

AN ABSTRACT OF THE THESIS OF

RICHARD ALBERT KIMERLE for the DOCTOR OF PHILOSOPHY
(Name) (Degree)

in ENTOMOLOGY presented on September 23, 1968
(Major) (Date)

Title: PRODUCTION BIOLOGY OF GLYPTOTENDIPES BARBIPES
(STAEGER) (DIPTERA:CHIRONOMIDAE) IN A WASTE
STABILIZATION LAGOON

Abstract approved: Redacted for Privacy
N. H. Anderson

Ecological studies of Glyptotendipes barbipes (Staeger) (Diptera:Chironomidae) were made in 1966 and 1967 in the 7.1 ha primary and 8.7 ha secondary waste stabilization lagoons of the city of Monmouth, Oregon. The purpose of the study was to evaluate the bioenergetic role of the midge population in the process of waste stabilization in lagoons.

Weekly production rates of the multivoltine midge G. barbipes were computed, based on frequent measurements of growth and biomass, during the period of definitive study, May, 1967 to November, 1967. Annual production of G. barbipes was 808 kcal/m^2 in Stratum I (a 3612 m^2 region around the shore containing 90 percent of the biomass) of the secondary lagoon (86903 m^2). Biomass data from both lagoons in 1966 and the primary lagoon in 1967 were utilized for production estimates using two methods: (1) by multiplying the mean yearly growth rate times the biomass and (2) by multiplying the turnover

ratio of 8.49 (calculated as the ratio of production/mean biomass from definitive data in 1967) times the mean annual biomass. The production estimates using the mean growth rate were more reliable than those obtained with the turnover ratio.

Chironomid production in the secondary lagoon was 459 kcal/m^2 in 1966 and 37 kcal/m^2 in 1967. Estimates of production in the primary lagoon were less: 165 kcal/m^2 in 1966 and 18 kcal/m^2 in 1967. The factors most responsible for these vast differences in production between years and lagoons were considered to be: (1) condition of the lagoon with respect to oxygen concentrations during the growing season, (2) percent of the total lagoon bottom inhabitable by midge larvae (related to water depth and oxygen concentrations) and (3) the unstable substrate caused by the accumulation of a sludge blanket as the lagoons aged.

The energy budget of G. barbipes was calculated from estimates of biomass, rates of production, emergence, mortality, respiration and assimilation. The total energy removed by emergence and respiration of G. barbipes was then compared to the fate of energy in other pathways in the lagoon, i. e., import of sewage, primary production, community respiration, storage and export. The energy removed by G. barbipes from the two cell system was 103×10^6 kcal in 1966 and 8.6×10^6 kcal in 1967. However, the dominant bioenergetic components of the lagoon ecosystem were the primary producers and decomposers. In 1966 G. barbipes removed 6.6

percent of the net primary production in the secondary lagoon, and 0.5 percent in 1967. It was therefore concluded that G. barbipes did not make a significant contribution to the degradation of energy in the lagoon ecosystem.

Production Biology of Glyptotendipes barbipes
(Staeger) (Diptera:Chironomidae) In a
Waste Stabilization Lagoon

by

Richard Albert Kimerle

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1969

APPROVED:

Redacted for Privacy

Associate Professor of Entomology
in charge of major

Redacted for Privacy

Chairman of the Department of Entomology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented September 23, 1968

Typed by Gwendolyn Hansen for Richard Albert Kimerle

ACKNOWLEDGEMENTS

I wish to express my appreciation to the following persons who each made a contribution to this thesis and my graduate education.

To Dr. N. H. Anderson, my major professor, for his assistance and guidance in conducting the research and preparation of the manuscript, and also for his contributions which broadened my education in general.

To Dr. P. O. Ritcher, chairman, Department of Entomology, for providing the facilities and atmosphere conducive to good graduate study.

To Dr. W. S. Overton for advice on sampling and analysis of data; to Dr. W. Nagel for advice and review of the manuscript; and to Dr. G. Davis and Dr. C. Warren for their guidance on the topic of production.

To fellow graduate students who participated in many hours of worthwhile and stimulating discussions.

To my wife, JoAnn, a special indebtedness is acknowledged for her patience and encouragement during my years in graduate school.

This research was supported by a grant from the U. S. Department of Interior, Grant No. 5R01 WP923, to Dr. R. L. Goulding and Dr. N. H. Anderson.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	4
Functional Aspects of Waste Stabilization Lagoons	4
Aquatic Insect Fauna of Sewage Lagoons	6
Production Studies	11
MATERIALS AND METHODS	14
Study Site	14
Chemistry and Energetics of Lagoon Waters	16
Solar Radiation	16
Energy in Bottom Sediments	17
Monitoring Dissolved Oxygen	17
Primary Production and Community Respiration	22
Biomass	24
Growth	28
Emergence	30
Respiration	31
Production	34
RESULTS AND DISCUSSION	35
Influent and Effluent Quality	35
Energy in Bottom Sediments	38
Performance of Dissolved Oxygen Monitoring Device	40
Dissolved Oxygen in Sewage Lagoons	42
Primary Production and Community Respiration	51
Biomass 1966 - 1967	57
Laboratory Growth Studies	63
Growth of Natural Population of <u>G. barbipes</u>	67
Emergence	70
Respiration	73
Production of <u>G. barbipes</u>	83
Comparison of <u>G. barbipes</u> Production with Other Studies	87
The Role of <u>G. barbipes</u> in the Lagoon Ecosystem	93

	Page
CONCLUSIONS	108
BIBLIOGRAPHY	112
APPENDIX	120

LIST OF FIGURES

Figure	Page
1. Map of waste stabilization lagoons at Monmouth, Oregon.	15
2. Water sampling apparatus for monitoring dissolved oxygen.	19
3. Sensing chamber of dissolved oxygen monitoring apparatus.	21
4. Respiration chamber used in measuring respiration rates of <u>G. barbipes</u> larvae in the laboratory.	32
5. Diurnal oxygen and temperature curves at surface, mid-depth and mud-water interface of secondary waste stabilization lagoon, Monmouth, Oregon, February 18-20, 1967.	43
6. Diurnal oxygen and temperature curves at surface, mid-depth and mud-water interface of primary waste stabilization lagoon, Monmouth, Oregon, May 19-20, 1967.	45
7. Calculation of primary production and community respiration from dissolved oxygen rate of change curve. Secondary lagoon, Monmouth, Oregon. April 1-7, 1967.	53
8. Monthly mean biomass of <u>G. barbipes</u> in primary lagoon, Monmouth, Oregon, 1966 and 1967.	58
9. Monthly mean biomass of <u>G. barbipes</u> in secondary lagoon, Monmouth, Oregon, 1966 and 1967.	59
10. Mean weight of <u>G. barbipes</u> larva grown under laboratory conditions, plotted on arithmetic scale.	64
11. Mean weight of <u>G. barbipes</u> larva grown under laboratory conditions, plotted on logarithmic scale.	64
12. Relative growth rates of <u>G. barbipes</u> cohort grown under laboratory conditions.	66

Figure	Page
13. Oxygen consumption rates of <u>G. barbipes</u> at 10° C with varying densities and environmental DO concentrations.	74
14. Oxygen consumption rates of <u>G. barbipes</u> at 15° C with varying densities and environmental DO concentrations.	75
15. Oxygen consumption rates of <u>G. barbipes</u> at 20° C with varying densities and environmental DO concentrations	76
16. Oxygen consumption rates of <u>G. barbipes</u> at 25° C with varying densities and environmental DO concentrations.	77
17. Monthly biomass compared with biomass of those individuals that died each month. Secondary lagoon, Monmouth, Oregon.	82
18. Biomass, production, accumulated production and accumulated emergence of <u>G. barbipes</u> in kcal/m ² in Stratum I of the secondary waste stabilization lagoon. Monmouth, Oregon, May 1967 to November, 1967.	85
19. Energy transfer in the secondary waste stabilization lagoon in kcal/m ² . Monmouth, Oregon, May, 1967 to November, 1967.	100
20. Energy transfer in the secondary waste stabilization lagoon in kcal/m ² . Monmouth, Oregon, 1966.	103
21. Energy transfer in the secondary waste stabilization lagoon in kcal/m ² . Monmouth, Oregon, 1967.	105
22. Energy transfer in the primary waste stabilization lagoon in kcal/m ² . Monmouth, Oregon, 1966.	106

LIST OF TABLES

Table	Page
1. BOD, flow rates and energy content of raw sewage, primary lagoon effluent and secondary effluent, Monmouth, Oregon. May, 1967 to November, 1967.	37
2. Dry weight and energy content of primary lagoon bottom sediments.	39
3. Primary production and community respiration in the secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.	55
4. Emergence of <u>G. barbipes</u> from the primary lagoon, Monmouth, Oregon, May, 1967 to August, 1967.	71
5. Emergence of <u>G. barbipes</u> from the secondary lagoon, Monmouth, Oregon, May, 1967 to October, 1967.	72
6. Monthly biomass respiration estimates of <u>G. barbipes</u> and respiration of those individuals (as biomass) that died each month. Secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.	80
7. Growth, biomass and production of <u>G. barbipes</u> in Stratum I of the secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.	84
8. Comparative production estimates of aquatic insects, snail and trout.	88
9. Comparison of production of <u>G. barbipes</u> in primary and secondary lagoons in 1966 and 1967.	90
10. Energy budget of <u>G. barbipes</u> in kcal/m ² in Stratum I and for all strata of the secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.	95
11. Energy budget of <u>G. barbipes</u> compared to other energy pathways in the primary and secondary waste stabilization lagoons in 1966 and 1967.	97
12. Energy removed by emergence and respiration of <u>G. barbipes</u> from the primary and secondary lagoons in 1966 and 1967.	98

PRODUCTION BIOLOGY OF GLYPTOTENDIPES BARBIPES
(STAEGER) (DIPTERA:CHIRONOMIDAE) IN A WASTE
STABILIZATION LAGOON

INTRODUCTION

Most natural aquatic ecosystems contain a complex association of many insect populations. Individuals within each population possess certain inherited attributes enabling them to perform necessary life functions and persist at some density. Population density, in a given habitat and time interval, results from the effective environment, past and present, interacting with the inherited properties of each individual in either a positive or negative manner. The ultimate objective of ecological investigations should be to explain how interactions between individuals and their environment affect the distribution, abundance, evolution and functional status of each population in the ecosystem.

Advances in ecology have been delayed, in part, because the complexity of natural ecosystems often limits meaningful interpretation of data. However, ecologists have gained some understanding of complex ecological problems by (1) studying selected natural populations and key environmental factors, (2) conducting controlled laboratory experiments to reduce the number of variables and (3) most recently, by methods of modeling and systems analysis.

Bioenergetic studies are a valuable method of reducing the parameters of the ecosystem to a common denominator, the calorie

energy unit. This approach enables analysis of the functional relationships between components (Odum, 1957; Teal, 1957; Odum, 1959; Davis and Warren, 1965; Phillipson, 1966). However, few natural systems have been studied bioenergetically since the introduction of this method more than 25 years ago by Juday (1940) and Lindeman (1941). Davis and Warren (1965) discussed a paradoxical problem of studying energy transfer in ecosystems. They felt that the large number of species usually found in ecosystems, make it difficult or impossible to adequately study each individual species. Yet, the purpose of ecosystem analysis is to formulate useful generalizations about complex associations of species. Furthermore, results from simple or artificial systems may not be generally applicable to other more complex ecosystems.

Waste stabilization lagoons are an ideal natural ecosystem for bioenergetic studies because of the lack of species diversity, and dominance exhibited by a few populations. The midge Glyptotendipes barbipes (Staeger) (Diptera:Chironomidae) is frequently the only aquatic insect to occur in appreciable numbers in some lagoons.

This thesis comprises a study of the production biology of G. barbipes in waste stabilization lagoons. It was believed that a definitive study of a single species, in the simplified lagoon ecosystem, would enable interpretation of results without the interferences of more complex environments. The specific objectives of this study

were (1) to estimate bioenergetically the biomass, growth rate, emergence, mortality, metabolism and assimilation rates of G. barbipes, (2) to calculate production of G. barbipes and (3) to determine the functional role of G. barbipes with respect to other energy pathways, i. e. , import of sewage and solar radiation, primary production, community metabolism, storage and export.

LITERATURE REVIEW

Functional Aspects of Waste Stabilization Lagoons

A waste stabilization lagoon is defined by Porges and MacKenthum (1963) as "a basin, natural or artificial, designed or used to treat organic waste by natural biological, biochemical, and physical processes, commonly referred to as self purification." Svore (1960) designated ponds which receive raw sewage as waste stabilization lagoons and ponds which receive, at least primarily settled sewage, as oxidation ponds.

The basic process of waste stabilization in lagoons results from a symbiotic relationship between bacteria and algae. Aerobic bacteria use the energy and nutrients of raw sewage for their life processes. Carbon dioxide and nutrients are released as bacteria grow and die. Algae utilize these materials to build new tissue through photosynthesis and release oxygen enabling aerobic bacteria to respire.

Satisfactory stabilization of waste can be achieved only by maintaining aerobic conditions (Bartsch and Allum, 1957). The primary source of oxygen is from photosynthetic activity of phytoplankton (Porges and MacKenthum, 1963; Horning, et al., 1964). Burgess and Northcraft (1965) indicated that atmospheric reaeration was also important at times when the oxygen concentration was below saturation. Failure of algae to supply the oxygen necessary for aerobic

decomposition can result from reduced solar radiation, reduced availability of nutrients, die-off of phytoplankton blooms, or harvesting of phytoplankton by zooplankton.

Sewage lagoons are of widespread use because of their low cost of construction and operation, and effectiveness of treatment. Numerous investigators have reported reduction of biochemical oxygen demand (BOD) of 80 percent and of coliform bacteria as high as 95 percent (Neel and Hopkins, 1956; Towne et al., 1957; Cooley and Jennings, 1960; Neel et al., 1961; Horning et al., 1964; Burgess and Northcraft, 1965). Sewage lagoons can also effectively remove nutrients. Neel et al. (1961), reported reduction of total nitrogen from 80 to 96 percent and total phosphorus from 33 to 97 percent. Towne and Horning (1960), Brinck (1960). Williamson (1960) and Atkins (1960) stated the degree of treatment achieved with properly functioning lagoons was at least equal to, and some times better than, that accomplished by conventional treatment facilities. Atkins (1960) also pointed out that lagoons can be designed to hold all water at times of low stream flow, thereby creating no pollution problems. Clare and Weiner (1960) compared the cost of building and operating primary and secondary treatment plants, waste stabilization lagoons and oxidation lagoons. Their general conclusion was that lagoons cost less and were economically more suitable for small communities.

The effect of lagoon effluents on receiving streams has not been

thoroughly investigated. After wastes are stabilized the effluent may, still contain large quantities of autochthonous organic matter in the form of algal cells (Ludwig et al., 1951). Living algae exert only a slight BOD, but when the cells die they exert a full BOD (Silva and Papenfuss, 1953). Burgess and Northcraft (1965) found that the BOD of unfiltered, heat-killed effluents increased from zero to 150 percent. In some tests the BOD of the effluent exceeded that of the influent.

Aquatic Insect Fauna of Sewage Lagoons

Organic enrichment of waters usually causes a reduction in species diversity and increase in the abundance of tolerant species (Bartsch, 1948; Gaufin, 1958; Hynes, 1963). Numerous investigators have reported nuisance problems near enriched bodies of water resulting from mass emergence of chironomid midges (Johnson and Munger, 1930; Felton, 1940; Bonnell and Mote, 1941; Hilsenhoff, 1962).

Waste stabilization lagoons are man-made environments designed specifically for organic enrichment. They have also created new insect problems by providing breeding sites for some species of mosquitoes and midges. Species lists of chironomids associated with waste stabilization lagoons and similar bodies of water were compiled by Grodhaus (1963), Fagan and Enns (1966), Kimerle and Enns (1968) and Sturgess (1968). Beadle and Rowe (1960) surveyed lagoons in midwestern and southwestern states for mosquito production.

Predominant species were Culex tarsalis Coquillett and the Culex pipiens complex, the primary vectors of encephalitic diseases. The abundance of mosquitoes was directly proportional to the amount of emergent vegetation.

Mosquito abatement procedures have been primarily concerned with reducing the favorability of lagoons as breeding sites (Beadle and Rowe, 1960; Curtis, 1963). Bay (1967) suggested that many naturalistic methods exist for controlling mosquitoes.

There are numerous reports of mass emergences of chironomid midges from sewage lagoons. Bay (1964) reviewed the status of nuisance chironomid problems in California. Grodhaus (1963) reported G. barbipes, Tendipes stigmaterus Say, Tendipes fulvipilus Rempel, Tendipes attenuatus Walker and Pelopia sp. as important nuisance species associated with oxidation ponds. In central Missouri, G. barbipes was observed to emerge in tremendous numbers (Kimerle, 1965; Fagan and Enns, 1966). Residents near four oxidation ponds (total area of 1266 acres) at Auckland, New Zealand were troubled with mass emergence of Chironomus zealandicus (Spiller, 1965).

Various methods have been investigated for control of chironomid midges. Chemical, biological and cultural methods were studied in small experimental ponds by Anderson et al. (1964). Granular formulations of some insecticides were effective in reducing the numbers

of larvae. Fagan and Enns (1966) suggested that 95 percent of the larvae could be controlled by applying a larvicide in a ten foot margin near shore. Spiller (1965) developed a strategic larviciding program by surveying the pest species and coordinating time and place of treatment with areas of maximum density. Effective control was achieved by aerial application of malathion. Bay et al. (1965), reported that adulticides used after an emergence had little effect on reducing larval populations of the next generation. Successful abatement was obtained by draining the ponds responsible for 90 percent of the adults. Carp, Cyprinus carpio Linnaeus and goldfish, Carrasius auratus stocked at 150 to 500 pounds per acre effectively reduced larval chironomids. The western mosquito fish, Gambusia affinis Baird and Girard, was ineffective because it is not a benthic feeder (Bay and Anderson, 1965, 1966).

The first detailed study of aquatic insects occurring in oxidation ponds was conducted at Concord, California (Usinger and Kellen, 1955). Fifty-two species were found in six cells, connected in series. The first two cells were septic and only contained species that used atmospheric oxygen, such as syrphids and ephydriids. Cells three through six contained a greater species diversity with G. barbipes being one of the dominant midges. This same midge has been reported to occur in sewage lagoons in Missouri (Fagan and Enns, 1966) and in Oregon (An ally in the war on waste, 1967).

The biology and morphology of G. barbipes has been studied by Fagan and Enns (1966) and Sturgess (1968). Each female lays an egg mass of 1500 to 2000 eggs. After deposition, the mass absorbs water and swells to form a gelatinous cylinder. Hatching occurs in about three days, depending on temperature. First-instar larvae are free-swimming. The larvae then settle to the bottom and construct tubes from salivary secretions. They feed indiscriminately on phytoplankton and suspended organic matter that adheres to the lining of their tube when water is pumped through the tube. There are four larval instars. Pupation occurs in the tube. The mature pupa swims to the surface and the adult escapes the pupal exuvae in 10 to 30 seconds. Fagan and Enns (1966) were not able to determine the exact number of generations per year because of the difficulty of identifying a single age class.

Fagan and Enns (1966) and Sturgess (1968) investigated the microdistribution of chironomid larvae on lagoon bottoms. G. barbipes was the dominant species in both studies. They concluded that thermal stratification and associated low concentrations of oxygen at lower depths could restrict the distribution of larvae to the upper zones of the sloped dikes. Sturgess (1968) also found larvae in the central region of a shallow lagoon. Kimerle and Enns (1968) reported that in lagoons ranging from one to 35 acres, G. barbipes larvae were largely restricted to a narrow band of five to eight feet on the slope

of dikes.

The density of G. barbipes has been reported to range from zero to 25,000 larvae/ft² (Kimerle and Enns, 1968). The same authors correlated the density of G. barbipes, Chironomus plumosus (Linnaeus), and Pelopia punctipennis Meigen with age and BOD loading rates of various lagoons in central Missouri. In lightly-loaded, newer lagoons the order of dominance was G. barbipes > C. plumosus > P. punctipennis, whereas in older, heavily-loaded lagoons the order was C. plumosus > P. punctipennis > G. barbipes.

The ability of some species of chironomids (bloodworms) to survive in waters with low DO has been attributed to haemoglobin pigment. Walshe (1950), concluded when DO is low the haemoglobin in C. plumosus transports oxygen, enabling larvae to continue filter-feeding and to recover at a faster rate from periods of oxygen lack. Larvae treated with carbon monoxide, to render haemoglobin non-functional, died sooner than did untreated larvae (Walshe, 1947, 1950). Sturgess and Goulding (1968) studied the tolerance of three species of chironomids to anaerobic conditions. G. barbipes was most tolerant, surviving for 19.5 days. Fagan and Enns (1966) reported that G. barbipes may be able to survive a certain amount of pollution (apparently meaning low concentrations of DO associated with higher BOD loading) but the species did not successfully overwinter in lagoons loaded in excess of 30 pounds of BOD per acre per day.

Few ecological studies have been conducted to determine the impact, role or functional status of chironomids in waste stabilization lagoons. Usinger and Kellen (1955) reported that in laboratory tests, midge larvae contributed to the degradation of energy by consuming large quantities of algae. This was beneficial in reducing the BOD of effluents. In addition, the larvae were responsible for extending the aerobic zone of decomposition into the substrate, as a result of their normal respiratory activity of pumping water through their tubes. Tubb and Dorris (1965) concluded that large amounts of energy were removed from oil refinery lagoons in the form of emerged adult midges. They also recognized that much energy would be expended in the growth of larvae prior to emergence. In general, many authors have supported the thesis that midges are beneficial to the stabilization of waste in lagoons and in improving the quality of the effluent (Kellen, 1953; Usinger and Kellen, 1955; Tubb and Doris, 1965; Kimerle, 1965; Fagan and Enns, 1966; Kimerle and Enns, 1968; Sturgess, 1968).

Production Studies

Production estimates of each species are a necessary prerequisite for complete bioenergetic studies. According to Ricker (1958) production is defined as "the total elaboration of new body substance in a stock in a unit time, irrespective of whether or not it

survives to the end of that time." Warren et al. (1964) and Hunt (1966) have incorporated the concept of negative production when there is a decrease in average weight of an individual over a period of time. Production is calculated arithmetically as the product of the instantaneous growth rate and mean biomass in some time interval (Clark, Edmondson and Ricker, 1946). Allen (1951) presented a graphical method of computing production which gives results nearly identical to the arithmetical method and offers additional benefits. Neess and Dugdale (1959) calculated production of chironomids by a method theoretically similar to Allen's method.

Numerous reliable estimates of production have been made for fish populations (Allen, 1951; Gerking, 1962; Warren et al., 1964; Hunt, 1966). Production estimates, based on actual measurement of growth and biomass, are also available for some invertebrates (Waters, 1966; Ernest, 1966; Kajak and Rybak, 1966; Azam, 1969). Many other estimates of "production" have been made, but they do not conform to the strict definition of production as stated above (Dugdale, 1955; Hayne and Ball, 1956; Anderson and Hooper, 1956; Hynes, 1961; Yount, 1966).

Waters (1966) discussed the use of the "turnover ratio" in calculating production. He defines it as the ratio of annual production rate to mean annual population density. It was also suggested that the turnover ratio of a species would vary within relatively narrow

limits if ecological conditions were similar. Lindeman (1941) calculated production by multiplying the mean annual population by the number of generations. However, as pointed out by Allen (1951) this approach does not account for production which took place and died, before the measurement was taken and for any potential production after the population estimate.

Waters (1966) felt that turnover ratios may be useful in energetic studies if they do remain constant for a species. The advantage would be that once determined for each species, researchers working under similar ecological conditions would not have to determine the growth rates. Production would equal turnover ratio times mean annual population.

MATERIALS AND METHODS

Study Site

This investigation was conducted at Monmouth, Oregon, where the waste stabilization lagoons for the city of approximately 4,000 persons, consist of a 17.5 acre (7.1 ha) primary cell and 21.4 acre (8.7 ha) secondary cell (Figure 1). The lagoons have been in operation since 1964. This site was chosen because of several factors; (1) the cooperation extended by city officials, (2) the lagoons were "typical" for this region of the state and met all the requirements of the Oregon State Board of Health (1965), with respect to construction, operation, maintenance and degree of treatment achieved, (3) the influent consisted only of municipal waste with no shock-loads from industry or agriculture and (4) the lagoons were known to contain large numbers of G. barbipes.

Raw sewage flows by gravity from the city sewerage lines to the pump house where the rate was monitored. At this point sewage is lifted to the inlet in the center of the primary cell. Water then flows by gravity to the secondary cell, and back to the pump house for chlorination. The effluent is then discharged into Ash Creek.

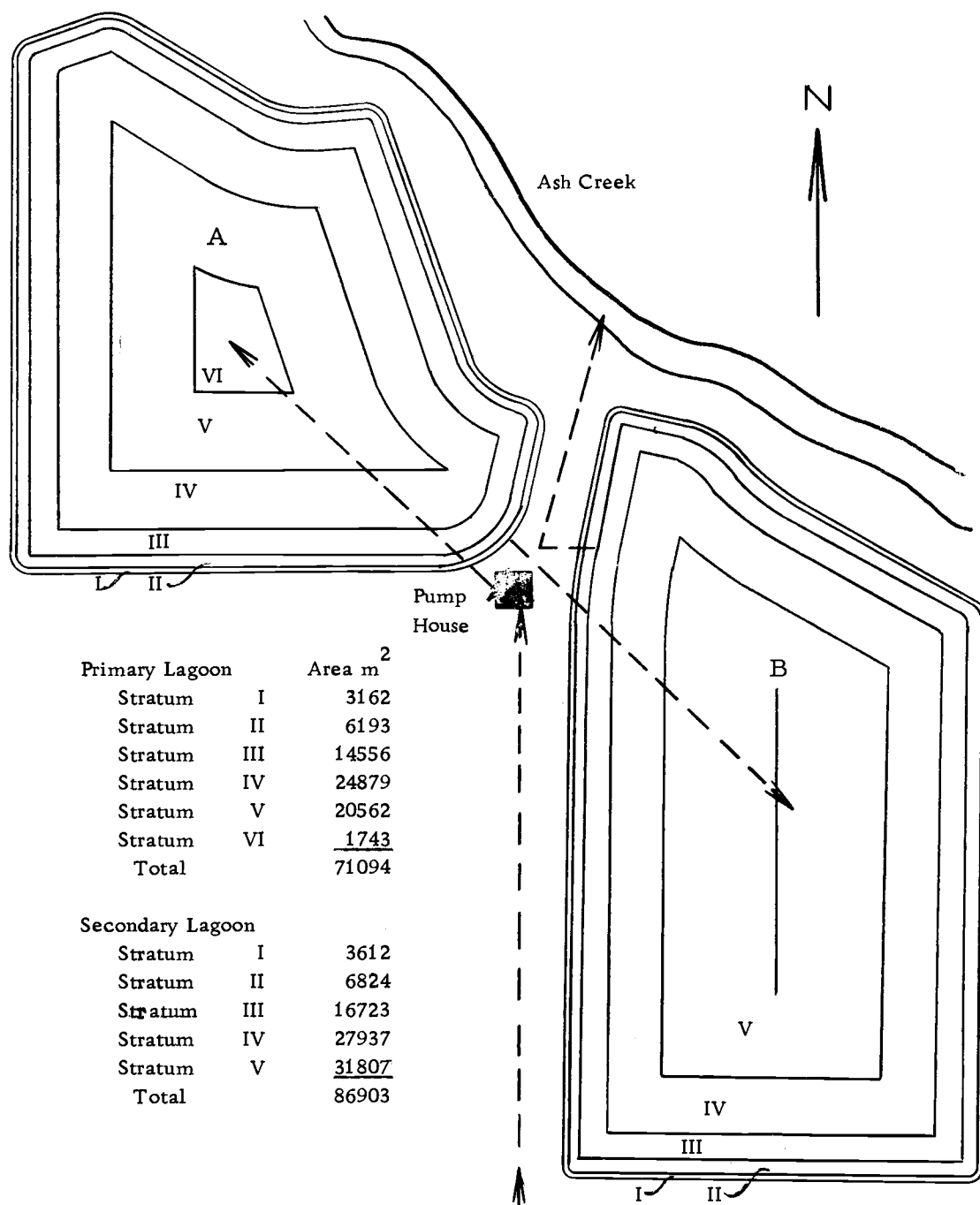


Figure 1. Map of waste stabilization lagoons at Monmouth, Oregon. Raw sewage to pump house, primary lagoon, secondary lagoon and discharges into Ash Creek. Strata in lagoons designate sampling areas.

Chemistry and Energetics of Lagoon Waters

Water samples and flow rates of the raw sewage (MI), primary effluent (MII) and secondary cell effluent (MIII) were taken monthly from November, 1966 to October, 1967. Each sample was analyzed for biochemical oxygen demand (BOD), most probable number of coliform bacteria (MPN), phosphate, chlorides and conductivity by the Oregon State Sanitary Authority. Total kjeldahl nitrogen (American Public Health Association, 1965) and nitrate were determined by the Agricultural Chemistry Department of Oregon State University. Eight quart samples of MI, MII and MIII were also taken to determine the energy content of water flowing through the lagoon system. The samples were frozen in cake pans. Dry weight was obtained after lyophilizing the samples, two at a time, in a freeze dry desiccator. Grams of residue per gallon in the monthly samples were converted to calories per gallon. All caloric determinations were made in a Bomb calorimeter by the Department of Animal Science, Oregon State University.

Solar Radiation

Estimates of incoming solar energy were not made at the lagoon site. It was necessary to use the data of a United States Weather Bureau Station located about 15 miles south of the lagoon.

Energy in Bottom Sediments

Three core samples of bottom sediments were taken, at each of ten stations, in the primary cell in January, 1967 and March, 1968. Samples were taken using a 1 ft. (0.3 m) by 1 1/2 in (4.4 cm) ID plastic tube attached to a 5 ft. (1.5 m) aluminum tube. The corer was driven into the substrate and then carefully lifted. The clay bottom of the lagoon usually sealed the core sample inside the tube.

Monitoring Dissolved Oxygen

Frequent dissolved oxygen (DO) readings, throughout the water column from the mud-water interface to the surface, were required to explain larval midge mortality and to estimate primary production and community respiration. Iodimetric methods of determining DO (American Public Health Association, 1965) were not considered feasible because of the frequency and duration of the required measurements. The apparatus described below was constructed to provide hourly DO and temperature data at three depths in the lagoons. It consisted of a dissolved oxygen analyzer, a continuous recorder and a water sampling device capable of sampling microstratifications.

The instruments employed were a galvanic cell oxygen

analyzer,¹ a thermistor² and dual channel recorder² located in a pump house between the primary and secondary lagoon cells. These instruments were connected by 50 m leads to their respective probes in the water sampling device near the shore of each lagoon. An extension cord supplied electricity to the sampler to operate a timer and pumps. The entire water sampling unit was portable and was alternated periodically between the primary and secondary cells.

The major components of the water sampling equipment (Figure 2) were: three water intake tubes suspended from a 2 m boom to different levels (Figure 2A), a submerged waterproof plastic box containing three oscillating pumps (Figure 2B), and a wooden box, supported just above the water, containing a microswitch timer and sensing chamber (Figure 2C). This apparatus operated once an hour, enabling separate measurements of DO and temperature to be taken at each depth. A 2-minute recording cycle for each depth was initiated when the microswitch activated a pump. A 5-minute interval separated each 2-minute pumping time between depths. About one liter of water was collected through one of the three samplers and lifted to the sensing chamber in each cycle. Water standing in the

¹ Catalog No. 68850, Precision Scientific Co. 3737 West Courtland Street, Chicago 47, Illinois.

² Tele-thermometer, Model 44 and Dual Channel recorder Model 81, Yellow Springs Instrument Co., Yellow Springs, Ohio.

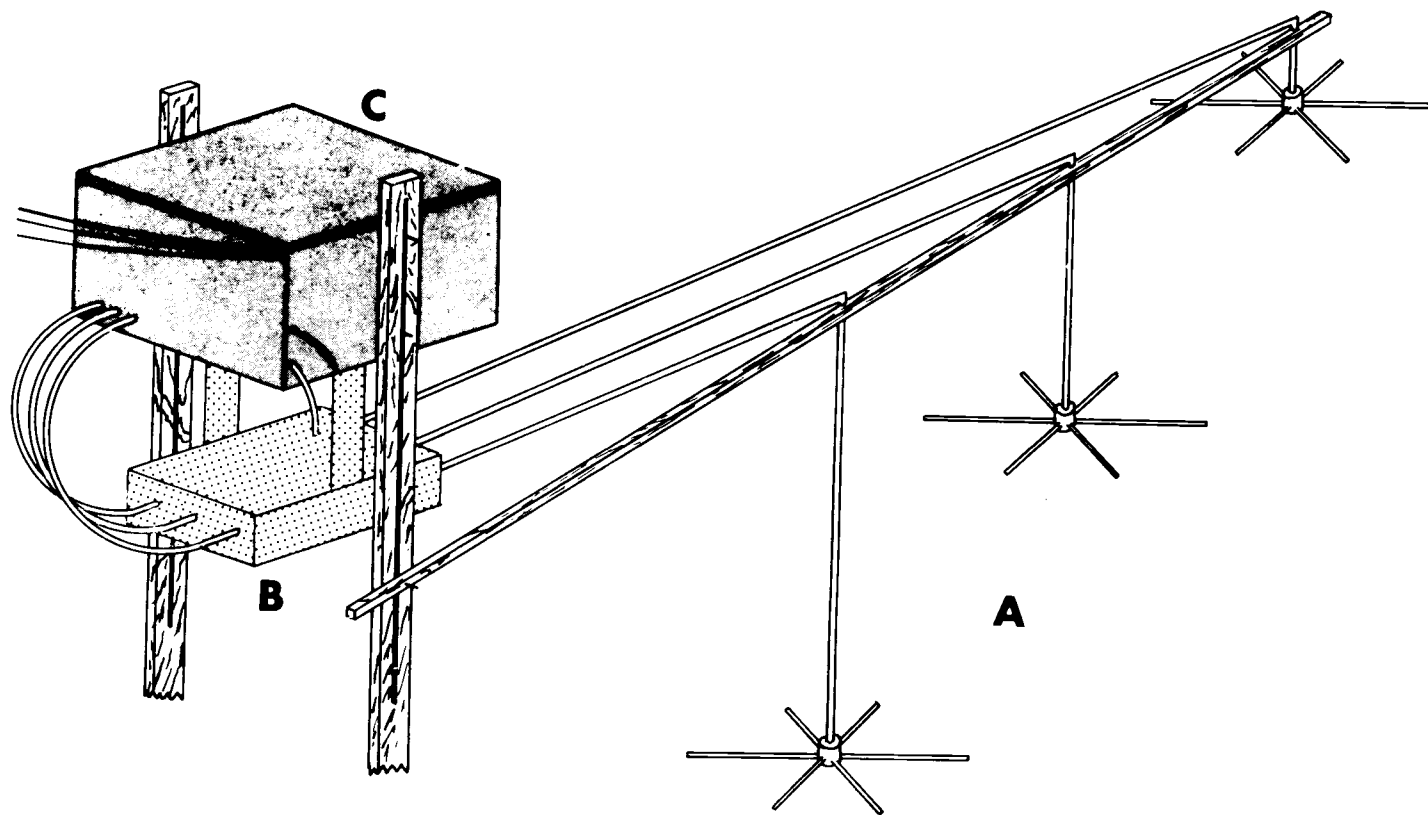


Figure 2. Water sampling apparatus for monitoring dissolved oxygen. A - water collecting tubes, B - submerged pumps, and C - box supported above water containing microswitch timer and sensing chamber.

system from the previous hour was flushed out in about 15 seconds.

Sampling at the surface, mid-depth and mud-water interface was through six acrylic plastic tubes arranged as 30.5 cm diameter spokes (Figure 2A). All spokes had a series of 0.635 mm holes drilled in the upper surface to admit water. Each 30.5 cm spoke was made of 2 different diameter tubes (not shown in detail); the distal half of 1/8 in. ID tubing and the proximal half of 1/4 in. ID tubing. The six spokes were connected to a 1 inch diameter hub. Each water collecting device was attached to a separate oscillating pump and the sensing chamber by 3/8 in. ID flexible plastic tubing. The size of tubing was kept as small as practical to minimize the volume of water removed from each depth.

The sensing chamber (Figure 3 C1, C2) with the oxygen probe (O) and temperature probe (T) was constructed of 1/8 in. clear plastic in two separate units. The first chamber (C1) filled and forced all air out of the system. Water entered the second chamber (C2, 2 × 2 × 1 in.) through the horizontal slit (HS). This slit provided the increased water velocity passing the probe that was required to obtain a valid DO reading. The entire sensing chamber filled in about ten seconds with water exiting the tubes at the upper and lower right. After the pump turned off, the sensing chamber emptied by gravity with air entering through the upper tube. A small hole in the partition between the first and second chamber (not shown) allowed

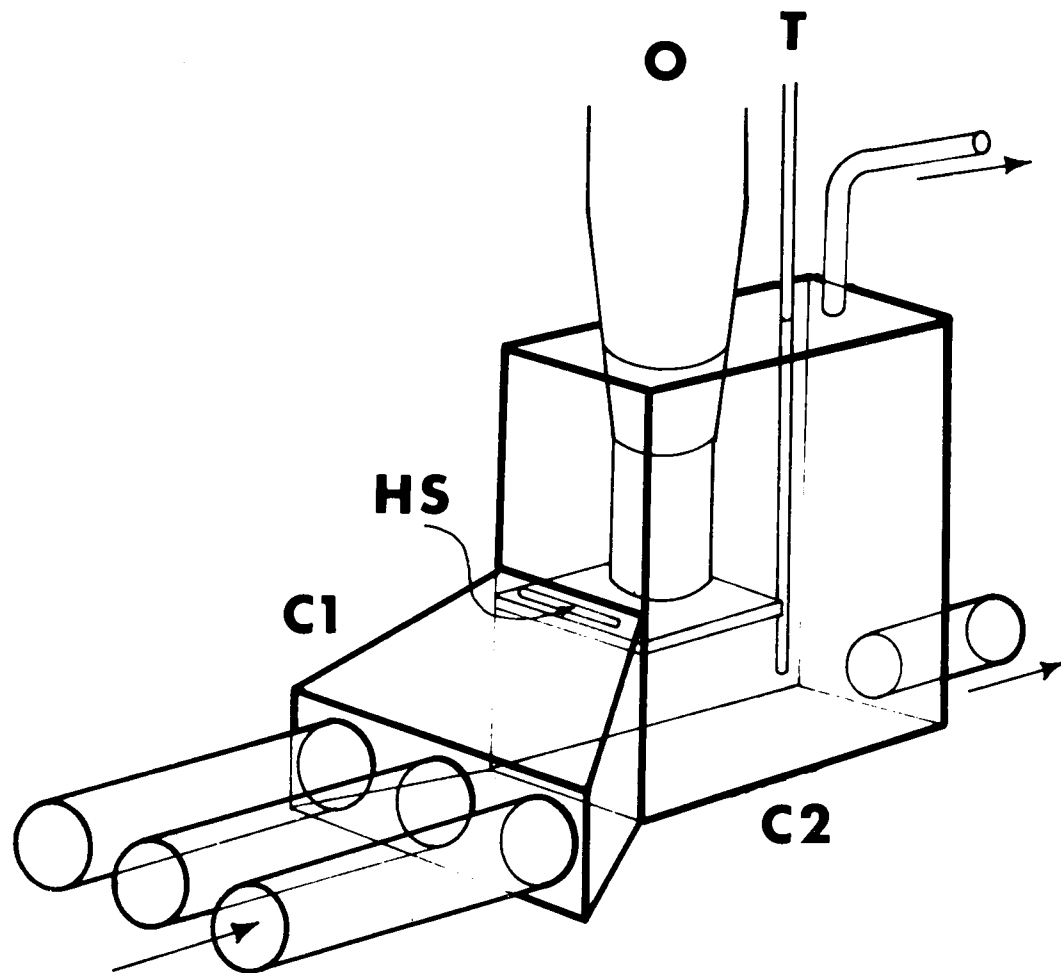


Figure 3. Sensing chamber of dissolved oxygen monitoring apparatus. First chamber C1 receives water, which flows through horizontal slit (HS) into second chamber C2, containing the oxygen probe (O) and temperature probe (T).

water to empty into the second chamber. The sensing chamber was designed to empty after a measurement was taken to minimize the growth of bacteria and algae on the oxygen probe membrane.

The apparatus required a minimum of maintenance. The oxygen probe was serviced with a new membrane and electrolyte about once a week. Calibration was performed daily when possible, by the Azide Modification of the Winkler Method (American Public Health Association, 1965) using tap water. Raw data were converted to mg/l DO using temperature correction and dissolved oxygen tables supplied by the manufacture of the DO analyzer. The water sampling device was alternated between the primary and secondary lagoons at approximately two week intervals and all tubes and the sensing chamber were flushed with fresh water to remove sediments at the time of transfer. Dissolved oxygen was monitored from February, 1967 to November, 1967.

Primary Production and Community Respiration

Estimates of primary production and community respiration were made using the diurnal oxygen curve method as described by Odum and Hoskin (1958). Although this method has been used extensively there are sources of error because of assumptions which must be made.

In this investigation no correction was made for oxygen transfer

at the air-water interface. It was felt that the only meaningful method of obtaining the oxygen transfer rate would have been to make frequent field measurements, and this was not attempted. Therefore the primary production estimates are minimal and intended to be only approximations of reality.

A mean weekly DO curve was calculated from the hourly profiles of seven consecutive days. These data were converted to a positive or negative rate of change per hour and plotted on 1 inch square graph paper with rate of change in grams of oxygen/ m^3 on the ordinate and hours on the abscissa.

The prescribed method of determining community respiration is to calculate the mean rate of change at night and assume that it is representative of the 24 hour rate. In this study an anomalous increase in DO occurred at certain hours of the night. The mean respiration rate was calculated excluding all positive rate of change data that occurred at night. The hourly rates most used were from 12 midnight through 3:00 A.M. A line was then drawn through the calculated mean respiration and extended to the entire 24 hours. Community respiration/ m^2 /day was equal to the mean rate of change per hour times 24 hours and times mean depth in meters.

Primary production in grams of oxygen/ m^2 /day was estimated by graphically integrating with a planimeter the area under the rate of change curve down to the mean respiration rate line. This figure

was multiplied by the mean depth in meters. Primary production and community respiration were converted to kilocalories using the relationship of 3.38 kilocalories/gram of oxygen.

Biomass

Absolute population estimates of G. barbipes larvae were made based on 1200 bottom samples taken with a 6 × 6 inch Ekman dredge. Both lagoons were sampled from July, 1966 to November, 1967 in 54 sampling dates. An emphasis was placed on measuring biomass from May, 1967 to November, 1967 because this was the period of intensive study with respect to measurements of emergence, growth, primary production and community respiration.

A stratified random sampling program was used because the distribution of larvae was known to be contagious. The greatest numbers were located within ten feet of shore. This sampling system enabled emphasis to be placed on those areas where the majority of the population occurred without neglecting areas of sparse populations.

Each lagoon was divided into concentric strata of known area (Figure 1). Smaller sections were delineated within each strata. At the intersection of each imaginary line, a wooden stake was driven into the lagoon substrate and marked above the water to identify its location. Concentric strata and subsections were

identified by Roman and Arabic numerals respectively.

To determine the number and location of samples required to obtain a total population estimate with an error of 15 percent, the following statistic was used:

$$(CV)^2 = \frac{1}{N} \left(\frac{\sum_{n=1}^{n=k} s \cdot A}{\sum_{n=1}^{n=k} \bar{X} \cdot A} \right)^2$$

or

$$N = \frac{\left(\frac{\sum_{n=1}^{n=k} s \cdot A}{\sum_{n=1}^{n=k} \bar{X} \cdot A} \right)^2}{(CV)^2}$$

where: CV = coefficient of variation
 N = number of samples
 s = standard deviation within each stratum
 \bar{X} = mean estimate within each stratum
 A = area within each strata
 k = number of strata.

After N was calculated, various percentages of N were assigned to each strata according to the percent of the total deviation which occurred in each strata. A sample calculation is given in the appendix. The N determined for sampling period X was used to dictate the number and location of samples needed for sampling period X + 1. In the summer of 1967 this procedure was not used

constantly, instead samples were taken as frequently as every three to five days.

Dredge samples were taken near shore by wading out and from a raft in deeper water. Each sample was emptied from the dredge into two nested brass screen buckets of 30 and 50 mesh/in. The material was swirled to remove sediments. The contents of both buckets were placed in quart containers. Each sample was reduced to 12.5 percent of the original volume using a sample splitter. The accuracy of the sample splitter was tested by comparing the number of larvae in a 12.5 percent sample with the actual counted total, divided by eight. Using the "t-test" for paired observations there was no significant difference between estimates and actual counts.

Samples were sorted immediately when possible. Other samples were either frozen or preserved with formalin. In the laboratory, midge larvae were sorted by instar, counted, dried at 58 to 60° C for 48 hours and weighed. Results were recorded as the number and dry weight (grams) of each instar in a 1/32 ft.². Dry weight biomass was converted to kilocalories by multiplying each gram by 4.977 kilocalories. This figure was obtained by burning midge larvae in a bomb calorimeter.

Data were submitted to the Computer Center of Oregon State University to be placed on punch cards. Analysis was performed in two separate operations. The first run gave an output of total number

and biomass for each instar, in each stratum, for each sampling period. Variances and standard errors were also calculated for each estimate so confidence limits could be assigned to estimates. It was then desired to express the data from July, 1966 to April 1967 in one month intervals and from May, 1967 to November, 1967 in biweekly intervals. A program was written to calculate a new mean and variance based upon the distribution of the sampling days within the specified time interval. Sample calculations are given in the appendix. The formula for the weighed biomass was:

$$\bar{B} = \frac{\sum_{n=1}^{n=k} C Y}{\Delta d}$$

where \bar{B} = weighted mean biomass
 C = coefficient of estimate Y
 Y = biomass estimates preceding, during, and following time interval specified
 k = number of Y estimates
 Δd = days in time interval

and the formula for weighted variance was:

$$V_{x\bar{B}} = \frac{\sum_{n=1}^{n=k} C^2 V_{xY}}{\Delta d^2}$$

where $V_{x\bar{B}}$ = weighted variance
 C = coefficient of estimate Y

V_{xY} = variance associated with biomass estimate Y
 k = number of Y estimates
 Δd = days in time interval

Confidence limits were assigned to each biomass estimate according to the formula:

$$CI = \pm 2 SE$$

where CI = 95 percent confidence limits
 SE = standard error

Growth

Various methods were tested for determining the growth rate of G. barbipes larvae. Laboratory growth studies were conducted in an aquarium to define the growth of each instar. The aquarium was held at 75° F with constant light. Food consisted of algae collected from the lagoon plus ground-up dry dog food. A sample of larvae was removed every one to three days to determine mean dry weights and lengths per individual in each instar. The relative proportion of each instar was also recorded.

Growth rates required for calculating production were determined by three different methods. Growth was determined directly from density and biomass data when there was little emergence and subsequent recruitment from new age classes. This procedure was used for the first three, and last four weeks of the production study.

During periods of emergence it was necessary to use a technique that eliminated recruitment. An attempt was made to measure growth of larvae reared in quart plastic containers but this proved unsatisfactory because growth at high densities was not comparable to that of similar densities under natural conditions. The final and most acceptable method was to conduct growth studies in clear plastic cylinders (5 in. OD \times 4 ft.) placed in the lagoon. A similar technique was used by Kajak (1966) to study growth of benthic organisms.

About 20 separate estimates of growth were obtained from studies over two or three day periods, from July, 1967 to October, 1967. Studies were conducted in undisturbed sections of Strata I of the secondary cell. The technique for using the tubes as growth chambers was: on day one, five plastic tubes were driven into the substrate. Tubes were placed obliquely to the shore, about one foot apart. A metal support stand held each tube in place. The substrate and organisms inside each tube were agitated and removed with a centrifugal pump. Water was added to the tube several times and the contents collected in a 50 mesh per inch screen bucket. The tubes were then relocated in the area between the first samples. Each tube was covered with screening and left for two or three days, after which time the contents were removed. The number of adults that emerged was recorded. Growth rates were determined on the basis of mean weight per larva at the beginning and end of each period. The

equivalent weight of adults, as pupae, was included in the final estimate.

Each mean daily relative growth rate was calculated according to the formula:

$$\frac{\frac{W_2 - W_1}{\frac{W_1 + W_2}{2}}}{t}$$

where: W_1 = mean weight per larva at time 1

W_2 = mean weight per larva at time 2

t = time 2 minus time 1

The results were expressed as mg/g/day. The growth was converted to cal/kcal/day using the relationship of 4.977 kcal/gram dry weight.

Emergence

Emergence rates of G. barbipes were estimated from May 1967 to October, 1967. Adults were collected in 2.5 ft.² (0.23 m²) staked box-traps designed specifically for this study. The use and efficiency of this emergence trap was compared to five other types and found to be superior in the lagoon habitat (Kimerle and Anderson, 1967).

Traps were placed in each strata of both the primary and secondary lagoons. However, an emphasis was placed on Strata I because few insects emerged from the middle of the lagoon. All traps

were serviced daily except on a few occasions when the interval was extended to 2, 3, or 4 days. Adults in each trap were counted by sex. Numbers/unit area were converted to grams dry weight and then to kilocalories using the relationship of 5.26 kilocalories for each gram dry weight.

Respiration

Field respiration experiments were attempted under natural conditions. However, reproducible results could not be obtained so these experiments were abandoned. Oxygen consumption by G. barbipes larvae was measured at different temperatures, densities and DO concentrations in laboratory experiments.

Oxygen consumption experiments were conducted in respiration chambers made of 1/8 in. (3.2 mm) clear plastic with inside dimensions of $2 \times 3 \times 7 \frac{7}{8}$ in. ($5.4 \times 7.6 \times 20$ cm) (Figure 4). The top of the chamber had a hole to admit the oxygen probe (O) of the galvanic oxygen cell analyzer. A stirring rod and paddle apparatus (S) was used to maintain the required water velocity past the probe membrane. Sterile sand was added to each chamber to adjust the volume of water to 0.333 liters and provide a substrate for midge larvae.

An attempt was made to simulate natural conditions and only measure respiration of larvae that had constructed tubes. Most

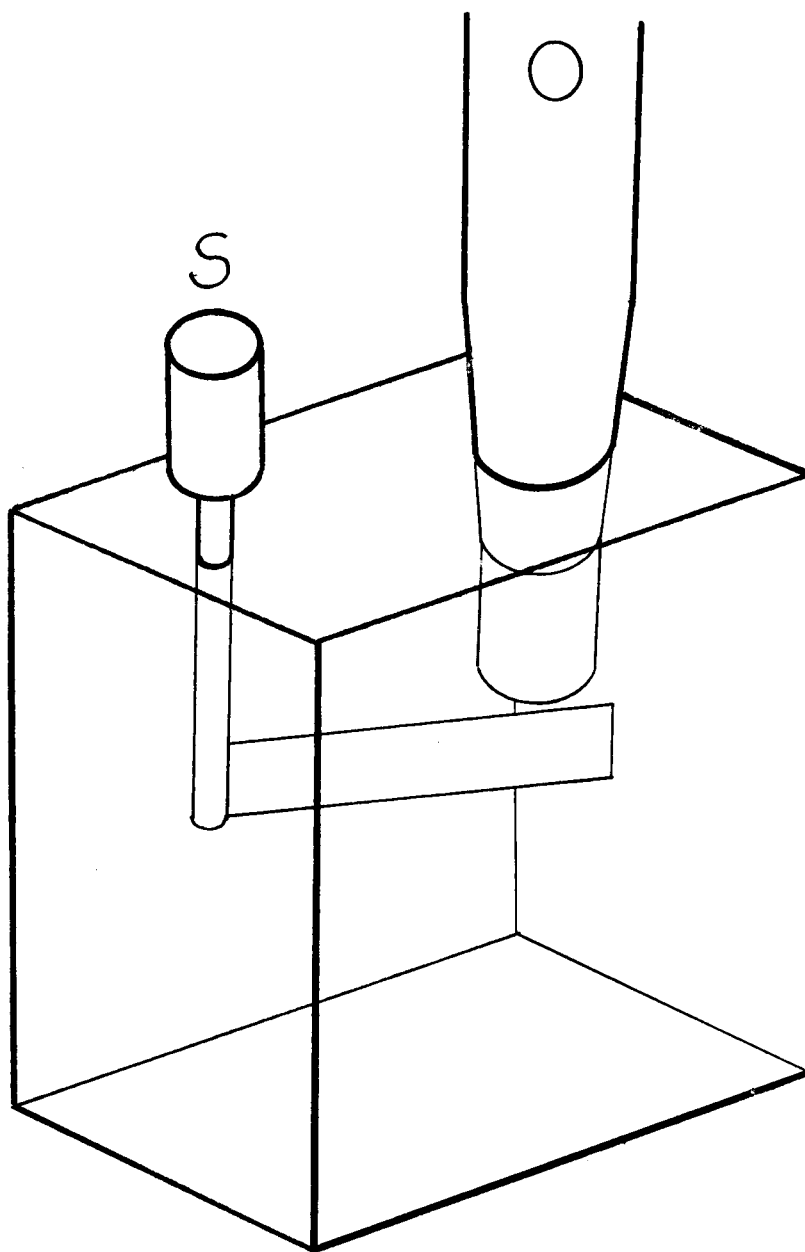


Figure 4. Respiration chamber used in measuring respiration rates of G. barbipes larvae in the laboratory: stirring rod (S) and oxygen probe (O),

larvae constructed tubes in the sand substrate and on pieces of screen placed in the respiration chamber during three hours of acclimation to test temperatures. Respiration chambers, without the tops, were submerged in an aquarium of distilled water inside a constant temperature cabinet. Larvae not established in tubes were allowed to swim out of the chamber. An experiment began after the tops were secured with rubber bands and the initial DO measurements were taken. Readings were made after one minute of agitation. A glass microslide was placed over the hole when the probe was removed. Dissolved oxygen measurements were taken every hour until the DO was reduced to less than 1 mg/l. The dry weight, of the larvae used in each chamber, was determined at the end of an experiment. Biomass used in each chamber was expressed in grams/m². Consumption was calculated as the difference in mg/l oxygen at the beginning and end of each hour.

Experiments were conducted at 10, 15, 20 and 25° C, with densities ranging from 2 to 54 grams/m². Consumption rates were expressed in class intervals of 0-11, 12-21, 22-32, 33-43 and 44-54 grams/m² for environmental DO values of > 5 mg/l; 3-4.99 mg/l; < 3 mg/l. Standard errors and 95 percent confidence limits were calculated for each estimate.

Respiration data were converted to energy burned using the oxycalorific coefficient of 3.38 calories for each mg of oxygen

consumed. Metabolism of the natural population, in Stratum I of the secondary lagoon from May, 1967 to November, 1967, was then estimated based on the mean weekly bottom temperature, environmental DO, and midge density.

Production

Production of G. barbipes was calculated weekly from May, 1967 to November, 1967 in Strata I of the secondary lagoon. The method of calculation was consistent with that of Ricker (1958) except that a relative growth rate was used instead of an instantaneous rate. The formula was:

$$P = \frac{k \cdot \bar{B} \cdot 7}{1000}$$

where: P = production/week in kcal/m²
 k = relative growth rate in cal/kcal/day
 \bar{B} = mean biomass in kcal/m²

The dates that growth rate estimates and biomass samples were taken did not coincide with the first and last day of each arbitrarily chosen weekly production interval. It was therefore necessary to calculate a weighted mean estimate of growth and biomass. Accumulated production from May, 1967 to November, 1967 was calculated as the sum of production each week.

RESULTS AND DISCUSSION

Influent and Effluent Quality

The rate of water flow through the entire lagoon system varied with seasons of the year. Storm drainage entered sewerage lines causing a flow rate of greater than five million gallons per day (mgd) on some days. During periods of reduced rainfall the sewage influent was as low as 0.1 mgd. Both cells had an operational depth of 1.5 m. However, the differential flow rate of the influent and losses to evaporation from both cells caused the mean depth to fluctuate. In summer the evaporation rate from the secondary cell exceeded the influent rate thus eliminating the effluent.

The validity of the results of chemical and energetic analysis of water samples during the winter months is questionable because of the infrequency of sampling and extreme variations in flow rates. Also the techniques for determining energy values were not completely worked out. The data during the period of intensive study, May, 1967 to November, 1967, are considered more reliable. Appendix Table 1 gives the results of chemical analysis.

The rate of flow of raw sewage into the primary cell was less than the discharge rate from April to June, 1967. There are two factors contributing to this anomaly: (1) sampling error resulting

from measuring flow rates of primary and secondary effluents only once a month, whereas raw sewage flow rates are monthly means. (2) in winter months the primary lagoon stored water, with the water level exceeding the height of the effluent pipe. When the influent rate decreased in the spring the primary effluent rate was maintained at a higher rate until excess water flowed into the secondary cell.

Energetic data of samples of raw sewage, primary lagoon effluent and secondary lagoon effluent from May, 1967 to November, 1967 are given in Table 1. The energy of raw sewage entering the primary lagoon was 632 million kilocalories. The secondary cell received 133 million kilocalories and the discharge to Ash Creek totaled 28 million kilocalories. Estimates for the entire year were made using the measured flow rates and mean values of kcal/gal. For 12 months the total energy of the raw sewage, primary effluent and secondary effluent were 1500, 400 and 120 million kilocalories respectively.

No correlation could be established between BOD and the energy content of water samples. Caloric determinations reflect the quantity of both living and dead organic matter, whereas the quantity of BOD results mostly from dead organic matter. Burgess and Northcraft (1965) established the fact that heat-killed samples exerted a higher BOD than living samples. The kcal/gal were highest in raw sewage (mean of 6.17 kcal/gal) with lesser quantities in the primary and

Table 1. BOD, flow rates and energy content of raw sewage, primary lagoon effluent and secondary effluent, Monmouth, Oregon. May, 1967 to November, 1967.

Month	BOD	grams/gal	kcal/g	kcal/gal	mgd	kcal 10 ⁶ /month
<u>Raw Sewage</u>						
May	180	4.46	2.68	11.97	.220	81.64
June	290	1.70	3.87	6.57	.125	24.64
July	320	1.66	2.49	4.13	.850	108.83
August	200	2.29	2.71	6.21	.540	103.96
September	325	1.53	2.97	4.55	.500	68.25
October	330	1.75	3.51	6.14	.230	43.78
November	308	2.18	3.04	6.64	1.010	201.19
Total						632.27
Mean	278	2.22	3.04	6.17		
Year Total						1500.00
<u>Primary Effluent</u>						
May	44	0.74	2.86	2.12	.554	36.41
June	34	1.20	3.46	4.15	.378	47.06
July	30	0.53	3.02	1.60	.142	7.04
August	22	0.83	2.50	2.08	.135	8.70
September	30	0.73	3.22	2.35	.065	4.58
October	38	1.04	2.60	2.70	.105	8.79
November	61	0.86	3.23	2.75	.244	20.13
Total						132.71
Mean	37	0.84	2.98	2.54		
Year Total						400.00
<u>Secondary Effluent</u>						
May	14	1.05	1.84	1.93	.430	25.73
June	15	1.19	1.21	1.44	.052	2.24
Total						27.97
Mean	14	1.12	1.52	1.68		
Year Total						120.00

secondary effluents, 2.54 and 1.68 kcal/gal, respectively. The mean energy content in the raw sewage and primary lagoon effluents were about the same, 3 kcal/g, while the secondary effluent contained about half as much energy per gram.

Allochthonous organic matter is the primary source of energy in raw sewage, whereas algal cells contribute most of the energy in the secondary effluent. It is suspected that the energy in the primary lagoon effluent would be somewhat intermediate with both algal cells and suspended organic matter. The secondary cell and effluent were never observed to contain appreciable amounts of organic matter.

Energy in Bottom Sediments

Variation in dry weight and caloric content of bottom sediments was quite high for samples taken at each station. Accurate estimates would require numerous transects across the lagoon, more stations and more samples at each station than were feasible in the present study.

The results of core sampling in the primary lagoon in consecutive years are given in Table 2. More material was deposited near the southeast shore than in the center or northwest corner. Observations on the amount of sludge present when Ekman samples were taken in all sections of the primary lagoon indicated that this

particular transect was not typical. Prevailing west and northwest winds often caused floating sludge and algal mats to accumulate against the southeast shore, sometimes extending out 30 to 50 feet. Deposition of the material in this region could account for the high values. Regions of greatest deposition should be near the influent pipe located in the center of the lagoon. Sturgess (1964) showed that sludge deposition increased with the distance from shore.

Table 2. Dry weight and energy content of primary lagoon bottom sediments.

Feet from shore	1967		1968	
	gram/sample	kcal/gram	gram/sample	kcal/gram
10	16.4	0.59	17.1	0.54
30	9.6	0.44	10.1	0.64
80	14.1	0.37	12.3	0.77
180	6.0	1.00	8.3	0.99
320	5.4	1.34	9.2	1.22
500	8.8	1.00	13.4	1.36
200	3.1	0.94	11.1	1.01
80	7.6	0.94	8.4	0.78
30	8.4	0.74	6.2	0.72
10	9.3	0.66	9.8	0.53

Caloric content per gram sludge increased with distance from shore. Raw sewage entering the lagoon near the middle contained 3 kcal/gram. Addition of this fresh material in the vicinity of the influent pipe could account for the higher caloric values. As sewage

becomes more dispersed with time it is subjected to bacterial decomposition, eventually reaching complete decomposition. Some of the caloric determinations made on samples were as low as 0.20 kcal/gram. This indicated that the material was probably near complete mineralization. All the samples were difficult to burn, necessitating the addition of known quantities of benzoic acid.

An estimate was made of the total energy deposited per year. The mean number of grams per sample (15.48 cm^2), after the lagoon had been operating for three years was 8.9, and after four years was 10.6. An approximate yearly increase was calculated as 2.5 g/sample/year, or $1614 \text{ g/m}^2/\text{year}$, or 115 million kilocalories for the entire lagoon.

Performance of Dissolved Oxygen Monitoring Device

The DO monitoring device previously described provided hourly measurement of DO and temperature at the surface, mid-depth and mud-water interface of the primary and secondary lagoons waters from February, 1967 to November, 1967.

The accuracy of the DO probe was compared to the Azide Modification of the Winkler Method (American Public Health Association, 1965) by determining DO of a single water source using both methods. The mean of five trials obtained by the Winkler Method was $9.34 \text{ mg/l} \pm 0.14 \text{ mg/l}$ and that of the oxygen analyzer was

9.37 mg/l \pm 0.05 mg/l. A comparison was also made between DO of the lagoon water before and after it had passed through the water sampling device. Six readings were taken by swirling the probe directly in the surface water. The probe was then placed in the sensing chamber and six more readings were obtained by activating the surface pump for two minutes in each trial. In all cases it was very difficult to detect any difference in the results as they appeared on the chart paper of the recorder. However, there was some loss of precision, from 0.05 mg/l to 0.13 mg/l, because of the necessity of reading and converting the data on the chart paper to mg/l. The advantages of recorded hourly DO and temperature data at three depths more than offset this loss of precision.

Extreme supersaturation of oxygen was frequent in lagoon waters during summer months. Under these conditions the oxygen analyzer was found to be superior because consistent results could not be obtained using the Winkler Method. It was thought that pumping might cause some oxygen to come out of solution. However, there was no measurable loss of oxygen. The pumps were submerged so there was no head to overcome by creating a vacuum over the water.

The most significant advantage of the device was that the spoked samplers permitted sampling of water for DO measurement from a thin layer. The tubes collected a small quantity of water (one liter)

from a large area (0.3 m^2) during two minutes of pumping. Flow patterns around a spoked sampler placed on the bottom of an aquarium were studied using India Ink. Observations indicated that water was being collected from a 1 cm layer just above the bottom sediments. Brundin (1950) discussed the importance of measuring oxygen concentrations within the respiratory environment of bottom fauna. He stated that the mud is rich in reducing substances which consume oxygen causing a sharp drop in oxygen concentration a few millimeters or centimeters over the mud surface. Thus with this device DO was measured within the respiratory microhabitat of midge larvae.

Dissolved Oxygen In Sewage Lagoons

Dissolved oxygen and temperature data indicated that sewage lagoons were extremely dynamic ecosystems. Seasonal and daily changes in oxygen evolved and consumed or lost from the lagoons were a function of day length, cloud cover, temperature, wind, algal density, zooplankton density and bacterial decomposition.

Figure 5 shows typical winter DO and temperature curves for the secondary lagoon with a moderate density of algae and a uniform temperature of 8°C . Dissolved oxygen decreased during night and increased in daylight. The rate of change in DO at the mud-water interface at night indicated that the respiration rate of the benthic

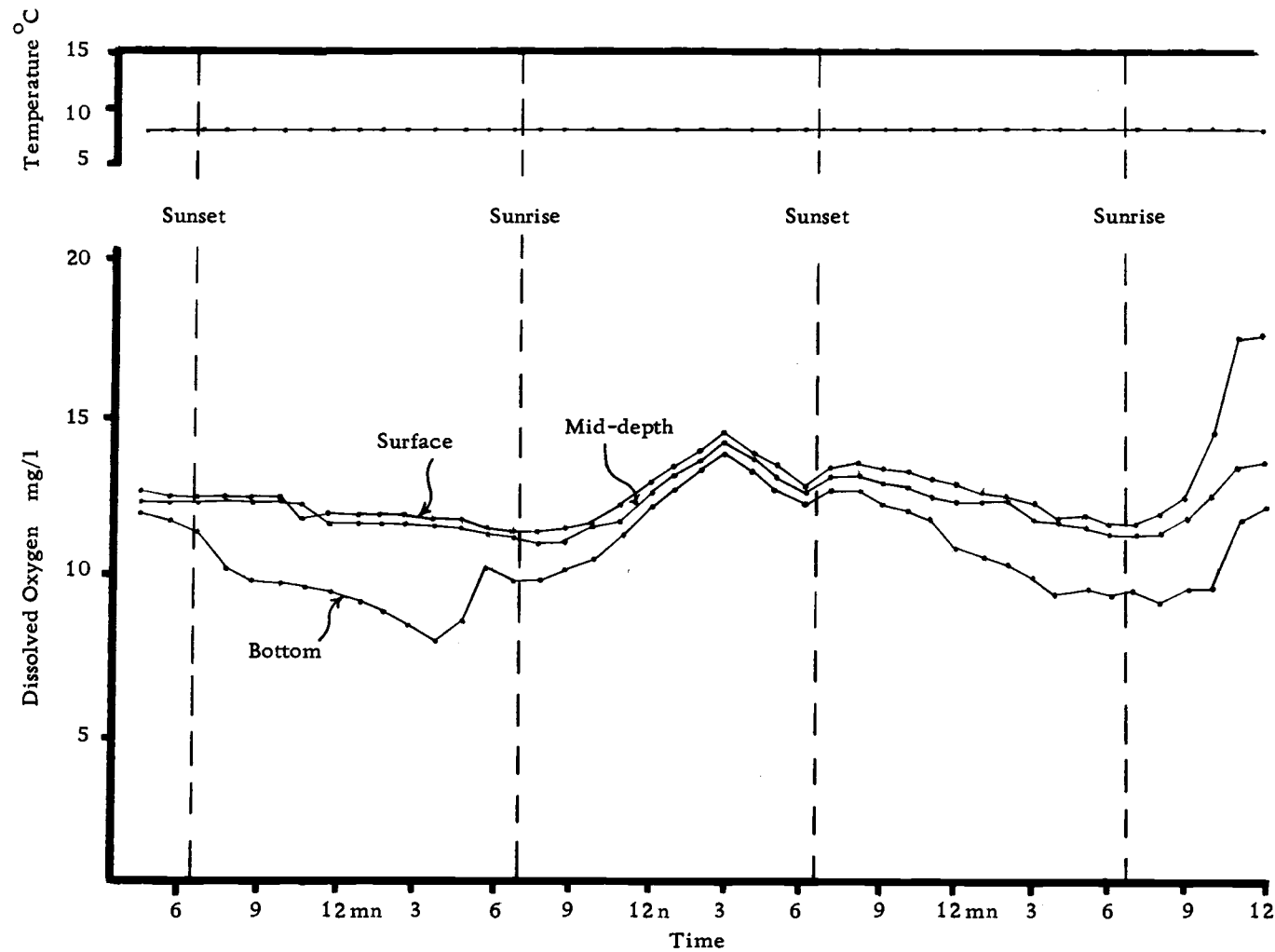


Figure 5. Diurnal oxygen and temperature curves at surface, mid-depth and mud-water interface of secondary waste stabilization lagoon, Monmouth, Oregon, February 18-20, 1967.

community was greater than that occurring in the middle and surface waters. Effective light penetration to the bottom of the lagoon is apparent in that DO at the mud-water interface increased during daylight hours. These types of DO and temperature curves were typical for both lagoons in winter and early spring. There were no extreme changes in DO and temperature from hour to hour and day to day. Algal blooms were not frequent.

In summer months, environmental conditions were conducive for extreme levels of oxygen production and consumption. Algal blooms persisted for two to three weeks and then either died or were cropped by zooplankton blooms. Figure 6 shows the extreme variations in DO and temperature which occurred in the primary lagoon during a summer month. These types of data were typical for many days in April, May and June. The most outstanding feature is the magnitude of the difference in DO and temperature from near 0 mg/l and 13° C at the bottom to 50 mg/l and 27° C at the surface.

Effective thermal stratification is evident in Figure 6 from 9:00 P. M. to 8:00 A. M. when the surface and mid-depth temperature were uniform while the bottom temperature was as much as 7° C lower. After 8:00 A. M. the surface water increased 10° C in six hours, thus creating layers of water with differences in density great enough to prevent mixing of surface, mid-depth and bottom water layers.

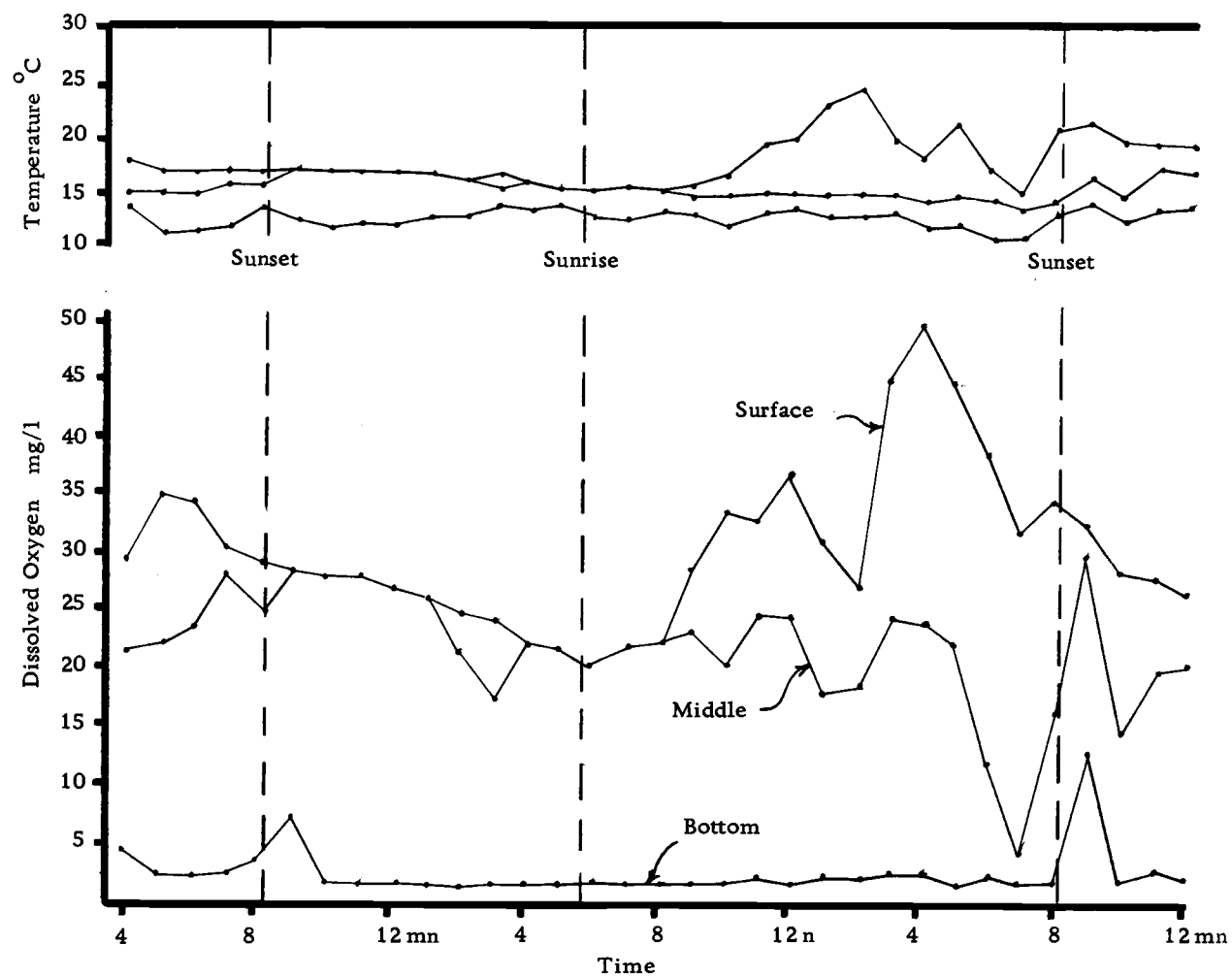


Figure 6. Diurnal oxygen and temperature curves at surface, mid-depth and mud-water interface of primary waste stabilization lagoon, Monmouth, Oregon, May 19-20, 1967.

Near-septic conditions prevailed on the bottom because phytoplankton turbidity limited light penetration and subsequent photosynthetic activity. Stratification also prevented rapid replacement of deoxygenated bottom waters. However, circulating layers of water must have carried some oxygen to lower depths or else the DO concentration at the mud-water interface would not have remained relatively constant at 2 to 3 mg/l.

Thermal stratification also affected the distribution of oxygen in the upper water layers. As with temperature, the DO at the surface and mid-depth was uniform from 9:00 P.M. to 8:00 A.M. (Figure 6). By 4:00 P.M. the surface DO was 25 mg/l greater than the mid-depth. From 4:00 P.M. to sunset the DO decreased, probably in response to algal respiration. Other fluctuations in DO could have been the result of diffusion between water layers and loss to the atmosphere. A mid-day depression of photosynthetic activity is shown for this day and was also evident in all other data.

Oxygen concentrations in the secondary cell, for the period of investigation, were consistently high except for about one week beginning on August 21, 1967. Algae died in the lagoon and the water lost the typical dark green color. For about three days the lagoon was septic and developed an odor. Numerous Western Mosquito fish, Gambusia affinis, were observed floating dead in the lagoon. Algal populations returned to normal levels after about two weeks, and the

DO concentration remained relatively high until the end of the investigation in November. Low concentrations of oxygen were recorded for short intervals in some days in September and October.

The primary lagoon contained an extremely dense concentration of algae in late May and early June, 1967. Dissolved oxygen concentrations ranged from 30 to 40 mg/l in late afternoon, to a few mg/l in early morning. Bottom DO values were usually near zero in the morning. On June 18, 1967 a bloom of Cladocera consumed the algae to the extent that the bottom could be seen to a depth of two feet. Windrows of Cladocera were observed. A day later the lagoon became septic and developed an offensive odor. The water turned black with suspended organic matter and pieces of sludge from the bottom were floating in the lagoon.

Some algae were observed in the lagoon after ten days but they did not completely become reestablished. By July 8, 1967 the lagoon was septic once again. For about six weeks the DO in the lagoon remained well below saturation with frequent periods of odor production. By late August oxygen production in the primary cell was normal.

An anomalous result encountered in this investigation was an apparent increase in DO at times of reduced light intensity and complete darkness. In Figure 5, under winter conditions, the DO at the mud-water interface increased beginning at 4:00 A.M. and then

leveled off at sunrise. Then, just after sunset, there was a slight increase in DO at all depths. Figure 6 shows a similar increase in DO at the mud-water interface and mid-depth just after sunset.

Initially, it was thought that the increase might be due to instrumental error. However, it was concluded that it was a real increase because of the consistency of its occurrence and because a single probe was used to measure DO at all depths but the increase occurred at only one or two of the depths.

The early morning DO increase was most prevalent in February, March and April with few PM increases. In summer months the increase after sunset was consistent in all data, while the morning increase was no longer detected.

A definite explanation of this phenomenon has not been found, although a number of possible explanations do exist. The most obvious source of oxygen would be from the oxygenated upper layers of water. The increase shown in winter in Figure 5 at the mud-water interface could have resulted from wind circulating oxygenated water downward. The temperature was 8° C at all depths. Therefore the wind could have easily mixed the water layers. However, the consistency of both presunrise and postsunset DO increases and the fact that effective thermal stratification sometimes prevented mixing of water layers, make it difficult to accept this explanation for all cases.

Eberly (1959) in studying metalimnetic oxygen maximum of a

lake found that the maximum oxygen production was at a point where the light intensity was about one to two percent of surface illumination. He correlated this oxygen production with the presence of a dense population of Oscillatoria agardhii Gomont, a filamentous blue-green alga that exhibited the ability to migrate vertically to the desired depth and light intensity. In carbon uptake studies conducted in light and dark bottles there was aphotic carbon assimilation in dark bottles (Eberly, 1964). He suggested that an explanation of this observation might be found in a supplemental metabolic pathway of Oscillatoria, possibly involving H_2S . Fogg (1953) in discussing the possibility of facultative chemotrophic algae assimilating carbon with H_2S as the hydrogen donor, indicated there would be no evolution of oxygen. These statements are contradictory but indicate that, with our present state of knowledge on the identification, physiology and role of aquatic micro-organisms, future investigations in this area may yield pertinent information. Oscillatoria agardhii was present in lagoon waters during winter and spring and may have been responsible for the production of some oxygen under conditions of reduced light intensities.

Another explanation could be that the oxygen causing the increase at night actually came from daylight photosynthetic activity but became "tied-up" and released after sunset. Oxygen gas could accumulate in the cells of algae or become trapped under benthic

algal mats. However, light penetration to the bottom rarely occurred except in some winter periods.

A final source of oxygen could be from some biochemical or chemical process occurring in or near bottom sediments. However, for this to be plausible some explanation would be needed to account for the periodic nature of the increase. The hydrogen ion concentration is a factor which might activate such a process. The pH decreases at night, because sewage lagoons have an excess of carbon dioxide which unites with water forming carbonic acid. Photosynthetic activity in daylight utilizes the CO_2 and the pH increases. Values for pH can range from a minimum of 7 in early morning hours to a maximum of 11.4 by late afternoon (Neel et al., 1961; Horning et al., 1964). Most chemical processes and the dissociation of compounds are affected by hydrogen ion concentrations. A change in pH from 11 in late afternoon to 8 or 9 by evening certainly would affect relative concentration of ions.

Sodium nitrate is often added to lagoons that become septic to eliminate offensive odors associated with anaerobic decomposition. Apparently this chemical dissociates, and the resulting denitrification releases oxygen within the lagoon. It therefore seems possible (although not highly probable with our present state of knowledge) that some similar reaction could be occurring naturally in sewage lagoons.

A definitive answer to the anomalous oxygen increases at night can only be found with an intensive study of all possibilities.

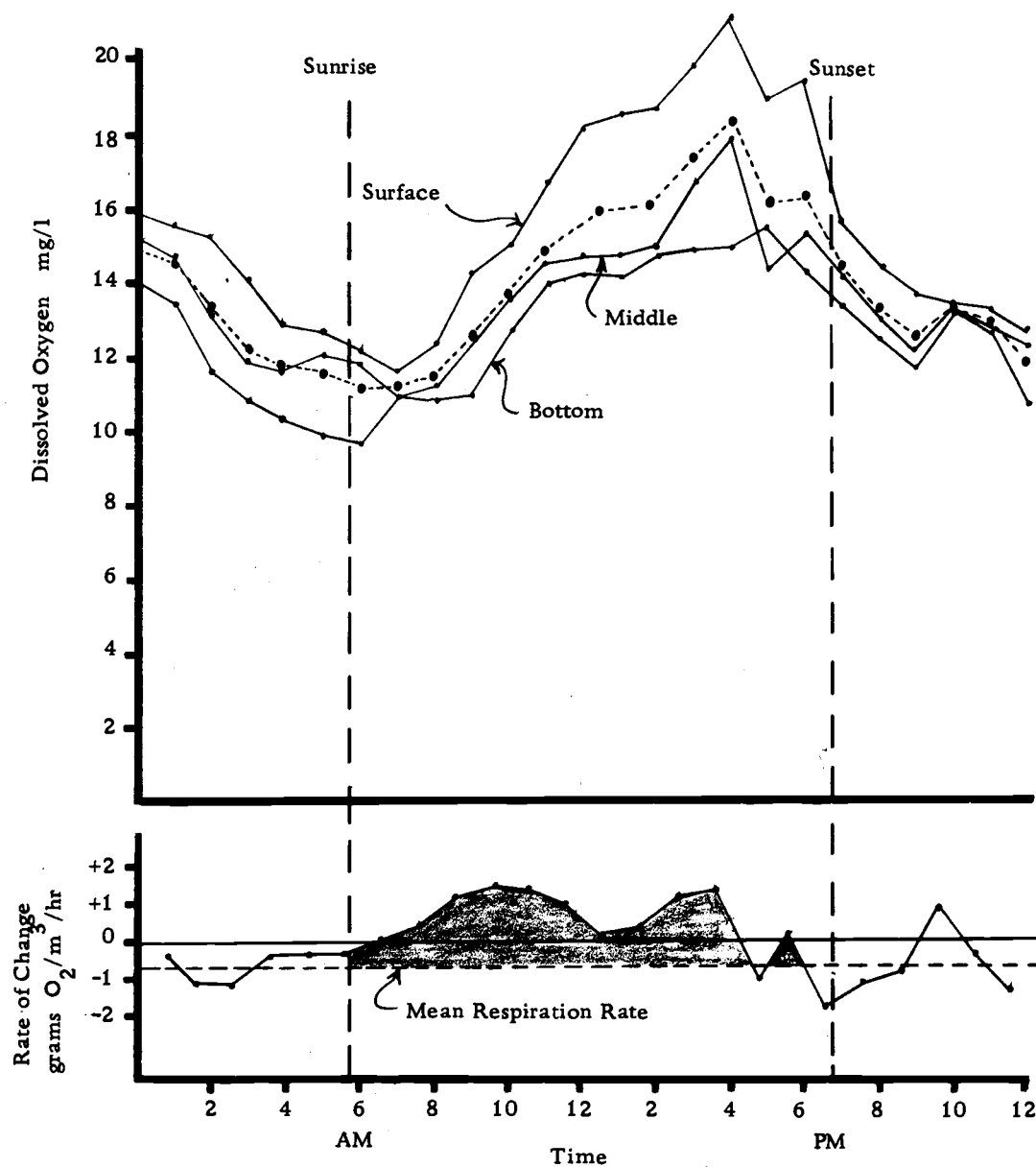
Primary Production and Community Respiration

Primary production and community respiration were calculated using the diurnal oxygen curve method. Figure 7 shows the mean weekly diurnal oxygen curve for bottom, mid-depth and surface waters in the secondary lagoon for April 1 through 7, 1967. The mean oxygen curve for the entire lagoon is shown by the dotted line.

One assumption of this method of calculating primary production is that the point of sampling is representative of the entire body of water, both horizontally and vertically. Dissolved oxygen was monitored at only one station in the lagoon. Point-source data is representative only if the lagoon is homogenous with respect to the distribution of oxygen. Figure 7 does show that the mean diurnal oxygen curve calculated from three depths is representative of vertical differences in DO.

The rate of change curve shown in Figure 7 was calculated from positive and negative changes each hour in the mean DO curve. Primary production and community respiration estimates were made from this rate of change curve as previously described. The data excluded from the mean respiration rate were from sunset to 11:00 P.M. Sample calculations of primary production and community

Figure 7. Calculation of primary production and community respiration from dissolved oxygen rate of change curve. Rate of change curve derived from mean weekly DO curve (dotted line). Secondary lagoon, Monmouth, Oregon. April 1-7, 1967.



Community Respiration = Mean respiration rate \times Hours \times Depth in m.
 = $0.79 \text{ grams } O_2/m^3/hr \times 24 \text{ hours} \times 1.5 \text{ meters}$
 = $28.44 \text{ grams } O_2/m^2/day$

Primary Production = Area shaded under rate of change curve down to mean respiration rate line \times depth in m.
 = $15.5 \text{ grams } O_2/m^3/day \times 1.5 \text{ meters}$
 = $23.25 \text{ grams } O_2/m^2/day$.

respiration are given in Figure 7.

Table 3 gives monthly primary production in grams of oxygen/ m^2 /day from May, 1967 to November, 1967 in the secondary lagoon. The range was from 6 to 54 grams of oxygen/ m^2 /day. The highest value obtained in this study was 83 g/ m^2 /day for three days in June in the primary lagoon.

As indicated previously, the primary production estimates are minimal because no correction was made for loss of oxygen to the atmosphere. However, primary production estimates in this study are similar to those reported in the literature. Odum (1959) summarized primary production estimates for numerous ecosystems. Values for polluted streams, sewage lagoons and enriched algae cultures, during short favorable periods, ranged from 23 to 57 g/ m^2 /day. Maximum rates for highly productive natural and cultivated ecosystems were estimated to be as high as 60 g/ m^2 /day. It is therefore concluded that primary production estimates in this study are of the correct order of magnitude, but necessarily low.

All primary production data indicated that the maximum rate of production occurred in late morning and mid-afternoon. About noon each day the rate of production was less, with respiration sometimes exceeding oxygen production. Bartsch and Allum's (1957) findings were similar. They attributed the lower production at mid-day to depletion of CO_2 .

Table 3. Primary production and community respiration in the secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.

	Primary production				Community respiration			
	grams $O_2/m^2/day$	grams organic matter/ m^2/day	kcal/ m^2/day	kcal/ $m^2/month$	grams $O_2/m^2/day$	kcal/ m^2/day	kcal/ $m^2/month$	P/R
May	54	51	183	5673	72	242	7502	.71
June	50	47	169	5070	50	169	5070	1.00
July	30	29	101	3141	43	145	4506	.70
August	14	13	47	1467	22	71	2200	.67
September	40	38	135	4060	58	196	5881	.69
October	13	12	44	1362	21	71	2200	.62
November	6	6	20	608	11	37	1115	.54
Total				21,383			28,474	
Mean	30	28			39			.77

Community respiration rates were calculated with the exclusion of positive rates of change in DO after sunset. These data were excluded because they masked the actual respiration rate, if respiration takes place at a more or less uniform rate throughout the 24 hour day, as is assumed when using the diurnal oxygen curve method. However, if the biological organisms stopped respiring, or drastically changed their respiration rate at the time of the increase in DO, then an error was made in excluding these data. It seems evident that the diurnal oxygen curve method is not a panacea for determining primary production and community respiration under all types of environmental conditions.

The ratio of primary production to community respiration (P/R ratio) is often used to designate whether an ecosystem is autotrophic or heterotrophic. By definition, heterotrophic environments consume more oxygen than they produce and eventually become anaerobic unless there is atmospheric reaeration. The mean P/R ratio from May, 1967 to November, 1967 in the secondary lagoon was 0.77. This indicates that the lagoon ecosystem was heterotrophic. At first this is easily accepted because organically enriched environments are generally considered to be heterotrophic. However, there are a few factors which make the P/R ratio of 0.77 implausible. Primary production estimates were minimal because oxygen produced in the lagoon and lost to the atmosphere was not included in

production estimates. The magnitude of this loss was unknown but on some afternoons when oxygen concentrations exceeded 300 to 400 percent saturation the loss was probably significant. If this loss could have been accounted for, the primary production, and the P/R ratio, would be considerably higher. Second, during May, June and July, DO concentrations of surface water usually exceeded saturation throughout the 24 hour day. Therefore no oxygen could be entering the lagoon from the atmosphere. Oxygen production in upper waters must have been sufficient to meet the oxygen demand of lower waters as long as anaerobic conditions did not develop. When this occurred the environment was autotrophic.

The general conclusion is that the dynamics of the sewage lagoon ecosystem make it difficult to apply the concept of heterotrophic and autotrophic communities. In the months of December and January the sewage lagoons were often septic for many days, and certainly heterotrophic. In any summer months the lagoon might change from autotrophic one day to heterotrophic the next day.

Biomass 1966 - 1967

Monthly means of total biomass in the primary and secondary lagoons from July, 1966 to November, 1967 are shown in Figures 8 and 9. The vertical lines through each estimate define the 95 percent confidence limits. The mean error for all estimates in the primary

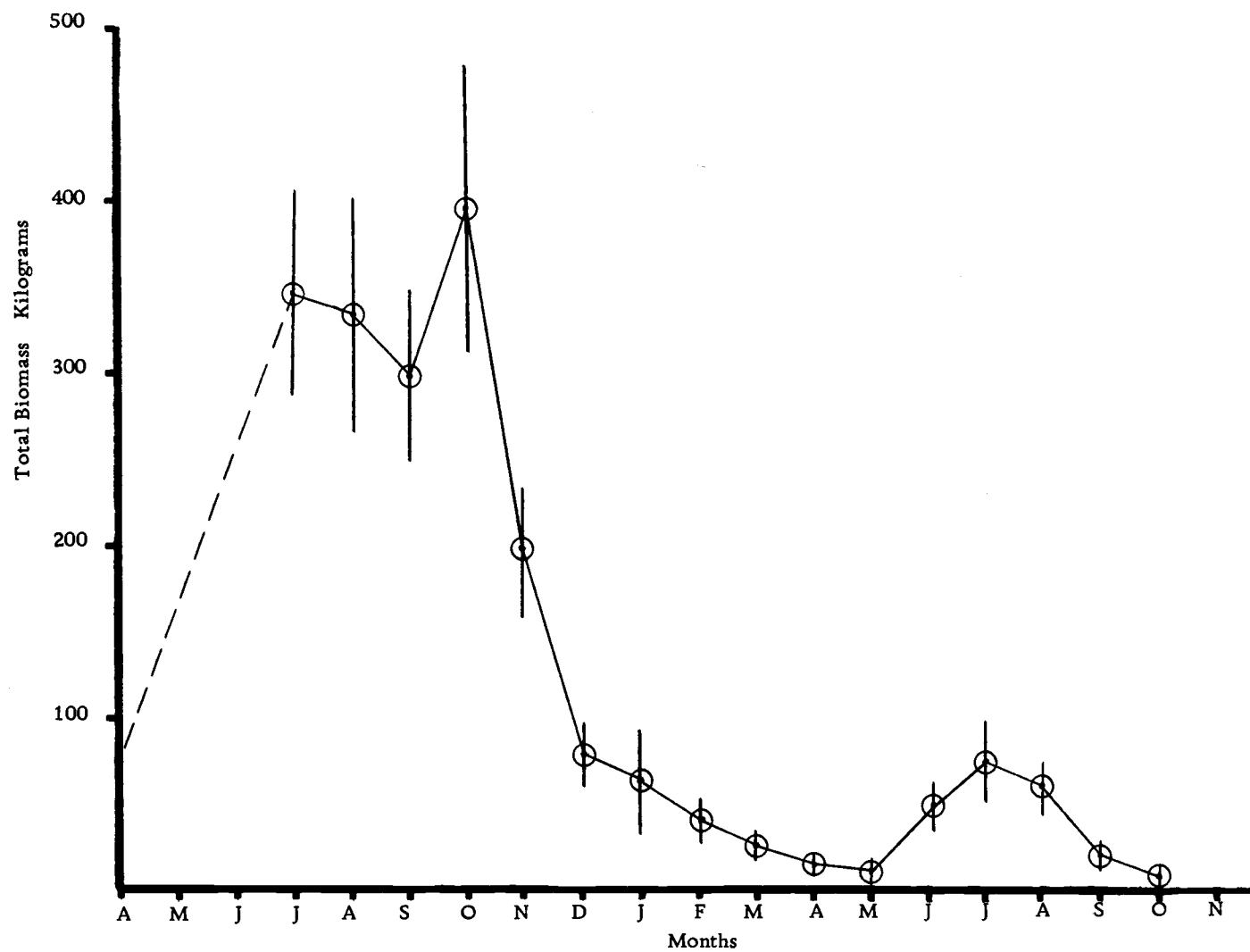


Figure 8. Monthly mean biomass of *G. barbipes* in primary lagoon, Monmouth, Oregon, 1966 and 1967. Vertical lines through biomass estimates are 95 percent confidence limits.

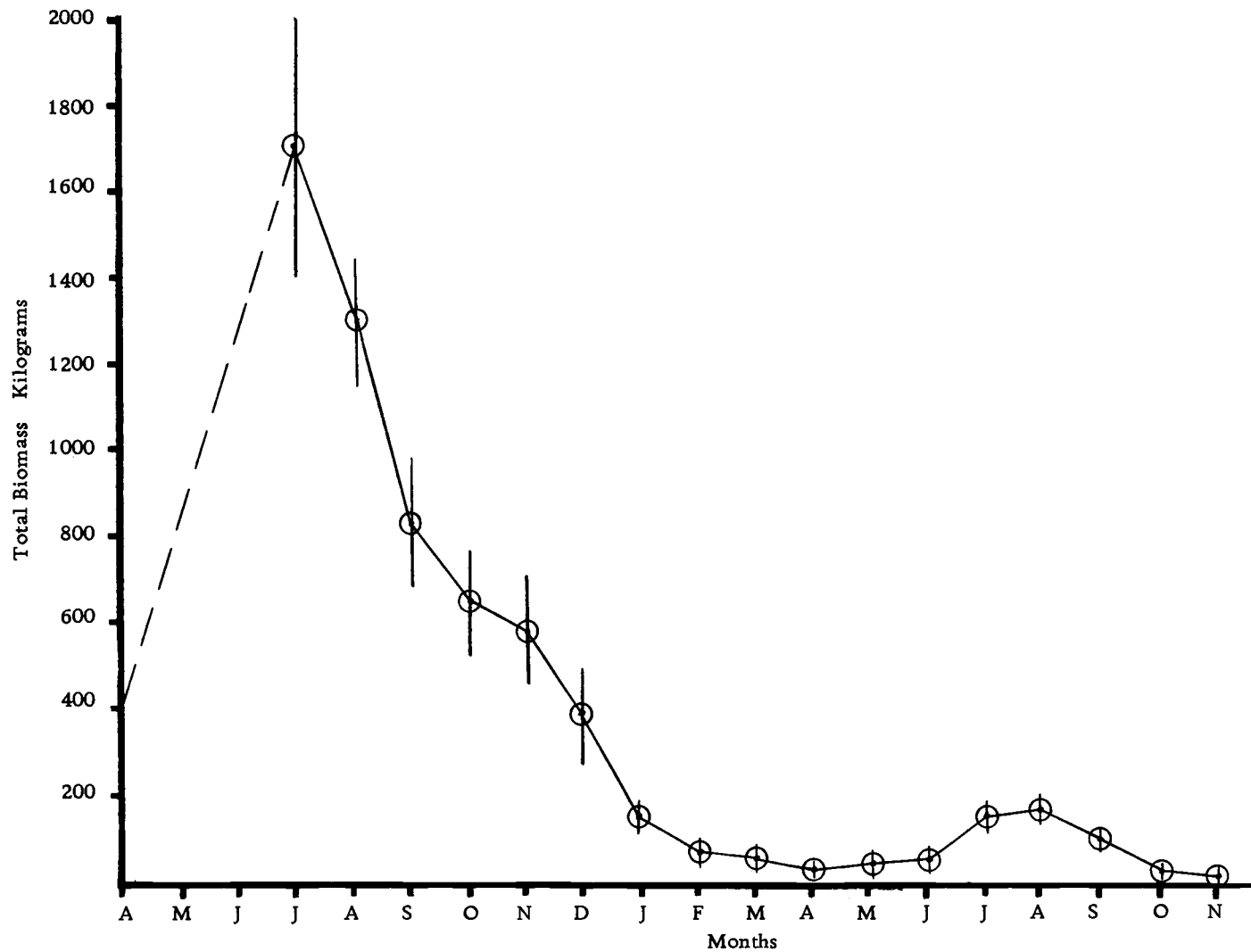


Figure 9. Monthly mean biomass of G. barbipes in secondary lagoon, Monmouth, Oregon, 1966 and 1967. Vertical lines through biomass estimates are 95 percent confidence limits.

and secondary cells were 15.6 and 14.7 percent, respectively, which is very close to the specified 15 percent of the sampling program. Some errors were as high as 20 to 30 percent in winter months because the sampling interval was five to six weeks. Sampling errors in summer months were about 10 percent because samples were taken every four to ten days. These biomass estimates are highly reliable considering the size of the sampling universe and variation in biomass which occurred within each stratum.

Biomass estimates prior to July, 1966 (dotted line in Figures 8 and 9) were not based on Ekman samples but merely put in to show the approximate increase which had to occur after low population densities in the winter.

The most outstanding feature of the biomass curves in Figures 8 and 9 are: (1) the decrease in total biomass in both cells from 1966 to 1967, and (2) the difference in biomass between the primary and secondary cells in both 1966 and 1967.

Total biomass in the primary and secondary cells in 1966 is not directly comparable. In the summer of 1966 the secondary lagoon was only about 1 m deep, while the primary cell was 1.5 m deep. Biomass per m² was actually higher in some regions of the primary lagoon, but was restricted to Stratum I. Midge larvae occupied Strata I, II, and III of the secondary cell in 1966, thus accounting for the higher total biomass. In the summer and fall of 1966 about 30

percent of the total biomass in the secondary cell was in Stratum I, whereas in 1967, Stratum I accounted for 90 percent of the total biomass. The shallow depth in 1966 apparently enabled larvae to successfully utilize a greater area of the lagoon. High DO levels at the mud-water interface in 1966 probably contributed to the success of the midge population. No measurements were taken of oxygen when the secondary cell was only 1 m deep. However, oxygen concentrations were probably higher at the bottom in 1966 than in 1967, because the trophogenic zone was closer to the bottom. Also oxygenated water could have been more easily circulated to the bottom.

Fagan and Enns (1965) studied the distribution of G. barbipes on the slope of a dike in a 1.2 m deep lagoon. They found that the lower limit of dense midge populations (0.8 m) was correlated with the depth of thermal stratification (0.8 m). Stratification caused the lower water layers to be anaerobic. Also, phytoplankton were found only in the epilimnion. Oxygen and food were readily available above this depth in the epilimnion.

In the present study, periodic oxygen depletion in both cells did affect larvae on the bottom. Growth and emergence decreased and mortality increased during periods of low DO. However, anaerobic bottom waters associated with thermal stratification never persisted for more than a few days and therefore could not have had a constant effect. Bottom DO values in June, July and August in the

secondary cell consistently ranged from 1 to 3 mg/l when there was no thermal stratification. The primary cell was more frequently septic on the bottom, especially at night. It is therefore concluded that low DO did restrict the distribution of larvae to upper areas of the dikes but thermal stratification was not necessarily the only factor responsible for low oxygen concentrations.

The decrease in biomass from 1966 to 1967 in both the primary and secondary lagoons was probably due to aging of the lagoon. Kimerle and Enns (1968) showed that the abundance of G. barbipes in sewage lagoons in central Missouri decreased with age and with higher BOD loading rates. Comparison of biomass samples taken in the primary cell in the fall of 1965 to the fall of 1966 and 1967 supports the above conclusion that there is a reduction in biomass of G. barbipes in successive years.

It is also possible for unfavorable environmental conditions in any one year to adversely affect the rate of biomass accumulation. Dissolved oxygen concentrations in the primary cell were very low and frequently zero during about eight weeks in the summer of 1967. The effect of the septic conditions were that the mortality rate increased and biomass never attained the level it might have if conditions had been better. Biomass in the secondary cell was unaffected by short periods of septic conditions, as occurred on August 21, 1967. Qualitative bottom samples taken in the primary cell in

August, 1968 indicate that the biomass will probably be higher in 1968 than it was in 1967, but not as great as in 1965 or 1966.

Laboratory Growth Studies

Growth of a cohort of G. barbipes under laboratory conditions was clearly defined in two exponential phases. The rate of weight gain per individual the first 16 days was higher than the last 40 days. Figure 10 gives the mean weights of larvae plotted on an arithmetic scale and Figure 11 the same data plotted on three cycle logarithmic paper. Figure 10 shows the two logarithmic growth curves. The dotted line is the projected growth per larva, if the rate during the first phase had been maintained. The validity of interpreting these data as two separate exponential rates is exemplified in Figure 11. Data in both phases fall very close to the straight lines of differing slopes. The point of inflection, day 16, was at the termination of the second-instar stadia and the beginning of the fourth-instar stadia. The presence of slower growing fourth instar larvae and lack of fast growing second instar larvae probably caused the rate of growth to change rapidly.

It was originally intended to give midge larvae a maximum food ration in the form of algae. However, after about 20 days of the study it became difficult to maintain a green color in the aquarium because algae were being consumed so rapidly. In addition to algae, ground

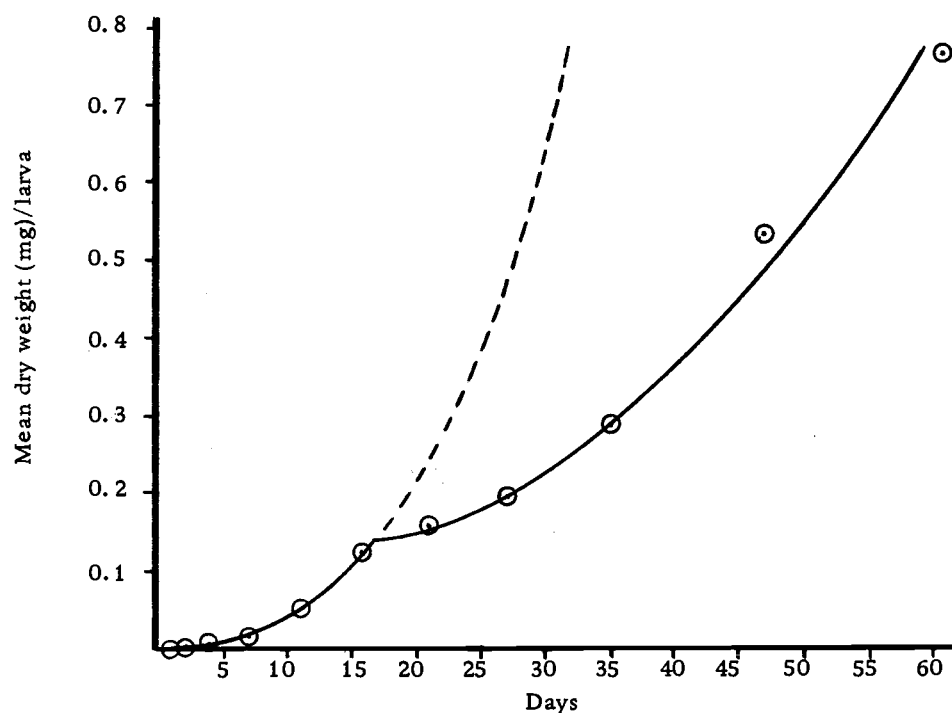


Figure 10. Mean weight of *G. barbipes* larva grown under laboratory conditions, plotted on arithmetic scale.

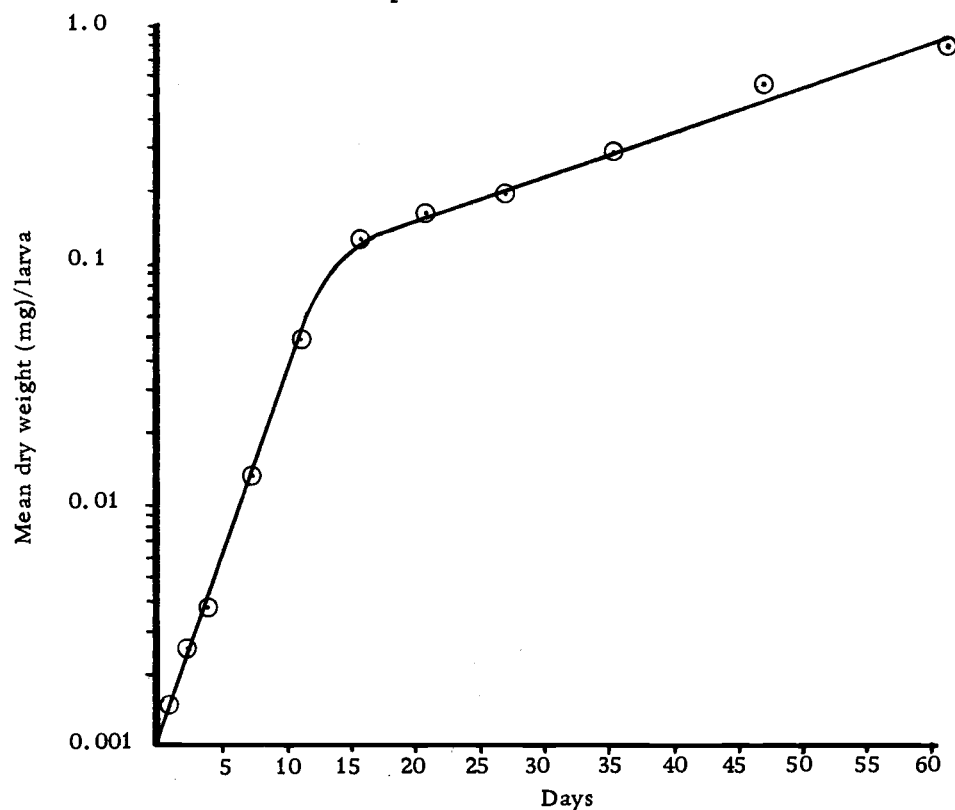


Figure 11. Mean weight of *G. barbipes* larva grown under laboratory conditions, plotted on logarithmic scale.

dry dog food was added to the aquarium water. Studies of feeding habits of benthic Chironomidae by Kajak and Warda (1968) indicated that midge larvae are able to select algal cells out of bottom sediments even though certain groups (green and blue-green algae) are difficult to assimilate. They found diatoms were most easily digested. Perhaps if larger quantities of algae would have been made available to G. barbipes in the present study, the high growth rate during the first 16 days would have been sustained, in which case the dotted line of Figure 10 would better describe the growth of G. barbipes larvae.

Figure 12 gives the relative growth rates of the G. barbipes cohort based on the mean weight per individual for all instars present. The horizontal lines designate the duration and the "bell-shaped" curve the relative abundance of individuals within each instar stadia. The relative growth rate varied considerably throughout the generation. First and second instar larvae grew extremely fast accounting for the high rate during the first ten days, 400 to 200 mg/g/day. When the quantity of larger, slow-growing individuals increased, the relative growth rate of the cohort decreased to 50 mg/g/day. Size distribution of individuals within a cohort therefore affects the growth rate of the cohort.

The variation which occurred in the relative growth rate within each instar stadia is not evident in Figure 12, except for about the

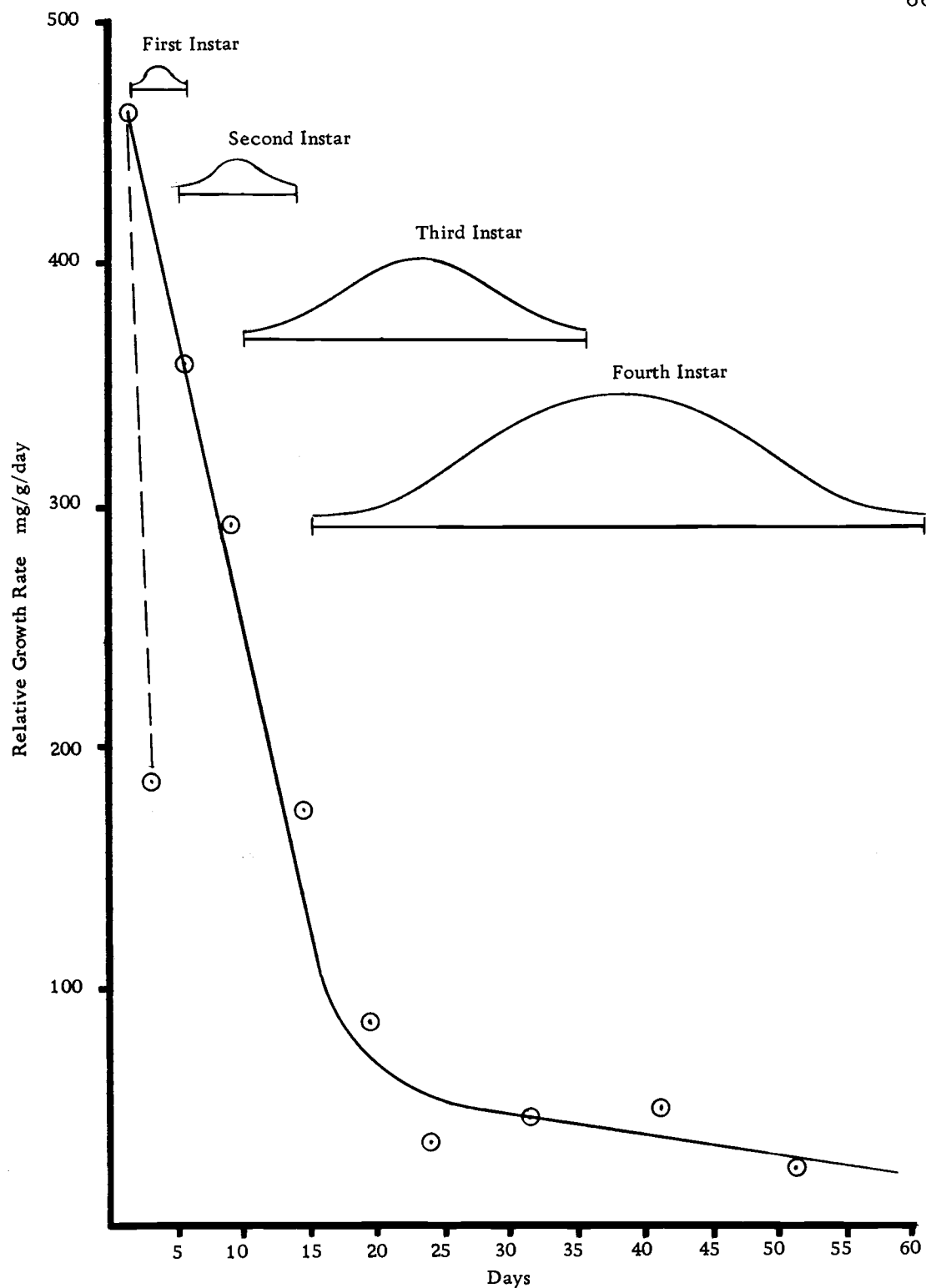


Figure 12. Relative growth rates of *G. barbipes* cohort grown under laboratory conditions. Horizontal lines indicate the approximate duration of each instar and the "bell-shaped" curve the relative abundance of larvae within each stadia. Temperature 75° F.

first week when only first instar larvae were present. The dotted line shows a decrease from 460 to 180 mg/g/day in the first four days. Separate calculations of growth within instar stadia were made. The rate of growth of each instar after molting was greater than the rate prior to molting. The relative growth rate of each newly molted instar larva was also greater than the rate at the end of the preceeding instar. Larvae in all instars went through a short period when the relative growth rate was negative. This coincided with the period when the majority of the individuals within the instar were preparing to molt. The ranges in growth rates within instars were: first, +461 to -111 mg/g/day; second, +322 to -64 mg/g/day; third, +101 to -29 mg/g/day and fourth, +63 to -33 mg/g/day.

The growth rates for each instar and for a cohort would have been impossible to measure if more than one age class had been present. This fact makes it impossible to study directly the growth of natural populations. The alternative procedure developed to eliminate recruitment was to conduct growth studies of natural populations in plastic cylinders, as previously described.

Growth of Natural Population of *G. barbipes*

Relative growth rates of the natural population of *G. barbipes* in Stratum I of the secondary lagoon varied from a single negative value of -4 mg/g/day to +96 mg/g/day. Weekly mean growth rates

in cal/kcal/day from May, 1967 to November, 1967 are given in Table 7.

The most apparent factors affecting growth in this study were oxygen concentrations, food supply, temperature and biomass density. It was difficult to determine whether DO concentration or limited food quantities were responsible for reduced growth rates because these two factors are related in two ways: (1) the quantity of phytoplankton is proportional to DO concentration and (2) for growth to take place both food and oxygen are essential. The quantity and quality of food is known to be important, as previously indicated. Also, experience in rearing G. barbipes has made it evident that algae is the best food for maximum growth. On August 21, 1967 the algae died in the secondary lagoon and anaerobic conditions developed. Growth rates decreased from 53 cal/kcal/day to 23 and 22 cal/kcal/day during the following two week period of low DO and food supply. This reduced growth rate probably resulted because both factors became limiting.

Biomass density has been demonstrated to have an indirect effect on growth. Growth rates studies were attempted in plastic container but growth ceased at high densities (20 to 25 g/m²). At first, growth was thought to be directly density dependent until it was realized that food supply was inadequate. However, it is felt that there are a set of circumstances occurring in natural populations,

and observed in the laboratory, which could result in growth being density dependent. At extremely high densities midge larvae build tubes on top of each other. This causes the larvae at lower depths to have their tubes effectively blocked at both ends. When this occurs the supply of food and oxygen would certainly be reduced, as compared to larvae which have access to the mud-water interface.

Temperature has been demonstrated to have an effect on developmental rates (Biever, 1965; Hilsenhoff, 1966), although no specific temperature-growth rate studies were undertaken. Growth rates decreased through September, October and November, 1967 apparently in response to lowering temperatures. Mean monthly temperatures decreased from 24° C in August to 18° C in September, 12° C in October and 9° C in November. However, algal blooms did not occur during these last three months so there could have been a reduction in phytoplankton food. Dissolved oxygen concentrations were never critical for extended periods throughout this time interval so this factor was probably not operating to reduce the growth rate. Therefore, temperature and reduction of food supply were considered responsible for the slow growth rates in the fall of 1967.

Numerous other factors could have affected the growth rate of G. barbipes, in addition to the more obvious factors discussed above. Biologically and chemically the bottom sediments are extremely active. Metabolic by-products of anaerobic decomposition such as ammonia,

hydrogen sulfide, methane gas and many of the organic acids must have been tolerated by G. barbipes, but at times may have adversely affected some physiological processes. These same factors could also be operating to limit the invasion of other less tolerant species of benthic insects.

Emergence

Emergence data for the primary and secondary lagoons are summarized in Tables 4 and 5. Number of adults per m^2 was converted to grams/ m^2 using the mean weight of 1.88 mg/adult. This figure was a mean weight from a sample of equal numbers of males and females collected from emergence traps. The mean energy content per gram dry weight was 5.26 kcal. The greater success of G. barbipes in the secondary lagoon compared with the primary is apparent in that five times more adults emerged from the secondary cell. The estimated emergence of G. barbipes as total number, grams dry weight, and pounds wet and dry weight is given in Tables 4 and 5.

Emergence rates were affected by adverse environmental conditions. Emergence almost ceased for two days when the secondary lagoon became septic on August 21, 1967. The number of adults emerging was reduced 3,000/ m^2 in the week of anaerobic lagoon water (Table 5).

Table 4. Emergence of G. barbipes from the primary lagoon, Monmouth, Oregon, May, 1967 to August, 1967.

Date		number/m ²	grams/m ²	kcal/m ²	Accumulated kcal/m ²
May	6-12	446	0.838	4.409	4.409
	13-19	138	0.259	1.362	5.771
	20-26	189	0.356	1.873	7.644
	27- 2	113	0.212	1.117	8.761
June	3- 9	226	0.425	2.234	10.996
	10-16	711	1.337	7.031	18.027
	17-23	15	0.028	0.149	18.176
	24-30	3130	5.880	30.950	49.130
July	1- 7	2077	3.900	20.540	69.670
	8-14	689	1.290	6.810	76.480
	15-21	408	0.770	4.040	80.520
	22-28	183	0.340	1.810	82.330
August	29- 4	30	0.060	0.300	82.630
	5-11	<u>0</u>	<u>0</u>	0	<u>82.630</u>
Total		8355	15.695		82.630

Total for the lagoon (7.1 ha)

29 million adults emerged
34,000 grams dry weight
75 pounds dry weight

500 pounds wet weight
261,276 kcal

Extremely warm surface water was also observed to adversely affect emergence. On a few days, surface waters reached 31 to 32° C by late afternoon. Bottom temperatures were 21° C. Adults never emerged from pupae which swam through this layer of warm water and dead pupae accumulated in one corner of the lagoon.

Table 5. Emergence of G. barbipes from the secondary lagoon, Monmouth, Oregon, May, 1967 to October, 1967.

Date	number/m ²	grams/m ²	kcal/m ²	Accumulated kcal/m ²
May	6-12	357	0.67	3.53
	13-19	186	0.53	5.36
	20-26	243	0.46	7.77
	27- 2	312	0.59	10.85
June	3- 9	247	0.46	13.30
	10-16	366	0.69	16.92
	17-23	344	0.65	20.32
	24-30	775	1.46	27.98
July	1- 7	1614	3.03	43.94
	8-14	861	1.62	52.46
	15-21	2894	5.44	81.08
	22-28	3755	7.06	118.21
	29- 4	4412	8.29	161.84
August	5-11	3508	6.59	196.52
	12-18	4734	8.90	243.43
	19-25	1754	3.30	260.68
	26- 1	2055	3.86	281.01
September	2- 8	3153	5.93	312.18
	9-15	2238	4.21	334.31
	16-22	904	1.70	343.25
	23-29	280	0.52	346.02
	30- 6	54	0.10	346.55
October	6-13	<u>0</u>	<u>0</u>	<u>346.55</u>
Total		35,048	65.89	346.55

Total for the lagoon (8.7 ha)

139 million adults
 164,000 grams dry weight
 361 pounds dry weight

2,410 pounds wet weight
 1.37 million kcal

Respiration

Respiration rates of G. barbipes larvae are affected by density, temperature, environmental DO concentrations and concentration of planktonic food particles. Only the first three variables were studied.

Larvae live in tubes constructed from salivary secretions. The habitat surrounding the larvae is certainly anaerobic in the lagoon environment. Therefore larvae must irrigate their tubes with an undulating motion to replenish their microhabitat with oxygenated water from above the mud-water interface. Water passing through the tube supplies the necessary oxygen and also the planktonic food particles, primarily algal cells. Walshe (1950) observed that Chironomus plumosus larvae spent varying amounts of time irrigating tubes, feeding and resting, depending on environmental oxygen concentrations. More time was allotted to irrigation and less to feeding and resting when DO decreased. In the present study, respiration rates were measured in chambers filled with distilled water and without food. Larvae rarely rested, even at high oxygen concentrations, possibly in response to the absence of food. The sand substrate might have affected their behavior, even though larvae readily built tubes.

Figures 13 through 16 give the results of oxygen consumption experiments conducted at 10, 15, 20 and 25° C. A best fit line was drawn through consumption estimates in each biomass class interval

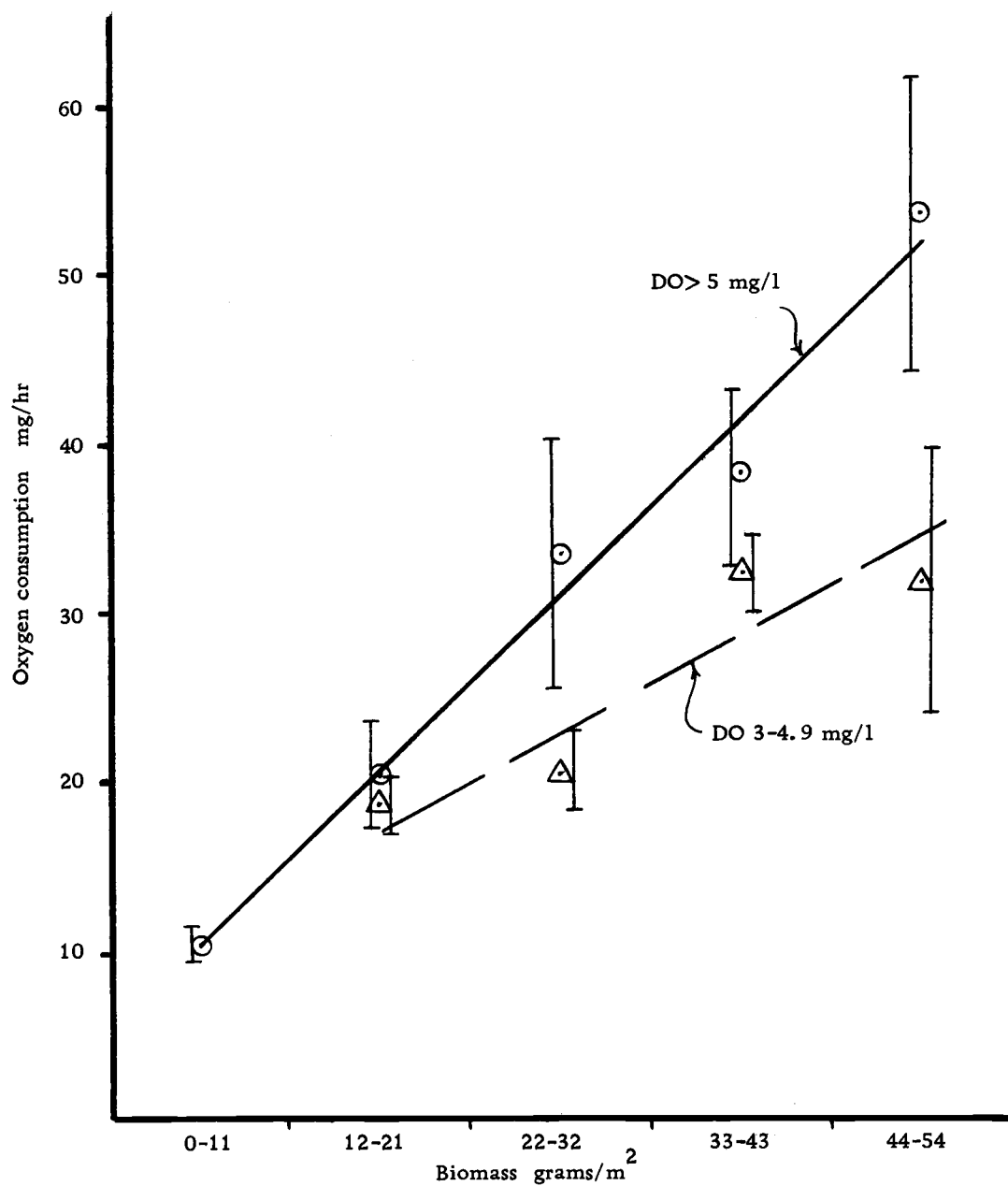


Figure 13. Oxygen consumption rates of *G. barbipes* at 10°C with varying densities and environmental DO concentrations.

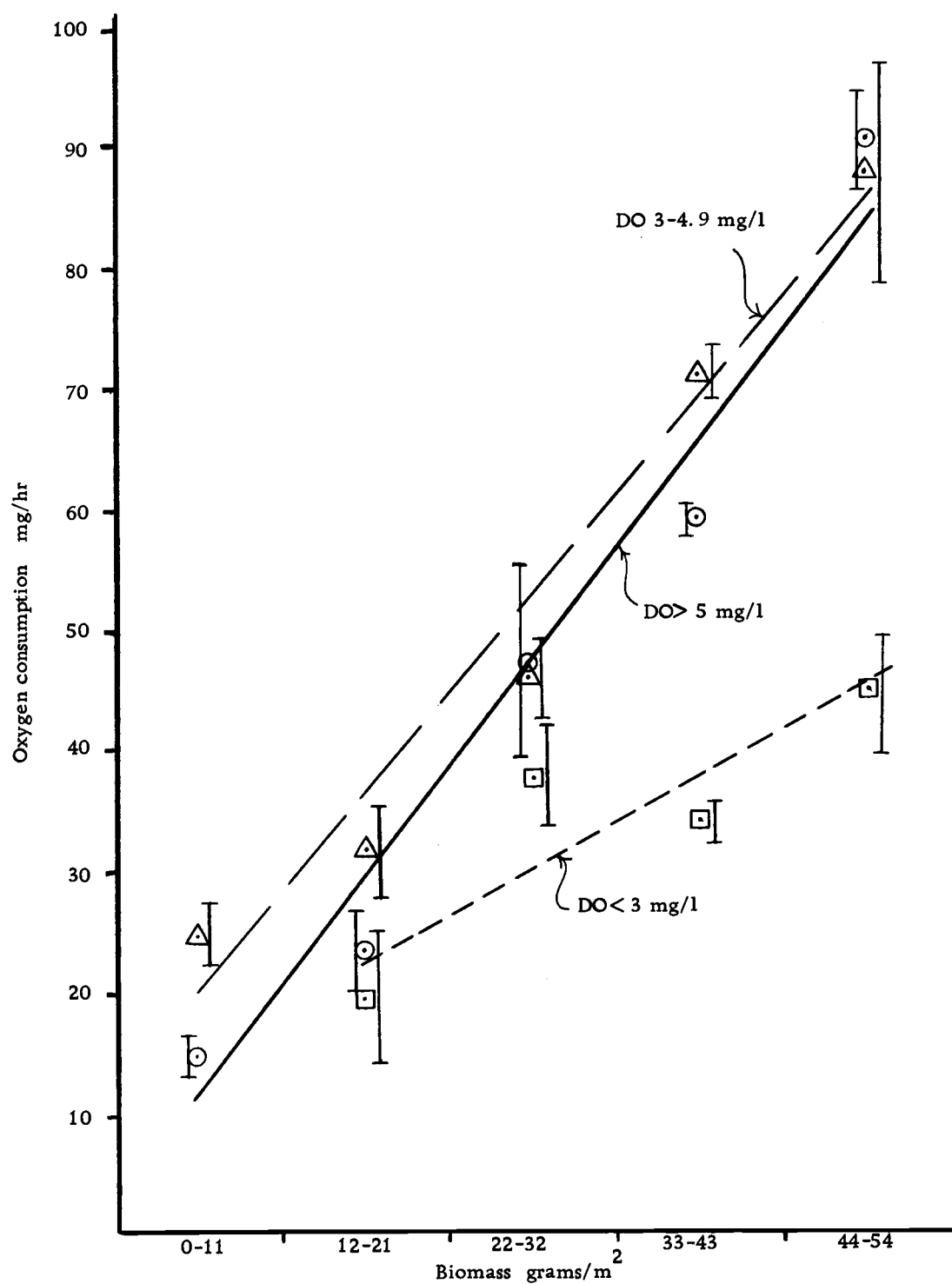


Figure 14. Oxygen consumption rates of *G. barbipes* at 15° C with varying densities and environmental DO concentrations.

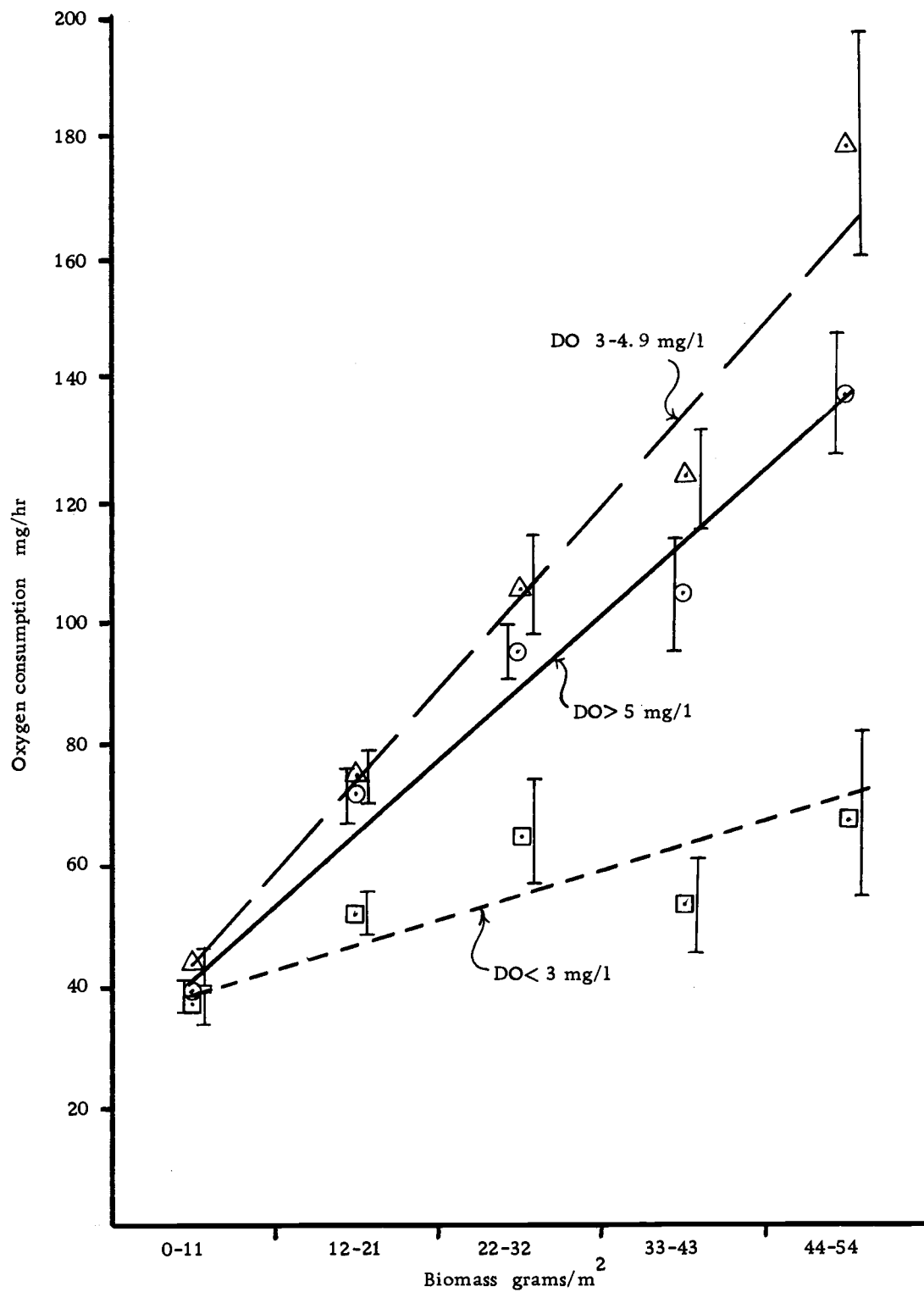


Figure 15. Oxygen consumption rates of *G. barbipes* at 20°C with varying densities and environmental DO concentrations.

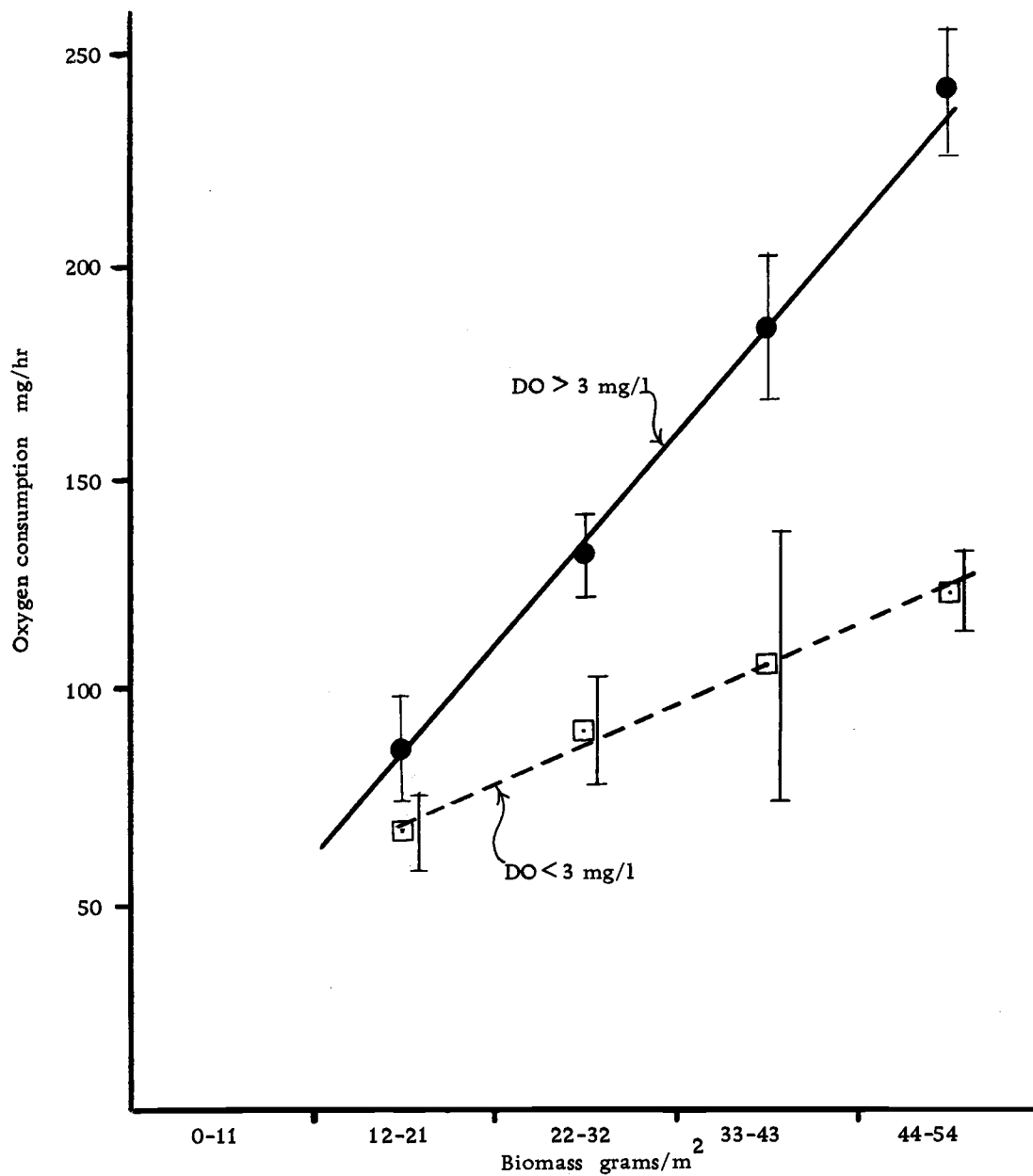


Figure 16. Oxygen consumption rates of *G. barbipes* at 25° C with varying densities and environmental DO concentrations.

at oxygen concentrations of $> 5.3-4.99$; and < 3 mg/l. The vertical line through each estimate indicates the standard error associated with each estimate.

The effects of increases in temperature and biomass concentration were about as would be expected. For each 10°C increase in temperature the respiration rate was about three times greater. There was a doubling in the respiration rate when biomass was doubled, except when environmental DO was less than 3 mg/l.

Results of oxygen consumption studies at different environmental DO concentrations indicated that at 10°C maximum consumption rates occurred when the DO was highest. At 25°C no difference in consumption rates could be seen above 3 mg/l. However, at 15° and 20°C the maximum consumption rate did not occur at highest oxygen levels. The oxygen consumption rate at 3 to 4.99 mg/l was higher than when the DO exceeded 5 mg/l (Figure 14 and 15). The difference in these lines was not statistically significant but the phenomenon could have biological significance for survival of G. barbipes larvae in environments of frequent low DO. The presence of haemoglobin is probably the factor responsible for higher consumption rates at lower oxygen tensions. Walshe (1947) showed that haemoglobin does not actively transport oxygen in Tanytarsus larvae when oxygen is greater than 25 percent saturation, but does transport oxygen in a range of 5 to 25 percent saturation. Ecologically, bloodworms are considered

to have an advantage over many other aquatic invertebrates, because they can survive in environments of low DO. However, they are not only able to survive in low oxygen concentrations, but their advantage is compounded by the fact that they physiologically function best at low oxygen concentrations.

The environment of a lagoon has a tremendous potential to support production of phytoplankton-feeding insects because of the extremely high primary production rates. Yet, this environment is not available to all plankton feeding species because most are unable to cope with the adverse conditions of low DO. Numerous species are often found in sewage lagoons but they never occur in great quantities like G. barbipes. These insects which merely survive in the lagoon environment under persistent low DO might not be able to effectively utilize the rich supply of food for growth because they are unable to transport sufficient quantities of oxygen to metabolize the food. On the other hand, a midge like G. barbipes can consume large quantities of food. Then with low oxygen tensions the haemoglobin actively transports oxygen in sufficient quantities to meet standard metabolic rates, plus activities of feeding and undulating, plus that needed to make the energy of food available for rapid growth.

Table 6 gives the total monthly respiration rate of G. barbipes in Stratum I of the secondary lagoon (1271.90 kcal/m^2). Monthly respiration is composed of two parts, respiration of biomass and

Table 6. Monthly biomass respiration estimates of *G. barbipes* and respiration of those individuals (as biomass) that died each month. Secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.

Month	Oxygen consumed mg/m ² /day	Energy burned ₂ kcal/m ² /day	Respiration of biomass ₂ kcal/m ² /month	Ratio of biomass to biomass that died each month x 100 %	Respiration of individuals that died kcal/m ² /month	Total respiration kcal/m ² /month
May	592	2.00	62.00	2.1	1.30	63.30
June	1574	5.32	159.60	11.8	18.83	178.43
July	3013	10.18	315.58	12.8	40.39	355.97
August	2658	8.98	278.38	16.5	45.93	324.31
September	1969	6.66	199.80	39.0	77.92	277.72
October	258	0.87	26.97	38.2	10.30	37.27
November	237	0.80	24.00	13.42	3.22	27.22
Total			1066.33		197.89	1264.22

respiration of individuals that died between intervals. Biomass respiration rates were obtained by applying field data on temperature, biomass and DO to the oxygen consumption rates determined in laboratory experiments (Figures 13-16). To include the respiration of individuals that died it was necessary to calculate the mean monthly biomass directly attributable to larval mortality.

There are three possible fates of tissue produced: (1) it can stay in the lagoon as biomass, (2) emerge or (3) die. The biomass which died each week was calculated by budgeting the difference of weekly production, positive or negative changes in biomass, and loss by emergence. Data necessary for these calculations were obtained from Tables 5 and 7.

Figure 17 gives the mean monthly biomass, loss of biomass resulting from individuals that died each month (cross-hatched) and the percentage that respiration of the biomass was underestimated. Respiration of biomass was calculated as previously described and is given in Table 6. Then the correction for mortality was made by multiplying the biomass respiration rates by the percent biomass not included in that estimate. Figure 17 shows that the amount of biomass lost to mortality increased from May to September, then decreased in October and November. The percent of biomass that died each month increased from June to October then decreased in November. If respiration of these individuals that died had not been included, the

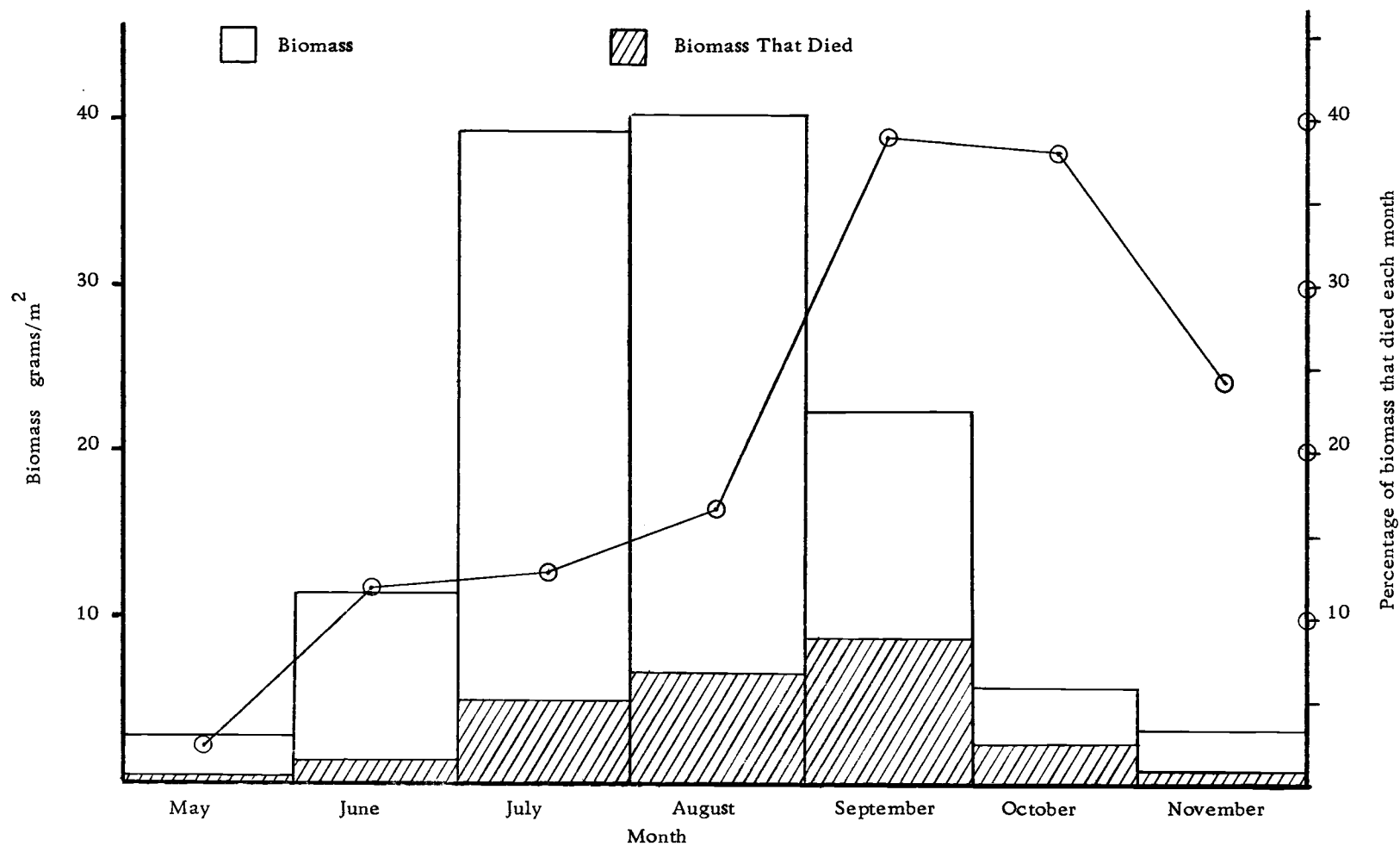


Figure 17. Monthly biomass compared with biomass of those individuals that died each month. Percent biomass mortality calculated as biomass of mortality/biomass \times 100. Secondary lagoon, Monmouth, Oregon.

respiration estimate would have been about 20 percent too low. Teal (1957), in budgeting the energy of respiration in the chironomid Calopsectra dives (Johannsen), also showed that respiration of animals that died was about 20 percent of biomass respiration.

Production of G. barbipes

Production of G. barbipes in the secondary lagoon is given in Table 7. Total production from May, 1967 to November, 1967 in Stratum I was 808 kcal/m^2 . The total production for the entire lagoon was 3.21 million kcal, assuming that Stratum I accounted for 90 percent of the total production as it did for biomass. Production for the entire lagoon on a square meter basis was 36.95 kcal/m^2 .

Figure 18 shows the relationship between biomass, production, and emergence. To discuss production one must include a discussion of these other related factors. From May 6 to June 16, the total accumulated production was only 43 kcal/m^2 or about five percent of the total for seven months. Growth rates were high during this period but biomass was so low that the quantity of new tissue elaborated was quite small. In the next week growth rate and biomass nearly doubled with production being 40 kcal/m^2 in the week. From June 23 to July 7, the biomass tripled as production increased. Emergence rates were quite low so biomass accumulated. Growth rates were probably underestimated during this time interval because calculated production

Table 7. Growth, biomass and production of *G. barbipes* in Stratum I of the secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.

Date	Growth cal/kcal/day	Biomass		Production kcal/m ² /wk	Accumulated production kcal/m ²
		grams/m ²	kcal/m ²		
May	6-12	73.00	2.16	10.76	5.50
	13-19	51.86	2.97	14.80	10.87
	20-26	36.01	3.58	17.84	15.37
	27- 2	36.29	4.36	21.70	20.88
June	3- 9	37.00	6.29	31.32	28.99
	10-16	53.85	7.97	39.65	43.94
	17-23	96.00	12.14	60.45	84.56
	24-30	49.12	26.94	134.06	130.66
July	1- 7	56.54	36.35	180.92	202.16
	8-14	44.02	39.06	194.41	262.14
	15-21	44.02	41.88	208.71	326.42
	22-28	40.00	40.27	200.44	382.54
	29- 4	37.43	38.54	191.80	432.80
August	5-11	77.72	34.82	173.28	527.07
	12-18	59.88	38.81	193.17	608.04
	19-25	53.43	44.02	219.09	689.99
	26-1	23.14	45.13	224.62	726.36
September	2- 8	21.71	39.83	198.22	756.49
	9-15	33.77	24.11	119.98	784.85
	16-22	13.04	14.89	74.11	791.61
	23-29	10.57	11.50	57.23	795.85
	30-6	5.29	8.68	43.22	796.45
October	7-13	11.29	7.14	35.54	800.26
	14-20	21.00	5.63	28.04	804.38
	21-27	13.72	4.61	22.94	806.58
	28- 3	4.00	3.24	16.14	807.04
November	4-10	4.00	3.24	16.14	807.49
	11-17	4.00	3.24	16.14	807.94
	18-24	4.00	3.24	16.14	808.39
Total					808.39
Mean	36.00		95.20		

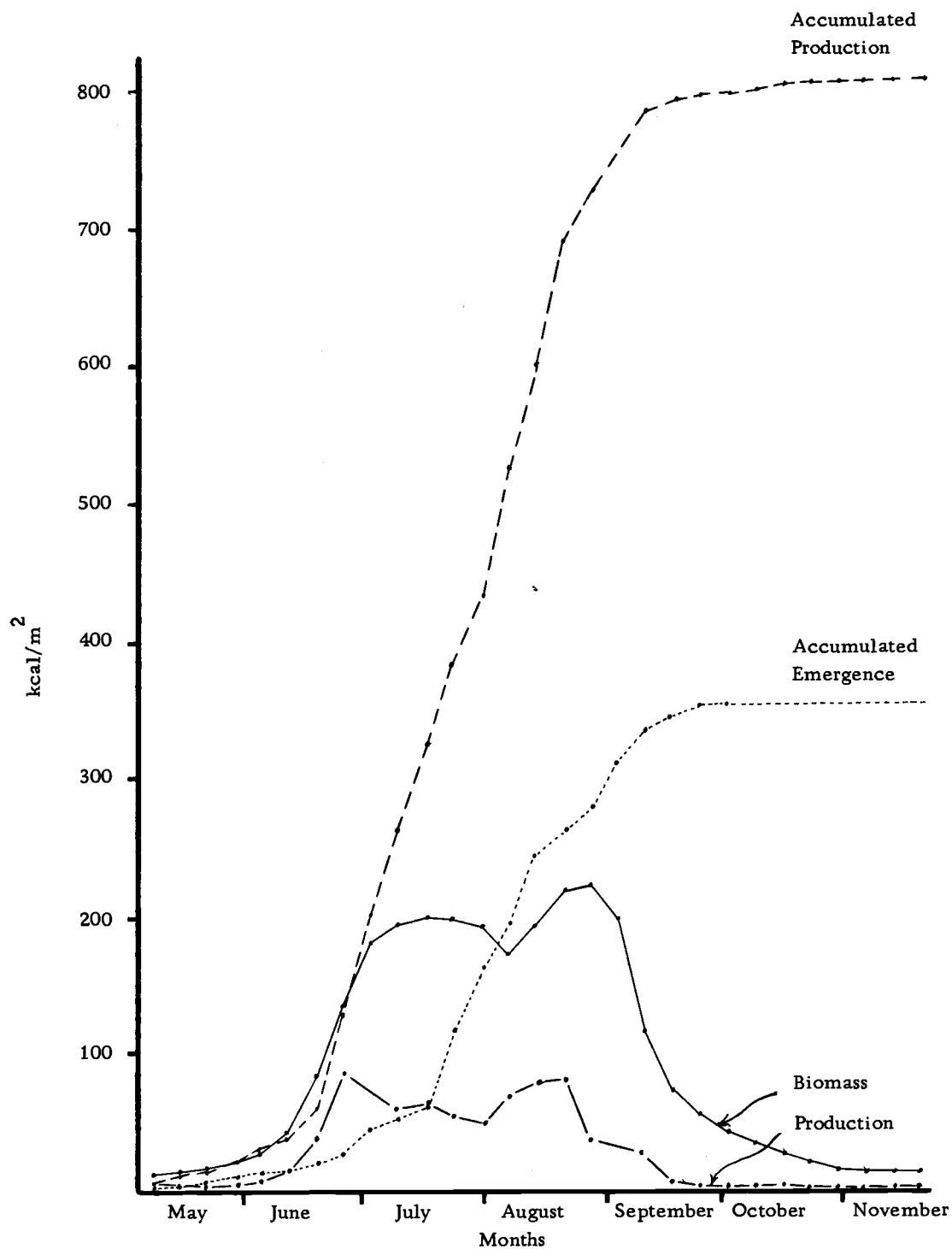


Figure 18. Biomass, production, accumulated production and accumulated emergence of *G. barbipes* in kcal/m^2 in Stratum I of the secondary waste stabilization lagoon, Monmouth, Oregon, May, 1967 to November, 1967.

did not account for the increase in biomass. It was at this time that growth rates determined in containers were found to be inadequate. After July 14 the plastic cylinders were used to measure growth of the undisturbed natural population.

A larger percentage of production went to emergence after the first of July, causing the biomass to level off at about 200 kcal/m^2 . The first mass emergence began on July 19, with about 40 kcal/m^2 emerging every week, until August 19. Biomass declined by the last week of July because the rate of biomass loss to emergence and mortality exceeded the production rate. In the first three weeks of August the growth rate increased, partly in response to removal of some biomass (possibly indicating density dependent growth) and partially due to the presence of young larvae, the progeny of adults that emerged during the preceeding three weeks. As demonstrated previously, the growth rate of the larval population is affected by the size distribution of the individuals in the population.

On August 21, the lagoon became anaerobic causing a decline in growth, production and emergence. Biomass was unaffected and actually continued to increase. This demonstrated that larvae are not directly affected by adverse environmental conditions of short duration. Emergence increased again during the recovery period, but growth and production remained low.

The decrease in biomass after September 1 resulted from a

set of conditions exactly opposite to those causing the biomass increase in the last two weeks of June. In June biomass was accumulating because production was much greater than emergence, whereas, in September a larger portion of production went to emergence. From June 17 to July 7, the ratio of production to emergence was 5.6:1; from August 26 to September 15 it was 1:3:1.

Adult emergence ceased after September 22. The decrease in biomass after that time resulted from larval mortality. Biomass was also affected by larval mortality throughout the year, but was only obvious when the biomass decrease exceeded the emergence rate. Ninety-two percent of the total production of G. barbipes (742 kcal/m²) took place in about half the growing season, June 17 to September 15.

Comparison of G. barbipes Production with Other Studies

Production of G. barbipes was extremely high when compared to production estimates of other aquatic animals (Table 8).

These comparisons indicate a general higher level of production in the herbivores (midges, mayfly and snail) than in the carnivores (Sialis and trout). G. barbipes production is the highest in the energy column. Production of O. silicula, on a dry weight basis was higher than G. barbipes because the weights were affected by the shell of the animal. Energy per square meter gives a better

Table 8. Comparative production estimates of aquatic insects, snail and trout.

Species and source	pounds/acre	grams/m ²	kcal/m ²
<u>Glyptotendipes barbipes</u>	7888 wet, Stratum I	162 dry	808
	1420 dry, Stratum I		
	366 wet, All Strata		37
	66 dry, All Strata		
<u>Microtendipes chloris</u> (midge) Kajak and Rybak, 1966	---	24 wet	---
<u>Oxytrema silicula</u> (snail) Earnest, 1967	3267* dry	366 dry*	333.43
<u>Baetis vagans</u> (mayfly) Waters, 1965	112 wet 19 dry*	12.6 wet 2.0 dry*	11.0*
<u>Sialis californica</u> (alderfly)	17 dry	1.88	10.14
<u>Sialis rotunda</u> Azam, 1969	23 dry	2.63	14.00
<u>Salmo trutta</u> (brown trout) Allen, 1952	500 wet	----	---
<u>Salmo clarki</u> (cutthroat trout) Warren et al., 1964	---	----	5.92
<u>Salvelinus fontinalis</u> (brook trout) Hunt, 1966	90.5 wet	----	---

* Conversion estimated.

indication of actual tissue elaborated.

Two alternative procedures were used to estimate production of G. barbipes in the primary and secondary lagoons in 1966 and primary lagoon in 1967 when growth rates were not measured. One method utilizes a turnover ratio and the other a yearly mean growth rate. Waters (1965) defined "turnover ratio" as the ratio of annual production to mean annual population density (Production/Mean Biomass). Once determined, production equals turnover ratio times the mean annual biomass. A turnover ratio of 8.49 was determined from definitive data on the production and biomass of G. barbipes. The second method was to use the yearly mean growth rate/day (36 cal/kcal/day) of G. barbipes (Table 7). Production was then calculated; (1) as the sum of the products of yearly mean growth rate (36 cal/kcal/day), monthly mean biomass and days in the month, and (2) by multiplying the yearly mean growth rate (36 cal/kcal/day), times the yearly mean biomass, times days in the season.

Table 9 compares the results of these "short-cut" methods of estimating production. Results obtained using the yearly mean growth rate were similar. The production estimates in 1966 using the turnover ratio are less than the estimates obtained by the mean growth rate methods. The accuracy of the techniques can only be inferred by comparison to the production of G. barbipes in the secondary lagoon in 1967. The 2.92×10^6 kcal is assumed to be correct because

Table 9. Comparison of production of *G. barbipes* in primary and secondary lagoons in 1966 and 1967. Calculations made using "short-cut" methods of turnover and mean growth rate.

	Actual measurements of growth rates and biomass in Stratum I	Total Production in kcal x 10 ⁶		
		Sum of mean yearly growth rate x monthly biomass	Mean yearly growth rate x mean yearly biomass	Turnover ratio x mean yearly biomass
1966				
Primary Cell (7.1 ha)	--	11.75	11.66	7.54
Secondary Cell (8.7 ha)	--	39.91	39.63	25.61
1967				
Primary Cell	--	1.19	1.19	1.53
Secondary Cell	2.92	3.05	2.89	3.35

it was based on frequent measurements of growth and biomass. Each of the "short-cut" methods have results similar to the best estimate, but the results of mean yearly growth rate times biomass are most acceptable.

The only application the "short-cut" methods have is when production of a species has been determined at one time or place, and it is desired to estimate production of the same species in another locality or at a different time. One can then forego the tedious work of measuring growth. However, the validity of the above techniques is contingent upon three facts: (1) the production was initially measured using valid estimates of growth and biomass, (2) that the turnover ratio or mean growth rate of a species is indeed relatively constant under similar ecological conditions and (3) that the habitats are ecological homologues.

Production of G. barbipes, calculated from the "short-cut" methods indicates that both the primary and secondary lagoons had about a 10-fold greater production in 1966 than in 1967. This great difference in production from 1966 to 1967 was as expected because the biomass in these two years differed by about the same order of magnitude.

Estimates of production rates of fish tissue have practical application in determining the amount of fish to be harvested without depleting the stock. Production data for aquatic insects are not as

obviously applicable to man's needs. However, if more estimates of production rates of aquatic insects were available, ecologists would have a more realistic basis for understanding and explaining the interactions of populations within the aquatic environment. An insight might also be gained on how to manipulate the effective environment of the species of interest to either maximize or minimize its production.

Production of aquatic insects is measured in bioenergetic studies to determine the fate of all tissue produced. Figure 18 shows that, of the 808 kcal/m^2 produced by G. barbipes, only 347 kcal/m^2 actually emerged as adults. The difference of 463 kcal/m^2 was recycled within the lagoon. Studies of biomass only and/or emergence would have given no indication of the rate of tissue elaboration. In addition there would have been no estimate of recycled production. To measure production of G. barbipes it was also necessary to measure growth, which in itself was valuable. Frequent measurement of growth resulted in a better understanding of how the effective environment (DO, temperature, food, emergence, density and etc.) operated to increase or decrease the size of the larval population.

The Role of *G. barbipes* in the Lagoon Ecosystem

In 1887 Stephen Forbes presented a paper to the membership of the Peoria Scientific Association describing the interdependency of all aquatic forms within a lake, referring to the lake as a "microcosm" (Forbes, 1925). Today, ecologists work within the conceptual framework of the ecosystem, analogous to Forbes's microcosm, in an attempt to understand the interrelations and exchange of materials which take place between organisms in nature.

A waste stabilization lagoon is a simplified ecosystem. Essential inorganic and organic matter enters the system in raw sewage at a rate much faster than occurs in natural ponds or lakes. Primary producers, in the form of unicellular planktonic algae, are extremely abundant. Consumers (midges and zooplankton) are present to utilize the vast quantities of algae. Energy is eventually made available to decomposers from the organic matter in raw sewage plus the autochthonous material that died within the lagoon. These components then function, with time, causing a cycling of nutrients and transfer of energy from one level to the next, with a loss of energy at each step. Self purification of organic wastes is achieved by the action of biological organisms and the loss of energy in respiration, but little is known of the actual contribution of each biological component affecting the total degradation of energy.

The approach used to evaluate the role of G. barbipes in the lagoon ecosystem was to determine the amount of energy expended in growth and emergence and compare this to the fate of energy in other pathways of the lagoon.

Table 10 gives the energy budget for G. barbipes in the secondary lagoon from May to November, 1967. The monthly data are presented as kcal/m^2 for Stratum I (3612 m^2) and as a total for all strata (86903 m^2). The grand total at the bottom of the table is the total amount of energy attributed to each component: emergence, mortality, production, respiration and assimilation.

Assimilation represents the amount of energy that was consumed by midge larvae. The assimilated energy was utilized to maintain physiological processes (respiration) and to build new tissue (production). Tissue produced either survived to the imaginal stage (emergence) or died as larvae or pupae (mortality). Table 10 shows that G. barbipes consumed 8.23×10^6 kcal, of which 5.02×10^6 kcal (60 percent) went to maintenance of physiological processes. Of the 3.21×10^6 kcal of tissue produced 40 percent reached the adult stage and 60 percent was recycled to the decomposers. Seasonally, most production occurred in June, July and August, with emergence being highest in July and August. Mortality was greatest in August and September.

The energy budget of G. barbipes during the definitive study

Table 10. Energy budget of *G. barbipes* in kcal/m² in Stratum I and for all strata of the secondary lagoon, Monmouth, Oregon. May, 1967 to November, 1967.

	Emergence kcal/m ²	Mortality kcal/m ²	Production kcal/m ²	Respiration kcal/m ²	Assimilation kcal/m ²
May					
Stratum I	10.85	1.19	20.88	63.30	84.18
Lagoon	0.50	0.05	0.95	2.89	3.84
June					
Stratum I	17.13	27.07	109.78	178.43	288.21
Lagoon	0.78	1.24	5.02	8.16	13.18
July					
Stratum I	90.23	100.54	251.88	355.97	707.85
Lagoon	4.12	4.60	11.52	16.27	27.79
August					
Stratum I	162.80	132.58	343.80	324.31	668.11
Lagoon	7.44	6.06	15.72	14.83	30.55
September					
Stratum I	65.01	175.19	69.49	277.72	347.21
Lagoon	2.97	8.01	3.18	12.70	15.88
October					
Stratum I	0.53	44.60	10.73	37.27	48.00
Lagoon	0.02	2.04	0.49	1.70	2.19
November					
Stratum I	--	2.17	1.81	27.22	29.03
Lagoon	--	0.10	0.08	1.24	1.32
Total per m ²					
Stratum I	346.55	483.34*	808.39	1264.22	2072.59
All Strata	15.83	22.10	36.95	57.80	94.74
Lagoon Total (kcal x 10 ⁶)	1.37	1.92	3.21	50.2	8.23

* Mortality, production and emergence do not balance because of probable low production estimates in June.

(May to November, 1967) is also given in Table 11 and compared with the gross energy budget of the entire lagoon ecosystem for two full years, 1966 and 1967. Adult emergence is the most obvious means by which G. barbipes directly removes energy from the lagoon. This component was 4.24 kcal/m^2 from the primary lagoon and 15.83 kcal/m^2 from the secondary lagoon in 1967. Emergence from both lagoons in 1966 was estimated using the relationships of the 1967 biomass, production and emergence data. The energy of emergence in 1966 was 66 and 184 kcal/m^2 from the primary and secondary lagoons, respectively.

The only other study on the removal of energy by chironomid emergence from an organically enriched environment was reported by Tubb and Dorris (1965). They found that chironomid adults removed 62,500 kcal from ten oil refinery ponds totaling $58,156 \text{ m}^2$, or about 1.1 kcal/m^2 . The energy removed by G. barbipes from the entire lagoon facility was much greater. In 1966 it was 131 kcal/m^2 and in 1967, 8.9 kcal/m^2 .

Although the quantity of energy lost from the lagoon ecosystem through emergence of G. barbipes was large, the energy burned in respiration was about four times greater. In the secondary lagoon in 1967, emergence totaled 15.83 kcal/m^2 but the energy consumed in larval development was 57.80 kcal/m^2 . Table 12 summarizes the removal of energy by emergence and respiration in 1966 and 1967

Table 11. Energy budget of G. barbipes compared to other energy pathways in the primary and secondary waste stabilization lagoons in 1966 and 1967, units are in kcal/m².

							<u>G. barbipes</u>				
	Solar radiation	Influent	Storage	Export	Primary Production	Community Respiration	Production	Emergence	Mortality	Respiration	Assimilation
Secondary Cell											
May-Nov. 1967	983,800	1530	460	322	21,380	28,468	36.95	15.83	22.21	57.80	94.70
Primary Cell											
1966	1,259,250	21099	1618	5626	35,000	49,000	165.30	66.00	99.16	262.61	427.91
1967	1,259,250	21099	1618	5626	35,000	49,000	17.71	4.24	13.08	26.58	44.32
Secondary Cell											
1966	1,259,250	4603	690	1381	27,790	37,000	459.00	184.00	275.00	730.00	1189.00
1967	1,299,280	4603	690	1381	27,790	37,000	36.95	15.83	22.21	57.80	94.70

Table 12. Energy removed by emergence and respiration of G. barbipes from the primary and secondary lagoons in 1966 and 1967.

	Numbers $\times 10^6$	Wet weight pounds	Energy kcal $\times 10^6$	Respiration kcal $\times 10^6$	Total kcal $\times 10^6$
1966					
Primary	575	10,000	4.70	18.67	23.37
Secondary	1,615	28,013	15.99	63.44	<u>79.43</u>
Total					102.80
1967					
Primary	29	500	0.30	1.89	2.19
Secondary	139	2,410	1.37	5.02	<u>6.39</u>
Total					8.58

from both lagoons. In 1966 the emergence of 38,000 pounds wet weight of G. barbipes adults removed a total of 103×10^6 kcal of energy. In 1967 the net effect of midges was much less; 2,910 pounds wet weight of adults accounted for the loss of 8.58×10^6 kcal.

The value of calculating the energy budget of the G. barbipes population in the sewage lagoon is that it enables the fate of assimilated energy to be defined and quantitatively partitioned into production, emergence, mortality and respiration (Table 10). The rate of energy transfer was affected by changes in the effective environment through the seasons. The general conclusion is that G. barbipes was directly responsible for the removal of tremendous amounts of energy from the lagoons (103×10^6 kcal in 1966 and 9×10^6 kcal in 1967). However, before judgment can be made as to the value of this energy loss, the energy budget of the midge has to be compared to the other energy pathways of the ecosystem.

Figure 19 is a simplified energy-transfer diagram of the secondary lagoon from May to November, 1967. The diagram compares the energy budget of the midge to the energy of primary production, community respiration and sewage influent. All possible fates of energy are not shown. No attempt was made to budget the energy of zooplankton, but it is recognized that they could be an important component. Other species of invertebrates such as Tubificidae worms (Annelida:Oligochaeta) and Corixidae (Hemiptera)

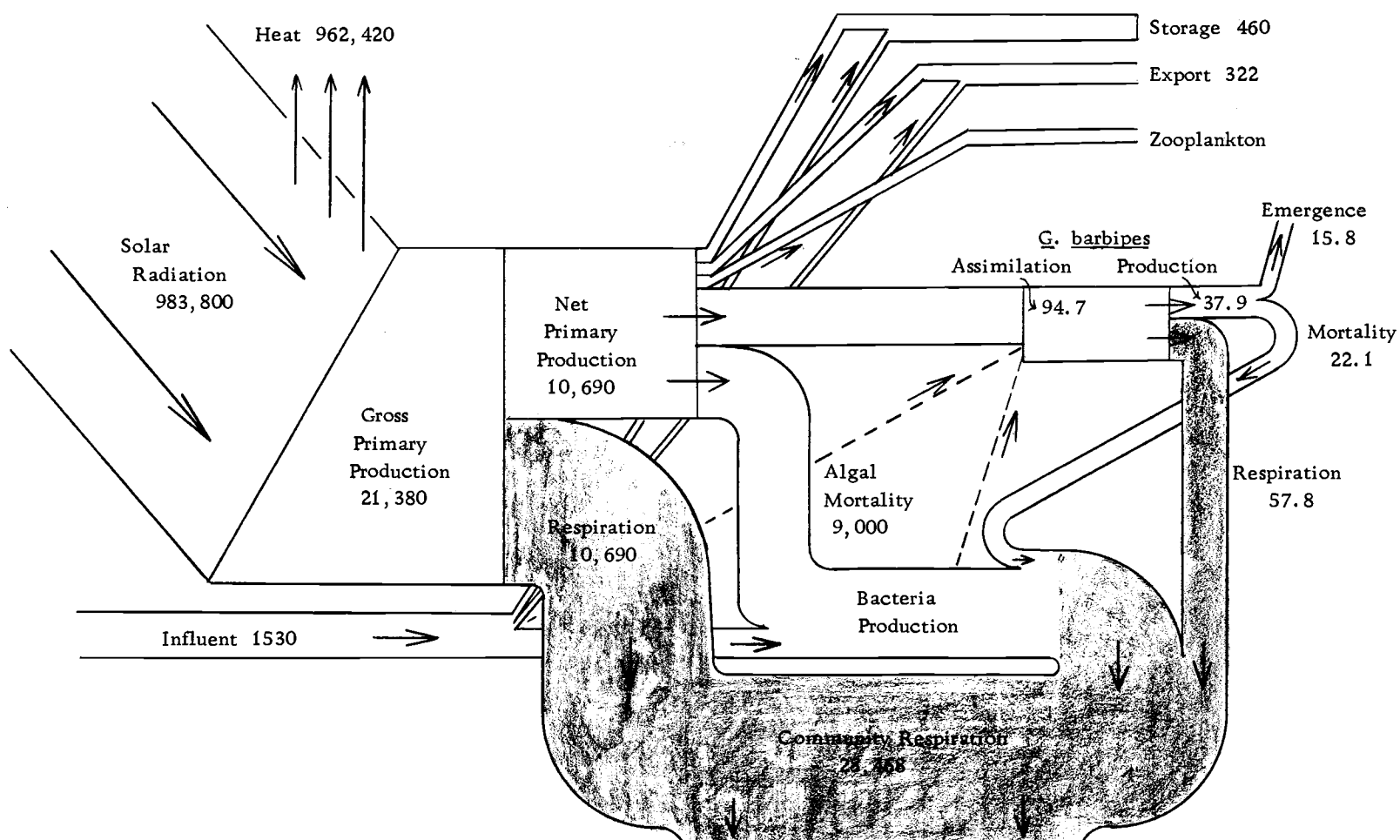


Figure 19. Energy transfer in the secondary waste stabilization lagoon in kcal/m². Monmouth, Oregon, May, 1967 to November, 1967.

were periodically abundant and also could have accounted for significant losses of energy.

The bioenergetic relationships shown in this diagram indicate that the contribution of G. barbipes to the degradation of energy in the sewage lagoon was negligible. Primary producers and decomposers were the dominant energetic components of the lagoon ecosystem. Primary production totaled $21,380 \text{ kcal/m}^2$, with an efficiency of utilization of solar radiation of about 2.2 percent. There is no way of determining the respiration of phytoplankton in nature so one half of primary production was arbitrarily channeled to respiration. Other authors have assigned various values of gross primary production to algal respiration (Odum, 1957; Teal, 1957; Davis and Warren, 1965). The transfer of $9,000 \text{ kcal/m}^2$ of the net primary production to algal mortality was also arbitrarily chosen and is probably too low an estimate. The significant relationship is that assimilation by midge larvae was only 95 kcal/m^2 , whereas, $10,690 \text{ kcal/m}^2$ of potential food was produced and died without being consumed, at least by midges.

It was shown by Usinger and Kellen (1955) that G. barbipes larvae significantly reduced algal populations in laboratory experiments. Many authors have agreed that midges are important because they remove algal cell material which otherwise would eventually end up in the effluent and add to the BOD load and pollution.

In this study G. barbipes removed 0.5 percent of the net primary production. It is therefore concluded that even though there was a dense population of midge larvae present in the lagoons, G. barbipes had no significant effect on the reduction of algal cells in the effluent.

It is also realized that under some circumstances the amount of algae removed by midge larvae could be a larger portion of the net primary production. It should be remembered that in this study, the dense larvae populations were restricted to less than five percent of the total area of the lagoon. In shallow lagoons, with dense concentrations of larvae distributed evenly over the bottom, the total effect of respiration and emergence would be much greater. The data for 1966 in the secondary lagoon indicate this possibility (Figure 20 and Table 11). In that year the total larval biomass was much greater than in 1967 because the larvae occupied about 30 percent of the lagoon bottom. Emergence and respiration removed 914 kcal/m^2 , or about 6.6 percent of the total net production. It is felt that under optimum conditions the contribution of midges, by the removal of energy, could exceed that reported in this study. The bioenergetic role of chironomids would be greatest in shallow oxidation ponds in some warmer regions of the country, especially if the growing season of the midge lasted most of the year. However, shallow depth and higher DO concentrations might also enable other species of insects to invade the lagoon environment and compete with G. barbipes,

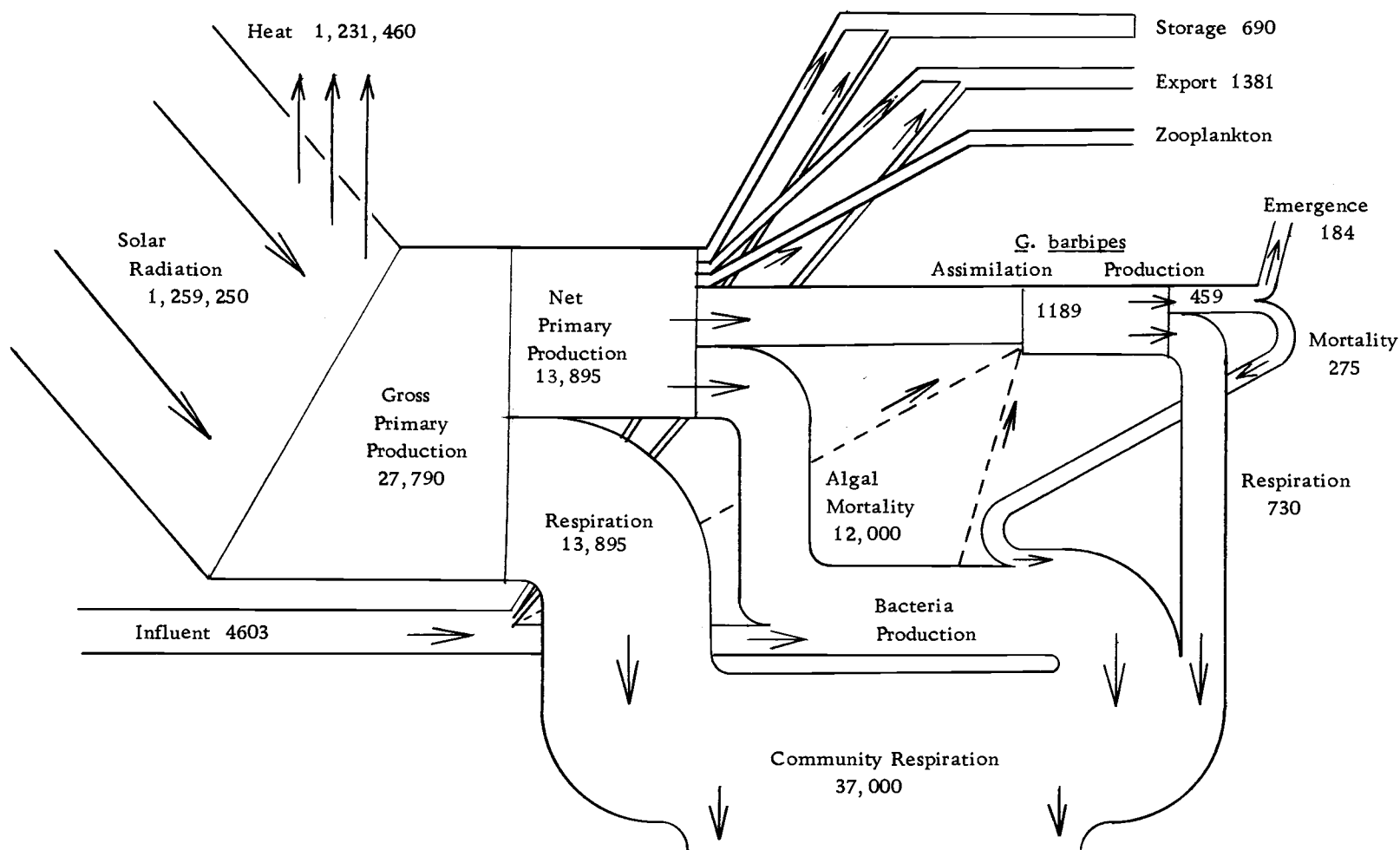


Figure 20. Energy transfer in the secondary waste stabilization lagoon in kcal/m².
Monmouth, Oregon, 1966.

thus negating any advantage gained by increasing the area of inhabitation. It is also possible that some optimum balance in the species composition would result in a greater reduction in the amount of energy in the effluent.

Figures 21 and 22 give the energy-transfer data of the secondary lagoon for 1967 and the primary lagoon in 1966, respectively. The midge data in 1967 are the same as those in Figure 19; however, the other energy pathways have been increased to account for all 12 months. Figure 22 shows that the raw sewage influent of $21,000 \text{ kcal/m}^2$ accounts for a sizable portion of the annual energy budget of the primary lagoon. The amount of energy being stored in bottom sediments was small, and therefore can not be considered as an important loss of energy. As indicated previously, bottom sediments are almost completely mineralized.

In addition to the beneficial aspects of removing energy from the lagoon system, midge larvae may contribute to the efficiency of waste stabilization by extending the aerobic zone of decomposition down into the substrate. Usinger and Kellen (1955) and Kimerle and Enns (1968) discussed the possible importance of this activity of midge larvae. However, in most raw waste stabilization lagoons with an operational depth of four to five feet, midge larvae are found only around the shores. Therefore, the effectiveness of larvae extending

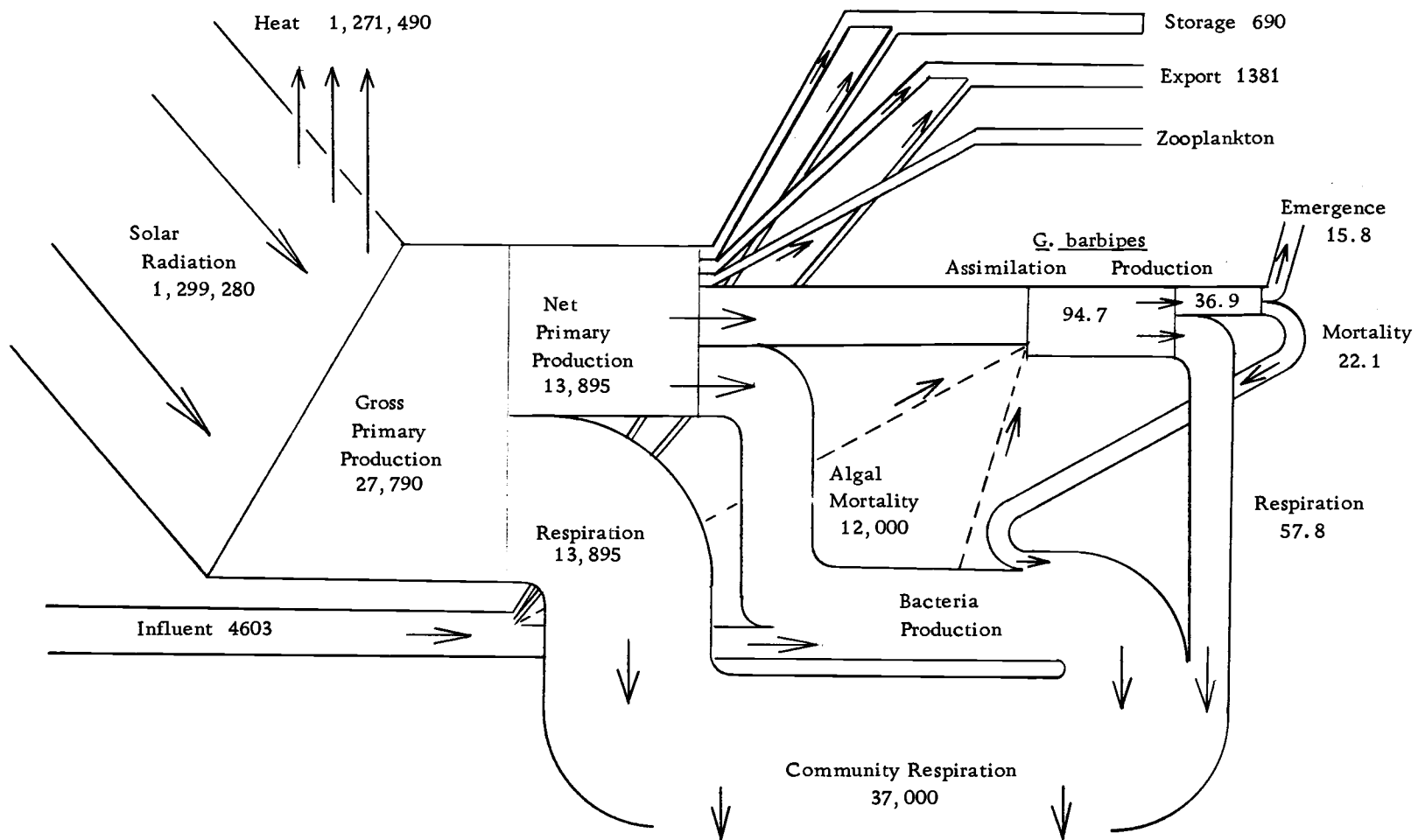


Figure 21. Energy transfer in the secondary waste stabilization lagoon in kcal/m². Monmouth, Oregon, 1967.

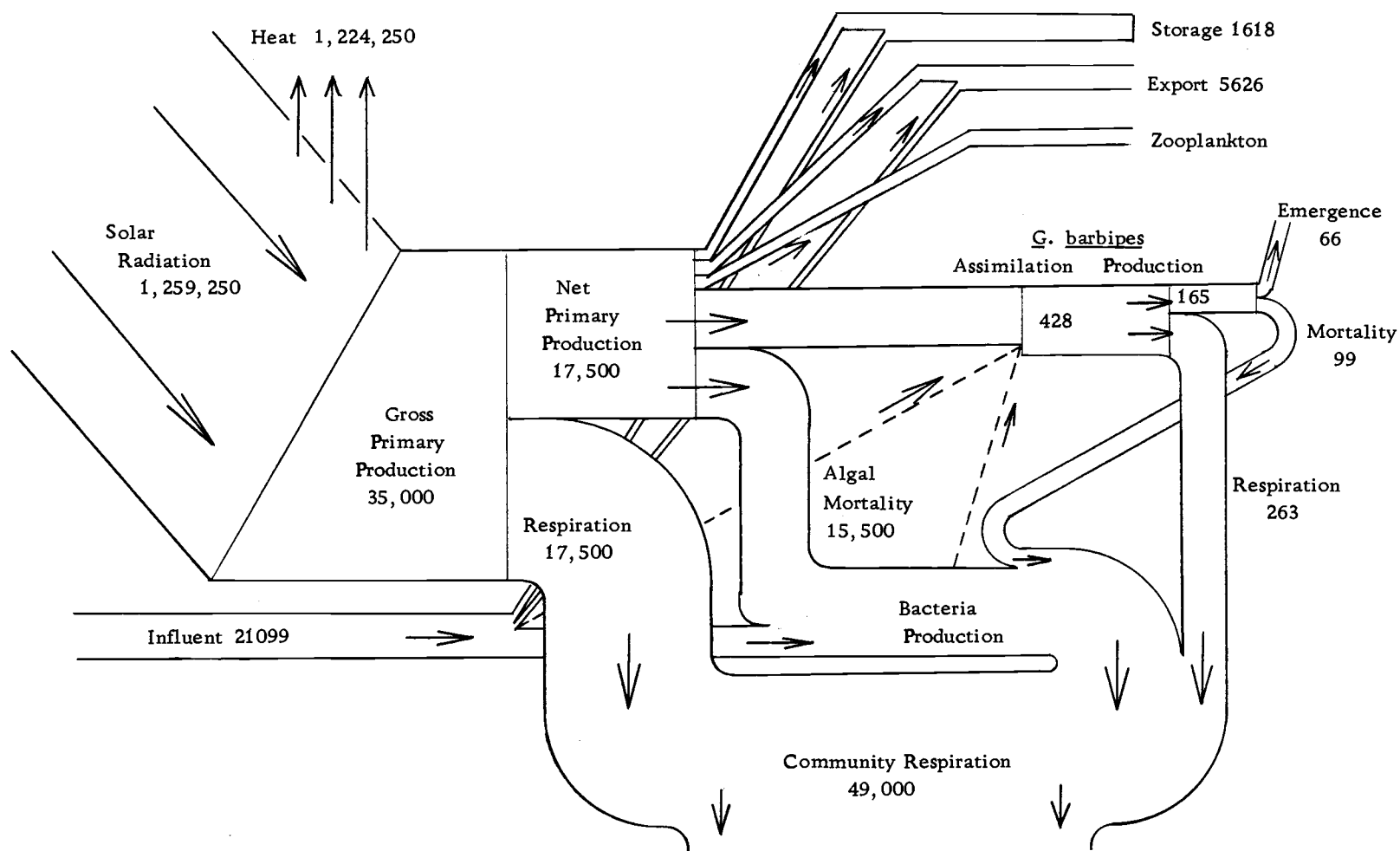


Figure 22. Energy transfer in the primary waste stabilization lagoon in kcal/m². Monmouth, Oregon, 1966.

the aerobic zone into the substrate would be increased if larvae were to occupy more area of the lagoon bottom.

CONCLUSIONS

This investigation is one of the first attempts to assess the bioenergetic role of a chironomid population in the process of waste stabilization in sewage lagoons. The energy budget of the dominant midge, G. barbipes was defined and compared with estimates of other energy pathways. It was also necessary to arrive at some understanding of how the effective environment operated to either increase or decrease the total energy removed by G. barbipes.

An energy budget of G. barbipes was calculated from measurements of growth rates, biomass, emergence rates and respiration rates. Included in the energy budget was an estimate of production, based on the frequent measurements of growth and biomass. Production rates of G. barbipes were very high. However, the lack of production estimates of other species chironomids, especially of multivoltine species, makes it difficult to compare G. barbipes production to other chironomid populations in various types of habitats.

Annual production of G. barbipes in 1967 was 808 kcal/m^2 in Stratum I (3612 m^2) of the secondary lagoon, or 36.94 kcal/m^2 over all strata (86903 m^2). Production in 1966 was about ten times greater than in 1967 in both the primary and secondary lagoons. It was lowest in the primary lagoon in 1967.

Daily and seasonal variations in growth rates, production and emergence reflect the instability of the lagoon environment. The major factor causing this instability, and having a pronounced effect on the biota of the lagoon, is DO. Periodical depletion of oxygen for short periods caused a cessation of growth and emergence of G. barbipes. Septic conditions persisted for six weeks in 1967 in the primary lagoon and drastically reduced production and emergence for the remainder of the season. Although periodic low concentrations of DO were sometimes unfavorable to G. barbipes, this same factor probably enabled the midges to exploit the lagoon environment. Apparently other benthic species that might compete with G. barbipes for the abundant food supply and limited space in the margin of the lagoon, were unable to tolerate the frequent low concentrations of dissolved oxygen.

The total amount of energy removed through emergence and respiration of G. barbipes in 1966 from the lagoon ecosystem was 103×10^6 kcal. In 1967 the total was 8.6×10^6 kcal. This seems to be a tremendous amount of energy, but when compared to other energy pathways of the lagoon it was a negligible amount. The dominant bioenergetic components of the lagoon ecosystem were the primary producers and decomposers. In 1966 G. barbipes removed 6.6 percent of the net primary production in the secondary lagoon and 0.5 percent in 1967. Therefore the bioenergetic role of G.

barbipes can not be considered important in the total degradation of energy in waste stabilization at Monmouth, Oregon.

The energy of the influents of both lagoons were less than the amount of energy fixed through photosynthetic activity. Energy of raw sewage was estimated to be about 60 percent of the primary production in the primary lagoon. The energy of the secondary lagoon influent was only about 20 percent of the total energy fixed by algae. However, the influents of both lagoons were the primary source of nutrients, and together with the nutrients recycled by bacteria, enabled the extremely high rates of primary production to occur.

The bioenergetic approach of analyzing the lagoon ecosystem and evaluating the role of G. barbipes is only one of several methods that could have been used. Waste treatment processes are designed to convert man's nocuous waste into an innocuous form suitable for discharge into the environment. Biologists and sanitary engineers are interested in the reduction of oxygen consuming organic matter, nutrients, bacteria and toxic materials. The bioenergetic analysis of the lagoon ecosystem reflected the reduction of oxygen consuming organic matter but no data were obtained on the fate of nutrients, and relative importance of different biological components affecting the reduction and recycling of nutrients. The calorie unit enabled all measurements to be reduced to a common denominator. It also

would have been possible to express data in terms of nitrogen or phosphorus. Analysis by this method may have indicated somewhat different relationships than did the bioenergetic data and given a more complete understanding of how waste are stabilized in the lagoon.

BIBLIOGRAPHY

- Allen, K. R. 1951. The Horokiwi stream: a study of a trout population. Wellington. 231 p. (New Zealand. Marine Department of Fisheries. Bulletin 10)
- American Public Health Association. 1965. Standard methods for the examination of water and wastewater. 12th ed. New York. 769 p.
- Anderson, L. D., E. C. Bay and A. A. Ingram. 1964. Studies of chironomid midge control in water spreading basins near Montebello, California. California Vector Views 11:13-20.
- Anderson, R. O. and F. F. Hooper. 1956. Seasonal abundance and production of littoral bottom fauna in a southern Michigan lake. Transactions of American Microscopical Society 75:259-270.
- An ally in the war on waste. 1967. Oregon's Agricultural Progress 14:14-15.
- Atkins, C. H. 1960. Critique. In: Proceedings of a symposium on waste stabilization lagoons, Kansas City, Missouri, August 1-5, 1960. Washington, D. C. p. 161-163. (U. S. Public Health Service. Publication 872)
- Azam, K. M. 1969. Life history and production of Sialis californica Banks and Sialis rotunda Banks (Megaloptera:Sialidae). Ph. D. thesis. Corvallis, Oregon State University. 111 numb. leaves.
- Bartsch, A. F. 1948. Biological aspects of stream pollution. Sewage Works Journal 20:292-302.
- Bartsch, A. F. and M. O. Allum. 1957. Biological factors in treatment of raw sewage in artificial ponds. Limnology and Oceanography 2:77-84.
- Bay, E. C. 1964. California Chironomids. Proceedings and papers of the 32d Annual Conference of the California Mosquito Control Association, p. 82-84.
- Bay, E. C. and L. D. Anderson. 1965. Chironomid control by carp and goldfish. Mosquito News 25:310-316.

- Bay, E. C., L. D. Anderson and J. Sugerman. 1965. The abatement of a chironomid nuisance on highways at Lancaster, California. *California Vector Views* 12:29-32.
- Bay, E. C. and L. D. Anderson. 1966. Studies with the Mosquito fish, Gambusia affinis, as a chironomid control. *Annals of the Entomological Society of America* 59:150-153.
- Bay, E. C. 1967. Potential for naturalistic control of mosquitoes. *Proceedings and papers of the 35th annual conference of the California Mosquito Control Association, 1967*, p. 34-37.
- Beadle, L. D. and J. A. Rowe. 1960. Sewage lagoons and mosquito problems. In: *Proceedings of a symposium on waste stabilization lagoons, Kansas City, Missouri, 1960*. Washington, D. C. p. 101-104. (U. S. Public Health Service. Publication no. 872)
- Biever, K. D. 1965. A rearing technique for the colonization of chironomid midges. *Annals of the Entomological Society of America* 58:135-136.
- Bonnell, D. E. and D. C. Mote. 1941. The Klamath midge. *Journal of Economic Entomology* 34:325.
- Brinck, C. W. 1960. Raw sewage lagoons in Montana. In: *Proceedings of a symposium on waste stabilization lagoons, Kansas City, Missouri, 1960*. Washington, D. C. p. 112-117. (U. S. Public Health Service. Publication no. 872)
- Brundin, L. 1950. The relation of oxygen microstratification at the mud surface to the ecology of profundal bottom fauna. In: *Report of the Institute of Freshwater Research (Drottningholm, Sweden)*. Vol. 32. p. 32-42.
- Burgess, F. J. and M. E. Northcraft. 1965. Final report: Wastewater lagoon criteria for maritime climates. Corvallis, Oregon State University, Engineering Experiment Station, Department of Civil Engineering. 80 numb. and 67 unnumb. p. 147.
- Clare, H. C. and D. J. Weiner. 1960. Economics of waste stabilization lagoons in Region VI. In: *Proceedings of a symposium on waste stabilization lagoons, Kansas City, Missouri, 1960*. Washington, D. C. p. 57-67. (U. S. Public Health Service. Publication no. 872)

- Clark, G. L., W. T. Edmonson and W. E. Ricker. 1946.
Mathematical formulation of biological productivity.
Ecological Monographs 16:336-337.
- Cooley, C. E. and R. R. Jennings. 1960. Study of the performance of a sewage stabilization pond at Farmville, Virginia. In: Proceedings of a symposium on waste stabilization lagoons, Kansas City, Missouri, 1960. Washington, D. C. p. 41-51. (U. S. Public Health Service. Publication no. 872)
- Curtis, L. C. 1963. Mosquito production in sewage lagoons. Proceedings of the Entomological Society of British Columbia 60:22-23.
- Davis, G. E. and C. E. Warren. 1965. Trophic relations of a sculpin in laboratory stream communities. Journal of Wildlife Management 29:846-871.
- Dugdale, R. C. 1955. Studies in the ecology of the benthic Diptera of Lake Mendota. Ph.D. thesis. Madison, University of Wisconsin. 99 numb. leaves.
- Eberly, W. R. 1959. The metalimnetic oxygen maximum in Meyers Lake. In: Investigations of Indiana lakes and streams. Vol. 5. Bloomington, Indiana University, Dept. of Zoology. p. 1-46.
- Eberly, W. R. 1964. Further studies on the metalimnetic oxygen maximum, with special reference to its occurrence throughout the world. In: Investigations of Indiana lakes and streams. Vol. 6. Bloomington, Indiana University, Dept. of zoology. p. 103-139.
- Ernest, R. D. 1967. Production of the snail Oxytrema silicula (Gould), in an experimental stream. Master's thesis. Corvallis, Oregon State University. 51 numb. leaves.
- Fagan, E. B. and W. R. Enns. 1966. The distribution and biology of aquatic midges in Missouri lagoons. Proceedings of the Entomological Society of Washington 68:277-289.
- Felton, H. L. 1940. Control of aquatic midges with notes on the biology of certain species. Journal of Economic Entomology 33:252-264.

- Fogg, G. E. 1953. The metabolism of algae. London, Methuen. 149 p.
- Forbes, S. A. 1925. The lake as a microcosm. Illinois Natural History Survey, Bulletin 15:537-550.
- Hayne, D. W. and R. C. Ball. 1956. Benthic productivity as influenced by fish predation. Limnology and Oceanography 1:162-175.
- Hilsenhoff, W. L. 1962. Granulated malathion as a possible control for Tendipes plumosus (Diptera:Tendipedidae). Journal of Economic Entomology 55:71-78.
- Hilsenhoff, W. L. 1966. The biology of Chironomus plumosus (Diptera:Chironomidae) in Lake Winnebago, Wisconsin. Annals of the Entomological Society of America 59:465-473.
- Horning, W. B., R. Porges, H. F. Clarke and W. B. Cooke. 1964. Waste stabilization pond study, Lebanon, Ohio. Robert A. Taft Sanitary Engineering Center. Cincinnati, Ohio. p. 48. (U. S. Public Health Service. Publication no. 999-WP-16).
- Hunt, R. L. 1966. Production and angler harvest of wild brook trout in Lawrence Creek, Wisconsin. Madison. 52 p. (Wisconsin. Conservation Department. Technical Bulletin no. 35)
- Hynes, H. B. 1961. The invertebrate fauna of a Welsh mountain stream. Archiv fur Hydrobiologie 57:344-388.
- Hynes, H. B. 1963. The biology of polluted waters. Liverpool, Liverpool University. 202 p.
- Gaufin, A. R. 1958. The effects of pollution on a midwestern stream. Ohio Journal of Science 58:197-208.
- Gerking, S. D. 1962. Production and food utilization in a population of bluegill sunfish. Ecological Monographs 32:31-78.
- Grodhaus, G. 1963. Chironomid midges as a nuisance. California Vector Views 10:27-37.
- Johnson, M. S. and F. Munger. 1930. Observations on excessive abundance of midges, Chironomus plumosus, at Lake Pepin. Ecology 11:110-126.

- Juday, C. 1940. The annual energy budget of an inland lake. *Ecology* 21:438-450.
- Kajak, Z. 1966. Field experiments in studies on benthic density of some Mazurian lakes. *Gewässer und Abwässer*, heft 41/42, p. 150-158.
- Kajak, Z. and J. I. Rybak. 1966. Production and some trophic dependences in benthos against primary production and zooplankton production of several Mazurian lakes. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 16:441-451.
- Kajak, Z. and J. Warda. 1968. Feeding of benthic non-predatory Chironomidae in a lake. *Annales Zoologici Fennici* 5:57-64.
- Kellen, W. R. 1953. Laboratory experiments in the role of insects in sewage oxidation ponds. *Journal of Economic Entomology* 46:1041-1048.
- Kimerle, R. A. 1965. A study of aquatic insects associated with waste stabilization lagoons. Master's thesis. Columbia, University of Missouri. 39 numb. leaves.
- Kimerle, R. A. and N. H. Anderson. 1967. Evaluation of aquatic insect emergence traps. *Journal of Economic Entomology* 60:1255-1259.
- Kimerle, R. A. and W. R. Enns. 1968. Aquatic insects associated with midwestern waste stabilization lagoons. *Journal of the Water Pollution Control Federation*, research sup., 40 (part II):R31-R41. February.
- Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. *Ecology* 23:339-418.
- Ludwig, H. F., W. J. Oswald, H. B. Gotaas and V. Lynch. 1951. Algae symbiosis in oxidation ponds. I. Growth characteristics of Euglena gracilis cultured in sewage. *Sewage and Industrial Waste* 23:1337-1355.
- Neel, J. K. and G. J. Hopkins. 1956. Experimental lagooning of raw sewage. *Sewage and Industrial Waste* 28:1326-1356.

- Neel, J. K., J. H. McDermott and C. A. Monday. 1961. Experimental lagooning of raw sewage at Fayette, Missouri. *Journal of Water Pollution Control Federation* 36:603-641.
- Neess, J. and R. C. Dugdale. 1959. Computation of production for populations of aquatic midges. *Ecology* 40:425-430.
- Odum, H. T. 1957. Trophic structure and production of Silver Springs, Florida. *Ecological Monographs* 27:55-112.
- Odum, H. T. and C. M. Hoskin. 1958. Comparative studies on the metabolism of marine waters. In: *Publications of the Institute of Marine Science. Vol. 5. Port Aransas, University of Texas, Institute of Marine Science.* p. 16-46.
- Odum, E. P. 1959. *Fundamentals of Ecology* 2d edition. Philadelphia, Saunders. 546 p.
- Oregon State Board of Health. State Sanitary Authority. 1965. *Criteria for design and utilization of domestic sewage lagoons or stabilization ponds.* Portland. 6 p.
- Phillipson, J. 1966. *Ecological energetics.* New York, St. Martin's. 57 p.
- Porges, R. and K. M. MacKenthum. 1963. Waste stabilization ponds: Use, function and biota. *Biotechnology and Bioengineering* 5:255-273.
- Ricker, W. E. 1958. *Handbook of computations for biological statistics of fish populations.* Ottawa. 300 p. (Canada. Fisheries Research Board. Bulletin 119)
- Silva, D. C. and G. F. Papenfuss. 1953. Report on a systematic study of the algae of sewage oxidation ponds. 35 p. (California. State Water Pollution Control Board. Publication no. 7)
- Spiller, D. 1965. Methods used for chironomid larva surveys of sewage oxidation ponds and natural waters at Auckland, New Zealand. *California Vector Views* 12:9-15.
- Sturgess, B. T. 1964. The ecology of insects associated with waste water lagoons. Master's thesis. Corvallis, Oregon State University. 58 numb. leaves.

- Sturgess, B. T. 1968. The ecology of chironomids associated with stabilization lagoons. Ph.D. thesis. Corvallis, Oregon State University. 96 numb. leaves.
- Sturgess, B. T. and R. L. Goulding. 1968. Tolerance of three species of larval chironomids to physiochemical stress factors occurring in stabilization lagoons. *Annals of the Entomological Society of America* 61:903-906.
- Svore, J. H. 1960. History of raw sewage lagoons in the midwest. In: *Proceedings of a symposium on waste stabilization lagoons*, Kansas City, Missouri, 1960. Washington, D. C. p. 2-6. (U. S. Public Health Service. Publication no. 872)
- Teal, J. M. 1957. Community metabolism in a temperate cold spring. *Ecological Monographs* 27:283-302.
- Towne, W. W., A. F. Bartsch and W. H. Davis. 1957. Raw sewage stabilization ponds in the Dakotas. *Sewage and Industrial Waste* 29:377-396.
- Towne, W. W. and W. B. Horning. 1960. Some observations on the growth, application and operation of raw sewage stabilization ponds. In: *Proceedings of a symposium on waste stabilization lagoons*, Kansas City, Missouri, 1960. Washington, D. C. p. 68-74. (U. S. Public Health Service. Publication no. 872)
- Tubb, R. A. and T. C. Dorris. 1965. Herbivorous insect populations in oil refinery effluent holding pond series. *Limnology and Oceanography* 10:121-134.
- Usinger, R. L. and W. R. Kellen. 1955. The role of insects in sewage disposal beds. *Hilgardia* 23:263-321.
- Walshe, B. M. 1947. The function of haemoglobin in Tanytarsus (Chironomidae). *Journal of Experimental Biology* 24:329-342.
- Walshe, B. M. 1950. The function of haemoglobin in Chironomus plumosus under natural conditions. *Journal of Experimental Biology* 27:73-95.
- Warren, C. E., J. H. Wales, G. E. Davis and P. Doudoroff. 1964. Trout production in an experimental stream enriched with sucrose. *Journal of Wildlife Management* 28:617-660.

- Waters, T. F. 1966. Production rate, population density and drift of a stream invertebrate. *Ecology* 47:595-604.
- Williamson, J. 1960. Impromptu remarks made. In: *Proceedings of a symposium on waste stabilization lagoons*, Kansas City, Missouri, 1960. Washington, D. C. p. 159-160. (U. S. Public Health Service. Publication no. 872)
- Yount, J. L. 1966. A method of rearing large numbers of pond midge larvae, with estimates of productivity and standing crop. *American Midland Naturalist* 76:230-238.

APPENDIX

Sample Calculation for the Number of Samples

Problem: To determine the number of samples needed to maintain an error of no greater than 15 percent for total population estimates of G. barbipes larvae. Also to determine the allocation of those samples in the strata. Results from one months calculations are used to dictate the number and location of samples to be taken the following month.

Raw Data:

Stratum Area in stratum, m ²	I 3612	II 6824	III 16723	IV 27937	V 31807
Number of larvae in each sample of 1 m ²	2140	320	16	1	0
	1485	85	7	6	0
	395	125	9	3	1
	740	70	11	0	0
	1148	220	13	1	0
	1200	105	2	4	1
	2640	215	6	0	0
	1955	410	1	5	2
	700	105	4	-	-
	415	-	-	-	-
Total	12818	1655	69	20	4
Number of samples taken	10	9	9	8	8
Mean \bar{X}	1281.8	183.8	7.7	2.5	0.5
Standard Deviation s	765.2	117.6	5.0	2.3	0.8
Mean times Area, $\bar{X} \cdot A$	4,629,862	125,425	128,767	69,842	15,904
$\Sigma \bar{X} \cdot A$			4,969,800		
Standard Deviation times area $s \cdot A$	2,763,902	802,502	83,615	64,255	25,446
$\Sigma s \cdot A$			3,739,720		
Percent of the total deviation occurring in each strata	74	21	2	2	0.7

$$N = \frac{\left(\frac{\sum_{i=1}^k n_i s \cdot A}{\sum_{i=1}^k n_i \bar{X} \cdot A} \right)^2}{(.15)^2} = \frac{\left(\frac{3,739,720}{4,969,800} \right)^2}{0.0225} = 25$$

Allocation of samples in
different strata

I 25 · 0.74 = 18
 II 25 · 0.21 = 5
 III 25 · 0.02 = 1
 IV 25 · 0.02 = 1
 V 25 · 0.01 = 1

samples 26

Sample Calculation For Determining Weighted Mean Biomass and Variance

Problem: Samples were taken on May 30, June 3, 7, 11, and 15,

1967. It is now desired to express the data over a time interval

which does not coincide to the dates of collections, June 1 to

June 13.

Raw Data:

Date	Accumulated Date			
	Biomass Estimate Y	Variance of Estimate Vx	Index of Estimate X	Specified Interval d
5/30/67	2	1	0	2
6/ 3/67	4	2	4	
6/ 7/67	6	3	8	
6/11/67	4	2	12	14
6/15/67	2	1	16	

Mean Biomass:

$$\bar{B} = \frac{\sum_{n=1}^{n=k} C Y}{d_2 - d_1} = \frac{C_1 Y_1 + C_2 Y_2 + C_3 Y_3 + C_4 Y_4 + C_5 Y_5}{d_2 - d_1}$$

Mean Variance:

$$\overline{V_x} = \frac{\sum_{n=1}^{n=k} C^2 V_{xY}}{(d_2 - d_1)^2} = \frac{C_1^2 V_{xY_1} + C_2^2 V_{xY_2} + C_3^2 V_{xY_3} + C_4^2 V_{xY_4} + C_5^2 V_{xY_5}}{(d_2 - d_1)^2}$$

Formulas for values of C are:

$$C_1 = \left(\frac{X_2 - d_1}{2} \right) \left[1 - \left(\frac{d_1 - X_1}{X_2 - X_1} \right) \right]$$

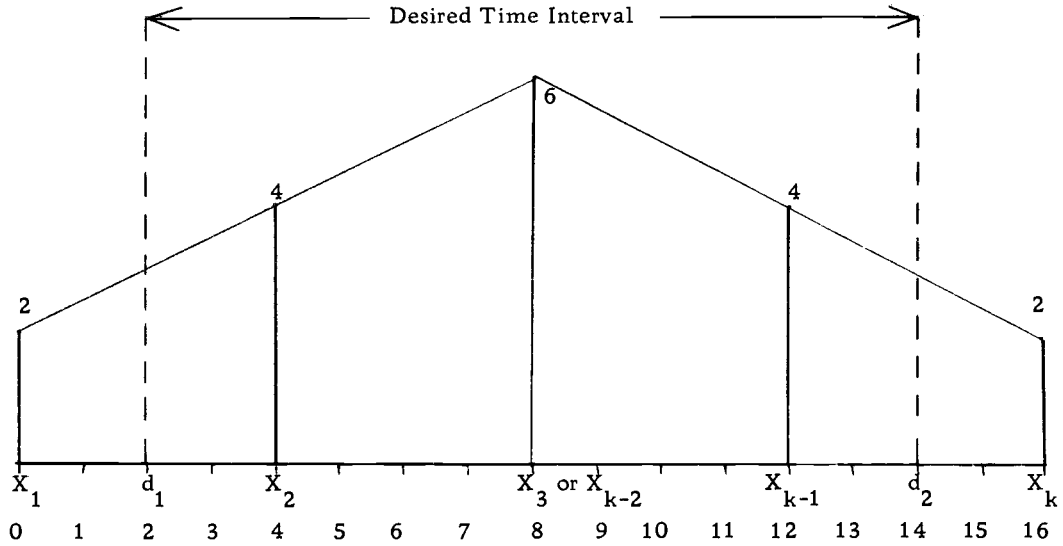
$$C_2 = \left(\frac{X_2 - d_1}{2} \right) \left[1 + \left(\frac{d_1 - X_1}{X_2 - X_1} \right) \right] + \left(\frac{X_3 - X_2}{2} \right)$$

$$C_3 \text{ or } C_t = \frac{X_{t+1} - X_{t-1}}{2} ; \text{ to } t=3, t=4, \dots, k-2$$

$$C_{k-1} = \left(\frac{X_{k-1} - X_{k-2}}{2} \right) + \left(\frac{d_2 - X_{k-1}}{2} \right) \left[1 + \left(\frac{X_k - d_2}{X_k - X_{k-1}} \right) \right]$$

$$C_k = \left(\frac{d_2 - X_{k-1}}{2} \right) \left[1 - \left(\frac{X_k - d_2}{X_k - X_{k-1}} \right) \right]$$

Diagram of Data



Calculations of C values:

$$C_1 = \left(\frac{X_2 - d_1}{2} \right) \left[1 - \left(\frac{d_1 - X_1}{X_2 - X_1} \right) \right] = \left(\frac{4-2}{2} \right) \left[1 - \left(\frac{2-0}{4-0} \right) \right] = \frac{2}{2} \left[1 - \frac{2}{4} \right] = 0.5$$

$$C_2 = \left(\frac{X_2 - d_1}{2} \right) \left[1 + \left(\frac{d_1 - X_1}{X_2 - X_1} \right) \right] + \left(\frac{X_3 - X_2}{2} \right) = \left(\frac{4-2}{2} \right) \left[1 + \left(\frac{2-0}{4-0} \right) \right] + \left(\frac{8-4}{2} \right) = 4.5$$

$$C_3 = \frac{X_{t+1} - X_{t-1}}{2} = \frac{12-4}{2} = \frac{8}{2} = 4.0$$

$$C_{k-1} = \left(\frac{X_{k-1} - X_{k-2}}{2} \right) + \left(\frac{d_2 - X_{k-1}}{2} \right) \left[1 + \left(\frac{X_k - d_2}{X_k - X_{k-1}} \right) \right] = \left(\frac{12-8}{2} \right) + \left(\frac{14-12}{2} \right) \left[1 + \left(\frac{16-14}{16-12} \right) \right] = 4.5$$

$$C_k = \left(\frac{d_2 - X_{k-1}}{2} \right) \left[1 - \left(\frac{X_k - d_2}{X_k - X_{k-1}} \right) \right] = \left(\frac{14-12}{2} \right) \left[1 - \left(\frac{16-14}{16-12} \right) \right] = 0.5$$

Calculation of Weighted Mean Biomass:

$$\begin{array}{rcl}
 C_1 \cdot Y_1 & = & 0.5 \cdot 2 = 1 \\
 C_2 \cdot Y_2 & = & 4.5 \cdot 4 = 18 \\
 C_3 \cdot Y_3 & = & 4.0 \cdot 6 = 24 \\
 C_4 \cdot Y_4 & = & 4.5 \cdot 4 = 18 \\
 C_5 \cdot Y_5 & = & 0.5 \cdot 2 = 1 \\
 & & \underline{\hspace{1cm}} \\
 & & 62
 \end{array}
 \qquad
 \begin{array}{l}
 \bar{B} = \frac{\sum_{n=1}^{n=k} C \cdot Y}{d_2 - d_1} \\
 \\
 = \frac{62}{12} = 5.167
 \end{array}$$

Calculation of Weighted Mean Variance:

$$\begin{array}{rcl}
 C_1^2 \cdot V_{xY_1} & = & 0.25 \cdot 1 = 0.25 \\
 C_2^2 \cdot V_{xY_2} & = & 20.25 \cdot 2 = 40.50 \\
 C_3^2 \cdot V_{xY_3} & = & 16.00 \cdot 3 = 48.00 \\
 C_4^2 \cdot V_{xY_4} & = & 20.25 \cdot 2 = 40.50 \\
 C_5^2 \cdot V_{xY_5} & = & 0.25 \cdot 1 = 0.25 \\
 & & \underline{\hspace{1cm}} \\
 & & 129.50
 \end{array}
 \qquad
 \begin{array}{l}
 \overline{V_x} = \frac{\sum_{n=1}^{n=k} C^2 \cdot V_{xY}}{(d_2 - d_1)^2} \\
 \\
 = \frac{129.5}{144} = 0.899
 \end{array}$$

Appendix Table 1. Chemical Data and Flow Rates; MI raw sewage, MII primary lagoon effluent and MIII secondary lagoon effluent.

		Flow	BOD	PO ₄	Kjeldahl	NO ₃	Cl ⁻	Conductivity
		mgd	mg/l	mg/l	Nitrogen	mg/l	mg/l	mhos/cm
November 1966	I	0.400	302	20.0	56.0	0.94	24.5	470
	II	0.208	61	14.0	19.2	14.97	24.4	295
	III	none	28	21.0	16.0	9.84	26.6	265
December	I	1.450	42	3.2	11.4	-	7.4	150
	II	0.525	28	13.5	9.5	-	15.0	192
	III	0.169	11	8.5	9.5	-	23.0	195
January 1967	I	0.802	85	7.0	19.0	0.12	8.3	167
	II	0.735	23	8.6	42.0	0.12	10.6	163
	III	0.285	33	7.0	29.0	0.12	15.0	164
February	I	0.840	38	4.6	8.0	1.50	6.5	172
	II	0.768	27	6.7	6.0	1.80	8.5	185
	III	0.495	21	2.9	3.0	3.30	11.0	183
March	I	0.870	44	7.0	10.0	0.36	9.3	245
	II	0.622	24	6.1	6.0	0.77	11.1	200
	III	0.472	19	2.1	3.0	1.00	11.1	176
April	I	0.460	117	23.0	30.0	-	20.1	340
	II	0.736	33	11.2	8.4	-	12.6	180
	III	0.487	16	6.2	8.7	-	15.5	170
May	I	0.220	180	25.0	46.0	0	26.0	340
	II	0.554	44	8.7	8.0	1.07	17.0	177
	III	0.430	14	6.2	3.0	1.80	13.4	154
June	I	0.125	290	32.0	30.0	-	22.0	500
	II	0.378	34	20.0	6.0	-	17.0	280
	III	0.052	15	6.1	3.0	-	13.5	205
July	I	0.850	320	-	-	0	-	-
	II	0.142	30	-	8.0	0	-	-
	III	none	-	-	-	-	-	-
August	I	0.540	200	28.1	3.0	0	26.0	440
	II	0.135	22	25.8	2.0	0	24.8	320
	III	none	-	-	-	-	-	-
September	I	0.500	325	35.0	20.0	0	24.0	540
	II	0.065	30	22.0	12.0	0	18.7	285
	III	none	-	-	-	-	-	-
October	I	0.230	330	-	-	-	-	-
	II	0.105	38	-	-	-	-	-
	III	none	-	-	-	-	-	-