# THE OBSERVATION OF FEEDING ABILITY OF *CULEX PIPIEN* FATIGANS LARVAE ON *ANABAENA*

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**Abstract**: Under the laboratory condition, the larvae of *Culex pipens* fatigans may feed mainly on *Anabaena* sp and complete their life cycle. The retention time of *Anabaena* sp. in larval intestine is about 6 hours while that of *Saccharomyces* sp. is about 5 hours, meaning that *Anabaena* sp. is not so digestible for the larvae. From hatching to pupation, the larvae fed with *Anabaena* sp. lasted 194 hours and those fed with *Saccharomyces* sp were 142 hours, showing that the duration for completing larval stage of the former is 52 hours, which is longer than those fed on *Saccharomyces*.

**Key words**: *Culex pipens* fatigans; *Anabaena*; Feed; Digest
**CLD number**: S942.3 **Document code**: A **Article ID**: 1000-3207(2005)05-0581-06

*Culex pipien* fatigans, Diptera, Insecta, is a kind of palish yellow, medium-sized mosquito generally distributed in the area from 30° to 33° north latitudes. Its populations constitute the majority of domestic mosquitoes in China, and have long been proved to be the media of spreading several human diseases. Great effort has been made for controlling this mosquito throughout the country. Due to the fact that the long-term use of chemical insecticides has produce chemical resistant strains of mosquitoes[1,2], remained us to pay attention to the studies and practices of the biological mosquito control[3~7]. Works demonstrated that *Culex pipien* fatigans is most sensitive to toxicity of *Bacillus*, of which *Bacillus thuringiensis* $H_{14}$ and *Bacillus sphaericus* are superior mosquito killing materials without remains and environmental pollution. Practically, an effective way to destroy mosquito populations is to use *Bacitlus thuringiensis* producing engineering *Anabaena* through biology technology. *Anabaena* belongs to Nostocales, Cyanophyceae, Cyanophyta. It can fix atmospheric nitrogen and add the content of available nitrogen to the land. The use of *Anabaena* has the advantages. It is one of the excellent micro-organisms to be the recipients for genu's grafting[8—14], and it is difficult to be digested by fish owing to the covering of keratinized membrane. However, there is little information dealing with the feeding and digestion of *Anabaena* by mosquito larvae. For the purpose of getting knowledge of food relationship between mosquito larvae and *Anabaena*, so as to enhance the practical application of mosquito control by means of engineering *Anabaena*, this study was undertaken.

## 1 Materials and methods

**1.1 Mosquito larvae and their rearing** Eggs or *Culex pipiens* fatigans were provided by Laboratory of Pesticide Microbiology in Department of Soils and Agricchemistry and hatched in dissecting dishes with tap water 1 ~ 1.2cm deep under temperature of 27 ± 2℃ and light intensity of 450 ~ 1000lx. After hatching, the larvae were fed with *Saccharomyces* or *Anabaena*.

**1.2 *Anabaena* and its cultivation** The strain of *Anabaena* was provided by Fishery College. The ingredient of culture medium (g/L)[15] was: $NaNO_3$, 1.5; $K_2HPO_4 \cdot 3H_2O$, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; Citric acid, 0.006; Iron Ammonium of Lemon, 0.006; EGTA, 0.001; $Na_2CO_3$, 0.02; Mixture of trace mineral A5 + Co, 1; distilled water, 1000ml; pH, 7.4. Mixture of trace mineral $A_5 + C_0$ Comprises (g/L); $H_3BO_3$, 2.86; $MnCl_2 \cdot$

**Received date**: 2004-11-03; **Accepted date**: 2005-06-20
**Foundation item**: supported by UNDP/Word Bank/WHO.
**Biogrphy**: Zhang Shiping(1954—), female, born in Queshan, Henan

$4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $Na_2MoO_4 \cdot 2H_2O$, 0.390; $CuSO_4 \cdot 5H_2O$, 0.079; $Co(NO_3)_2 \cdot 6H_2O$, 0.0494. For use, 500ml medium was sterilized at 0.11MPa, 121℃ for 30min. Then *Anabaena* was inoculated and cultured in incubator at 26 ± 1℃ and 450 ~ 1000lx.

**1.3 Observation of *Anabaena* in larval gut** The intestine of a larva was pulled out with a dissecting needle under a dissecting microscope. The degrees of food content in pre-intestine and in entire gut were determined. The color and digestive condition of food ingested were observed as well.

**1.4 Examination of feces of mosquito larvae fed on *Anabaena*** Mosquito Larvae and *Anabaena* were put into a little beaker containing tap water for five hours. Feces egested by the larvae were carefully removed out with a pipette and cultured in the medium at 28℃ for 3—5 days, then checked the survivorship of *Anabaena* in the feces.

**1.5 Determination of digestion speeds of larva fed on *Anabaena* and *Saccharomyces*** Two beakers each contained 20 larvae, to which *Anabaena* and *Saccharomyces* were provide respectively. After full eating, all larvae were removed to other beakers containing tap water only. In every successive larva was taken out from each group to examine its digestive under dissecting microscope

**1.6 Comparison of growth and developmental speed between larvae fed on *Anabaena* and *Sccharomyces***

Two groups of 20 newly hatched larvae were put into two beakers respectively containing *Sccharomyces* and *Anabaena* in high concentrations (over $8.5 \times 10^5$/ml). The duration of their exuviations and pupation were carefully observed and recorded.

## 2 Results and analyses

### 2.1 *Anatomical* observation

27 larvae of different instars fed with *Anabaena* were examined under dissecting microscope. It showed that the fore-gut of larvae was filled with greenish granular matter by *Anabaena* 2 hours after feeding (Fig.1). When splitting by dissecting needle, there was much *Anabaena* spilling out and its structure is clear (Fig.2). Their mid-and hind-gut were full of yellow granularity, which was undigested *saccharomyces* and feces (Fig.3). By four hours, the mid-gut appeared greenish, but the structure of *Anabaena* was vague, meaning that the *Anabaenas* was partly digested (Fig.5). By six hours, all the intestines were full of green ball-like material (Fig.6). When splitting the hind-gut, a strip of material with fully digested *Anabaena* spilled out with structure no longer recognizable (Fig.6,7).

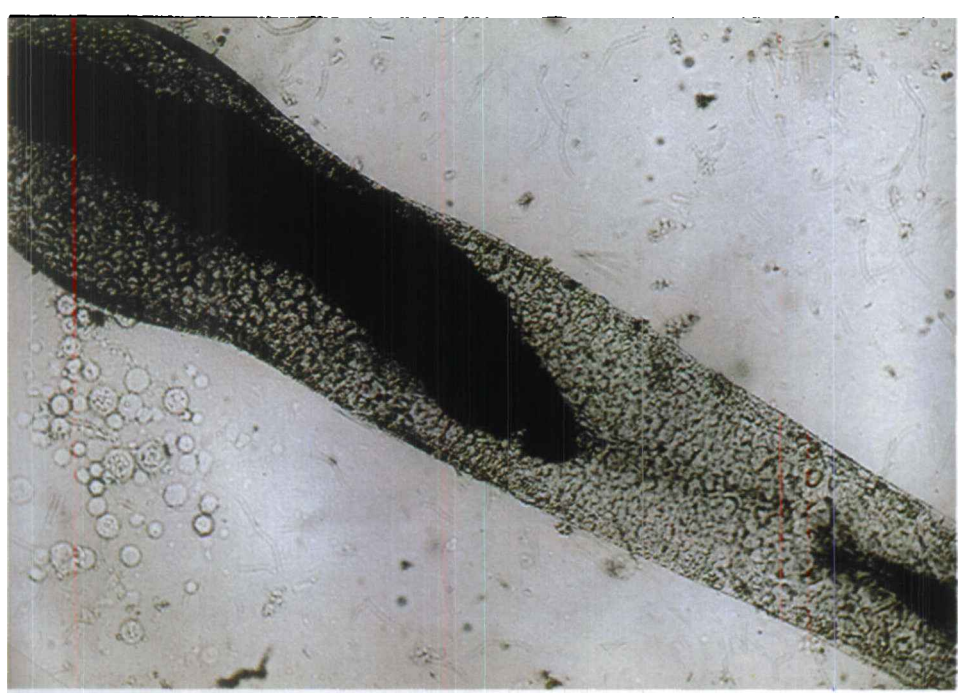

Fig.1 Two hours feeding, fore-gut full of ball-like material, × 40

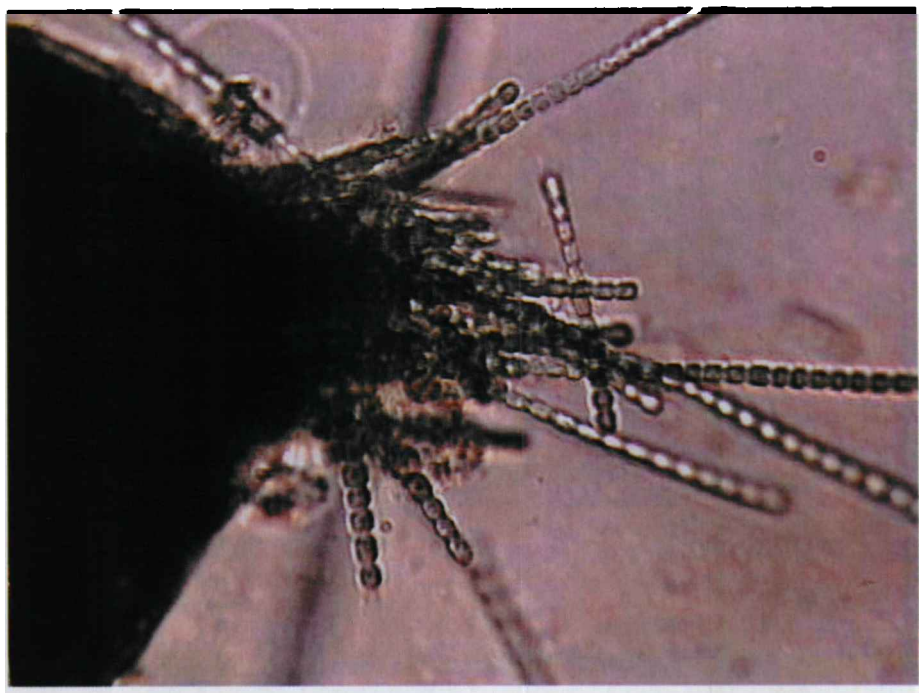

Fig.2　By four hours later, fore-gut and mid-gut full of green ball-like

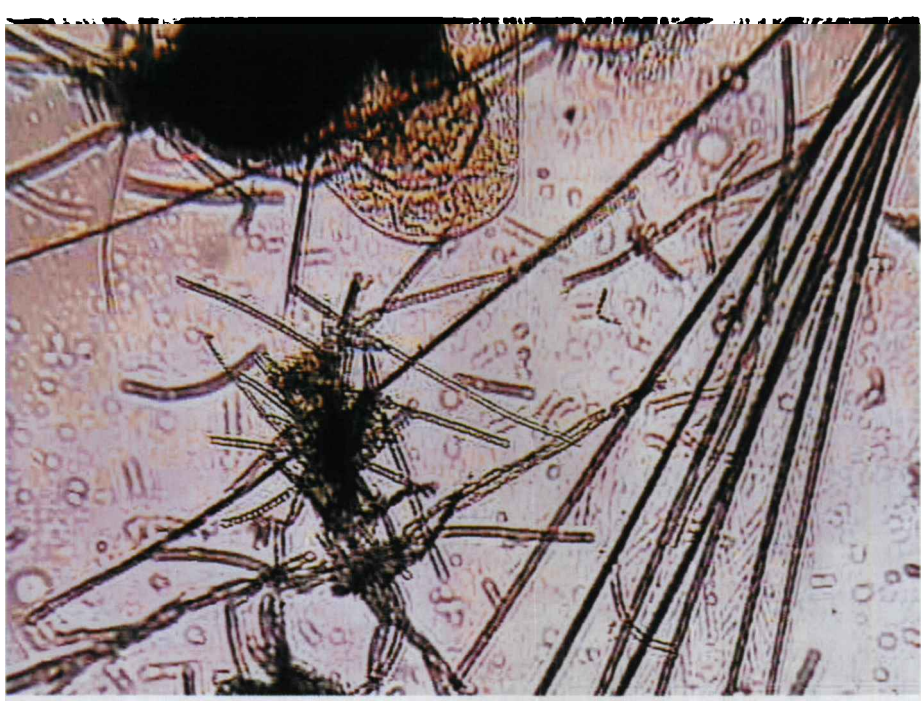

Fig.3　Intestine of larva fed on *Saccharomyces*, × 40

**2.2　Survivorship of *Anabaena* in larval feces**

After culturing larval feces in medium for 3—5 days, no surviving *Anabaenas* was found, meaning that *Anabaena* was completely digested by the larvae.

**2.3　Digestive speed comparison between larvae fed on *Anabaena* and *Saccharomyces***

Tab.1 shows that mosquito larvae enabled to digest *Anabaena* in 6 hours and *Saccharomyces* in 5 hours or so. It seemed that *Saccharomyces* was easier to be digested than *Anabaena*.

**2.4　Duration of different growth stages of mosquito larva fed on *Anabaena* and *Saccharomyces***

Fig.4 By six hours, entire gut full of ball-like material, × 40

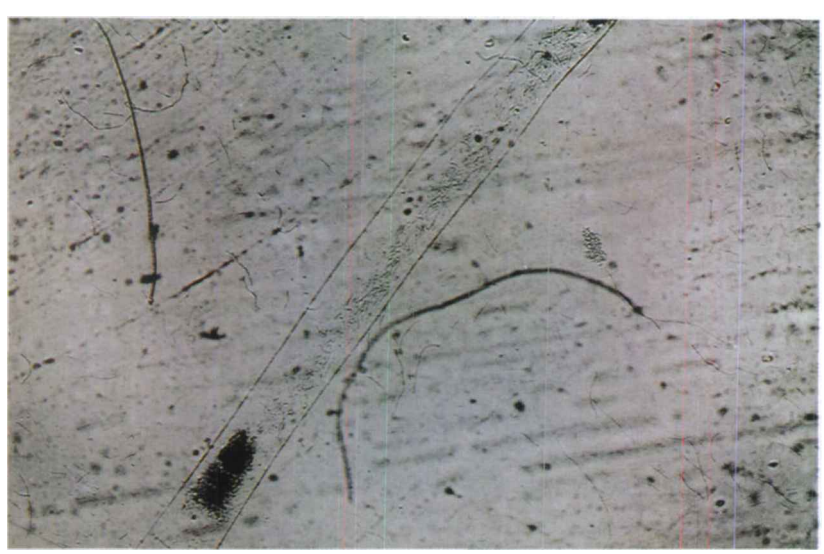

Fig.5 Much *Anabaena* spill out from gut and the structure of *Anabaena* is visible, × 40

**Tab.1 The retention time of *Anabacna* and *Saccharomyces* in larval intestine**

| Time(d) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| *Saccharomyces* | ++++ | +++ | ++ | + | 0 | 0 | 0 |
| *Anabaenas* | ++++ | ++++ | +++ | ++ | + | 0 | 0 |

* *the number of "+" indicates relative quantity of food in guy.*

**Tab.2 Duration of different growth stages of mosquito larva fed on *Anabaena* and *Saccharomyces***

| | The First molt | The Second molt | The Third molt | Pupation |
|---|---|---|---|---|
| *Anabaenas* | 40 | 76 | 124 | 194 |
| *Saccharomyces* | 40 | 64 | 94 | 142 |

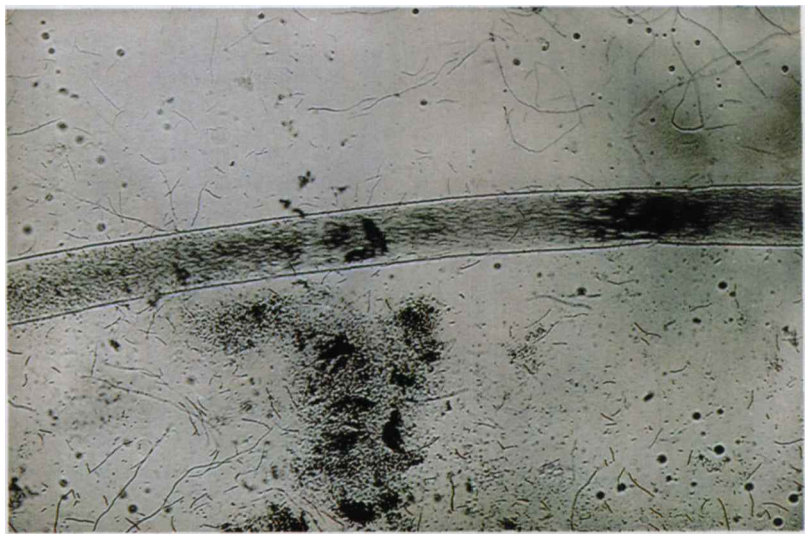

Fig.6　*Anabaena* in mid-gut is under digestion

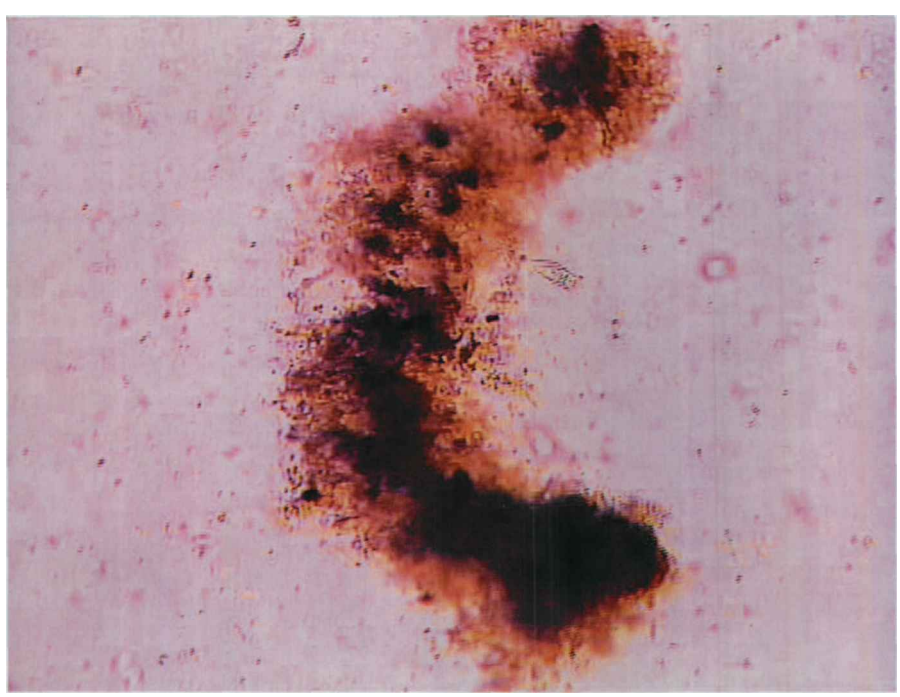

Fig.7　Feces of larvae fed with *Anabaena*, × 40

It can be seen from Table 2. that forty hours was needed for larval to reach their first exuviations no matter they were fed with *Anabaena* and *Saccharomyces*. Then the growth in larvae fed with *Anabaena* were obviously slower than those fed with *Saccharomys*. Compared with the molting of larvae fed with *Saccharomyces*, the *Anabaena* feeding larvae needed a time 12 hours longer to reach the second molt; 18 hours to the third molt and 22 hours to pupation. From first molt to pupation, the larvae fed with *Anabaena* required 52 hours longer than those fed with *Saccharomyces*.

### 2.5　Conclusion and Discussion

The present study demonstrates that *Anabaena* is a suitable food for mosquito larvae. It can be digested and uptaken by the larvae. Although it seems not so effective to feed larvae as the yeast, its nutrient substance and energy can still ensure the growth and development of the larvae and complete their life cycle. Thus, using engineering *An-*

*abaena* for mosquito control is a possible way. In nature, however, *Anabaena* is not always abundant, where many kinds of food such as bacteria, human and other algae are available for mosquito larvae. This condition is in sharp contrast to that in the laboratory. For application, studies referring to the food selectivity of the larvae and whether the larvae can feed on great amount of *Anabaena* should be carried out.

**Acknowledgements**

We are indebted to Liang Yanling who's the Prof. of Institute of Hydrobiology Chinese Academy of Sciences, and for valuable comments to Wang Ming who's the chief engineer of Huazhong Agriculture University.

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# 致乏库蚊幼虫摄食和消化鱼腥藻的观察

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**摘要**: 在实验室条件下, 致乏库蚊幼虫可大量摄食鱼腥藻, 并能消化利用, 完成生活史。鱼腥藻在蚊幼虫肠道中滞留时间约6h, 酵母约5h, 鱼腥藻比酵母稍难消化。蚊幼虫至化蛹时期相比, 饲喂鱼腥藻为194h, 而饲喂酵母的为142h, 饲喂鱼腥藻比饲喂酵母延缓了蚊幼虫期52h。

**关键词**: 致乏库蚊; 鱼腥藻; 摄食; 消化