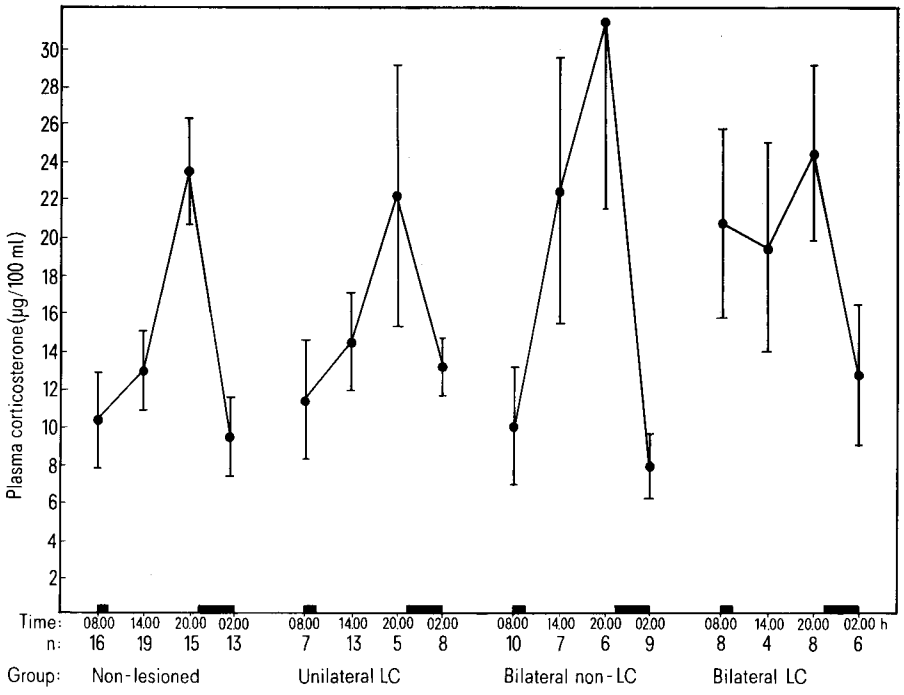

Circadian rhythms of plasma corticosterone in male Swiss mice implanted bilaterally and either lesioned or sham lesioned 48 h before decapitation. Times of day and lesion groups are shown on the abscissa, accompanied by the numbers of animals in each time lesion category. The vertical lines represent ±SEM. The dark bars along the abscissa indicate the periods of darkness (21.00–09.00 h).

tion of the corticosterone diurnal rhythm produced by bilateral LC-lesions is consistent with an inhibitory role for an ascending system impinging directly upon the basal hypothalamus. It is also conceivable that the lesions could influence neural input to the adrenals without altering ACTH secretion.

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PRO EXPERIMENTIS

An 'ecobox' with a discontinuous temperature gradient and a continuous light intensity gradient

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Summary. An apparatus is described for culturing micro-algae in a discontinuous temperature gradient and a continuous light intensity gradient. The apparatus provides 100 different combinations of these abiotic factors at 1 time. The cross-gradient culture apparatus is called 'ecobox'.

In studies on the cyanobacterium *Spirulina platensis*[1], cultures grown at different temperatures and light intensities were compared. To save time and space and to avoid variations in experimental results due to variable conditions over long time intervals, it was considered appropriate to culture the cyanobacterium at various combinations of temperature and light intensity at one time.

Edwards and Van Baalen[2] described an apparatus for the culture of benthic marine algae under varying regimes of temperature and light intensity. The apparatus described here involves an improvement, especially concerning heat transport and temperature regulation.

Materials and methods. Description of the apparatus (figure 1). A colourless anodized aluminium plate (15 mm thick) with 2 longitudinal borings, through which water of different temperatures is pumped, is used to achieve a temperature gradient. Aluminium is used because it is light, strong, non-toxic, easily machined, non-magnetic and

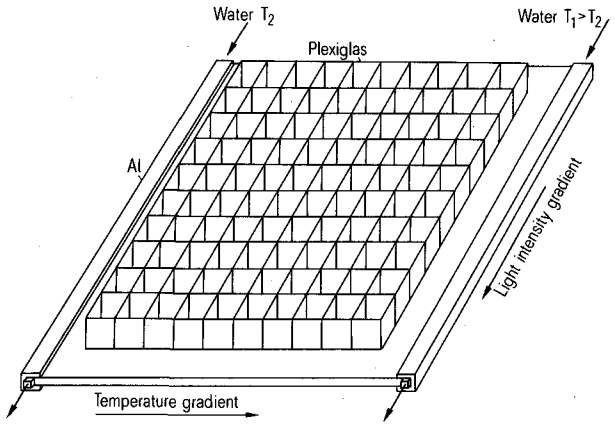

Fig. 1. The ecobox; an aluminium plate with sealed Plexiglas framework.

highly corrosion-resistant. It is anodized in order to obtain a coating highly reflective for visible light and radiant heat; further, the coating soon forms a thin layer of the protective oxide and does not deteriorate. The thermal conductivity of aluminium ranges from 2.36 W/cm °C at 0 °C to 2.40 W/cm °C at 77 °C. A Plexiglas framework consisting of 10×10 squares is sealed to the aluminium plate with silicon kit. Length and width of the framework are about 20% smaller than those of the plate to prevent temperature side effects. The actual dimensions of the framework are $40 \times 40 \times 4.0$ cm forming 100 compartments with inner dimensions of $3.6 \times 3.6 \times 4.0$ cm each. A glass plate is used to cover the framework. The glass plate can be treated with Dri-Film™ SC-87 (Pierce, Rockford, Illinois, USA) to prevent condensation at temperatures higher than the ambient temperature. 2 waterbaths (temperature accuracy, in the ranges used, 0.2 °C) with circulation pumps are used to obtain a linear temperature gradient. The light intensity gradient is achieved by fluorescent lamps (Philips TL33, 40 W) suspended above the plate, with arrangements of lowering, raising and moving the lamps backwards and forwards. The 'ecobox' is insulated with polystyrene strips. Temperatures were measured with thermocouples; light intensities were estimated in lux using a Yokogawa luxmeter type 3281.

Results and discussion. Experiments with water only did not reveal any rise in temperature in the compartments due to the fluorescent lamps. Therefore, it was not necessary to cover the framework with an IR. reflecting glass plate. The temperature in any compartment was constant within 0.5 °C; that means that there was no temperature range in a single compartment. The temperature constancy is certainly due to the high thermal conductivity of aluminium and the very low thermal conductivity of Plexiglas. Convection of the medium in the compartment resulted in the temperatures obtained. For the apparatus as a whole, it means that a discontinuous linear gradient of discrete temperatures was achieved (figure 2).

With 2 fluorescent lamps, it was possible to obtain a continuous light intensity gradient from 0.4 to 10.5 klx at the level of the growing cultures.

Figures 2 and 3 show the results of an experiment with *Spirulina platensis* at a continuous light intensity gradient given by the 'smooth' solid curve and at a discontinuous temperature gradient indicated by the scalariform solid curve; from figure 2 it is obvious that the maximum light intensity (7.6 klx) was provided at the 7th row from above and that the temperature increased stepwise from 16 to 38 °C from left to right. The 7th row was chosen for maximum light intensity for 2 reasons: in order to obtain a clearly pronounced maximum and distinct minimum in the 1st row. The optimum temperature for the strain tested was 31.0 ± 0.5 °C. The maximum light intensity of 7.6 klx did not yield the maximum dry weight/compartment (figure 3). The apparatus can be used in ecological experiments with photosynthetic microorganisms to study combined effects of temperature and light intensity on growth and yield, as well as on morphology and ultrastructure, and is, therefore, indicated in short as 'ecobox'.

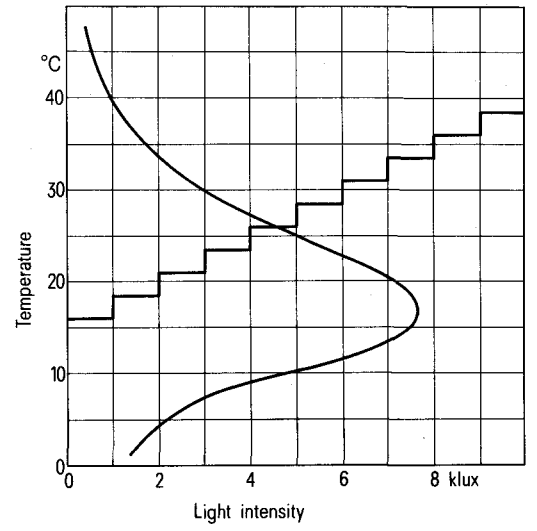

Fig. 2. Topview of the ecobox with the temperature gradient indicated by the scalariform curve and the light intensity gradient given by the smooth curve.

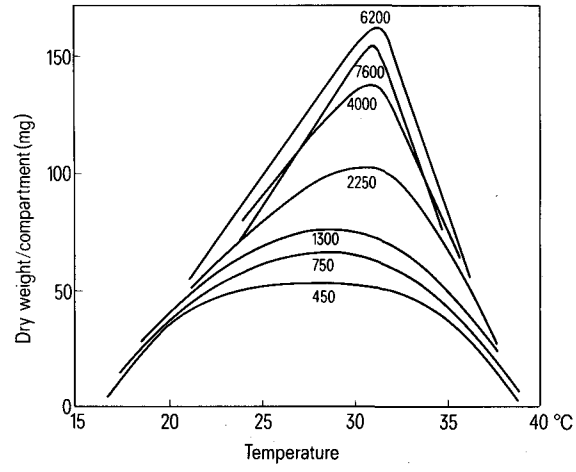

Fig. 3. Dry weight per compartment in relation to temperature; 7 curves with increasing light intensity. Curve 450 lx: 1st row, 750 lx: 2nd row, 1300 lx: 3rd row, 2250 lx: 4th row, 4000 lx: 5th row, 7600 lx: 7th row, 6200 lx: 6th row. Note: the maximum light intensity is not the optimum light intensity as shown by dry weight measurements; the optimum temperature for growth shifts to higher temperatures with increasing light intensity until the optimum is reached.

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