## Water exchange between mother and larvae in scorpions

M. Vannini, M. Balzi, A. Becciolini, I. Carmignani and A. Ugolini

*Istituto di Zoologia, v. Romana 17, I-50125 Firenze, and Laboratorio di Radiobiologia, v.le Morgagni, I-50134 Firenze (Italy), 11 March 1985*

*Summary.* Scorpion larvae spend their first larval stage settled on their mother's back. Isolated larvae can also survive, but their survival probability is lower than it is if they remain on the back of the mother. Experiments with tritiated water suggest that a water exchange must take place between mother and larvae.
*Key words.* Maternal care; scorpions; water exchange.

It is a well known fact that the newborn of all scorpions climb onto their mother's back just after birth and settle there for all their larval period, i.e. until their first molt, when they change into nymphs. The larvae are scarcely mobile and whitish, look much stouter than the adults and do not actively feed or drink; on the other hand the nymphs are mobile and colored; they resemble miniature adults and prey actively.

Three hypotheses have been made regarding the adaptive value of the scorpions' maternal care[2-6]: a) defense against predators, b) continuous selection by the mother of the optimal microclimate for the larvae (this may be particularly important because the larvae lack an epicuticle of the adult kind[7,8]), c) some sort of trophic exchange between mother and larvae. Obviously these hypotheses do not necessarily exclude one another, but, in any case, no experimental demonstration seems to exist.

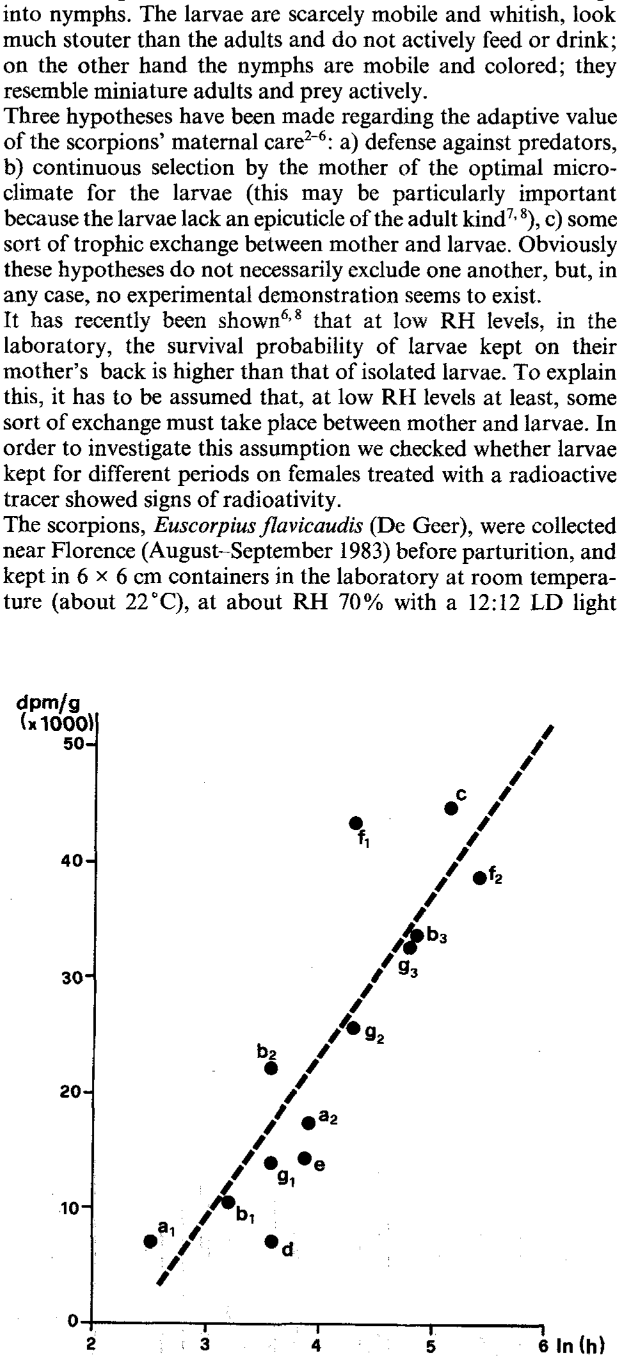
It has recently been shown[6,8] that at low RH levels, in the laboratory, the survival probability of larvae kept on their mother's back is higher than that of isolated larvae. To explain this, it has to be assumed that, at low RH levels at least, some sort of exchange must take place between mother and larvae. In order to investigate this assumption we checked whether larvae kept for different periods on females treated with a radioactive tracer showed signs of radioativity.

The scorpions, *Euscorpius flavicaudis* (De Geer), were collected near Florence (August–September 1983) before parturition, and kept in 6 × 6 cm containers in the laboratory at room temperature (about 22°C), at about RH 70% with a 12:12 LD light cycle. The animals were never fed. Starting before parturition, three females were offered tritiated water while seven others were given normal water. HTO (NEN, sp. act. 1 mCu/g) was used. Tritiated water was given by gently dispensing single drops directly onto the scorpion's chelicera or mouth orifice from a pipette. A further drop was administered only when the previous one had been fully absorbed. For the first treatment 50 µl of the basic solution was used, then 50 µl of 1:2 diluted solution was used for subsequent applications. The drinking schedule was as follows: female A, three times (7 and 13 days after the first time), female B, three times (7 and 11 days after the first time) and female C, four times (4, 8 and 14 days after the first time). Females A and B gave birth 2 days after the first treatment, and female C after 15 days. As soon as birth had taken place, the larvae from the treated females were removed and replaced by larvae from untreated females. It has been shown[5,9] that the behavior of both mother and larvae is unaffected by this manipulation.

After varying periods on the females' backs, the larvae were killed and their radioactivity measured. Each litter was weighed and then kept in hyamina for 48 h, at 45°C until completely dissolved. 2 ml of this solution were added to 10 ml of Biofluor (NEN) and then counted in a scintillator (Beckman, LS 9000). Results are given in dpm/g of tissue (table).

The overall relationship between time spent on the treated females' backs and radioactivity shown by the larvae is highly significant (fig., $r = 0.866$, $df = 11$, $p < 0.001$). It was not possible to determine exactly how the tritium concentration varied in the females' bodies with time. Nevertheless comparison of results from litter b (b1, b2, b3) with those from litter g (g1, g2, g3) indicates an analogous relationship between time spent on females and radioactivity, in spite of the fact that female C (carrier of litter g) was treated for a period about twice as long (18–22 compared to 8–12 days) as female A (carrier of litter b).

The transfer of tritium thus demonstrates the existence of some kind of exchange between mother and larvae. If (at low RH levels) the presence of the mother gives to the larvae a higher survival probability, this can be due either to a water exchange




Relationship between time of permanence on the treated females and radioactivity shown by the larvae.

Radioactivity shown by the larvae kept on tritiated females. Litters a and f were split into 2 and litters b and g into 3 batches

| Treated females | Larvae batch | No. of larvae | Hours on back | ln (h) | dpm/g (*1000) | Days after 1st treatment |
|---|---|---|---|---|---|---|
| A | a1 | 10 | 12 | 2.48 | 7.0 | 5 |
| | a2 | 3 | 48 | 3.87 | 17.2 | 7 |
| | b1 | 8 | 24 | 3.18 | 10.3 | 8 |
| | b2 | 10 | 35 | 3.56 | 22.2 | 8 |
| | b3 | 8 | 120 | 4.79 | 33.8 | 12 |
| | c | 4 | 168 | 5.12 | 44.8 | 20 |
| B | d | 3 | 36 | 3.58 | 7.6 | 6 |
| | e | 3 | 48 | 3.87 | 14.1 | 7 |
| | f1 | 10 | 72 | 4.28 | 43.8 | 14 |
| | f2 | 3 | 216 | 5.38 | 38.8 | 14 |
| C | g1 | 10 | 36 | 3.58 | 14.0 | 18 |
| | g2 | 10 | 72 | 4.28 | 26.8 | 20 |
| | g3 | 6 | 120 | 4.79 | 32.8 | 22 |

(compensating larval water loss) or a wax exchange (reducing larval water loss). Consequently two possible mechanisms can be hypothesized: 1) the tritium could have been incorporated in some of the females' epicuticular lipids which in turn could have been transferred to the larvae merely by contact, or 2) the tritium could have simply left the female and been absorbed by the larvae just as liquid water or steam.

Long term direct observations failed to reveal any signs of water exchange straight from the mouth. The larvae never approached the buccal area of the mother, nor was the mother seen to actively move the larvae toward its mouth. The amount of water that scorpions can lose by evaporation is in any case quite high. At 10% RH, in laboratory, adult *Euscorpius* can lose 20% of their total weight in a week, without appearing to be damaged[11]. On the other hand, it has also been shown[12, 13] that hydrocarbon biosynthesis in adult scorpions is very slow, and that the cuticular lipid turnover depends more on dietary hydrocarbon than on autonomous synthesis. Furthermore, it has been pointed out[14] that the maximum waterproofing effectiveness is provided by long-chain, highly viscous molecules, which presumably cannot easily be transferred from the female to the larvae simply by contact. In conclusion, the hypothesis that water is exchanged directly through the cuticle seems the most plausible for the moment.

1 We thank Prof. L. Pardi and Prof. A. Ercolini for their critical reviews of the manuscript. This study was supported by the Ministero della Pubblica Istruzione (60% contributions).
2 Williams, S. C., Proc. Calif. Acad. Sci. *37* (1981) 1.
3 Maury, E. A., Physis *29* (1969) 131.
4 Alexander, A. J., XVth int. Ethol. Conf., Bielefeld 1977.
5 Vannini, M., Ugolini, A., and Marucelli, C., Monit. Zool. Ital. *12* (1978) 143.
6 Vannini, M., and Ugolini, A., XVIIth int. Ethol. Conf., Oxford 1981.
7 Pavan, M., Boll. Zool. *21* (1954) 283.
8 Kwartinikow, M. A., Acta zool. bulg. *17* (1981) 5.
9 Ugolini, A., Vannini, M., and Carmignani, I., J. Arachnol. (in press).
10 Vannini, M., and Ugolini, A., Behav. Ecol. Sociobiol. *7* (1980) 45.
11 Ugolini, A., and Vannini M., Boll. Zool. *45* (1978) 247.
12 Hadley, N. F., and Hall, R. L., J. exp. Zool. *212* (1980) 373.
13 Hall, R. L., and Hadley, N. F., J. exp. Zool. *240* (1982) 195.
14 Hadley, N. F., in: Biology of Integument, p. 841. Eds J. Bereiter-Hahn et al. Springer, Berlin–Heidelberg 1984.

0014-4754/85/121620-02$1.50 + 0.20/0


## An extremely cadmium-sensitive strain of *Chlorella*

E. Kessler[1]

*Institut für Botanik und Pharmazeutische Biologie der Universität, D-8520 Erlangen (Federal Republic of Germany), 15 April 1985*

*Summary.* Growth of 14 strains from five *Chlorella* species is rather insensitive towards cadmium. One strain (211–1a) of *C. saccharophila*, however, was found to have a sensitivity towards this toxic heavy metal about 100 times higher than that of the other strains of *C. saccharophila*.
*Key words. Chlorella;* cadmium; growth.

The species of the genus *Chlorella* exhibit a surprising diversity of biochemical and physiological properties[2]. Some of them are able to grow under rather extreme conditions of salinity and acidity[3]. Therefore, it seemed interesting to study also their ability to grow in the presence of environmentally important toxic heavy metals[4]. In the course of this work we found that the common and fast-growing species[3], i.e. *C. vulgaris, C. sorokiniana, C. saccharophila, C. fusca* var. *vacuolata*, and *C. kessleri* (three strains each), show rather uniform and fairly low sensitivities (as measured in growth) towards lead, copper, and cadmium (unpublished results).

There was one striking exception, however. Strain 211–1a of *C. saccharophila* has a sensitivity towards cadmium about 100 times higher than that of the other strains of *C. saccharophila*. It shows good growth only up to a concentration of 1 µmole cadmium, in contrast to a limit of 100 µmoles for the other strains (table). It is interesting to note that strain 211–1a had earlier been found to differ rather considerably in some biochemical properties from the other, typical strains of *C. saccharophila*[2], so that its taxonomic position within the '*C. vulgaris* group' (which includes *C. sorokiniana, C. vulgaris*, and *C. saccharophila*) remained somewhat problematic.

Our present results, therefore, are of interest both taxonomically and for a possible utilization of these microalgae for growth in heavy metal-contaminated water[5–7]. Whereas most *Chlorella* species, due to their low sensitivity, appear to be suitable for growth in and purification of heavy metal-polluted water, strain 211-1a might serve as a highly sensitive indicator organism for cadmium contamination. It should be stressed that its extreme sensitivity seems to be restricted to cadmium; only with the chemically related mercury does it exhibit a slightly increased sensitivity, whereas it appears perfectly normal in the presence of lead and copper.

Growth of *Chlorella saccharophila* (Krüger) Migula, strains 211-1d, 211-1b, and 211-1a from the culture collection at Göttingen, in the presence of various concentrations of cadmium. Production of dry mass ($g \cdot l^{-1}$) after 14 days at 25 °C and 6000 lux (continuous illumination; air + 2% $CO_2$) in culture medium[4]. Mean values from 4 experiments (n.d. = not determined)

| Strain | $Cd(NO_3)_2$ ($\mu mol \cdot l^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.5 | 1 | 2 | 5 | 10 | 20 | 50 | 100 |
| 211-1d | 0.77 | n.d. | n.d. | n.d. | 0.58 | 0.60 | 0.54 | 0.52 | 0.40 |
| 211-1b | 0.73 | n.d. | n.d. | n.d. | 0.55 | 0.66 | 0.53 | 0.56 | 0.47 |
| 211-1a | 0.54 | 0.50 | 0.44 | 0.23 | 0.06 | 0 | 0 | 0 | 0 |

1 Acknowledgment. For excellent technical assistance I am indebted to Mrs U. Knoch and Mrs E. Weitemeyer.
2 Kessler, E., Prog. phycol. Res. *1* (1982) 111.
3 Kessler, E., Algol. Stud. *26* (1980) 80.
4 The culture medium (pH 6.4) of Kessler, E., and Czygan, F.-C., Arch. Mikrobiol. *70* (1970) 211, contains (the NaCl was omitted): $KNO_3$ (0.81 g/l), $NaH_2PO_4 \cdot 2H_2O$ (0.47 g/l), $Na_2HPO_4 \cdot 12H_2O$ (0.36 g/l), $MgSO_4 \cdot 7H_2O$ (0.25 g/l), $CaCl_2 \cdot 2H_2O$ (0.014 g/l), $FeSO_4 \cdot 7H_2O$ (0.006 g/l), $MnCl_2 \cdot 4H_2O$ (0.5 mg/l), $H_3BO_3$ (0.5 mg/l), $ZnSO_4 \cdot 7H_2O$ (0.2 mg/l), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.02 mg/l), and EDTA ('Titriplex III') (8.0 mg/l).
5 Jennett, J. C., Hassett, J. M., and Smith, J. E., Miner. Envir. *2* (1980) 26.
6 Rai, L. C., Gaur, J. P., and Kumar, H. D., Biol. Rev. Cambr. Phil. Soc. *56* (1981) 99.
7 Aaronson, S., and Dubinsky, Z., Experientia *38* (1982) 36.

0014-4754/85/121621-01$1.50 + 0.20/0