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Light-induced photoreceptor sensitivity loss and recovery at 4°C and 14°C in *Mysis relicta* Lovén (Crustacea: Peracarida) from Pojoviken Bay (Finland)

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Keywords : Mysidaceae, vision, photoreceptors, temperature, damage, lake-specific adaptations.

Specimens of *Mysis relicta* from Pojoviken Bay (Finland) were divided into two groups, one kept at 4°C, the other at 14°C, in total darkness. Immediately following a 1 h exposure to noon sunlight both 4°C and 14°C animals displayed strongly reduced visual sensitivities. In both groups pre-exposure levels were regained in about two days, but apparently along slightly different routes. Slopes of V/log I curves hardly changed throughout the time of observation in the 14°C material, suggesting an adaptation to brighter light levels without undue stress responses. In the 4°C animals, however, there appears to have been not only a longer initial delay before recovery commenced, but slopes of V/log I curves indicated that these animals had reacted with depression to the bright light and needed time to regain their pre-exposure value. The results suggest that recovery is a two-stage process in which biochemical reactions and structural phenomena interact. The results, when compared with similar observations on Lake Pääjärvi specimens, underline the view that different localities may have populations of *Mysis relicta* which differ from each other in photophysiological characteristics.

Perte de sensibilité induite par la lumière des photorécepteurs et récupération de cette sensibilité à 4°C et 14°C chez *Mysis relicta* Lovén (Crustacea : Peracarida) de la baie de Pojoviken (Finlande)

Mots clés : Mysidacés, vision, photorécepteurs, température, lésions, adaptations lacustres

Des spécimens de *Mysis relicta* provenant de la Baie de Pojoviken (Finlande) ont été divisés en 2 groupes et maintenus dans l'obscurité, l'un à une température de 4°C et l'autre à 14°C. Après 1 h d'exposition en pleine lumière, les 2 lots d'animaux ont montré des sensibilités visuelles réduites. Dans les 2 groupes, deux jours ont été nécessaires avant le retour à l'état initial, mais apparemment selon des modalités légèrement différentes. Les pentes des courbes de V (amplitude de réponse) en fonction du log I (intensité du stimulus) changent à peine en fonction du temps pour les animaux conservés à 14°C suggérant une adaptation à des niveaux supérieurs de lumière vive, sans réponses de stress excessif. Pour les animaux conservés à 4°C, cependant, il apparaît un délai plus long avant le retour à l'état initial, mais aussi les pentes des courbes de V en fonction du log I sont déprimées à la lumière vive; le délai pour retrouver leur valeur (initiale) est long. Ces résultats préliminaires suggèrent que le retour à la normale est un processus en 2 étapes, dans lesquelles des réactions biochimiques et des phénomènes structuraux interagissent. Les résultats, après comparaison avec des observations similaires réalisées à partir d'échantillons du lac Pääjärvi, soulignent le fait que différentes localités peuvent avoir des populations de *Mysis relicta* qui diffèrent par leurs caractéristiques photophysiolo-

1. Introduction

Whether or not morphological and physiological differences in animals belonging to the same species, but occupying separate and geographically isolated lakes, are due to polymorphism or the result of lake-specific evolutionary adaptations is an old, yet ever-timely question. Lindström & Nilsson (1984) have shown that in Finland different populations of the opossum shrimp *Mysis relicta* possess different spectral sensitivity peaks, which are attuned to the light transmission maxima of the water-bodies the animals live in.

A further physiological difference between at least two populations of the same species was related to photoreceptor sensitivity recovery, following prolonged exposures to bright light: specimens from the deep, brown lake Pääjärvi ((ca.61°05'N; 25°05'E; maximum depth 87 m: Ruuhijärvi 1974) showed either no or only a very slow recovery, whereas specimens from the much shallower Pojoviken Bay (ca. 60°00'N; 23°30'E) regained their pre-exposure photoreceptor sensitivities much faster (Lindström & Nilsson 1988).

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Using specimens from Lake Pääjärvi alone, Lindström et al. (1988) had then examined whether the temperature in which the animals were kept had any effect on photo-induced damage and recovery. The results clearly showed that original, pre-exposure light sensitivities were not regained in the group of animals that were maintained at the higher temperature of 14°C, but that recovery was possible within two days in the 4°C group. The alternative study, based entirely on specimens from the Pojoviken Bay population of *M. relicta*, had been wanting till now. The new results not only permit us to assess the physiological differences between the two populations more fully, but additionally shed light on the recently reviewed complex problem of light-induced sensitivity loss in crustacean photoreceptors, generally (Meyer-Rochow 1994).

2. Material and methods

Experimental animals were collected during the day in early May from 12°C (surface temperature) warm Pojoviken Bay at a depth of 37 m. Captured specimens were taken back to the laboratory under the protection of a black tarpaulin and divided into a 4°C and 14°C group. Each group was maintained in thermostat-controlled aerated water under total darkness, all necessary manipulations, preparations, etc. being carried out in infrared light with the aid of a «Find-R-Scope»-infrared viewer. While control animals of both temperature regimes remained in the dark throughout the entire series of experiments two days following capture, two subsets, representing the 4°C and the 14°C groups, were exposed in a large Petri dish (30 cm diameter) with the respective temperatures kept constant for 1 h at midday to direct sunlight (60,000 - 80,000 lux).

Following exposure to sunlight, animals were immediately returned to their dark environments of their respective groups with exposed ones being kept separate from the non-exposed controls. Tests of post-exposure visual sensitivity commenced 1 h after the animals' return to the dark and continued at several hour intervals. Since no apparent morphological differences between eyes of males and females were noted or have been reported (Hallberg 1977), data from male and female individuals could be pooled.

Experimental procedures followed Lindström & Nilsson (1983). During preparation, using infrared image converters mounted on a Wild-5 stereo-microscope, each animal was illuminated by light that had passed through 2 Kodak Wratten 87 gelatin filters and sometimes a heat filter as well, which were inserted in the ray path of white light coming from a microscope lamp. The incident light, perpendicular to the eye surface, was centered around the hole through which the recording electrode was lowered 40-50 µm into the eye. The light spot made by the stimulating flash covered the entire eye. Always the same region of the eye was aimed for when inserting the electrode. Following preparations, which on average did not take longer than 5-10 minutes, the test animals were given 30 min in total darkness to recuperate from the operation. The system for stimulation consisted of an extended source (Osram 6V, 15 W microscope lamp, powered by a constant voltage device) and all recordings were made in the AC- rather than DC-setting.

All experimental stimulus intensity/response amplitude data pairs were entered into an Exzel TP2 computer in such a way that response amplitudes were expressed in µV and stimulus intensities in neutral density units. Resulting curves were printed by a Panasonic KX-P1091P printer in a double log plot, but are (as is the usual practice) referred to simply as 'V/log I-curves' rather than 'logV/logI'. Similar double logarithmic plots were used to demonstrate response amplitude/time and stimulus intensity/time relationships for fixed neutral density and response amplitude, respectively. Useful intensity/response amplitude runs were obtained from a total of 21 animals, 11 of which represented the 4°C subgroup, the remainder of 10 came from the 14°C subgroup. The rather small number of post-exposure tested animals is a reflection of the difficulty of the experiment, its time-consuming nature, and the problems associated with maintaining the populations in good health.

The statistical analysis of serially-collected data such as ours presents problems, which have been addressed by Matthews et al. (1990). Firstly, all V/log I curves underwent a curve-fitting exercise, following

$$y = p1 + p2x + p3x^2 + p4x^3$$

and, thus, had their slopes 'p' determined individually. The slope 'p' characterizes each curve and is an important indicator of the state of adaptation the photoreceptor is in at the time of recording (Eguchi & Horikoshi 1984). The latter authors, however, used a different method to determine slopes which is why our 'p'-values are not immediately comparable to the Eguchi & Horikoshi's data. Where necessary, Student's t-test and a significance level of 95 % were employed.

3. Results

All electrophysiologically recorded responses were typical of crustacean eyes, generally, and consisted of rapid hyperpolarizations whose peak amplitudes varied according to the intensity of the stimulus light. In animals not previously exposed to any light, threshold levels of 10 µV response amplitude usually correspon-

ded to stimulus intensities of at least -5.0 log I units. The largest amplitude recorded (9500 μV) came from a male 4°C animal, but no inference must be drawn from this as the second largest amplitude (5000 μV) belonged to a 14°C female.

Response amplitude/stimulus intensity relationships of all (4°C as well as 14°C) animals were plotted in the usual double log way (Figs. 1 & 2) and formed the basis for all further statistical considerations and discussions. Since sensitivity thresholds remained the same in both 4°C and 14°C control animals and no statistically significant differences could be detected in the slopes of 4°C (2.47 ± 0.53) and 14°C controls (2.62 ± 0.48), we have to conclude that, at least over the period the animals were observed, temperature alone did not interfere with the dark-adapted state of the eye of *M. relicta*. Moreover, in all cases in which spectral sensitivity was measured (e.g. the controls of 4°C and 14°C populations as well as 4°C 2 h and 40 h post-exposure animals) the resulting curves were of similar shapes and all displayed a peak at about 550 nm. Finally, no statistically significant difference was noticeable when the means of the slopes of all V/log I curves from both 4°C (2.32 ± 0.71) and 14°C material (2.25 ± 0.46) were compared with each other.

Immediately after the exposure to bright light absolute visual sensitivities were, however, dramatically reduced in both 4°C and 14°C animals (Figs. 1 & 2). In agreement with the situation in the freshwater crayfish *Procambarus clarkii* (Cronin & Goldsmith 1984) and Lake Pääjärvi specimens of *M. relicta* (Lindström et al. 1988) the process of photopigment regeneration apparently proceeds in complete darkness as evidenced by the steady post-exposure rise in absolute sensitivity in both temperature groups (Figs. 1 & 2). When response amplitude values to fixed stimulus intensities (log I = -0.5, -1.0, and -1.5) on log/log axes were analyzed, no obvious deviation from a regularly progressing impro-

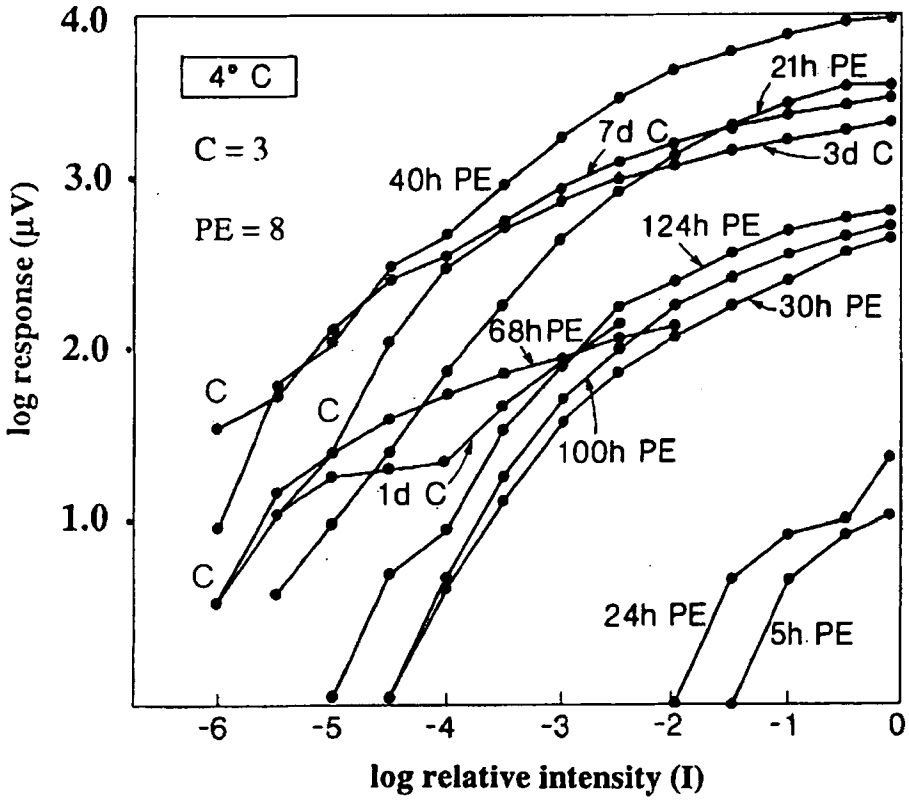

Fig. 1. Stimulus intensity [I]/response amplitude [V] curves of 4°C animals plotted on log - log axes, showing gradual increases in sensitivity to control levels (curve moves to the left with time). Numbers alongside the curves refer to post-exposure times in hours (h) or control animals (C) kept in the dark for 1-7 days (d).

Fig. 1. Les courbes Intensité du stimulus (I)/Amplitude de réponse (V) des animaux à 4°C sont figurées sur des axes log-log. Elles présentent une augmentation progressive de la sensibilité par rapport aux niveaux des témoins (courbes déplacées vers la gauche en fonction du temps). Les nombres ou lettres le long des courbes représentent les temps de post-exposition en heures (h) ou les animaux témoins (C) conservés à l'obscurité pendant 1-7 jours (d).

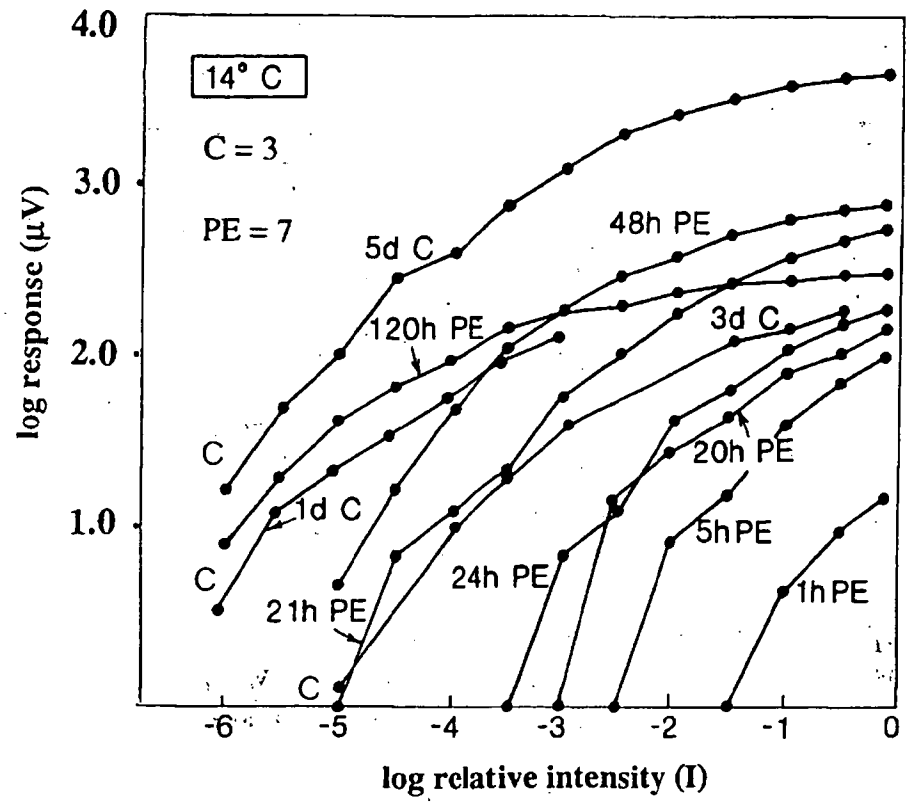

Fig. 2. Stimulus intensity [I]/response amplitude [V] curves of 14°C animals plotted on log - log axes, showing gradual increase in sensitivity to control levels (curve moves to the left with time). Numerals and letters alongside individual curves as in Fig. 1.

Fig. 2. Les courbes Intensité du stimulus (I)/Amplitude de réponse (V) des animaux à 14°C sont figurées sur des axes log-log. Elles présentent une augmentation progressive de la sensibilité par rapport aux niveaux des témoins (courbes déplacées vers la gauche en fonction du temps). La signification des nombres et des lettres le long des courbes est la même que pour la Fig. 1.

vement in sensitivity to pre-experimental levels appeared to be apparent (Fig. 3), suggesting that the latter could be regained in approx. 2 days by both 4°C and 14°C animals.

In order to test whether the changes in threshold and overall absolute sensitivity following exposure to light were due simply to a horizontal shift of the V/logI curves along the x-axis and, thus, indicative not of physiological stress, but of regular adaptive behaviour based on photopigment densities alone (Meyer-Rochow & Eguchi 1985), the slopes of the V/log I curves of 4°C and 14°C material were plotted against postexposure time (Fig. 4). A regression analysis clearly shows that overall the slopes of the V/log I curves in the 14°C animals change very little with time, but those of the 4°C animals keep on increasing with time. The difference is statistically significant (P=0.0281) and shows that sensitivity recovery is not identical in the two populations. In the 14°C animals an improvement in sensitivity is achieved by a horizontal 'sliding' along the x-axis of the V/log I stimulus/response amplitude relationship and does not alter the actual shape of the curve. The situation for the 4°C material, however, was different and suggested that following the exposure to bright light, V/log I curves had become flatter, but were, within two days post-exposure, capable of regaining their pre-exposure shapes. A similar behaviour of the stimulus/response amplitude relationship had previously been noticed in relation to stress indicative of imminent photoreceptor damage (Meyer-Rochow & Tiang 1984, Lindström et al. 1988).

4. Discussion

The observations demonstrate that photopigment recovery and/or synthesis following light-induced bleaching are indeed affected by temperature, a conclusions reached earlier by Barnes & Goldsmith (1977) for the lobster eye through ERG-studies and by Larrivee & Goldsmith (1982) for the eye of the freshwater crayfish *Procambarus* on the basis of in vitro studies of extracted photopigments. Turning specifically to

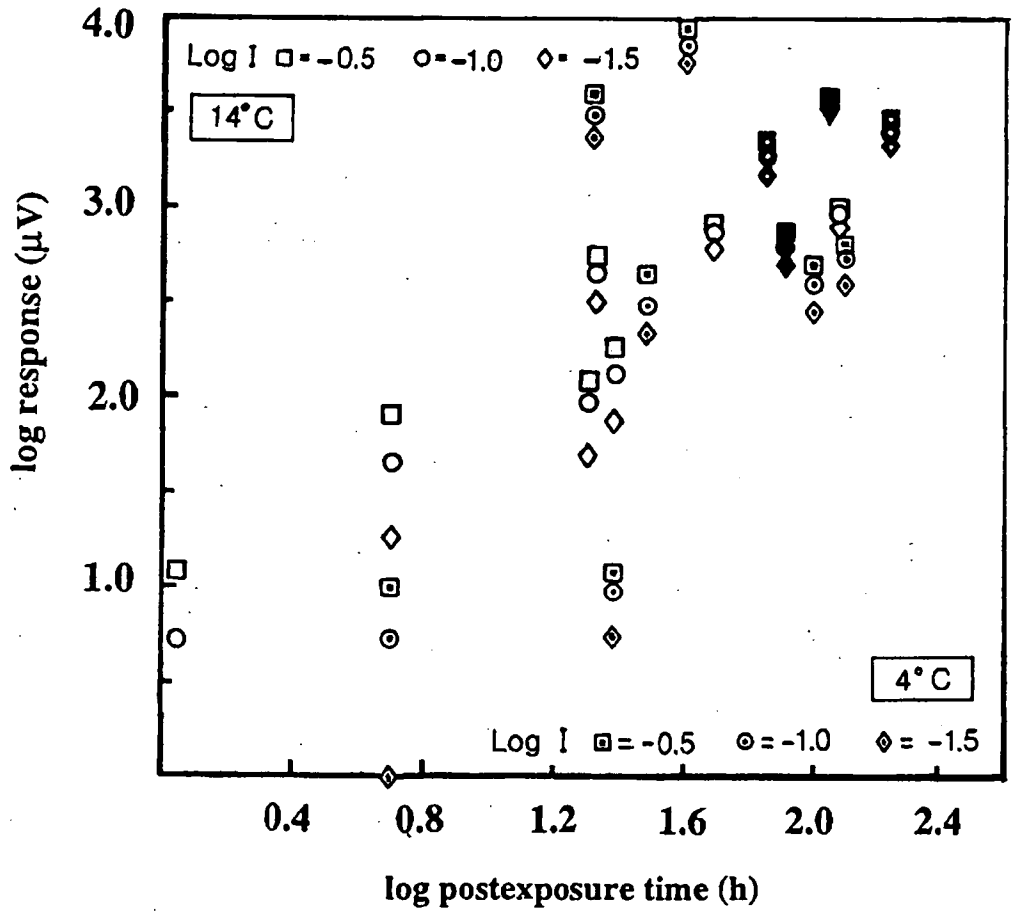

Fig. 3. Response values to fixed stimulus intensities (log I = -0.5, -1.0, and -1.5) from animals of 4°C (symbols with central spot) and 14°C (symbols without central spot) environments. Following 2 days of recovery (log post-exposure time 1.5), sensitivities of both 4°C and 14°C animals have almost reached normal (= control) levels (black symbols with and without spot). However, there is apparently a greater delay of recovery in 4°C specimens.

Fig. 3. Valeurs des réponses pour des intensités de stimulus fixées (log I = -0.5, -1.0, et -1.5) des animaux à 4°C (symboles avec un point central) et à 14°C (symboles sans point central). Après 2 jours de récupération (log du temps de post-exposition 1,5) les sensibilités des animaux à 4°C et à 14°C ont presque atteint des niveaux normaux (= contrôle) (symboles noirs avec ou sans point central). Cependant il y a apparemment un délai de récupération plus grand pour les échantillons à 4°C.

our Pojoviken Bay material, the results show that the 14°C specimens regain pre-exposure sensitivities through a more or less steady 'left-shift' along the x-axis of the V/log I relationship, which undoubtedly signifies an increase in excitatory photopigment (Meyer-Rochow & Eguchi 1985). The 4°C material, on the other hand, had to undergo a two-phase sensitivity recovery, consisting firstly of an increase of V/log I slope value to 'normal' magnitude and secondly, of the necessary left shift of the V/log I curve towards lower visual thresholds.

According to Matic & Laughlin (1981) the depression of peak amplitudes and the flattening of the V/log I curves could be explained by voltage-sensitive K^+ conductance and a reduction in the ratio of channels to photons occurring during the response. Other ions, most notably Ca^{2+}, are known to be involved in phototransduction mechanisms and dark/light-adaptational processes (Hardie & Minke 1995). Since membrane properties must ultimately be held responsible for the performance of the photoreceptor as a whole, the finding that fatty acid composition and ultrastructure of photoreceptive membranes do, indeed, change under conditions of thermal and photic stress, takes on special signifcance (Kashiwagi et al. 1997).

Homeoviscous adaptations of membranes (Sinensky 1974), however, are not immediate, which is why it is entirely possible that «even minor decreases in tempe-

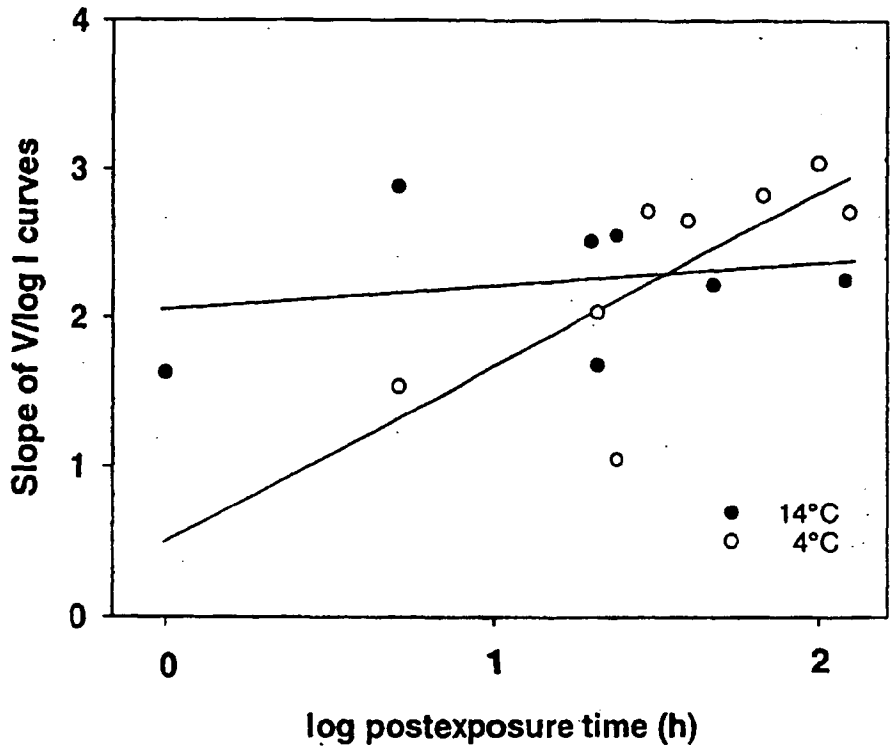

Fig. 4. When the slopes of individual V/log curves are plotted in relation to post-exposure time, it becomes apparent that 14°C material largely retained the normal (= pre-exposure) V/log I curve slopes (i.e. 2.62). In 4°C material, however, curves first became flatter following the exposure to bright light, and then gradually build up V/log I slopes to normal magnitude again.

Fig. 4. Quand les pentes des courbes individuelles V/log I sont figurées en fonction du temps de post-exposition il devient visible que le matériel à 14°C conserve une pente de courbe (V/log I) normale (= pré-exposition) (i.e. 2,62). Cependant pour le matériel à 4°C, les courbes deviennent plus étalées suivant l'exposition à la lumière vive et ensuite la pente de V/log I en fonction du temps reprend progressivement une valeur normale.

rature could present permeability problems» (Pruitt 1990) - especially when combined with an assault of very bright light. Very recently it has been reported that 11-cis retinyl esters, i.e. building blocks of photopigment, can be stored in the eyes of crustaceans within fatty acids generally and docosahexaenoate in particular (Srivastava et al. 1996). The latter acid was identified by Kashiwagi et al. (1997) to slowly increase in material acclimated to cold, dark conditions, but to decrease when subjected to illumination by bright light! We think it is, therefore, feasible that if this light-induced decrease overtakes synthesis and incorporation of the acid into the visual membranes, photopigment stores will begin to run low. In combination with the incomplete restructuring of the membrane, due to ongoing homeoviscous adaptation, this could cause the reduction in the V/log I slopes and the delay in sensitivity recovery seen in our 4°C material. Parallels to the scenario outlined above for the *M. relicta* eye can be found amongst some vertebrate eyes: in the bullfrog, according to Sillman et al. (1978), dark-adaptation is also a two-stage process in which the two phases can be separated by altering the temperature of the retina.

Metabolic demands of the 4°C and 14°C photoreceptors under conditions of testing also differ. In invertebrate eyes oxygen consumption of the photoreceptors rises steeply during periods of light exposure (Tsacopoulos 1995) and there is evidence that under stress, at least in the crayfish eye, the metabolized substrate changes (Taylor & Meyer-Rochow 1997). A water temperature of only 4°C in the month of May, is arguably more stressful than one of 14°C, especially during exposures to bright light and the switch from one to another metabolic substrate could affect the initial speed of visual sensitivity recovery in the 4°C animals.

The admittedly somewhat preliminary results suggest to us that Pojoviken Bay specimens can cope much better with higher temperature and are much less vulnerable to photo-induced membrane damage than Lake Pääjärvi specimens. Obviously, the Pojoviken Bay environment is an overall brighter and warmer one, in which greater natural fluctuations in temperature and brightness are the rule. Since Lake Pääjärvi and Pojoviken Bay shrimps behaved similarly only with regard to photoreceptor recovery at 4°C, but quite differently when 14°C warm water was used, we conclude that our findings, generally, support the view that different localities have populations of *M. relicta* with different visual charcateristics. Visual pigment polymorphism, known to exist in vertebrates from fishes (Archer et al. 1987) to primates (Jacobs & Neitz 1987) has to be ruled out, of course. Knowing that only rhodopsin is present in the eyes of *M. relicta* (Lindström et al. 1988), we have demonstrated with our tests that photophysiological differences of isolated populations can be identified when carefully designed experiments are being used to probe the limits of what the eyes in terms of brightness and temperature are able to tolerate.

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