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MUTUALISM BETWEEN THE MIDGE *CRICOTOPUS* AND THE ALGA *NOSTOC*[1]

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INTRODUCTION

Larvae of the dipterous family Tendipedidae are found in practically every aquatic habitat from the polar regions to the tropics. Some, of the genus *Spaniotoma,* live symbiotically with mayfly or stonefly nymphs, either under the wing-covers or clinging to the appendages (Johannsen 1937), whereas others live in freely movable cases of definite form, in fixed cases, or in mud (Edwards 1929). Many are leaf miners and may be restricted to a particular species of plant, either in terrestial plants (Needham *et al.* 1928) or in aquatic plants, such as cattails and reeds (Walshe 1951). A few members of the genus *Cricotopus* are known to invade the pondweed, *Pontamogeton* (Berg 1950).

The two species of *Cricotopus* considered in this paper have been studied in their relation to the colonial blue-green alga *Nostoc parmelioides.* Heretofore no investigation has been made of this relationship; the only recorded observation of the association was noted by Johannsen (1937). He mentioned finding small chironomid (tendipedid) larvae coiled in disks of the alga *Nostoc parmelioides* and ascribed them to the genus *Spaniotoma.* Adult midges reared for this project were sent to W. W. Wirth, at the National Museum, for identification. He noted two types present and described both as new species. He examined the material used by Johannsen and stated that they were indistinguishable from *Cricotopus.* Thienemann (1954) did not mention the midge or the alga in his classic work on fresh-water biology.

Nostoc parmelioides was first described in 1843, by Kützing. In his description there is no mention of a tendipedid larva. Forti (1907), Lemmermann (1910), Tilden (1910) and Geitler (1932) all described this species but did not mention the presence of an insect larva. Drouet (1939, 1942, 1943) referred to the species but wrote only of its distribution.

The filaments of *Nostoc parmelioides* are held together by a gelatinous matrix so that a flat, vertical thallus is formed, measuring about 12 mm in height and 1 mm in thickness (Figure 1). All *Nostoc* disks observed were found to contain a tendipedid larva or they showed signs that the larva had been present. The larvae are host-specific and have not been found in a free-living state.

The family name Tendipedidae is used throughout this study since it has priority over the better known name Chironomidae. The term "mutualism" as used in this paper means the living together of two organisms with benefit to both members.

This study was made at the University of California's Wildlife and Fisheries Experimental Station, at Sagehen Creek about 15 miles north of Truckee, California.

METHODS

Monthly larval collections, made between September, 1954, and May, 1955, were all obtained from the same immediate area in Sagehen Creek; the scarcity of algal pads during other months necessitated collecting from a greater area of stream. The ratio of the 4th instar larvae to the number of pupae was obtained for each month in

[1] Sincere thanks are extended to Drs. Robert L. Usinger, Paul R. Needham, George F. Papenfuss, and Charles R. Goldman for their advice. Dr. Willis W. Wirth identified the two tendipedid species involved in this study. The use of facilities at the Sagehen Creek Station was made possible through the financial assistance of the Max C. Fleischmann Foundation of Nevada.

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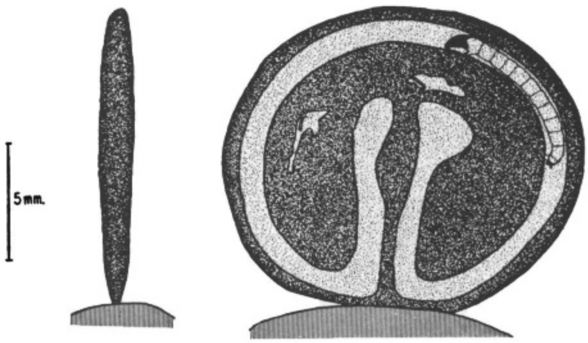

FIG. 1. Larval stage in the alga (end view and side view).

order to establish the major period of emergence for the tendipedid. Direct preservation of the algae in 10% formalin prevented the loss of pupae and larvae through emergence or through openings in the algae created during the process of collecting. This style of collecting gave a method for making comparisons among pads with larvae, pads with pupae, and pads that had been vacated.

The length of the pupal stage was determined by gathering 4th instar larvae that were ready to pupate and placing them in hatchery troughs which were fed by a flow of spring water. The dates of pupation and emergence were then noted. The swelling of the alga and the increased size of the tendipedid's thorax were signs that the time of transformation was near. The adults were captured and reared in small screen cages placed over the algal pads.

Determining the shape of the larval passageway

The excavation formed by the tendipedid always had the same characteristic shape. The older algal pads, being thin and transparent from the removal of matrix tissue by the feeding actions of the larvae, readily showed this passageway. Many of the younger pads were opaque, and if the passageway could not be seen by holding the pad in front of a light source, the algae were placed in a tightly closed jar, where they suffocated. With the death of the colony the algal pigments dissolved, leaving the blue pigment, phycocyanin, trapped throughout the tendipedid's tunnel and thus clearly showing the extent of the cavity.

Measurements of head capsules

Head widths were measured with an ocular micrometer. Measurements were made on the dorsal side of the capsules at their widest point near the posterior end. Capsule widths measured from newly hatched larvae verified the first instar peak. Occasional findings of cast skins from previous instars in the algal passageway with the larva helped to separate the stages. Shed capsules were not too reliable, however, since they were split in the act of molting, thereby losing their original shape. Immediately after a molt, the new head capsule was light in color and disproportionately large, so that a recently molted larva was easily recognized. The swollen thorax, characteristic of the late 4th instar stage, helped to identify this group as the last instar.

ALGAL LIFE CYCLE

Multiplication in the family Nostocaceae is either by means of specially differentiated resting spores called akinetes or by means of hormogonia (parts of trichomes). The cells divide within the spore forming a filament that later, after being released, develops into a colony. Hormogonia become separated from the trichomes when a weakening occurs in the chain of vegetative cells. The breakages occur most often at points where heterocysts (enlarged specialized cells) and vegetative cells join (Fritsch 1945). It is possible that the feeding activities of the tendipedid also create these breakages. After floating to a new location, the hormogonium comes to rest and grows directly into a new thread. The young colonies of *N. parmelioides* were spherical; those smaller than 2 mm did not contain tendipedid larvae. When the colonies reached a size of 3.5 mm or more, the larvae were usually present. At this size, the pads that were destined to stand erect became laterally flattened. Occurring less frequently was a 2nd form of *N. parmelioides,* the mature size of which was smaller than the vertical form. This was a horizontally flattened thallus with a verrucose surface, an irregular shape, and a swollen peripheral border wherein the tendipedid was always found. According to Chapman (1941), the form a colony takes will depend on the effect of the environment, which may determine the consistency of the mucilage. West and Fritsch (1927) state: "In no class have there been so many records of polymorphism as in the Cyanophyceae."

After the tendipedid had spent its larval stages within the alga and had pupated, the algal tissue surrounding the larva began to swell. The cortex was weakened and separated, creating an exit through the otherwise leathery algal sheath. The pupa forced its way through this exit and swam immediately to the surface of the water. Following the emergence of the tendipedid, the alga continued to decompose. All algae that were reobserved in the stream after the pupae emerged were completely disintegrated at the end of 2 weeks. Apparently, the death of the alga is caused by the removal of large amounts of algal tissue by the feeding of the larva, combined with the access of water through the break in the cortex. Experiments were tried to test the survival of the algae without the presence of the tendipedid, but the results were inconclusive because the methods of removing the larva usually injured the algal colony. However, during October, 1955, when many midge larvae were killed by an infestation of mermithid nematodes (see "predators"), the algal pads containing these dead larvae were yellow, thin, loosely attached to the rocks, and in a state of decomposition.

In Sagehen Creek *Nostoc* was most often found attached to rocks, which varied in size from pebbles (with just enough surface for the attachment of one algal pad) to large boulders densely covered with numerous pads. The alga was infrequently found attached to stationary sticks or branches in the water. At one location, where a hard clay bed prevailed, algal pads were found attached to the stream bottom. The depths at which the algae were found varied from directly below the surface, covered by only a small layer of flowing water, to the bottom of pools 3 feet deep. The algae were observed in both lentic and lotic waters, although there was a preference for the latter. *Nostoc* was frequently found growing on the elges of waterfalls and in the spillways. Colonies in swift running waters were frequently covered with simuliid larvae and pupae. There was no orientation of the alga to the direction of the current.

Development of the Insect Stages

Eggs

On April 24, 1955, a day after the emergence of 6 *Cricotopus* adults in captivity, 2 sticky egg masses were noticed in the water; one egg bunch was floating at the surface while the other was stuck to the bottom of the container. Two days after these adults emerged, a female was observed laying eggs, a process that took about 20 minutes. In the mass there were fewer than 100 orange, oval eggs. Their incubation period was not determined, since no males had been present and the eggs had not been fertilized. Fellton (1940) mentioned that female tendipedids emerging in the laboratory deposited eggs even though they had not mated. The incubation period for this subfamily is usually just a few days, according to Johannsen (1937).

Three egg bunches found attached to the upper edges of *Nostoc* pads on August 8, 1955, consisted of elongated gelatinous strings wound back and forth within an irregular mass. The largest of these groups had fewer than 500 eggs. Although these eggs were found in August, the breeding season is assumed to be in March and April, when the emergence period occurred. This cycle may fluctuate from year to year since the breeding season varies with weather and seasonal changes; there is a direct relation between water temperature and transformation (Sadler 1935). During March the maximum water temperatures at Sagehen Creek were between 36° and 46°F.

Larvae

The 2 species here concerned, *Cricotopus nostocicola* Wirth and *C. fuscatus* Wirth, can be separated from each other and from other species only in the adult stages. According to Wirth (1957), larvae of *Cricotopus* can be identified to groups only, the groups being based on larval structures and feeding habits. He recognizes 4 larval groups in this genus; the *Nostoc*-dwelling larvae belong to the 4th, previously unrecognized, group.

Cricotopus larvae were present in nearly all the algal pads examined, and there was never more than one mature larva per pad. Pads lacking a larva always showed the typical passageway that it had excavated at one time. Pupae were found at all times of the year, but the peak of emergence was in March and April. Therefore a large percentage of vacated pads was recorded in May (Figure 2). Observations of *Nostoc* in Sagehen Creek in 1955 indicated that algae were abundant from January through April, and scarce in July and August. This paucity of the alga is correlated with the tendipedid emergence period.

The typical tendipedid passageway found in every pad, and the position of the larva within the alga, are shown in Fig. 1. The tunnel followed the periphery of the pad until it reached the base and from there the 2 passages moved parallel to one another to the center. These 2 central passages were not connected at any point.

Larvae removed from the algae were very slow and awkward. The undulatory swimming motions usually characteristic of other tendipedids were entirely lacking. They showed no ability to wriggle or to crawl when out of their algal passageway. In the passageway, however, the tendipedid moved very rapidly. When the larva moved forward it would seize the tissue of the alga with its mandibles and arch the thorax over its head. When

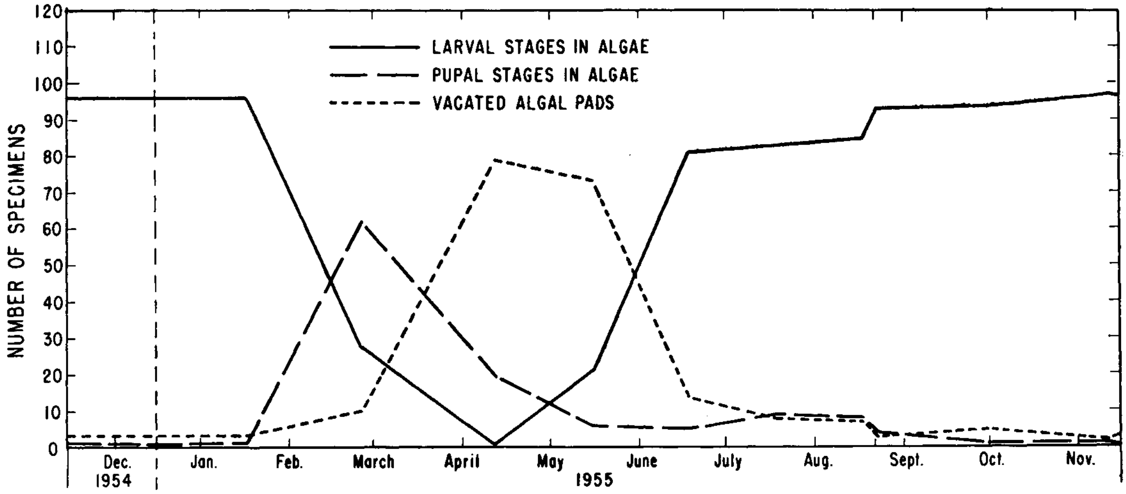

FIG. 2. Seasonal abundance of larvae and pupae.

the mandibles were released the anterior prolegs would seize the alga. The head would then be pointed forward and the mandibles would again grasp the alga. When the larva reached the end of the passageway it would reverse its position by sharply turning on itself in order to start off again with the anterior end forward.

In addition to the one large larva present in every mature algal pad, there could also be found several earlier instar *Cricotopus* larvae. These small larvae were distributed throughout the pad, where they created their own tunnels by eating their way through the alga (Fig. 1). Algae with one small larva were most abundant; pads with more than 10 larvae were less frequent. An algal colony sometimes included 50 larvae or more. In such a situation it was suspected that an egg mass may have been deposited on the algal pad and that the larvae, after hatching, had entered the nearest available plant. Berg (1950) noted similar occurrences in *Cricotopus elegans,* which mines in the aquatic *Potamogeton.* He said (p. 89): "Since all young larvae present in a group of several leaves floating together usually are concentrated in only one or two of the leaves, it seems likely that eggs are deposited in masses on or near *Potamogeton* leaves, and that newly hatched larvae tend to enter the nearest, most desirable, or most accessible leaf, and roam for short distances in search of fresh food as they grow older." He found that there was no restriction on the number of mature larvae of *C. elegans* per leaf, but that there was a limit on the number of penultimate-stage larvae—no more than 3 per leaf. More than 20 first instar larvae, in contrast, might riddle a leaf with an irregular network of criss-crossing mines.

There are several possibilities by which *Cricotopus* may invade the algal pads within a given locality. When an egg mass is deposited on an alga, the larvae that hatch will invade that pad. Larvae, on emerging from the egg mass, might be distributed by being swept downstream. It was noticed that, when small buds formed at the base of older algal colonies, they were occupied by young larvae. If these buds should separate from the parent plant, they would then already contain a tendipedid. Also, as Berg (*op. cit.*) mentioned, when the tendipedids grow older they start to roam in search of food. Young larvae removed from their algal pads and placed in a container along with available pads soon re-entered them. The larva entered the alga anywhere on its surface, by eating a hole into the cortex and channeling through the matrix.

In addition to being found in the large *Nostoc* pads, the first 3 instars were observed as solitary individuals in very small algal colonies. In small pads up to 3.5 mm in height, the tendipedids formed a semi-circular passageway following the contour of the pad. When the plants were about 4 mm in diameter the ends of the tunnels were extending up from the base toward the center.

While living within the alga, the tendipedid fed exclusively on the algal matrix; *Nostoc* filaments were abundant in the stomachs of the larvae. Many other aquatic species of tendipedids living as leaf miners produce currents through their burrows by dorsoventral undulations of their bodies, thus securing plankton. *Cricotopus,* however, had no exit holes along any part of the pathway. Larvae that were placed in glass tubing of the same diameter as the algal passageway showed no undulatory movements nor revealed any attempts to construct nets or webs. A mining larva subsisting on the tissue of the plant that shelters it must extend its excavation as it feeds or abandon it and make a new excavation. Fourth instar *Cricotopus* larvae apparently never moved from pad to pad in search of food. After the mines were extended to a certain point, the larvae would start to increase the diameter of the tunnel. Doubtless, some algal tissue was regenerated. *Cricotopus fuscatus* and *C. nostocicola* larvae are assumed to host-specific since they have not been found in a free-living state or in any plants other than *Nostoc parmelioides*.

No traces of excrement were found in the tendipedid's passageway. Needham *et al.* (1928) mentioned terrestrial leaf miners that eject all their frass through a hole in the epidermis of the leaf. They state that there are other leaf miners (certain Lepidoptera and Diptera larvae) that have their mines almost filled with frass pellets, resulting in a "very messy appearance." On one occasion a larva that had been removed from the alga was observed to eject a small amount of yellow fluid. Since no fecal material was found inside, and exits from the passageway were lacking, it is probable that the waste material consists of this yellow fluid, which diffuses between the filaments after it is ejected.

According to Leathers (1922), the larval stages of the Tendipedidae extend over a period varying from 25 to 30 days to all winter, depending on food and weather.

From the insects collected at Sagehen Creek it was established that there were 2 species, both of them new (Wirth 1957). The adults of the 2 species (*Cricotopus nostocicola* and *C. fuscatus*) are dissimilar in appearance, but their larvae do not have any obvious characters whereby they may be separated.

Johannsen (1937) described certain species of the subfamilies of Tendipedidae, and in one description mentioned finding a small larva coiled in the disk of the alga *Nostoc parmelioides*. This larva was designated by him as *Spaniotoma* sp. G. In comparing 2 of Johannsen's larval slides of specimens from Cos Cob, Connecticut, and one larval slide from *Nostoc* in the Callowash River, Oregon, Dr. Wirth concluded that the specimens determined by Johannsen as *Spaniotoma* sp. G were indistinguishable from the larvae of *C. nostocicola* and *C. fuscatus*.

Head capsule measurements are used as a basis for determining the number of instars since they remain fairly constant while within the instar, but increase in size, with each succeeding molt. In all, 360 head capsules were measured. The 4 different peaks in Figure 3 indicate the modes of 4 different head capsule sizes. The range of variation for the 4 instars is 0.01 mm, 0.05 mm, 0.10 mm, and 0.11 mm, respectively.

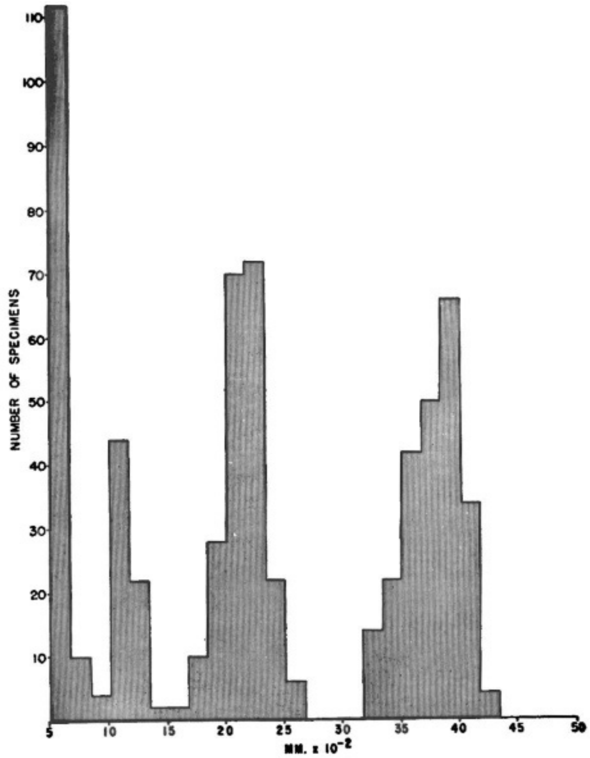

FIG. 3. Distribution of larvae according to widths of head capsules.

First instar

The head capsule of the newly hatched larvae were disproportionately large; the head width at that time measured 0.07 mm. The first column in Fig. 3 represents the width of the head capsule immediately after hatching. The head capsule widths measured 0.07 mm to 0.08 mm. The mean head width was 0.071 mm. The body of the larvae of this instar had a violet pigmentation and the digestive tract had a visible yellow coloration. The head capsule was dark brown. The average length of the larvae was 0.5 mm. This stage was found in the randomly built passageways within the large *Nostoc* pads and also in the very small algal nodules.

Second instar

The head capsule widths measured from 0.10 mm to 0.15 mm. The mean was 0.12 mm. The abdomen was a light violet, in contrast to the darker violet pigmentation of the thorax. The yellow coloration of the digestive tract was still visible. The head capsule was black. The body lengths ranged from 1.00 mm to 1.50 mm. Larvae of this instar were found in small *Nostoc* pads as solitary individuals or in large algal pads, where they occupied the small random passageways.

Third instar

The head capsule widths ranged from 0.17 mm to 0.27 mm. The mean was 0.22 mm. In this instar the violet pigmentation was retained by the thorax whereas the abdominal coloration was replaced by a bright yellow. The head capsule was black. The body lengths ranged from 1.00 mm to 2.75 mm. Larvae of this stage were found as solitary individuals in small algal colonies or in large pads, where they were not in the main peripheral passageway. This instar, as well as the 4th instar, was occasionally infected with a nematode.

Fourth instar

During the 4th larval stage the head width and body length increased greatly. Head widths ranged from 0.32 mm to 0.43 mm with a mean of 0.38 mm. Body lengths ranged from 3.50 mm to a maximum larval length of 8.25 mm. The head capsule was black and the entire body was a bright yellow. This instar always inhabited the peripheral passageway of the alga. Just before pupation the thorax would become greatly enlarged (Figure 4), signifying that transformation was about to take place. Simultaneously the larva would excavate a small cavity in the alga, which later served as the exit pathway for the pupa. This short path was always excavated only to the cortex of the alga, and never through that layer. At the same time a change would take place in the shape and structure of the alga. The entire area surrounding the larva would swell (side view, Fig. 4) and continue to swell until the cortex was weakened and broken, allowing some of the softer inner parenchyma tissue to protrude (Figure 5). This break in the outer layer provided an exit for the pupa.

Pupal Stage

The length of the pupae ranged from 4.50 mm to 7.25 mm. The color varied from a bright yellow to a deep orange. The orange was characteristic of a female containing eggs in the abdomen. Many of the pupae had the abdomen contracted within the pupal skin, leaving the last few segments empty.

A very much enlarged upper portion of a *Nostoc* pad indicated the presence of a tendipedid pupal stage (Fig. 5). The entire pupal period was spent within the alga. This period varied from 5 to 9 days, with a mean of 6.7 days. When the pupa was mature and ready for its final molt, it left the alga. This action was probably similar to the behavior of miners in *Potamogeton* (Berg 1950) which created vertical undulations of their bodies until they were able to break through the plant tissue. After leaving the alga, the pupa presumably swam to the surface of the water, where the pupal skin was split along the dorsum of the thorax, allowing the adult to emerge. The pupal exuviae floated freely on the water; those of the last larval instar remained in the passageway.

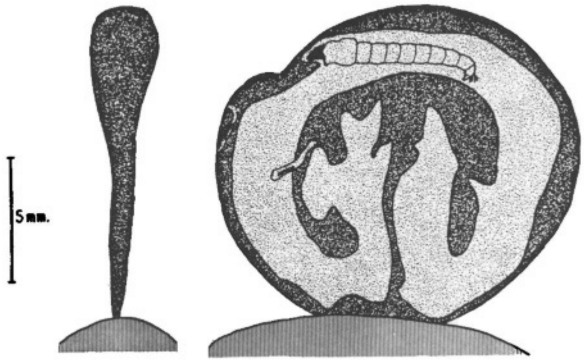

FIG. 4. Late larval stage in the alga (end view and side view).

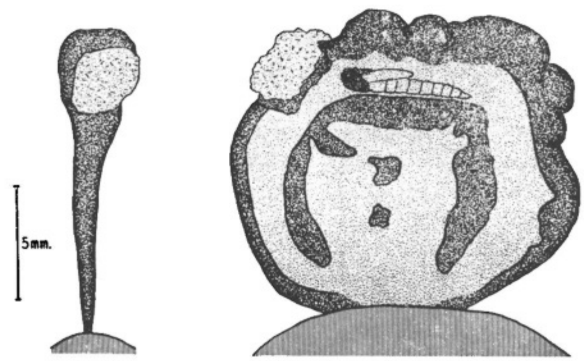

FIG. 5. Pupal stage in the alga (end view and side view).

Adult Midges

Wirth (1957) stated: "Although adults of the two species do not have the prominent white-banded legs, pale-banded abdomen, or whitish genitalia characteristic of *Cricotopus* in the strict sense, the presence of dense pubescence on the eyes, absence of macrotrichia on the wing, absence of distinct erect hairs arising from obvious punctures on the mesonotum, and presence of a complete fringe of hairs on the squama place both species in *Cricotopus,* Group C of Edwards (1929). . . ." The fact that most of the adults that were reared lived only one day after emergence was probably due to their confinement. According to Leathers (1922), the longevity of the adult stage of tendipedids is brief but lasts 5 to 10 days.

Predators of the Tendipedid

Tendipedids constitute an important food item in the diet of many predacious animals. In the larval and pupal stages they are devoured by aquatic beetle larvae, waterbugs, the nymphs of dragonflies, damselflies, and stoneflies and by caddisworms, salamanders, and fish (Leathers 1922). Ping (1917) observed that the larvae of tendipedids were preyed upon by the copepod crustacean *Cyclops* and that the adults were preyed upon by bats, which were seen to fly across the swarms. Fish may be predacious on *Cricotopus,* but other dipterous larvae and parasitic nematodes are known to be the main predators.

Other Tendipedids

A 2nd type of tendipedid with a life cycle that may be very similar to that of the *Cricotopus* species was occasionally found in the *Nostoc* pads. This tendipedid also belonged to the subfamily Hydrobaeninae but had a much larger, less tapering, brown head, with longer hairs on the body. Most often they were found in their own passageways, which were distributed at random in the same fashion as those of young instars of *Cricotopus,* but occasionally they were observed in the large passageway. When this situation prevailed, the dead body of a *Cricotopus* larva or pupa was also present in the channel. It is therefore assumed that this tendipedid may be predatory on *Cricotopus,* though this could not be established in the laboratory by placing the 2 larvae, free of the alga, in a common container.

The 2 larvae appeared superficially similar but were easily separated by their behavior. *Cricotopus* moved very slowly when out of the alga, whereas the other tendipedid was very active and had the ability to wriggle. When they were examined more closely, the 2nd type was distinguished by its much longer anal hair tuft, longer and less tapering head capsule, more clearly separated body segments, and a slightly darker coloration; the mature larval size of the 2nd tendipedid was much smaller.

On August 12, 1955, five gelatinous egg masses found attached to *Nostoc* colonies were brought into the laboratory. Five days later, when all the young larvae had emerged, it was noted that 2 types of tendipedids were present. One type showed a very active wriggling motion, a very long anal bristle, a transparent white body, and reddish eyes. *Cricotopus* could be told by its slow, awkward motions, much shorter anal bristles, violet and yellow body coloration, and black eyes and head capsule. The life cycle of the 2nd tendipedid may be similar to that of *Cricotopus,* since its egg masses have been found attached to the upper edges of the *Nostoc* pads and its larvae have been found eating their own pathways through the algae; it is also known to pupate within the alga.

Cranefly Larvae

Cranefly larvae of the genus *Antocha* were often noticed in collections of algae brought into the laboratory. These larvae were most frequently associated with the horizontal form of *Nostoc,* where they were found inhabiting the area under the alga. Since it is known that tipulid larvae are predatory on small aquatic organisms, there is a possibility that they may be predators of *Cricotopus.* Only in one instance was an *Antocha* larva found inside the tendipedid passageway; no midge larva was present. Once again, no evidence of predation could be established by placing the 2 types of larvae together in a container.

Parasitic Nematodes

In reference to leaf miners and parasites, Needham *et al.* (1928) state: "In the beginning, the mine may have been a place of comparative security from parasites, but it is not so now. Once the parasite has learned how to effect an entrance, the miner is worse off than its free-living ancestor, having no means of escape. A very high percentage of parasitism is the rule among leaf-miners."

Of the predators found, the one that was responsible for the highest mortality was a roundworm belonging to the family Mermithidae, a group common as insect parasites. Certain tendipedid larvae collected during the course of the year were light green instead of bright yellow; this green was due to the presence of the mermithid parasite. Some of the midge larvae were just as active as the uninfected larvae, whereas in others the presence of the roundworm had slowed their activities so that the only movement present was that caused by the writhing of the nematode within. The latter condition probably meant that the nematode had undergone its last molt and was now ready to leave the tendipedid. The length of the nematode was usually 3 or 4 times that of the midge larva. This excessive length meant that

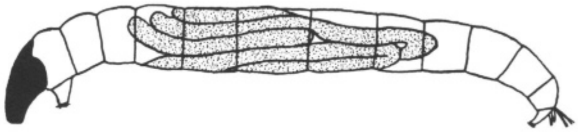

FIG. 6. Parasitic nematode within the midge larva.

the roundworm was curled back and forth within the larva occupying nearly the entire body cavity (Figure 6). The digestive tract of the tendipedid was pushed to one side, against the body wall.

The nematodes were found in the 3rd and 4th instar larvae as well as in the pupae. In August and September of 1955 they were present in small numbers, but in October of that year they had become very abundant. In a random sample of 100 larvae, 9 were infected. Those algal pads containing infected or dead larvae were very thin and yellow toward the base and loosely attached to the rocks. The dead tendipedids were no more than the larval skins that the nematode had left behind when it escaped. Infected larvae were removed from the algal pads and placed in a separate container. After the roundworm had escaped from the larvae, they were removed and placed in a container of mud and water, where they were allowed to mature. These roundworms were removed one month later and preserved for identification.

According to Filipjev and Stekhoven (1941), the adult mermithids are free-living and occur either in aquatic habitats or in the soil. They state that the host dies either from exhaustion caused by the food consumption of the parasite or by secondary bacterial wound infection.

Christie (1942) and Hyman (1951) both gave accounts of a mermithid found within a tendipedid larva. The life cycle of *Paramermis contorta*, a nematode found in a *Chironomus* sp., is probably very similar to the life cycle of the nematode in *Cricotopus*. The host can become infected either by eating the mermithid eggs or through being penetrated by the juvenile worms. The young nematode can better penetrate a larva that has recently molted and has a soft cuticle. With *Cricotopus*, the nematode possibly has a method of entrance into the alga before it penetrates the tendipedid. All infected *Cricotopus* larvae had only one roundworm present. Christie and Hyman go on to state that the nematode dwells in the haemocoel, where it is very destructive to the viscera and body fat, killing the host or rendering it incapable of metamorphosis or reproduction. After the parasite has spent its juvenile stages in its invertebrate host it leaves and does not feed in the adult stage. The parasite may exit through the anus or force its way directly through the body wall, the majority emerging just before their insect host would normally pupate.

Fish

Tendipedid larvae and pupae constitute one of the staple food items in the diet of nearly all carnivorous young fish. They are used as food for young fish in hatcheries, and the use of *Chironomus tentans* was recommended by Sadler (1935) in a publication in which he described special methods for its propagation. One can therefore understand why the habitat occupied by *Cricotopus* is beneficial to the species, although it is likely that *Nostoc parmeliodes* may be taken occasionally. Hildebrand and Towers (1927) surveyed the food of trout in Fish Lake, Utah, and reported that "rounded egg like masses of dark green *Nostoc* colonies are common on gravelly shallows, and are not uncommon in fish stomachs." They further stated that investigation of the ingested material indicated that the globular colonies of *Nostoc* were fed on rather regularly. They observed that there was a considerable variation in the size of the colonies taken but that the average diameter was about 4 millimeters. Dr. Richard Gard, working at Sagehen Creek, informed me that he found no *Nostoc* pads in examinations of more than 100 stomachs from rainbow, eastern brook and brown trout taken at all times of the year. The only time that *Cricotopus* would be directly available as food for fish would be in its adult stage, when it flies near the water, especially during oviposition, or during its emergence period, when the pupa leaves the alga and swims to the surface of the stream.

TENDIPEDID'S ABILITY TO ERECT THE ALGAL PAD

When algal pads were torn from their original holdfast, the tendipedid had the ability to erect them to an upright position and refasten them to the substrate. If the alga was so large that the larva was unable to place it upright, the pad would be fastened in a horizontal position. This process was observed under artificial conditions when the pads were torn from the rocks and placed in hatchery troughs. During the spring snow melt, when freshets cause the rolling of rocks and the scouring of the stream bottom by debris, there is a noticeable lack of *Nostoc* in Sagehen Creek. It may be at this time of the year that the larvae benefit the alga by reattaching the pads to the stream bottom.

Algae were gathered from the stream and brought into the laboratory for observation of the reattachment procedure. Removal of the alga from the rock usually created a break near the base of the pad. If these algal disks were placed in a dish of water and left undisturbed, the tendipedid would protrude its head and thorax through the hole in the pad. If the container was jolted in the slightest way, the larva would retract itself and not come out again until the water had settled. While raising the algal pad to a vertical position, the tendipedid never left the pad; instead, it projected the anterior half of its body from the pad and sought a secure hold with its mandibles. When the hold was established, it pulled the pad over to the point. While the larva held fast to the substrate, it started to erect the pad. During the struggle to gain control of the alga, the larva caused the pad to sway back and forth. When the alga was vertical and under control, the tendipedid cemented the edges of the torn area to the substrate. In a few minutes the pad was so secure that it could be probed with a needle without being broken loose.

Some of the larger pads, and a few of the smaller ones, were raised only to a 45° angle or not raised at all; in the latter instance they were cemented to the substrate in a horizontal position. The larva would usually create a hole at 2 different locations and cement the edges to the substrate. On many occasions when quantities of *Nostoc* were collected and placed in one container, the algae were fastened to one another by the action of the larvae. In one experiment, 34 pads were placed in an enamel pan and only 2 failed to become reattached. Of the 2 pads that were not attached, one had been vacated by the larva.

Distribution of the Alga and Insect

The distribution of the alga was established from personal observations, from specimens of the alga in the University of California Herbarium, and from the literature. Most of the herbarium specimens of *Nostoc parmelioides* that were examined readily showed the typical tendipedid passageway and the larva. If the features were not immediately noticed, because of opaqueness caused by the blue and green pigments, the alga was held up to a light source to help reveal the channel and the tendipedid. In other pads the presence of the larva could be detected by the prominence of the head capsule. When the algal pads had been dried on paper, the plant tissues as well as the body of the larva had contracted but the head capsule retained its shape, forming a readily noticeable protrusion in the algal pad.

The alga and its associated larva are found throughout California, but most of the records are from the cold streams of the high mountains in the Sierra. Outside of California there are scattered records of the alga in many states, all the way to Connecticut. It was not established whether the tendipedids were present in the *Nostoc* reported to occur outside the United States. The material that Kützing used for his original description was collected from a mountain stream in the Thuringian Forest, near Suhl, Germany. Thienemann (1954) did not mention the midge or the alga as occurring in Europe. Geitler (1932) described the alga and stated that its distribution was Europe, the Antilles, North America and Africa. In none of these accounts was the presence of a tendipedid mentioned. Among the specimens of algae examined in the herbarium there was one collection from the Kropbach River, a tributary of the Dos River near Geroldsau, Baden, Germany. These pads were too small to detect the presence of a tendipedid.

Advantages of the Interrelationship

The advantages of this symbiotic association are more obvious for the tendipedid than for the alga. The tendipedid, living within the alga, receives shelter and protection from adverse conditions. It is partially protected from fish and predatory insects. It has a constant food supply since it feeds on the alga. When the stream level drops, the alga may be an insurance against the immediate desiccation of the tendipedid, since algal pads were observed above the water level with larvae still alive inside. The alga may thus help keep the larva moist until the stream level rises again.

The main advantage of the relationship to the alga is found in the ability of the larva to reattach the colony to a firm substrate. If it were not for the midge larva the pad would continue to drift downstream. By erecting the *Nostoc* pad to a vertical position, the larva may help to expose the alga to a greater amount of sunlight. By its feeding activities upon the trichomes, the larva helps to create more reproductive filaments. Smith (1933) mentioned that hormogonia are formed by breakage of trichomes, and that "breaking may result from animals feeding on the filament. . . ." He also stated, in reference to *Nostoc*, that "the large number of trichomes within the adult colony is largely the result of development of these hormogones into new trichomes without their being liberated from the colonial sheath." Thus, when the tendipedid pupates, its influence in breaking the algal cortex may be an aid to the release of hormogonia. Although the parent colony is destroyed, many potential new colonies are liberated.

Since both the tendipedid and the alga apparently benefit from the intimate symbiotic association, the more specific designation "mutualism" may appropriately be applied to it.

Summary

Midge larvae living in the blue-green alga *Nostoc parmelioides* Kützing (1843) taken from Sagehen Creek, Nevada County, California, were reared and found to represent 2 new species. The tendipedids, *Cricotopus nostocicola* Wirth (1957) and *C. fuscatus* Wirth (1957), can be separated only in the adult stage since no characters were found to distinguish the larvae. Measurements of head capsule widths of the mixed populations revealed the presence of 4 larval instars, each with a distinctive color pattern. There was never more than one last instar larva in an algal pad. Every algal pad examined contained the larva or showed the characteristic passageway created by the larva in feeding. Younger instar larvae were also found in the larger pads, but their passageways were irregular in shape and distributed at random. Pupation always took place within the alga; pupae were found during all seasons of the year but the peak of emergence occurred in March and April. Just before pupation, the larva created a small excavation toward the cortex. This channel was later used by the pupa as an escape exit. Simultaneously the algal tissue surrounding the larva began to swell and enlarge, until the leathery surface layer of the alga was weakened and broken. When the pupa was ready to leave the pad, it pushed its way through this area of weakened tissue, swam to the surface, and emerged. After the tendipedid had departed, the alga continued to decompose.

Although such a niche is of protective value to the tendipedid larva, it by no means offers complete safety, since a parasitic nematode played an important role in larval mortality. For the tendipedid the main advantages are shelter, food, and protection from most enemies. *Nostoc* gains from the relationship because the larva is able to cement the alga to a firm substratum whenever it is dislodged. The tendipedid's presence may also enhance the reproductive methods of the alga. Hence, the relationship of the midge and the alga has mutual advantages—a case of mutualism.

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