Odonatologica 17(3): 227-233 *September 1, 1988*

THE IMPORTANCE OF MYCOBACTERIA FOR THE NUTRITION OF LARVAE OF *LEUCORRHINIA RUBICUNDA* (L.) IN BOG WATER (ANISOPTERA: LIBELLULIDAE)

K. SOEFFING

Zoologisches Institut und Museum, Martin-Luther-King-Platz 3, D-2000 Hamburg-13, Federal Republic of Germany

Received March 10, 1988 / Accepted May 16, 1988

Mycobacterium chelonei, M. flavescens, M. fortuitum and *M. sphagni* were recorded in *Sphagnum* bogs, inhabited by *L. rubicunda*. Experiments with tritium labeled mycobacteria indicate that these are accumulated by the cladocerans. The *L. rubicunda* larvae acquire the mycobacterian flora through ingestion of Cladocera. It is shown that a direct correlation exists between the mycobacteria concentration in the bog water and the larval growth of *L. rubicunda*.

INTRODUCTION

Sphagnum vegetation provides suitable conditions for the maintainance and multiplication of mycobacteria (KAZDA, 1977; MÜLLER et al., 1980; IRGENS et al., 1981; MÜLLER & KAZDA, 1988; KAZDA & COOK, 1987). Rapidly growing mycobacteria frequently occur in *Sphagnum* vegetation in Europe (Germany, Sweden, Norway), and in North- and South America (KAZDA, 1981, 1983; MÜLLER & KAZDA, 1988). Recently, a high occurrence of mycobacteria in *Sphagnum* vegetation from New Zealand has also been reported (KAZDA & COOK, 1987) Some species, like e.g. *Mycobacterium sphagni* can be regarded as a common microflora of *Sphagnum* vegetation (KAZDA, 1980).

The most favourable condition for multiplying mycobacteria has been found in hydrophilic *Sphagnum* species (KAZDA, 1981) that have a close contact to the ponds situated in the bogs, a situation resembling the surface of the pools in moorlands (COOK & KAZDA, 1988). The world-wide distribution of *Sphagnum* species and their association with moorland water ponds play an important role in the occurrence of mycobacteria in moorland waters.

During the study of the ecology of the larvae of *Leucorrhinia rubicunda*, a high concentration of mycobacteria in these larvae was observed (SOEFFING, 1986). The questions that arise concern the nature of the accumulation of the mycobacteria and their possible role in the nutrition of the larvae. The bog ponds are characterised by a low concentration of nutritives, yet, in spite of this, they support the growth of a number of dragonfly species. The results are described in this paper.

MATERIAL AND METHODS

The investigations were carried out in the "Nienwohlder Moor", northwestern Germany (N 53°88', E 10°12').

Larvae of *L. rubicunda* were caught by the trap described by SOEFFING (1987). At the same time samples of *Sphagnum* and of Cladocera (Phyllopoda, Crustacea) were taken.

The larvae were homogenized in an Ultra-turrax homogenisor. *Sphagnum* samples were pressed out in a 10 ml syringe; the fluid was used for examination in the same as the water samples that had been taken directly from the pond.

The examination for mycobacteria was done by using smears stained after Ziehl-Neelsen. The cultivation from material as described above, was made on conventional media for mycobacteria, Löwenstein-Jensen and Middlebrook 7H10 agar, incubated at 31° C for six weeks (KAZDA & COOK, 1987). The mycobacterial strains were identified through standard methods (KAZDA & COOK 1987; COOK & KAZDA, 1988).

The incorporation of tritium-uracil was carried out in 90 ml liquid Middelbrook 7H9 medium by adding 150 μCi in the labelled substance. After an incubation of 14 days at 31° C, mycobacteria were harvested, the labelled medium was washed off in phosphate buffer (pH 7.0) and the bacteria were centrifuged 5 times at 4000 r.p.m. Labelled *Mycobacterium sphagni* and *M.fortuitum* were added to filtered sterile bog water in a concentration of 10^6/ml (step 1). Cladocera *(Daphnia magna, D. pulex, Ceriodaphnia reticulata)* were exposed to this medium (step 2), and after two days caught through a net and cleaned twice in tap water. Then they were fed to *rubicunda* larvae (step 3), housed in individual glass tubes. All these steps were carried out at a constant temperature of 15° C.

The radioactivity of the water (1 ml/sample) and of the Cladocera (10/sample) was measured every two days. The larvae were examined six weeks after the beginning of feeding.

For measurements, the head, cuticula, fat and gut of the larvae were dissected out and together with the samples of the Cladocera, dissolved in lumasolve and left for five days at 31° C.

The faeces of all larvae (N=14) were collected every two days and prepared for measuring as described above. The radioactivity was measured using a Betaszint BF 5000 scintillation counter.

In the course of the experiments relative to growth, first instar larvae, housed singly in glass tubes at 20° C (+/— 1° C), were exposed to a constant rhythm of 16 hr light and 8 hr of darkness (SOEFFING, 1986).

Three different conditions were selected for the aqueous medium: (1) bog water enriched with *Mycobacterium sphagni* at a concentration of $> 10^6$/ml; — (2) bog water with natural concentration of mycobacteria (10^4-10^5/ml); — (3) lake water with natural concentration of mycobacteria ($< 10^2$/ml).

The larvae were fed on Cladocera, food being available all the time. After 25 and 50 days, the growth of the larvae was checked by measuring the width of the head (HARVEY & CORBET, 1985). For measuring an ocular scale with a magnification of 20x was used.

RESULTS

The *Mycobacterium* species found in the larvae as well as in the moss were *M. chelonei, M. flavescens, M. fortuitum,* and *M. sphagni.*

It was found that the concentration of the bacteria in the ponds increases from spring to autumn. Thus, in April 1987 mycobacteria were found in concentrations of less than 100/ml, up to 4.4×10^5/ml were found in June (17-VI-1987) while in August (28-VIII-1987) a concentration of 6.1×10^6/ml was reached.

An increase in mycobacteria during the year could also be noticed in samples of larval *L. rubicunda.* While in spring 1987 fewer than 100/ml were found, in summer the bacteria reached a level of 2.5×10^5/ml (6.VII-1987), increasing to 3.6×10^6/ml in autumn (10-IX-1987). Randomly sampled larvae of *Sympetrum danae, Libellula quadrimaculata* and *Leucorrhinia dubia* showed comparable numbers of mycobacteria at these times.

Samples of the Cladocera *(Ceriodaphnia reticulata, Polyphemus pediculus)* from the pond revealed the same *Mycobacterium* species as found in the *Sphagnum* and in the larvae. This evidence led us to the tentative assumption that mycobacteria are acquired by dragonfly larvae through ingestion of Cladocera.

The experiments with tritium-marked mycobacteria are illustrated in Figure 1. In water enriched with mycobacteria average radioactivity was 177 c.p.m./ml during the experiments. The Cladocera quickly accumulate the tritium-labeled mycobacteria to a high concentration.

When larvae of *L. rubicunda* feed on Cladocera containing labeled mycobacteria they incorporate most of the radioactivity. Samples of the faeces showed

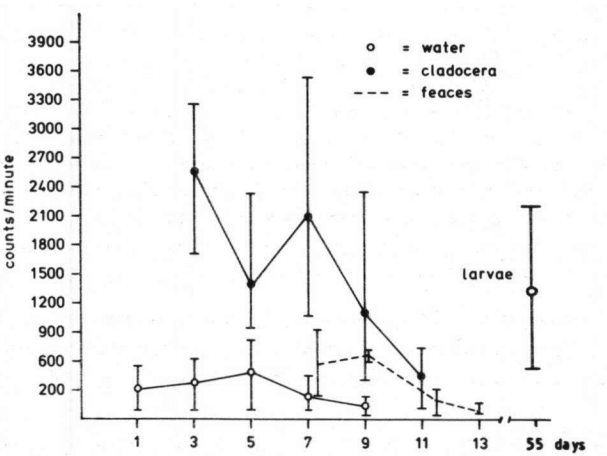

Fig. 1. Nutritive chain between mycobacteria, Cladocera and larvae of *Leucorrhinia rubicunda.* The radioactivity of mycobacteria in water (1 ml sample), of Cladocera (10 specimens sample), of faeces, and the incorporated radioactivity of larvae are shown.

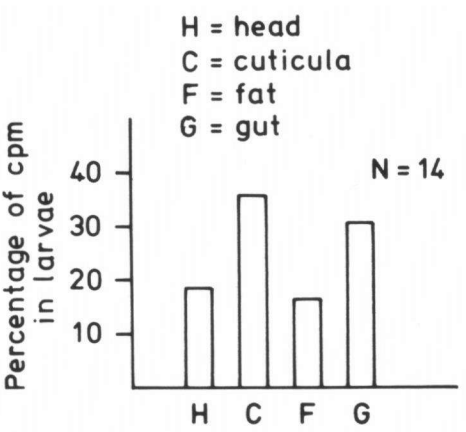

Fig. 2. Radioactivity distribution in different parts of the larva of *Leucorrhinia rubicunda.*

only little radioactivity, the average value per larva being 1340 c.p.m. Dissection showed that most of the tritium had been taken up in the cuticula and gut. Nearly 20% of radioactivity was localized in the fat (Fig. 2).

Measurements of the head width suggest a direct correlation between concentration of mycobacteria and larval size ($p < 0.002$, Mann-Whitney U-Test; Fig. 3)

The larvae exposed to lake water were the smallest. Only after 50 days had they reached the head width that the other larvae had already after 25 days. The best conditions for growth, thus, exist in bog water enriched with *Mycobacterium sphagni.*

DISCUSSION

Numerous studies on the odonate fauna of oligotrophic bogs show a great number of species and a high population density (e.g. PAJUNEN, 1962; SCHMIDT, 1964). This feature has been mainly explained by the absence of fish (e.g. CORBET, 1983; MACAN, 1964). However, the role of microorganisms in the developement of dragonfly larvae has been neglected for a long time.

The existence of habitual mycobacterial flora in *Sphagnum* bogs of Europe, North- and South America and New Zealand has been evidenced by KAZDA (1977, 1981), MÜLLER et al. (1980), IRGENS et al. (1981) KAZDA & COOK (1987) and by COOK & KAZDA (1988). The best conditions for mycobacterial growth have been found in hydrophilic *Sphagnum* vegetation which communicates with bog water (MÜLLER et al., 1980; IRGENS et al., 1981). The amino acid composition of mycobacteria shows a similar picture to that of dragonflies. Alanin, arginin, glutamin, glycin, tyrosin and valin are generally present, in mycobacteria as well as in dragonflies (REIF, 1957; WIGGLESWORTH, 1975; KNOPF, 1977). This fact leads to the tentative assumption that mycobacteria represent a fundamental nutrition for the Odonata in oligotrophic bogs.

Bacteria as a food resource for aquatic invertebrates have been established by BAKER & BRADNAM (1986) and FREDEEN (1964). In the guts of *Simulium* and *Chironomus* larvae, Baker & Bradnam found bacteria in concentrations of

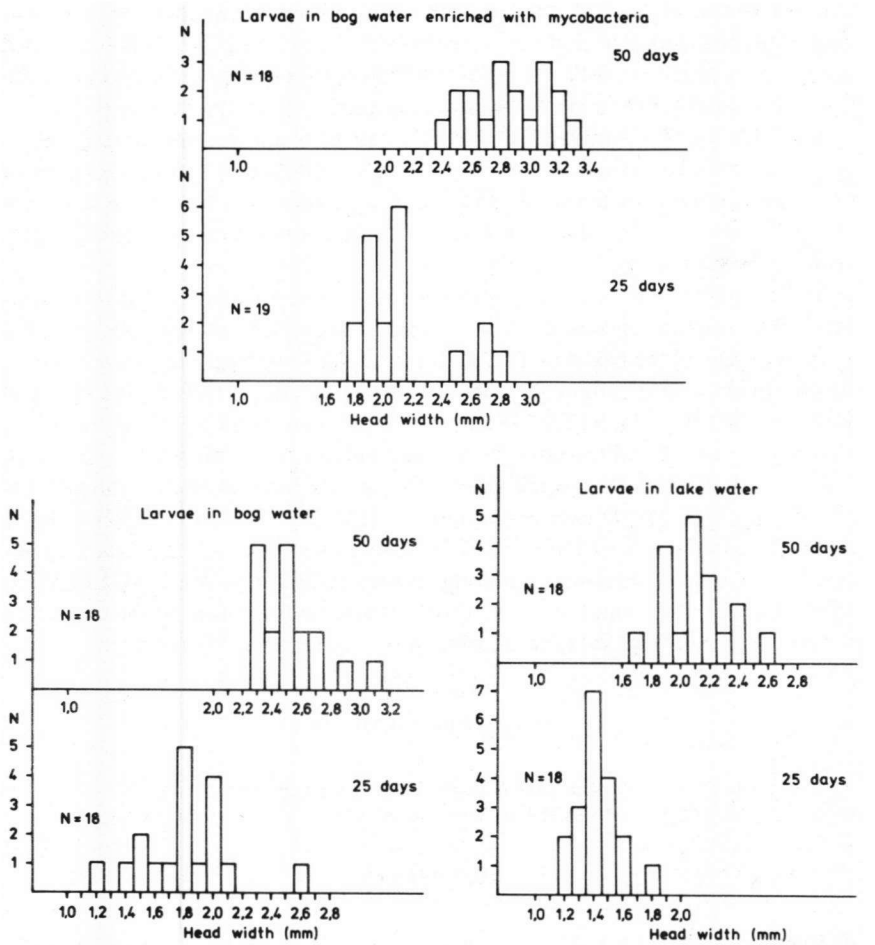

Fig. 3. Larval growth of *Leucorrhinia rubicunda* under different concentrations of mycobacteria. — ($p < 0.002$, Mann-Whitney-U-test).

10,000-100,000/ml and they assumed that the assimilated bacteria had an important nutritive value. Fredeen was even successful in raising *Simulium* on an exclusive diet of suitable bacteria.

The present work with labeled material shows that mycobacteria are accumulated by the cladocerans. Cladocera exposed for two days to a medium containing labeled bacteria showed a nearly ten times higher concentration of labeled tritium than the medium.

Experiments proved that the larvae take up most of the accumulated bacteria.

After a period of feeding only small concentrations of radioactivity were measured in the faeces. The findings on the dissected larvae indicated that the labeled compounds of the mycobacteria have been incorporated into fat and cuticula. Thus, it seems that the mycobacteria are digested and metabolized by the larvae.

LAWTON (1970) and THOMPSON (1978) demonstrated a correlation between size of larvae and that of prey. BLOIS (1985) found that young larvae of *Aeshna cyanea* mostly feed on Cladocera. These observations may point towards the importance of mycobacteria accumulated by small Cladocera for the nutrition of young larvae.

The nutritive value was also experimentally demonstrated. The direct correlation between the concentration of mycobacteria and the larval size indicates the nutritive value of the bacteria (Fig. 1). It is possible that dragonfly larvae take up amino acids and vitamins of the mycobacteria (BUCHNER, 1953; WIGGELSWORTH, 1975; STRÜMPEL, 1983. On the other hand, it is also possible that mycobacteria and the odonate larvae maintain a kind of specific symbiosis.

It is well known that some dragonfly species have evolved strategies for ovipositing over *Sphagnum* vegetation (SCHMIDT, 1964; PAJUNEN, 1962; CORBET, 1983; SOEFFING, 1986). A reason for this may be the faster development of eggs in heat-accumulating mosses (CORBET, 1983; SOEFFING, 1986). On the other hand, it could also be that here the young larvae can reach food of high nutritive value in an otherwise oligotrophic environment.

ACKNOWLEDGEMENTS

For numerous helpful comments and critical reading of the manuscript the author is thankful to Professor Dr KAZDA. Dr N. BÜRKEL improved the English version of the manuscript.

REFERENCES

BAKER, J.H. & L.A. BRADNAM, 1976. The role of bacteria in the nutrition of aquatic detrivors. *Oecologia* 24: 95-104.

BARKSDALE, L. & K.-S. KIM, 1977. Mycobacterium. *Bacteriol. Rev.* 41(1): 217-372.

BLOIS, C., 1985. The larval diet of three anisopteren (Odonata) species. *Freshw. Biol.* 15: 505-514.

BUCHNER, P., 1953. Endosymbiose der Tiere mit pflanzlichen Mikroorganismen. Basel-Stuttgart.

COOK, B.R. & J. KAZDA, 1988. Mycobacteria in pond waters as a source of non specific reactions to bovine tuberculin in New Zealand. *N. Z. vet. J.* [In press].

CORBET, P., 1980. Biology of Odonata. *A. Rev. Ent.* 25: 189-217.

CORBET, P., 1983. *A biology of dragonflies.* Classey, Faringdon.

FREDEEN, F.J.H., 1964. Bacteria as food for blackfly larvae (Diptera: Simuliidae) in laboratory cultures and in natural streams. *Can. J. Zool.* 42: 527-548.

HARVEY, J.F. & P.S. CORBET, 1985. Territorial behaviour of larvae enhances mating success of male dragonflies. *Anim. Behav.* 33: 561-565.

IRGENS, L.M., J. KAZDA, K. MÜLLER & G.E. EIDE, 1981. Conditions relevant to the occurrence of acid-fast bacilli in sphagnum vegetation. *Acta pathol. microbiol. scand.* (B) 89: 41-47.

KAZDA, J., 1977. The importance of sphagnum bogs in the ecology of mycobacteria. *Zentbl. Bakt. Hyg.* (I/B) 165: 323-334.

KAZDA, J., 1980. Mycobacterium sphagni sp. nov. *Int. J. syst. Bacteriol.* 30: 77.

KAZDA, J., 1981. Growth of the mycobacteria in sphagnum peat substrates; nutritive requirements of some cultivable and non cultivable mycobacteria with special reference to Mycobacterium leprae. *Proc. Int. Peat Symp.*, Bemidji, Minnesota, p. 335.

KAZDA, J., 1983. The principles of the ecology of mycobacteria. *Biol. Mycobact.* 2: 323-341.

KAZDA, J. & B. COOK, 1987. Unusually high densities of slowly growing mycobacteria on sphagnum moss in New Zealand. *Int. Peat J.* 1987: 119-125.

KNOPF, K.W., 1977: *Protein variation in Gomphus (Odonata: Gomphidae).* PhD thesis, Univ. Florida, Gainesville.

LAWTON, J.H., 1970. Feeding and food energy assimilation in the larvae of the damselfly Pyrrhosoma nymphula (Sulzer) (Odonata: Zygoptera). *J. anim. Ecol.* 39: 669-689.

MACAN, T.T., 1964. The Odonata of a Moorland fishpond. *Int. Revue ges. Hydrobiol.* 49(2): 325-360.

MÜLLER, K., J. KAZDA & L.M. IRGENS, 1980. Zum Vorkommen von Mykobakterien in Mooren. *Telma* 10: 245-252.

MÜLLER, K. & J. KAZDA, 1988. Zum Vorkommen von Mykobakterien in der Sphagnum--Vegetation der Paramo-Moore Südkolumbiens. [Telma, in press].

PAJUNEN, V.I., 1962. Studies on the population ecology of Leucorrhinia dubia v.d. Lind. (Odonata: Libellulidae). *Annls Zool. Soc. zool.-bot. fenn. Vanamo* 24: 1-78.

REIF, W., 1956. Untersuchungen über die Zusammensetzung der Mycobacterienproteine. *Jber. ForschInst. Borstel* 1956: 689-699.

SCHMIDT, EB., 1964. Biologisch-ökologische Untersuchungen an Hochmoorlibellen. *Z. wiss. Zool.* 169: 313-386.

SOEFFING, K., 1986. Ecological studies on eggs and larvae of Leucorrhinia rubicunda (L.) (Odonata: Libellulidae). *Jber. ForschInst. Borstel* 1986: 234-237.

SOEFFING, K., 1987. Eine Wasserfalle für Libellenlarven. *Libellula* 6(3/4): 102-104.

STRÜMPEL, H., 1983. Homoptera (Pflanzensäuger). *Handb. Zool.* Berlin-New York.

THOMPSON, D.J., 1978. Prey size selection by larvae of the damselfly, Ischnura elegans (Odonata). *J. anim. Ecol.* 47: 769-785.

WIGGLESWORTH, V.B., 1955. *Physiologie der Insekten.* Basel.