. For example, Munchberg (12) obtained 412 cu mm/hr/g for a 63-mg *Lestes sponsa* (Hansem.) at 19°C and 285 cu mm/hr/g for a 65-mg *Lestes virens* (Charp.), at 20°C. At 20°C, Harnish (32) obtained values of 263.2, 267.8, 87.2, and 112.46 cu mm/hr/g live weight for *Agrion* sp.

Both of the Plecoptera species tested possess thoracic gills by which they obtain oxygen from the water. Movement of the gills and undulatory body movements aid these insects in maintaining a flow of water past their gills. The species tested normally exist in

nature clinging to the underside of large rocks or rubble in streams. Although the upper strata of the stream may possess a rapid flow, Knight and Gaufin (33) believe that the velocity of the current in a stream rapidly decreases as it approaches the bottom and underside of rocks and rubble. They used fluorescein dye to test this hypothesis and found that this dye moved very slowly or not at all for periods in these habitats. The value for a 40-mg *Perla abdominalis* appears to be approximately 10 cu mm in Balke's work (12) using 41 specimens. This is considerably higher than the 4.45 cu mm obtained from this study's regression line for a 40-mg specimen of *P. media*. Pattee (34), with the Winkler method at 20.5°C, obtained a consumption rate of 1,714 cu mm/hr/g dry weight for an 80-mg *Perla bipunctata* and 1,460 cu mm for a 113-

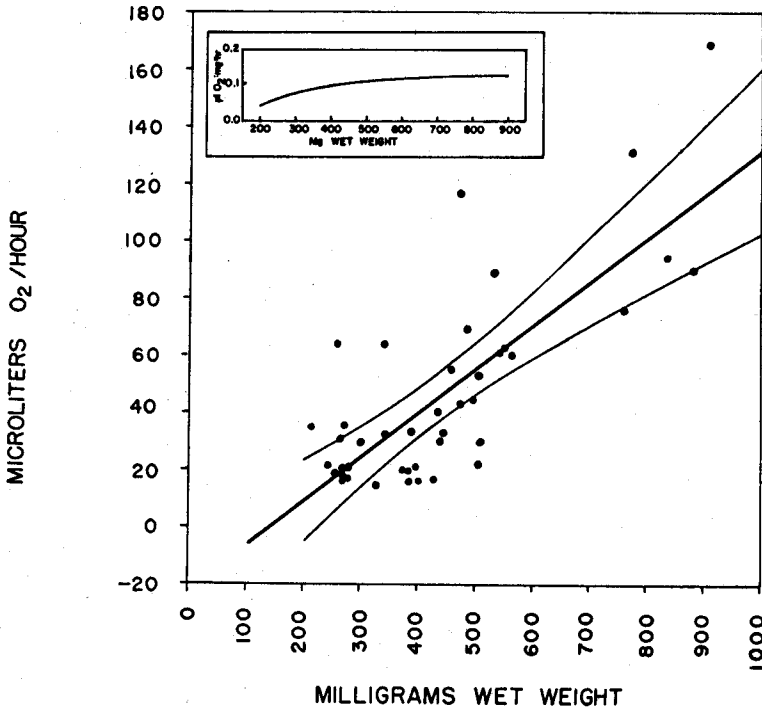


FIGURE 9.—Relationship of oxygen consumption (ordinate) to live body weight of 44 *Pteronarcys pictetii* naiad specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = -21.20870 + 0.15274X$ (SE slope = 0.01931). Inset: Oxygen consumption rate in relation to live body weight.

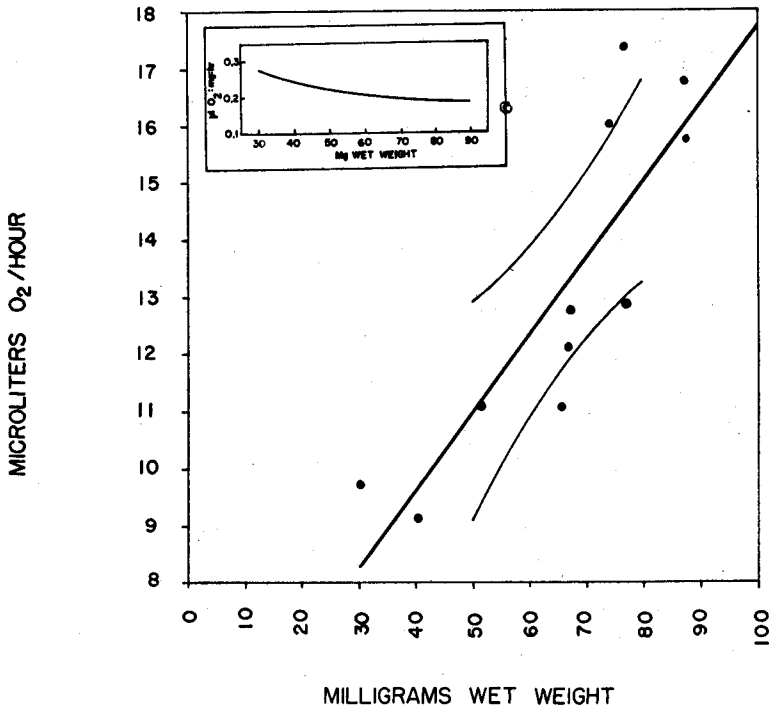


FIGURE 10.—Relationship of oxygen consumption (ordinate) to live body weight of 11 *Macronemum zebratum* larval specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 4.22615 + 0.13508X$ (SE slope = 0.02714). Insert: Oxygen consumption rate in relation to live body weight.

mg specimen. Liebmann (6) lists this species as occurring in the oligosaprobe (clean-water) zone. For a 3-mg specimen of *Isoperla grammatica*, Pattee (34) obtained a rate of 4,220 cu mm/hr/g dry weight. Knight and Gaufrin (33), with the Winkler method at 10°C, obtained a standard oxygen-consumption rate of about 300 cu mm/hr/g dry weight for *Pteronarcys californica* when the oxygen content of the water was 6 cu cm/l and about 600 cu mm/hr/g dry weight when the oxygen content was 11 cu cm/l. The mean oxygen consumption rate we obtained for *P. pictetii* at 20°C was 99.6 cu mm/hr/g live weight (Table II).

Both of the Trichoptera species tested possess respiratory gills, but evidently the larvae use cutaneous respiration for the most part. Morgan and O'Neil (35), using the Winkler method, found that amputation of the

gills on *M. zebratum* reduced oxygen consumption slightly and sometimes not at all. They concluded that the gills are only accessory organs and that the oxygen intake occurs through the body integument. Normal larvae eliminated carbon dioxide more rapidly than larvae without gills. Both of the species we tested are case-building forms, and undulatory movements of the abdomen create a current of water through the case. The average rate obtained (Table II) for *P. guttifer* was somewhat lower than the average (147.4 cu mm/hr/g wet weight) Collardeau (36) obtained with *Limnophilus rhombicus* with the Winkler method at 20°C. This may be due to species difference, method variation, or the fact that Collardeau used three larvae per test. The averages obtained by him were very similar for the three genera tested. Hiestand

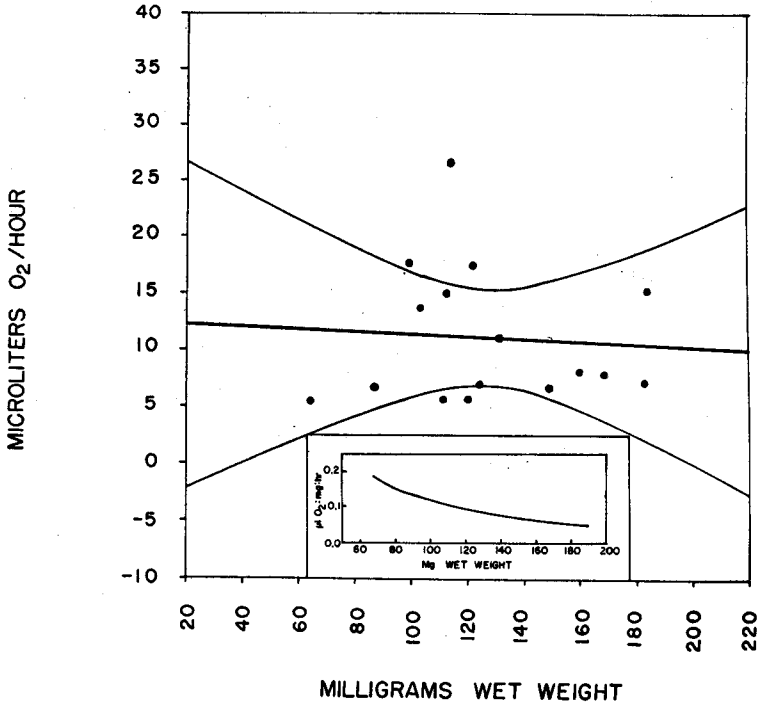


FIGURE 11.—Relationship of oxygen consumption (ordinate) to live body weight of 16 *Pycnopsyche guttifer* larval specimens at 20°C with the Warburg method. Regression line (with 95-percent confidence limits): $Y = 12.55815 - 0.01146X$ (SE slope = 0.04745). Insert: Oxygen consumption rate in relation to live body weight.

(37) also studied the respiration rate of *L. rhombicus* by the Winkler method, but did not weigh the larvae, so his results cannot be compared with those of others. Norris *et al.* (22), using 3 genera of Trichoptera with the Warburg method at 21°C, obtained the highest rate with

the smallest species, $1,030 \pm 50$ cu mm/hr/g wet weight. The rates they obtained were considerably higher than those obtained for Trichoptera in this study, but may have been due in part to their using multiple specimens and removing the larvae from their cases. Ambuhl (38) using a circulating-water

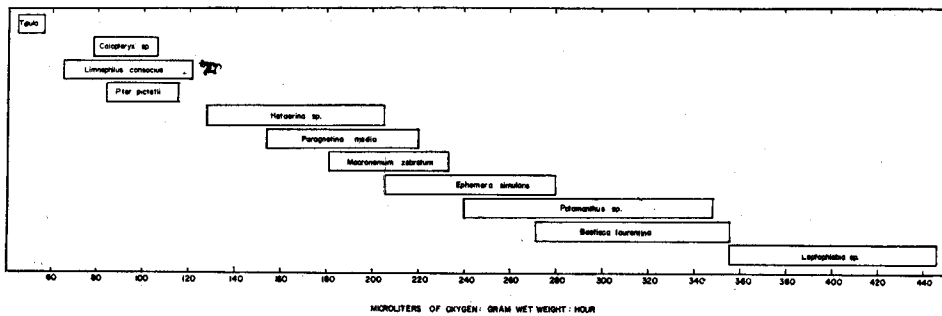


FIGURE 12.—A comparison of the mean (± 2 S.E.) microliters of oxygen used by the test insects per gram wet weight per hour.

* SHOULD BE *P. GUTTIFER*

respirometer, obtained lower rates for Trichoptera than Ephemeroptera at velocities of 0.3 and 0.7 cm/sec and oxygen saturation at 8 mg/l, but the reverse at velocities of 1.65 and 2.8. He concluded, as did Knight and Gaufin (33), that water current is a very important ecological factor in the life of aquatic insects, especially those which do not possess structures enabling them to maintain a current of water over their bodies.

Summary

The objective of this study has been the determination of oxygen-consumption rates of common benthic insects in order to obtain information which may aid in their classification as members of an index-organism community. Individual insects of various weights were used with the Warburg method, and the regression of the oxygen used per hour against the live body weight has been calculated. The insects reported consist of: Diptera (*Tipula* sp.), Ephemeroptera (*Baetisca laurentina* McD., *Ephemerella simulans* Walker, *Leptophlebia* sp., and *Potamanthus* sp.), Odonata (*Calopteryx* sp. and *Hetaerina* sp.), Plecoptera (*Paragnetina media* Walker and *Pteronarcys pictetii* [Hagen], and Trichoptera (*Pycnopsyche guttifer* [Walker] and *Macronemum zebratum* [Hagen]).

As has been pointed out by workers of human basal metabolism rates, it appears that there is not a single lowest value for a particular species, but a variable quantity that can be standardized but not absolutely fixed. Smaller specimens usually had higher rates than larger specimens of the same species, and in general, the rates appear to be related to their usual index-organism classification. It thus appears that studies of the oxygen consumption of individual species can assist in the refinement of the index-organism classification of a given species.

Acknowledgment

Acknowledgment is hereby given for the financial support from the Research Grants Division (WP-320) of the National Institutes of Health of the U. S. Department of Health, Education, and Welfare.

Reference

1. Hynes, H. B. N., "The Biology of Polluted Waters." Liverpool Univ. Press, Liverpool, England (1960).
2. Kolkwitz, R., and Marsson, M., "Ecology of the Saprobic Plants." *Ber Detsch. Bot. Gesell* (Germany), **26**, a, 505 (1908).
3. Kolkwitz, R., and Marsson, M., "Ecology of the Saprobic Animals." *Intl. Rev. ges. Hydrog. u. Hydrog.*, **2**, 126 (1909).
4. Kolkwitz, R., "Ecology of the Saprobic Organisms, Publication of the Association for Water, Soil- and Air Hygiene." No. 4. Piscator-Verlag, Stuttgart, Germany (1950).
5. Fair, G. M., and Whipple, M. C., "Revision of The Microscopy of Drinking Water by G. C. Whipple." 4th Ed., John Wiley & Sons, Inc., New York, N. Y. (1948).
6. Liebmann, H., "Handbook of Freshwater and Wastewater Biology." Vol. 1, Oldenbourg, Munich, Germany (1951).
7. Gameson, A. L. H., and Griffith, S. D., "Six Months' Oxygen Records for a Polluted Stream." *Water and Waste Trt. Jour.* (Brit.), **7**, 198 (1959).
8. Needham, P. R., and Usinger, R. L., "Variability in the Macrofauna of a Single Riffle in Prosser Creek, California, as Indicated by the Surber Sampler." *Hilgardia*, **24**, 383 (1956).
9. Macan, T. T., "Freshwater Ecology." John Wiley & Sons, Inc., New York, N. Y. (1963).
10. Wigglesworth, V. B., "The Principles of Insect Physiology." E. P. Dutton & Co., Inc., New York, N. Y. (1950).
11. Edwards, G. A., "Respiratory Mechanisms" and "Respiratory Metabolism." In "Insect Physiology." John Wiley & Sons, Inc., New York, N. Y. (1953).
12. Balke, E., "The O₂-Consumption and the Inner Surface of the Tracheae With Relation to Body Size in Insect Larvae Breathing Through Tracheal Gills." *Zeits. vergl. Physiol.* (Germany), **40**, 415 (1957).

13. Patton, R. L., "Introductory Insect Physiology." W. B. Saunders Co., Philadelphia, Pa. (1963).
14. Keister, M., and Buck, J., "Respiration: Some Exogenous and Endogenous Effects on Rate of Respiration." In "Physiology of Insects, 3." Academic Press, New York, N. Y. (1964).
15. Boothby, W. M., Berkson, J., and Dunn, H. L., "Studies of the Energy of Metabolism of Normal Individuals: a Standard for Basal Metabolism, with a Nomogram for Clinical Application." *Amer. Jour. Physiol.*, **116**, 468 (1936).
16. Robertson, J. D., and Reid, D. D., "Standards for the Basal Metabolism of Normal People in Britain." *Lancet* (Brit.), **1**, 940 (1952).
17. Florentino, R., and De Guzman, P., "The Selection of Data for Basal Metabolic Rate Standards." *Phil. Jour. Sci.*, **93**, 307 (1963).
18. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometric Techniques." 4th Ed., Burgess Publ. Co., Minneapolis, Minn. (1964).
19. Ludwig, H. F., Oswald, W. J., and Gotaas, H. B., "Manometric Technique for Measurement of BOD." Sanitary Engineering Lab., Univ. of California, Berkeley, Calif. (1951).
20. Fox, H. M., Wingfield, C. A., and Simmonds, B. G., "The Oxygen Consumption of Ephemerid Nymphs from Flowing and from Still Waters in Relation to the Concentration of Oxygen in the Water." *Jour. Exp. Biol.* (Brit.), **14**, 210 (1937).
21. Edwards, G. A., "The Influence of Temperature upon the Oxygen Consumption of Several Arthropods." *Jour. Cell. Comp. Physiol.*, **27**, 53 (1946).
22. Norris, W. E., Jr., Arnold, C. R., and Edwards, S. W., "Oxygen Consumption by Caddisfly Larvae." *Texas Jour. Sci.*, **16**, 72 (1964).
23. Sayle, M. H., "Factors Influencing the Rate of Metabolism of *Aeshna umbrosa* Nymphs." *Biol. Bull.*, **54**, 212 (1928).
24. Gaufin, A. R., and Tarzwell, C. M., "Aquatic Invertebrates as Indicators of Stream Pollution." *Pub. Health Rept.*, **67**, 57 (1952).
25. Gaufin, A. R., and Tarzwell, C. M., "Environmental Changes in a Polluted Stream during Winter." *Amer. Midland Naturalist*, **54**, 78 (1955).
26. Fox, H. M., Simmonds, B. G., and Washbourn, R., "Metabolic Rates of Ephemerid Nymphs from Swiftly Flowing and from Still Waters." *Jour. Exp. Biol.* (Brit.), **12**, 179 (1935).
27. Eriksen, C. H., "Evidence of a Spring Rise in Metabolic Rate in the Burrowing Mayfly *Ephemera simulans* Walker." *Hydrobiologia* (Netherlands), **23**, 506 (1964).
28. Pennak, R. W., and McColl, C. M., "An Experimental Study of Oxygen Absorption in Some Damselfly Naiads." *Jour. Cell. Comp. Physiol.*, **23**, 1 (1944).
29. Munchberg, P., "Comparative Investigation About the Respiratory Intensity of the Zygoptern-Larvae, With an Experimental Contribution to the Functional Importance of Their Anal Lamella. (Odonata)." *Beit. z. Entomol.* (Germany), **12**, 243 (1962).
30. Pattee, E., "On the Role of Caudal Lamellae in the *Calopteryx*." *Bull. Biol. France et Belgique* (France), **90**, 106 (1956).
31. Harnisch, O., "Investigation on the Respiratory Physiology of the Larvae of *Calopteryx* (Odonata)." *Zool. Anz.* (E. Germany), **161**, 291 (1958).
32. Harnisch, O., "Investigation of the Anal Gills of the Larvae of *Agrion*." *Biol. Zbl.* (E. Germany), **77**, 301 (1958).
33. Knight, A. W., and Gaufin, A. R., "Relative Importance of Varying Oxygen Concentration, Temperature, and Water Flow on the Mechanical Activity and Survival of the Plecoptera nymph, *Pteronarcys californica* Newport." *Utah Acad. Sci. Arts Letters*, **41**, 14 (1964).
34. Pattee, E., "Influence of a Change in Temperature on Oxygen Consumption and Habits of Some Aquatic Larvae of Insects." *Bull. Biol.* (France), **90**, 369 (1956).
35. Morgan, A. H., and O'Neill, H. D., "The Function of the Tracheal Gills in Larvae of the Caddis Fly, *Macronema zebratum* Hagen." *Physiol. Zool.*, **4**, 361 (1931).
36. Collardeau, C., "Influence of Temperature on the Oxygen Consumption of Some Trichoptera Larvae." *Hydrobiologia* (Netherlands), **18**, 252 (1962).
37. Hiestand, W. A., "The Influence of Varying Tensions of Oxygen upon the Respiratory Metabolism of Certain Aquatic Insects and the Crayfish." *Physiol. Zool.*, **4**, 246 (1931).
38. Ambuhl, H., "The Importance of the Current as an Ecological Factor." *Schweiz. Zeits Hydrol.* (Switzerland), **21**, 133 (1959).