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The Effect of Calcium Sulfide on the Hactchability of Chironomous Riparius Meigen Eggs

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THE EFFECT OF CALCIUM SULFIDE ON THE HATCHABILITY OF CHIRONOMUS RIPARIUS MEIGEN EGGS

by

Gerald S. Wegner

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

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INTRODUCTION

The members of genus Chironomus Meigen (Diptera, Chironomidae) figure prominently in the ecology of the world's fresh water lakes, ponds, rivers, streams, and estuaries. The abundant larvae constitute a major portion of the diet for young and small fish, and their morphology and numbers reflect the condition of the waters they inhabit.

The natural history of the group is well known. The gravid female envelopes 200 to 2000 eggs measuring 0.3 to 0.5 mm long in a gelatinous matrix to form an egg mass of species-characteristic size and shape. Development of the embryo is typical of suborder Nematocera. In a few days the eggs hatch into minute, transparent, aquatic larvae. The larvae, which feed upon detritus, zooplankton and phytoplankton, grow rapidly and pass through four instars within tubes constructed of fine particulate matter held together by a silken secretion. The 12-segmented larvae possess a pair of anterior and posterior prolegs, four anal gills and often two pairs of ventro-lateral gills for more efficient respiration in oxygen-deficient waters. The hemolymph of most species contains hemoglobin.

The full grown larvae transform into pupae of the simple exarate type which rise to the water's surface at the time of adult emergence. The adults are similar to mosquitos
in general appearance but lack functional mouth parts in most cases. The males possess plumose antennae, large compound eyes, and long segmented palpi. Mating occurs in swarms, usually at dawn or dusk.

The species used in this study, *Chironomus riparius* Meigen, was chosen for its ability to colonize under laboratory conditions, and for the ample egg content of its egg masses.

It is crucial to the balance and safety of fresh water ecosystems to determine the tolerance of these organisms to pollutants entering the system. Therefore, I have chosen to study the effect of an industrial waste solubility product, namely, calcium sulfide, on the hatchability of *Chironomus* eggs. Sulfide ions are known to leach into fresh water in relatively high amounts from air-cooled blast furnace slag used in landfill.¹ The purpose, then, of this thesis is to determine the specific concentration of sulfide that interferes with embryogenesis in a species of *Chironomus*.

¹A. Kozlowski et al. 1974. Slag and Slag Thermal Effects on Hatchability of Select Lake Michigan Organisms. Unpublished final report on research supported by the National Science Foundation, Student Originated Studies.
REVIEW OF THE LITERATURE

The literature consulted during the preparation of this thesis dealt mainly with four areas of specialization within the study of Chironomidae, namely, the taxonomy, natural history, embryology, and ecology of this family.

The Taxonomy of Chironomidae with Emphasis on Genus Chironomus

Abundant studies have been done on chironomids, and much of the literature dates back to the early 19th Century. The genus Chironomus was originally described by Meigen (1800) in his work on dipteran genera. The group was defined again by Van der Wulp (1874), and more recently by Edwards (1929). Great progress concerning the classification of both immature and adult forms was made by Thienemann (co-author, 1916) and his associates Lenz (1921), Zavrel (1921), and Goetghebuer (1912, 1928) in Europe, and by Johannsen (1905, 1936, 1937) and Mallock (1915) in North America. Current work on the taxonomy of Chironomidae is being done by Wülker, Sublette, and Martin (1971).

The Natural History of Chironomidae

Miall (1900) pioneered work in this area with his elucidation of the structure and life history of the harlequin fly, Chironomus dorsalis Meigen. Johannsen (1905),
Needham (1906), Mallock (1915), and Branch (1923, 1928, 1931) described the formation, physical nature, and deposition of the gelatinous egg masses unique to the Chironomidae.

Johannsen (1905), Mallock (1915), and Thienemann (1954) have added greatly to the knowledge of the biology of the Chironomidae in addition to their contribution to the taxonomy of this family. Konstantinov (1952a) wrote on the biology and life cycle of C. dorsalis. Oliver (1971) compiled an excellent discussion of the classification and life history of Chironomidae.

The Embryology of Chironomidae


Much work has been done on abnormal embryogenesis and experimentally-initiated defects. Among these, Yajima (1960), and Overton and Raab (1967) observed the effects of centrifugation on the eggs of Chironomus sp. Oelhafen (1961), Yajima (1964), and Kalthoff (1972) have tested the effects of ultraviolet radiation on embryonic determination in mosquito and chironomid eggs.

The Ecology of Chironomidae

A variety of ecology-related literature has been written on the Chironomidae. Most of the research effort has been concentrated on the larvae, which are considered to have the greatest practical importance, and comparatively little work has been done on the ecology of the eggs, pupae, and adults.
Such activities as larval distribution in particular bodies of water, feeding, respiration, growth, development, behavior and responses to various environmental factors (natural and induced) are commonly studied.

Decksbach (1928), Berestov (1937), Chernovski (1938), Linevich (1948), Alekseyev (1955), Kalugina (1957), and many others (see Konstantinov, 1971a,c) have described the chironomid larvae and their distribution in the reservoirs and natural waters of the U.S.S.R. Stahl (1959), Hilsenhoff (1966, 1967), Hilsenhoff and Narf (1968), and others have done similar studies on the chironomid fauna of inland waters of the United States.


Respiration in chironomid larvae has been studied under a variety of conditions. Grandilevskaja and Decksbach (1939), Vinberg (1939, 1948), and Konstantinov (1958b) shed light on the various ecological adaptations by larvae to
conditions of oxygen depletion, and peculiarities of larval respiration following anaerobiosis and in microaerophilous conditions. Luferov (1958) and Lukanin (1962) described the effect of temperature on larval respiration. Kasatkina (1960) correlated the rate of respiration to body weight in ecologically different species of chironomids. Lavrovsky (1966) wrote on the role of hemoglobin in the oxygen transport in *Chironomus* larvae. Konstantinov (1971b) found that the magnitude of respiration in chironomid larvae depends on the species, age, state, and environmental condition. Other studies on the measurement of larval respiration include Gorodetskaja (1948), Kamlyuk (1964), and Konstantinov (1968b, 1969b).

On the topics of growth, development, and behavior, Konstantinov (1958a,c, 1962) conducted studies on the weight to size relationships in larval growth; Konstantinov and Luzina (1961) investigated the cytological basis of larval development; Miseiko (1965), Downe and Caspary (1970, 1971, 1973), and Downe (1973) described swarming and mating behavior of chironomids under natural and laboratory conditions.

Research conducted on the responses of the immature stages of chironomids to various environmental conditions has proven important in demonstrating the chironomid's ability for survival. The great majority of experimentation in this area has been done on the larvae using chemical pollutants, pH, salinity, temperature, and radiation as
Rhode (1912) categorized several species of the Chironomidae according to the aquatic situations to which they had adapted. He found that the larvae of *Trichocladius halophilus* Kieffer and *Tanytarsus excisus* Kieffer thrived in what he termed "inorganically foul waters" which contained a high salt content (i.e., 3 to 5.5% NaCl by weight). Rhode also observed a variety of chironomid larvae in "organically foul waters."

Pause (1918) tested the effects of certain chemicals and conditions on the larvae of *Chironomus gregarius* Kieffer. Larvae were exposed to solutions of a strong base (potash lye), alcohol, anesthetics (chloroform and cocain), and acids (citric and acetic). He found that a strong solution of citric acid caused larval death after 21.5 hours, and that a 0.1% solution of glacial acetic acid caused almost instantaneous death to larvae. Pause also observed a strong larval resistance to high concentrations of hydrogen sulfide in the water. He noted that younger larvae responded to these chemical reagents more quickly than did the older larvae.

Skadovski (1924) demonstrated that *C. plumosus* larvae could survive indefinitely in a weak HCl solution; however, at pH 2 they could live only a few days. The maximum alkalinity that these larvae were able to tolerate was found to be pH 12. Cherfas (1939) wrote on the toxicity of copper salts given off by hydroelectric plants. Gromov
(1941) studied the effect of paper mill pollutants on the bottom fauna of the Kama River in the U.S.S.R. Stroganov and Pazhitkof (1941) and Tauson (1947) studied copper salt toxicity on chironomids and also determined that early instar larvae were better able to adapt to an acidic salt environment than later instar larvae. Tauson (1955) found that C. plumosus larvae could live only 1.5 days in a 0.01N solution of K_2CrO_4, and even less if the K_2CrO_4 was more concentrated. Nitric acid salt, Pb(NO_3)_2, was shown to be less toxic; in a 0.089N solution, larvae of C. plumosus survived for up to 3 days. He also found that H_2SO_4 was more toxic to the larvae as an acid than in its salt form.

Konstantinov (1953a, 1967, 1968a,c) surveyed the benthos of certain rivers in connection with pollution from oil refineries and other sources. Konstantinov (1960) described the toxic action of certain inorganic acids, salts, and bases on two species of chironomid larvae, Chironomus annularius Meigen and C. dorsalis. He found CuSO_4 to be effectively toxic at a 0.001% (10ppm) concentration, ZnSO_4 to be toxic at a 0.005% (50ppm) concentration, and FeSO_4, H_2SO_4, HCl, and NaOH to be toxic at 0.01% (100ppm) concentrations, the latter four given in order of decreasing toxicity with respect to time. The larvae of C. annularius were generally more resistant to these substances than those of C. dorsalis, and the younger larvae of both species were generally more resistant to these toxins than older ones. Belyavskaja, et al. (1968) wrote on the toxic action of certain
herbicides on water invertebrates and fish. Thornton (1972) conducted a study on the physiological effects of NaCl on Chironomus attenuatus Walker. Luferov (1958b), Biever (1968), and Konstantinov (1958c, 1971b) described the relationship between temperature and larval respiration and growth.

Experimentation on the eggs of chironomids thus far has been restricted to normal embryological studies (see above), centrifugation (Yajima, 1960; Overton and Raab, 1967), point exposure to ultraviolet radiation (Yajima, 1964; Kalthoff, 1971), and temperature effects (Biever, 1968).

The literature discussed here represents only a small portion of the available published work on the Chironomidae and related nematocerans. For more comprehensive listings of works in this field, I suggest Brehm (1926), Johannsen and Butt (1941), Konstantinov (1971a,c), Counce and Waddington (1972), and Johannsen (1973).
MATERIALS AND METHODS

A standing colony of *Chironomus riparius* was maintained using the method outlined by Biever (1965) with slight modifications on the apparatus (Fig. 1).

Six small rearing units were constructed from one-pint Corning Pyrex-ware® storage containers (Figs. 3 & 4). An aperture 7 cm in diameter was made in the plastic lid of each unit to serve as an exit for the emergent adults; a small hole was made in the side of the lid for the air stone tubing. A cylindrical enclosure 10 cm high and 11 cm in diameter was made to fit atop the plastic lid. This was constructed of fine plastic screening (#40 mesh) held together with silicone caulk. Each unit contained 25 mm clean, fine sand and 400 ml water.

One large rearing unit was prepared from an 18.92 liter (5 gallon) aquarium (Fig. 2). A tight-fitting, #40 mesh screen cover was made to contain the emergent adults; a small perforation was made near one edge of the cover to accommodate the air stone tubing. The large unit was filled to 25 mm with fine sand, and to 10 cm with water.

A mating cage was constructed from a 9 in. x 3 in. Ekcoloy Spring Form® cake pan (Fig. 5). The removable bottom of the pan was inverted within the rim of the pan so
that the central column of the bottom piece projected downward. The pan was then placed upon a 23 cm high support tripod, with the central column fitting through the ring of the tripod. A cylindrical glass jar 9.5 cm high and 6.5 cm in diameter was used as an oviposition vessel. The jar, filled with water, was held in place within the central column of the pan bottom by an inverted 1000 ml beaker. A small mirror was positioned at a 45° angle within the support beaker to facilitate observation of the jar contents from beneath. A screen floor was caulked to the upper surface of the pan bottom, and a 6.5 cm hole was cut in the screen to accommodate the mouth of the oviposition jar.

A cylindrical enclosure, 30.5 cm high and 22.5 cm in diameter, was constructed of #40 mesh screening, and placed into the pan. A 2.5 cm hole was cut in the screen enclosure, just above the pan rim, to accommodate a 2.5 cm length of 2.2 cm (7/8 in.) glass tubing. The glass tube was inserted halfway through the hole in the screen enclosure and caulked into place. The portion of the tube lying outside the enclosure was wrapped with black plastic tape to fit the inside diameter of an entomological aspirator tube for use in transfer of adults from the rearing units to the mating cage.

Emergent adults of *Q. riparius* in the rearing units were captured with an aspirator and transferred to the mating cage. Mated females were compelled to deposit their egg masses into the oviposition jar provided. The jar was checked several times a day for egg masses, and the solution
in the jar was changed every 24 hours.

All of the water used in the study was tap water which had been aerated for at least 24 hours at 21°C.²

Individual control and test egg masses were examined immediately following discovery for number of eggs, and transferred to 2-dram glass vials. Eggs were counted individually by following the back and forth windings of the gelatinous ribbon in which they were laid down, from one end of the egg mass to the other (Johannsen, 1905; Branch, 1923, 1928, 1931) (Figs. 7 & 8). The vials were stoppered with cotton, and regular checks were made on the egg masses within for viability (Fig. 6).

Twelve egg masses, each containing approximately 475 eggs, were used as controls in order to determine the mean percent hatchability under normal laboratory conditions. The oviposition jar in which the egg masses were deposited, and the vials in which they developed, were filled with plain aerated tap water at 21°C.

Graduated test solutions (Am. Publ. Health Assoc., 1965) of technical calcium sulfide (CaS) in aerated tap water were prepared a liter at a time, at concentrations of 1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, and 100.0 mg/l, for the test egg masses. The oviposition jar was filled with

²Some variability in the organic and inorganic ion content of tap water may be expected with respect to its source and treatment. Therefore, artificial pond water may be substituted for tap water at the experimenter's discretion.
fresh test solution every 24 hours so that the eggs would be introduced directly to the specific sulfide concentration at the time of egg mass deposition. Each egg mass was transferred with the proper sulfide dilution to its own 2-dram vial. The solutions in the vials were changed every 24 hours to insure relatively stable sulfide levels for the duration of the 72 hour period allowed for embryogenesis and larval emergence. Six egg masses were tested per sulfide concentration. The arcsine (angular) transformation of percent hatchability for test eggs was plotted against the initial sulfide concentration in mg/l $\text{H}_2\text{S}$ and $\text{HS}^-$. A 0.95 percent confidence of limits and best curve fit were performed using this data, and the $\text{TL}_m$ 72 interpolated (Fig. 9). Test eggs that failed to hatch were chosen at random and examined for abnormalities by comparing them to control eggs at critical periods of development.

Determinations for pH and dissolved oxygen were made at 0 hours and again at 24 hours for each sulfide concentration used. The solutions were tested in 1 liter quantities that were prepared with aerated tap water at $21^\circ\text{C}$. These liter bottles were kept sealed for the entire period between readings. Determinations were made using a Corning Model 12 pH meter with Sargent S-30072-15 combination electrode, and a YSI Model 54 oxygen meter with YSI $\text{O}_2$/Temp.-sensing probe.
RESULTS

The mean value for hatchability in the control group was 94.23%. Based on this figure, an average of 5.77% of the eggs in any given viable egg mass will fail to hatch under normal laboratory conditions.

Among the test egg masses, the mean hatchability for 1.0 mg/l sulfide was 92.48%; for 1.8 mg/l, 93.80%; and for 3.2 mg/l, 93.30%. Clearly, these values do not vary significantly from that of the controls. These results suggest that little or no toxic effect was sustained by the embryos at the lowest sulfide dilutions.

The mean hatchabilities for 5.6 mg/l and 10.0 mg/l sulfide were 60.23% and 46.77% respectively. Development of the fertilized eggs in the egg masses tested at these two concentrations proceeded normally up to the point of hatching. However, the numbers of larvae that hatched successfully were significantly lower than in controls.

The egg masses tested at 18.0 mg/l sulfide exhibited a mean hatchability of 12.61%. The majority of the embryos developed into larvae, but most of these failed to hatch at the end of the 72 hour period. Movement was observed in many of the unhatched larvae.

At 32.0 mg/l sulfide, a mean hatchability of 1.42% was established. Usually less than half of the eggs in each egg
mass contained embryos developed to the point of external delineation of segments. Of those that did, usually less than a third reached the larval stage, and even fewer succeeded in hatching.

A mean hatchability of 0.45% was recorded for egg masses tested at 56.0 mg/l sulfide. Formation of the blastoderm occurred in most of the eggs; the processes of gastrulation and elongation of the germ band were evident in some of the eggs; external delineation of segments was observed in only a few eggs. Successful hatching of larvae rarely occurred.

At a concentration of 100.0 mg/l sulfide, a mean hatchability of 0.00% was recorded. Little or no development beyond the formation of the blastoderm was observed. Degeneration of yolk and nuclei occurred in many eggs.

All mean hatchability values were converted to arcsine values according to the equation \( \theta = \arcsin \sqrt{p} \), where \( p \) is equal to the percent hatchability (Table 3). This method is employed for transforming percentages and proportions into a variable meeting the assumptions of the analysis of variance. These results were plotted against the sulfide concentration on a semilog scale, and the TL\(_{72}\) was interpolated (Fig. 9). This value corresponded to a concentration of 9.15 mg/l sulfide. 0.95 percent confidence limits were established for all hatchability values (Fig. 9), and the equation best fitted to the resulting curve was \( y = a(e^{bx}) \), where \( y \) is the arcsine value, \( x \) is the sulfide conc., \( a \approx 9.68 \), \( b \approx -8.37 \times 10^{-2} \), and \( e \) (the natural log) \( \approx 2.72 \).
DISCUSSION

Difficulties in the Colonization of C. riparius

C. riparius was chosen as the subject for this study because of its ability to reproduce under laboratory conditions. Unfortunately, the ample content of the egg masses (approximately 200 to 600 eggs per mass) and the large number of egg masses produced daily by a medium-sized laboratory colony provide misleading figures for the useful yield of this species. I found only a fraction of any given day's yield of egg masses to contain a significant number of fertilized eggs. Most of the egg masses which were deposited in the oviposition jar proved to be totally non-viable; other egg masses exhibited only partial viability. In those egg masses where only some of the eggs were fertilized, these eggs were localized at the ends of the gelatinous mass; the central region of the mass contained only unfertilized eggs.

This condition of localized fertilization was manifest in both control and test yields alike, and could only be attributed to some difficulty in oviposition, since fertilization of the eggs occurs at that time. The frequency of non-viability and localized fertilization was much lower in egg masses which had been deposited in the rearing units before the adults could be transferred to the mating cage.
This observation leads me to believe that the adults of *C. riparius* preferred the conditions of the rearing unit to those of the mating cage for copulation and oviposition.

Unlike the still water in the oviposition jar of the mating cage, the water in the rearing units was in constant motion due to the bubbling of the air stones within. This gentle rippling action of the surface water, combined with a local increase in humidity from the bubbling action, proved conducive to mating and oviposition behavior.

As a result of this discovery, emergent adults were removed from the rearing units at 3-day intervals to allow some mating of adults to occur in the units, under the more favorable conditions. Some egg masses were deposited in the units due to this period of adult retention; these egg masses were used as simultaneous controls. Most of the adult females were transferred to the mating cage before oviposition had occurred. This method provided a greater yield of viable egg masses.
Reactions of Calcium Sulfide in Tap Water

The plotted results (Fig. 9) clearly show that percent hatchability of *C. riparius* eggs declines as the initial concentration of dissolved sulfide is increased. This inverse relationship correlates the increase in frequency of terminated development with the addition of greater amounts of CaS to the test solutions, but gives no indication concerning what has actually occurred in the water to bring about such an event. It was necessary, therefore, to elucidate the chemical chain of events that occur when CaS is added to aerated tap water, and allowed to react over a period of 24 hours, in order to determine the true limiting factor (or factors) to embryogenesis in *C. riparius*.

When CaS is added to tap water which has been aerated (saturated with atmospheric oxygen and carbon dioxide) for 24 hours at 21°C, the following reactions occur in the approximate order in which they are given below.

Initially, the calcium sulfide reacts with water and carbon dioxide to form calcium carbonate and hydrogen sulfide:

\[
\text{CaS} + \text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{CaCO}_3 + \text{H}_2\text{S}.
\]

The hydrogen sulfide then partially dissociates to give a sulfhydryl ion plus a hydrogen ion. The sulfhydryl ion may dissociate further into a sulfur divalent anion plus two hydrogen ions:

\[
\text{H}_2\text{S} \leftrightharpoons \text{SH}^- + \text{H}^+ \leftrightharpoons \text{S}^- + 2\text{H}^+.
\]
The extent of this dissociation depends on the pH of the aqueous environment. The pH of the test solutions lay between 8 and 9.5 (Table 1). At a pH of 8, the three possible states of dissociation shown above would be present in the following ratio (at equilibrium):

$$\frac{[\text{SH}^-]}{[\text{H}_2\text{S}]} = 10.8 \quad ; \quad \frac{[\text{S}^= \text{]} }{[\text{SH}^-]} = 10^{-7}.$$ 

At pH 9, this ratio would be

$$\frac{[\text{SH}^-]}{[\text{H}_2\text{S}]} = 108 \quad ; \quad \frac{[\text{S}^= \text{]} }{[\text{SH}^-]} = 10^{-6}.$$ 

The hydrogen sulfide present reacts with water to yield elemental sulfur plus hydrogen gas:

$$\text{H}_2\text{S} + \text{H}_2\text{O} \rightarrow \text{S}^0 + \text{H}_2\uparrow + \text{H}_2\text{O}.$$ 

The trace amounts of sulfur divalent anion combine with oxygen to give sulfate ions:

$$\text{S}^= + 2\text{O}_2 \rightleftharpoons \text{SO}_4^{=}. $$

Sulfhydryl ions combine with oxygen to form hydrogen sulfate ions, which dissociate into sulfate and hydrogen ions to the point of equilibrium:

$$\text{HS}^- + 2\text{O}_2 \rightleftharpoons \text{HSO}_4^- \rightleftharpoons \text{SO}_4^{=} + \text{H}^+.$$ 

Sulfate ions will combine with any calcium divalent cations present to form calcium sulfate:

$$\text{SO}_4^{=} + \text{Ca}^{++} \rightarrow \text{CaSO}_4.$$
Sulfur divalent anions, elemental sulfur, and sulfhydryl anions will react with free or combined oxygen to form thiosulfate anions:

\[ \text{S}^2- + O_2 \rightarrow \text{S}_2\text{O}_3^2-; \]
\[ \text{S}^0 + O_2 \rightarrow \text{S}_2\text{O}_3^2-; \]
\[ \text{HS}^- + O_2 \rightarrow \text{S}_2\text{O}_3^2-. \]

The thiosulfate can react with water in the presence of chlorine (remaining from treatment of tap water) to yield sulfuric acid plus hydrogen ions and chlorine anions:

\[ \text{S}_2\text{O}_3^2- + 4\text{Cl}_2 + 5\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{SO}_4^- + 8\text{H}^+ + 8\text{Cl}^- . \]

The thiosulfate ions may also convert to other species:

\[ 2\text{S}_2\text{O}_3^2- \leftrightarrow \text{S}_4\text{O}_6^2- + 2\text{e}^- . \]

Many more reactions are possible in the chemical interaction of CaS with tap water, but the most important reactions have been discussed here. The major chemical species present in the test solutions at the close of the 24 hour period include: CaCO₃, CaSO₄, S⁰, S₂O₃²⁻, SH⁻, H₂S, and H⁺.

Despite the release of hydrogen ions by these reactions, which would tend to lower the pH of the test solutions, sufficient CaCO₃ was formed to not only counteract the acidity (i.e., as a buffer: CaCO₃ + H⁺ → Ca⁺⁺ + HCO₃⁻), but actually cause an increase in pH over the 24 hour period.
(Table 1). Also, since many of the reactions with sulfur involve oxygen uptake (i.e., in the formation of $SO_4^{2-}$ from $S^-$ or $HS^-$, and in the formation of $S_2O_3^{2-}$ from $S^-$, $S^0$, or $HS^-$), there was a marked decrease in dissolved oxygen (D.O.) in the test solutions over the 24 hour period between changes, especially in those solutions with high initial concentrations of sulfide (Table 2).
Significance of Results

It is clear from the results of the pH tests (Table 1) that conditions of extreme acidity or alkalinity were not responsible for the termination of embryogenesis in the *C. riparius* eggs. However, the findings for the end-products of the chemical reactions, and the results of the D.O. tests (Table 2) suggest that increased levels of dissolved sulfur products (i.e., sulfide, thiosulfate, and sulfate) and decreased levels of dissolved oxygen contributed significantly to this end.

The occurrence of sulfide (including H$_2$S, HS$^-$, and S$^-$), thiosulfate, and sulfate in fresh water bodies is a natural and quite common event. H$_2$S is generated by most heterotrophic bacteria as a by-product of protein metabolism and protein decomposition products. However, this process can take place only in deoxygenated regions of low redox potential, such as in the hypolimnion and sediments of productive lakes. For example, the anaerobic sulfate-reducing bacteria of genus *Desulfovibrio* use sulfate, sulfite, hyposulfite, thiosulfate, and elementary sulfur as a hydrogen acceptor in metabolic oxidation to produce H$_2$S (Hutchinson, 1957). In the waters of mineralized lakes, hundreds of mg/l of H$_2$S are formed by the bacterial reduction of sulfates. Therefore, very large quantities of the gas may be present in solution at quite high pH values, provided that the alkalinity is great (Hutchinson, 1957).
Studies done on the later instar larvae of *Chironomus* have shown that they are a major component of the benthos fauna in productive lakes and ponds, where they feed on detritus and certain microorganisms. These larvae are capable of withstanding high concentrations of \( \text{H}_2\text{S} \) in the course of normal development (Pause, 1918). *Chironomus* larvae also are capable of surviving in oxygen-deficient waters due to the presence of hemoglobin in their hemolymph. The hemolymph percolates through specialized anal and ventrolateral gills which provide a surface for \( \text{O}_2 \) diffusion. Wave-like body movements create a continuous flow of water past the gills for even greater respiratory efficiency.

There is a significant difference between the water constituency at the habitat of the older larvae, and that at the site of oviposition. The females of most chironomid species deposit their egg masses at the surface of the water, and anchor them firmly, by means of a rather short gelatinous cord, to some stationary object such as a protruding branch or rock. Therefore, the egg masses always remain within a few centimeters of the surface, where diffusion of atmospheric \( \text{O}_2 \) is a constant occurrence, and where sulfide ions cannot exist under natural conditions. This situation provides the eggs with an ample oxygen supply and comparatively mild chemical conditions for the duration of embryonic development. It is not until the larvae hatch and occupy deeper waters with successive molts that they become exposed to sulfide and low \( \text{O}_2 \) concentrations. Under natural
conditions the embryo developing within the egg has no need of specialized morphological and physiological adaptations to cope with such harsh conditions, and therefore, are unsuited for survival in the same habitat with the larvae.

The presence of significant levels of sulfide in the surface waters of a fresh water body would necessitate the constant introduction of that substance from some outside source, for sulfide is oxidized readily to thiosulfate and sulfate in the well-aerated surface waters of productive lakes, rivers, and ponds. However, industrial wastes could, and often do, serve as a perpetual source of sulfur compounds, sulfide being among them. Kozlowski et al. (1974) found that sulfide, in the form of CaS, is a constituent of air-cooled blast furnace slag (a by-product of iron smelting). The CaS was found to leach into solution, in relatively high amounts, when the slag was immersed in water over a 24 hour period.  

Precautions should be taken to prevent the introduction of sulfur-bearing pollutants to fresh water systems. Concentrations of sulfide that are harmful to chironomid embryos may prove equally harmful to other organisms which occupy a surface (or clean water) habitat in productive waters, and to those organisms which depend upon chironomid larvae for food.

3 No slag land fill site water analysis information is available at this writing.
EXPLANATION OF FIGURES 1 AND 2

Fig. 1 *C. riparius* colonization apparatus

Fig. 2 Large rearing unit
EXPLANATION OF FIGURES 3 AND 4

Fig. 3 Small rearing unit

Fig. 4 Components of a small rearing unit
EXPLANATION OF FIGURES 5 AND 6

Fig. 5 Mating cage and aspirator

Fig. 6 2-dram testing vials
EXPLANATION OF FIGURES 7 AND 8

Fig. 7 *C. riparius* egg mass

Fig. 8 *C. riparius* egg mass construction (diagramatic)
EXPLANATION OF FIGURE 9

Fig. 9  Arcsine transformation of percent hatchability versus the initial sulfide concentration in mg/l $\text{H}_2\text{S}$ and $\text{HS}^-$
Fig 9

Sulfide Conc. (mg/l)

Arcsine Transformation (θ)
EXPLANATION OF TABLES 1 AND 2

Table 1 pH of the sulfide (H₂S and HS⁻) test solutions at 0 hours and 24 hours, at 21°C.

Table 2 Dissolved oxygen (D.O. in mg/l) of the test solutions at 0 hours and 24 hours, at 21°C.
<table>
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<th>Initial Sulfide Conc. (mg/l)</th>
<th>pH (at 21°C)</th>
<th>0 Hrs.</th>
<th>24 Hrs.</th>
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<tbody>
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<td>9.50</td>
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Table 1

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<tr>
<th>Initial Sulfide Conc. (mg/l)</th>
<th>D.O. (mg/l at 21°C)</th>
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<th>24 Hrs.</th>
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Table 2
EXPLANATION OF TABLE 3

Table 3  Mean percent hatchability values for control and test eggs, and corresponding arcsine transformation values.
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<tr>
<th>Initial Sulfide Conc. (mg/l)</th>
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<th>Arcsin $\sqrt{p}$ (θ)</th>
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<tr>
<td>100.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
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Table 3
LITERATURE CITED


40


________. 1953c. Prospect of Chironomid Cultivation for Fish Feeding. Trudy Vsesozn. konferens. no voprosam ribnogo khoz-va.


________. 1955. Instruction to the Propagation of Chironomids as a Fish Food. Moskow.


Stroganov, N.S. and A.T. Pazhitkov. 1941. Deistvie stochnikh promishlennikh vol na volnie organizmy. *Uch. zap. MGU*, No. 60; also *Tr. Labov. gidrobiol.*, v. 4.


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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

Date

5-19-1975

Director's Signature