DISEASES OF THE CLEAR LAKE GNAT *CHAOBORUS ASTICTOPUS* (DIPTERA: CHAOBORIDAE)

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INTRODUCTION

The Clear Lake "gnat," *Chaoborus asticotopus* Dyar and Shannon, is a non-biting midge which spends all but its adult stage in a lentic environment. In Clear Lake, which has about 41,000 surface acres and contains about 1 million acre feet of water, the midge breeds in large numbers and in past years has been a serious deterrent to the full recreational use and development of lakeshore property. Hazeltine (1963) reported that collections of about 45 million gnats per light trap per evening were common during the summer months when control was not exercised. An excellent report on the economic importance and history of the control of the midge was reviewed by Cook (1965).

At the present time, about $40,000 are spent annually for chemical treatment, bringing the gnat under virtually 100 percent control for the past 6 years in Clear Lake. In order to find another method to aid in the control, attention has been given to the diseases of *Chaoborus*. The studies reported here are concerned with microorganisms pathogenic to the gnat. The ultimate goal is to utilize naturally occurring, host specific microorganisms such as bacteria, fungi, protozoa, nematodes and viruses in microbial control. If achieved, such control would eliminate the problem of toxic residues, destruction of beneficial insects, and could provide self-sustaining, long-term benefits.

PSEUDOMONAS AERUGINOSA MIGULA

Order: Pseudomonadales
Family: Pseudomonadaceae
Locality encountered: Sonoma County, California

Determination. The determination was made by G. M. Thomas, Division of Invertebrate Pathology, University of California, Berkeley. This strain of *Pseudomonas aeruginosa* produces a rose-colored pigment, is motile by a single polar flagellum occurring at either one or both poles of the cells, is gram negative, and cytochrome oxidase positive. It grows very slowly on nutrient agar, and is even less vigorous on enriched culture medium, such as brain-heart infusion agar with .2 percent yeast extract. Growth on AC medium indicates a preference for aerobic conditions.

Signs and symptoms. The major sign is the change from the normal transparency of the larval body to opacity. The first noticeable sign is an opaque white color of the entire larval body. In a day or two the white color intensifies and in the final stage infected larvae are dull milky white. The gradual color change is combined with a progressive loss of vigor. The dead larvae are pinkish red. The bacterium enters the body cavity most likely through the alimentary canal, where it produces general septicemia that kills the host.

Effects on the gnat. Preliminary laboratory tests were conducted with first, second, third and fourth instar larvae. Five to eight hundred larvae of each stage were tested. The mortality of first instar larvae averaged 75 percent with a dose of $2X10^7$ spores per cc and 56 percent with a dose of $2.5X10^6$. Second and third instars were less susceptible with an average mortality of 59 and 31 percent when exposed to the same dose of bacterium as first instar larvae. Fourth instar larvae demonstrated a high degree of resistance even at the highest dose used. The virulence of this strain rapidly declines after several transfers on artificial medium.

In the field, the natural incidence of infection in the several ponds surveyed ranged from a fraction of 1 percent to 20 percent. The ponds with a high num-

ber of infected larvae normally had a small population of gnats. The impact of the disease on the gnat was most noticeable during early spring, just before the pupation and emergence of adults. The infected larvae collected were in second, third and fourth instars; the first stage larvae were not examined. Larvae with apparent infections usually, with a minor exception, succumb before reaching the next developmental stage. In the laboratory a few fourth instar larvae reached the adult stage, which after death became pinkish red. The normal adult is light gray.

Field and laboratory data showed that this strain of *Pseudomonas aeruginosa* is capable of causing fatal disease of young instars and weakening fourth instar larvae.

It was concluded from this study that even though *Pseudomonas aeruginosa* was not observed to cause an epizootic, it is responsible for a considerable cumulative mortality throughout the season.

BACCILUS SP.

Order: Eubacteriales
Family: Bacillaceae

Determination. Rods, 3.6 to 4.6 by 1.1 to 1.27 μ. Gram positive, catalase positive, aerobic spore former. Sporangium swollen by spherical spore. Spores 1.5 to 2.0 μ by .8 to 1.0 μ. Pathogenic for *Chaoborus astictopus*. Growth on nutrient agar at pH 6.8–7.2. Locality encountered: Lake County, California.

Signs and symptoms. The first detectable signs of infection are decline in vigor and an opaque white color of larvae. As the disease progresses the larvae assume a milky white color. The gradual color change is combined with a progressive loss of vigor. The bacterium enters the body cavity, probably through the alimentary canal. The bacterium produces general septicemia. Microscopic examination revealed a large number of bacterial vegetative cells and spores throughout the body cavity.

Effects on the gnat. The natural incidence of infection in the ponds and lakes surveyed was normally low, ranging from a fraction to several percent. Preliminary laboratory tests were conducted with first and fourth instars. The mortality of first instar larvae ranged between 74 and 85 percent with the dose of 3.6×10^7 spores per cc during 48 hours exposure. The fourth instar mortality attributable to *Bacillus* sp. infection ranged from 8 to 27 percent with the same dosage as used for first instar larvae and exposure of 13 days.

It can be concluded that this particular strain is capable of causing a distinct disease in the gnat and presumably is responsible for cumulative mortality throughout the year; however, the high reproductive rate of the gnat during the summer season can completely mask the effect of the bacterium on the population.

DISEASES CAUSED BY STREPTOCOCCAE

Order: Eubacteriales
Family: Lactobacillaceae
Genus, species undetermined

Cells spherical or elongate, usually occurring in pairs or in chains. None of the species grows well on solid media. Microaerophilic to anaerobic. Catalase negative. Localities encountered: Lake, Mendocino and Sonoma counties.

Signs and symptoms. I. Bacteria normally in long chains.

The midguts and hindguts from freshly collected larvae are reddish-brown. The epithelial cells of midgut and the midgut itself are usually enlarged. The enlargement of the midgut may extend throughout its length or form irregular swellings in portions of it. The color of the digestive tract is most intense in freshly collected larvae and fades after a few days' incubation at 5° C. The vigor of infected larvae varies from normal to moderate.

II. Bacterial cells in pairs. The bacterium grows poorly on artificial media; the cultures are difficult to maintain even in an anaerobic environment.

The major signs are the yellow-red color of the midgut and hindgut and enlargement of epithelial cells of the gut. Other

symptoms are similar to those given above for bacteria associated in long chains.

Effects on the gnat. This group of bacteria is associated with the digestive tract of the gnat larvae, where they multiply. Normally, a dense growth of bacteria occurs in the gut, but the vigor of such larvae appears to be little affected by their presence. Only occasionally could mortality associated with the disease be attributed to streptococcal invasion of the gut. More often, streptococcal-caused diseases are accompanied by diseases caused by other microorganisms, which in turn may be able to infect only already weakened larvae.

Even so, streptococcal diseases characteristically produce low larval mortality. They do, however, have two notable features which may be important from a microbial control point of view: 1) The diseases caused by the bacteria are very infectious under natural conditions. On numerous occasions ponds were located in which entire populations were almost completely infected by one or the other member of the family Lactobacillaceae. 2) Infected larvae are also more susceptible to secondary attacks by other microorganisms.

It could be speculated that disease-weakened larvae would be more susceptible to chemical control and would require a lower dose of insecticide than would healthy larvae.

SPIRILLUM SP. (TENTATIVE IDENTIFICATION)

Order: Pseudomonadales
Family: Spirillaceae

Spiral shaped cells. Spirals consisting of 1½ to 7 complete turns. The bacterium is an obligate parasite and is probably not host specific.

Localities encountered: Lake and Mendocino Counties, California. A closely related bacterium was isolated from mosquito larvae and sent to us by Dr. William R. Kellen (State of California Department of Public Health, Bureau of Vector Control, Fresno, California) for determination of its effects on the gnat.

Signs and symptoms. Both bacteria are morphologically unseparable, as far as we were able to determine, and produced similar effects on gnat larvae. Infected larvae were sluggish in movement and succumbed in 7 to 14 days after infection. Normally, the symptoms of the disease are so unspecific that all the larvae believed to be infected had to be examined microscopically for final determination of the causative agent. The problem of diagnosis was further complicated by frequent contamination with other bacteria, which masked the already difficult-to-recognize symptoms.

Effects on the gnat. *Spirillum*-infected larvae were collected infrequently and were found sporadically during routine examination of weak larvae brought from the field. Since several attempts to culture this organism on artificial media failed, dead infected mosquito larvae obtained from Dr. Kellen were used as the source of inoculum for a few infectivity tests conducted in the laboratory. During a period of 14 days, 20 to 50 percent of a total of 60 fourth instar larvae tested became infected. The larvae infected in the late fourth instar may pupate and emerge as infected adults. In such cases the infection may cause a reduction in fertility of females. From the data so far available, it can be speculated that the bacterium has a limited impact on the gnat population.

VORTICELLA SP.

Order: Peritricha
Family: Vorticellidae
Species: Probably *V. campanula*

Inverted bell-form; colorless; peristome wide, with a contractile, unbranched stalk, peristome 53–133 μ long. Locally encountered: Sonoma County, California.

Signs and symptoms. The protozoa cling to the external surfaces of almost all parts of the larval body. The stalk of each organism is attached directly to the cuticle of the larvae. In spite of the fact that *Vorticella* is not parasitic on the larvae, since it does not take food from them, most infected larvae examined in the

laboratory were in poor vigor and usually succumbed in a few days.

Discussion. Association of *Vorticella* with the larvae of *C. astictopus* is incidental, since aquatic plants collected from the same pond also had *Vorticella* attached to them. The poor vigor of larvae attributable to *Vorticella* adhesion was caused by immobilization of the larvae by the great numbers of *Vorticella,* which were even attached to mouth parts, eyes, setae, etc. The *Vorticella*-infected larvae normally can be collected during winter months when the water temperature is low.

THELOHANIA CORETHRAE

Order: Microsporidia
Family: Nosematidae
Species: *T. corethrae*
Localities encountered: Sonoma and Mendocino Counties, California.

Determination. *Thelohania corethrae* was first described by Shuberg and Rodriguez (1915) from *Corethra* (*Sayomyia*) *plumicornis,* and was later found associated with *Chaoborus flavicans* (Fantham *et al.* 1941). The original description of this protozoan as given by Schuberg and Rodriguez does not include measurements of the parasite. This information was provided by Fantham *et al.* In 1965, for the first time, *Thelohania corethrae* was observed infecting larvae of *C. astictopus* in several ponds near Healdsburg, Sonoma County, California. Presence of eight sporoblasts places the microsporidian parasitizing this midge species in the genus *Thelohania.* Measurements of fresh spores are 6.8x2.8 μ, which is in the size range reported for *T. corethrae.* Measurements of other stages also correspond with the description given by Fantham *et al.* (1941).

Signs and symptoms. In the early stages of parasitization, i.e. during schizogony, infected larvae are difficult to recognize even with the aid of a dissecting microscope. The infected areas first become recognizable during sporogeny. At this time infections acquire a conspicuous dull-white appearance. This condition is typical of patent infection and was apparent in larvae examined in the laboratory.

At first the sites of infection look like white dots barely visible to the naked eye. In a week or two the infections increase in volume and frequency in number to a point where the large spore masses may eventually occupy ⅓ of the abdominal cavity. In general, larvae with light infections often reach the pupal and adult stage. It is not uncommon, however, to see pupae with ⅓ of the abdominal segments filled with spore masses. Adults that develop from heavily parasitized pupae are weak and incapable of flying. Dead adults, partially emerged from the pupal skin, are frequently the result of *Thelohania* infections. Spore masses of parasitized adults can only be seen with dissection and microscopic examination. *T. corethrae* is tissue-specific to oenocytes, as indicated by Fantham *et al.* (1941); the spore masses are hypertrophied cells packed with spores (Figure 1).

Effects on the gnat. Mortality caused by *Thelohania* is cumulative, that is, only a portion of infected larvae of a given instar reach the next developmental stage. The highest mortality takes place in the fourth instar. Under laboratory conditions the death of parasitized fourth instar larvae averaged 82 percent as compared to 11 percent in controls in tests using a total of 314 infected and 242 healthy larvae. It was observed that death occurs most frequently just before or during pupation. A high percentage of infected adults are weak and incapable of flying, and consequently they do not mate.

The youngest larvae observed with patent infections were in the early third instar. The spore masses in this instar are usually small and interfere little with development from the third to fourth stage; mortality was lowest at this transition.

This species of *Thelohania* can be transmitted transovarially, and larvae develop patent infections in 16 to 24 days at 20° C. Peroral infection of larvae was not demonstrated experimentally in the laboratory, but it is believed that larvae can be infected when spores reach a certain but as yet undetermined stage.

FIG. 1.—Spore masses of *Thelohania corethrae* infecting a fourth instar *Chaoborus astictopus*.

In the field the natural incidence of infection in the several ponds surveyed ranged from 1 to 20 percent. However, the percentage of infected larvae varies with the season. The highest percent of larvae with apparent infections were collected from the overwintering population when the temperature of the water was low and the larvae were exposed to the parasite for the longest time. This period was followed by a gradual decline in the frequency of patent infection as the water temperature began to rise and adults began to emerge. During the summer months the number of larvae with patent infection dropped to its lowest point. Summer generations of *C. astictopus* require the shortest time to complete their life cycle; Snell and Hazeltine (1963) reported a period of 36 to 48 days from egg to adult in Lake County, California. During autumn the percentage of larvae with patent infection gradually increased as the larval period became progressively longer with decreasing water temperature. In the last part of October the emergence of adults ceased.

ADELINA SP.

Order: Coccidia
Family: Adeleidae
Species: Probably a new species and a new host record.
Localities encountered: Pinto Lake, Santa Cruz County; and several ponds near Healdsburg, Sonoma County, California

Determination. The final determination was made by Gerard M. Thomas, Division of Invertebrate Pathology, Berkeley, California. The slides, stained with Haematoxylin from Santa Cruz County, were deposited at the Division of Invertebrate Pathology, University of California, Berkeley, California.

Signs and symptoms. Heavily parasitized larvae have a granular appearance caused by masses of oocysts which are visible through a transparent cuticle. The cysts are freqently so tightly packed against the cuticle that the larvae resemble plastic bags filled with glass beads. The color of such larvae is creamy gray. Most larvae examined had cysts in all thoracic and ab-

dominal segments. Often the cysts were also located in head capsule.

The response of infected larvae to an external stimulus, such as touching the larvae with a dissecting needle, was similar to normal larvae. It was only in the final stage of the disease that the larvae declined in vigor prior to death.

Microscopic examination revealed the presence of cysts in various stages of development mainly in the fat body and floating free in the blood. Cysts were not observed in the alimentary canal, muscle or cuticle.

Effects of the parasite on its host. The youngest parasitized larvae observed were in the third instar, but the majority of diseased larvae collected were in the fourth instar.

The natural incidence of infection in ponds surveyed ranged from a fraction of 1 percent to several percent, with the exception of one pond in Sonoma County, California, where over 50 percent of the larvae were estimated to be parasitized by *Adelina*. It is interesting to note that *Adelina*-infected larvae were usually found in ponds rich in organic matter.

In the early stage of infection, the presence of the protozoan can be established only by dissection of the larvae, since at this stage infected larvae do not show any noticeable symptoms.

In the late stage of infection, the fat body reserves become exhausted and metamorphosis becomes impossible. Infected pupae were not found. Parasitized larvae maintained in the laboratory also succumbed before reaching the pupal stage.

DISEASE OF UNKNOWN ORIGIN (PROBABLY VIRUS CAUSED)

Localities encountered: Lake, Mendocino and Sonoma Counties, California.

Determination. In this laboratory 32 different bacteria were isolated from the blood and the alimentary canals of diseased larvae. The isolates, which consisted of aerobic and anaerobic bacteria, were cultured on various media and their effects on the 1st and 4th instar larvae were tested. Since none of the bacterial isolates produced typical symptoms of the disease, the presence of a virus in the diseased larvae was suspected. Infected larvae were sent to Dr. T. B. Clark, University of California —State Department of Public Health for electron microscope examination. The conclusion of the examination was that inclusion bodies of virus origin were not present.

Symptomatology. For convenience, the external symptoms of the disease are divided into three stages of its development, the initial, intermediate and final stages.

The initial stage is characterized by a slight color change of the midgut; the normal transparent color of this organ becomes a faint opaque white. This color change can best be observed by placing the larvae in clear water on a black background. The development of the disease is slow at first and the vigor of the larvae differs little, if any, from normal. Under confinement at room temperature this condition may continue for several days.

The intermediate stage is marked by a gradual shrinking of the larvae. Infected larvae become shorter, appear broader than normal, and they almost continually move the head back and forth. At this stage the larvae remain permanently on the water surface. With the process of shrinking, the change of the midgut color becomes gradually more and more masked by the constriction of the abdominal segments. The duration of this stage is only 1 to 2 days.

The final stage can be easily distinguished from the above stages by size of the larvae, which are reduced from ¾ to less than ½ of the normal length. The larval bodies are wrinkled and only slight trembling indicates that they are still alive. The duration of this stage is a day or two. Dead larvae remain constricted for one day and then turn white, becoming somewhat longer than live larvae in this final stage.

Effects on the gnat. The disease is most common during the summer months and least prevalent during winter. The highest number (50 percent) of infected larvae

were collected in July, 1966 in Blue Lake, Lake County, California.

In the infectivity tests, healthy fourth instar larvae were exposed to an inoculum prepared from macerated infected larvae. Three infected larvae were ground in a porcelain mortar and the resulting pulp was suspended in a beaker with 100cc of Clear Lake water. Five larvae were placed in each beaker; controls were free of inoculum. The experiments were terminated in 30 days. One hundred and seventy larvae were used in the tests.

The results of the tests showed that 25 to 37 percent of the larvae become symtomatic and that the disease is infectious.

The larvae collected in the field were, in the majority of cases, in the intermediate stage and reached the final stage of the disease development after incubation in the laboratory for 1 to 3 days. The larvae in the final stage endured the disease for a day or two, but their vigor was so low that microscopic examination was needed to determine if the larvae were dead or alive. Recovery of the larvae from the intermediate or final stage of the disease was never observed. Larvae from the area with high incidence of disease were weak and susceptible to attacks by other microorganisms.

Discussion. At the outset of this study no information was available dealing with diseases of the Clear Lake gnat. It is established in this article that the gnat is vulnerable to attack by microorganisms of all major groups known, with the exception of nematodes and rickettsia. The microorganisms capable of inducing diseases range from very specific obligate pathogens to organisms that produce disease only when vigor is reduced by other environmental conditions.

Bacteria, such as *Pseudomonas aeruginosa* and *Bacillus* sp., are able to produce diseases in first, second, and in weak third and fourth instar larvae. The impact of these diseases is most noticeable on overwintering larvae, which are exposed to bacterial attack from September to May. During that period the gnat does not reproduce and the mortality attributable to these bacteria can be established. In the early spring, just before pupation and emergence of adults, the larvae are more susceptible to bacterial attack than at any other time. At this time the pupation period is the longest, and naturally occurring ruptures which accompany a change from fourth instar to pupae provide a port of entry for bacteria. Although mortality caused by facultative pathogenic bacteria is low, they are believed to be responsible for a considerable cumulative mortality throughout the season.

Laboratory tests, in which fourth instar larvae were exposed to *Pseudomonas aeruginosa* suspensions ranging from 2.5×10^6 to 2×10^7 spores/cc, indicate that the mortality of larvae did not increase to any considerable extent even with the highest dose used. This suggests that the invasive capacity of this bacterium is low and that penetration into the body cavity is dependent on the gut rupturing during molting, as is suggested by field observations.

Diseases caused by streptococci and related genera are usually not fatal to the larvae, although the number of bacteria in symptomatic larvae is usually very high. The major feature of this disease, from the microbial point of view, is that the disease is very infectious under natural conditions. It is speculated that disease-weakened larvae would be more susceptible to chemical control and would require a lower dose of insecticide than healthy larvae.

A disease believed to be viral in origin produces considerable mortality during summer months, but its effect on the gnat population is masked by the high rate of reproduction associated with this season. It is interesting to note that the larvae from the area with a high incidence of this disease are weak and more subject to invasion by other microorganisms. Also, larvae from a local lake where this disease is prevalent have demonstrated over the years to be generally more sensitive to chemical toxicants than are larvae from other sources.

Diseases caused by protozoa are limited

mainly to the two obligate parasites, *Thelohania corethrae* and *Adelina* sp. *Thelohania* can be transmitted via the egg and perpetuated in that way from generation to generation. Peroral infection, even though it was not demonstrated experimentally, was apparent from two years of field data. Thus, once established, this parasite plays its part in every generation of the gnat. The parasite is transovarially transmitted during the summer when the life cycle of the gnat is completed in 5 to 7 weeks and they are exposed to *Thelohania* spores for the shortest time. During the winter, larvae are exposed to spores for several months and they become infected perorally. Even though in the field the natural incidence of infection in the several ponds surveyed only ranged from 1 to 20 percent, it is believed that when the infectious stage of the spores becomes known, this protozoan may prove to be of vital importance in controlling the Clear Lake gnat.

The disease caused by *Adelina* is normally limited to a small percentage of larvae in areas surveyed. In addition, the life cycle of this species is poorly known. It was demonstrated that *Adelina* produces fatal disease of third and fourth instar larvae, but any reduction in the gnat population parasitized by the protozoan was not observed in natural conditions.

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