ผลของรังสีอุลตราไวโอเลตบีต่อตัวอ่อนปลานิล (*Oreochromis niloticus*)

Effect of Ultraviolet-B Radiation on Nile Tilapia Larvae (*Oreochromis niloticus*)

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บทคัดย่อ

ผลสืบเนื่องอันไม่พึงประสงค์หลายประการที่เกิดจากภาวะโลกร้อนเป็นสิ่งที่ผู้คนต่างกังวลอยู่ในขณะนี้ การเพิ่มระดับของรังสีอุลตราไวโอเลต (UV) ซึ่งก่อผลเสียหายแก่สิ่งมีชีวิตหลายชนิดก็เป็นหนึ่งในปัญหาอันยากแก่การยอมรับได้เหล่านั้น สัตว์มีกระดูกสันหลังในน้ำจัดเป็นสิ่งมีชีวิตที่มีความเสี่ยงสูงต่อรังสีUV งานวิจัยนี้จึงมุ่งศึกษาถึงผลกระทบของรังสี UV-B ต่อตัวอ่อนปลานิล *(Oreochromis niloticus*) ได้เลี้ยงตัวอ่อนอายุ 9 วัน ภายใต้แสงยูวีในช่วงความยาวคลื่น UV-B (312 nm) ความเข้มแสง 194 $\mu W/cm^2$ โดยให้แสง 3 และ 5 ชั่วโมง/วัน ส่วนกลุ่มควบคุมได้รับเฉพาะแสงจากหลอดนีออนธรรมดา พบว่าตลอดระยะเวลา 20 วันของการได้รับรังสี UV-B ตัวอ่อนที่ได้รับรังสี 3 และ 5 ชั่วโมง/วัน มีการตายสะสม 57 % และ 67% ตามลำดับ ผลการศึกษาทางเนื้อเยื่อวิทยาพบว่าตัวอ่อนที่ได้รับรังสียูUVทั้ง 2 กลุ่มมีความหนาของผิวหนังชั้นนอกมากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (P<0.05) และบางบริเวณมีการตายแบบ necrosis และมีการหลุดลอกของเซลล์ผิวหนัง นอกจากนี้ยังพบว่าเซลล์สร้างสีชนิดเมลาโนไซท์ที่ผิวหนังบริเวณหัวของตัวอ่อนที่ได้รับรังสียูวีทั้ง 2 กลุ่มมีการหดตัวและเสียสภาพ

Abstract

Global warming has now raised pubic concerns for its unwanted consequences. Increasing UV-Radiation which harmfully affects a variety of organisms is one of those inconvenient problems. Aquatic vertebrates are among high UV-risk organisms. This research aimed to investigate the impact of UV-B radiation on larvae of Nile Tilapia *(Oreochromis niloticus*). Nine-day-old larvae were exposed to radiation in the wavelength of UV-B (312 nm), intensity 194 $\mu W/cm^2$. Two groups of UV-B exposure, 3 and 5 hrs were conducted. Control group was exposed to only visible light. The results showed that the cumulative mortalities in larvae exposed to UV-B radiation for 20 days were 57% and 68% for 3 hrs/days and 5 hrs/day exposure respectively. Histological examination revealed that epidermis of UV-B exposed larvae in both groups were significantly thicker than those of controls (P<0.05) accompanying with epidermal necrosis and sloughing in some region. Moreover, the skin melanocytes at the head area were shrunken and deformed.

Introduction

The harmful effects of solar radiation on terrestrial and animals including man have been documented in the large body of literature (Young *et al*., 1993). In aquaculture solar radiation is a well known environmental hazard for salmonids in shallow ponds leading to necrosis of the skin and dorsal fin (Bullock, 1988). Aside from lesion-related infections, UV-radiation may also depress the immunity and increase the organism's vulnerability to other pathogens (Knowles, 1992). In aquatic environments radiation is effectively absorbed by dissolved substances and suspended particles in the water (Scully and lean, 1994; Kirk, 1994). Thus in deeper water layers and particularly in turbid freshwater aquatic organisms are protected from UV-radiation. However, in oligotrophic water, near the surface of water organisms are often exposed to high UV intensities. The sensitivity to UV-radiation is species-specific (Kaweewat and Hofer, 2001) and depends on the developmental stages of the fish. The sensitive stages are eggs and larvae (Eisler, 1961; Hunter *et a*l., 1979; Dey and Damkaer, 1990). The skin damage is particular relevance for early life stages of many fish species since their skin are incomplete or even lacking pigmentation (Kaweewat and Hofer, 1997). Due to recent evidences of stratospheric ozone depletion and global warming situation which lead to increasing levels of UV-B radiation, it is necessary to improve our understanding of the impact of this hazard radiation on freshwater ecosystem. The aim of our study, thus, was to investigate the sensitivity of nile tilapia larvae to UV-B radiation by evaluation of mortality, histology of the skin and pigmentation.

Materials and Methods

Animals

*Experiments were performed with 2, 4, 6 and 9-day-old larvae of Nile tilapia (*O. niloticus*). The larvae were obtained from Faculty of Fisheries Technology and Aquatic Resources, Mae Jo University. For each experimental condition three replications with 100 larvae were transferred to black containers with a total water column of 2.5 l and a water level of 5.5 cm. Twice daily swim up larvae were fed with commercial fish diet and accumulated residues were removed.*

UV Experiments

UV-radiation was provided by UV lamps (Cole-Parmer Instrument Co.) emitting UV-radiation at UV-B wavelength (312 nm, intensity 194 $\mu W/cm^2$) and supplemented with visible light (Dura-Test, Philips Ltd, 36 W; 12 $h.day^{-1}$).Two groups of UV-B exposure, 3 and 5 hrs were conducted with 50 cm. UV-lamps above the water surface. Control group was exposed to only visible light. During 20 days of the experimental period dead larvae were counted and removed daily. At the end of the experiment, the 30 survivors of each group were sampled for histological and pigment examinations.

Histology and Pigmentation

Fifteen larvae were fixed in Bouin's solution for histological examination using a routine histology technique. 6 μm longitudinal sections were stained with hematoxyline and eosin. The rest 15 samples were fixed in 5% buffered formalin and whole mount preparation was performed using a routine histology technique. Histopathological changes of the skin and melanocytes of the dorsal side at head area were examined under the light microscope.

Statistics

Data were expressed as mean ± standard deviation. The significance of difference between the means was determined by one-way analysis of variance (ANOVA). The observed significance was then confirmed using the least significant difference (LSD) test.

Results

Mortality

The cumulative mortality in larvae exposed to UV-B radiation for 3hrs/day was 68% and for 5 hrs/day was 57% while control fish showed no mortalities (Fig 1.).

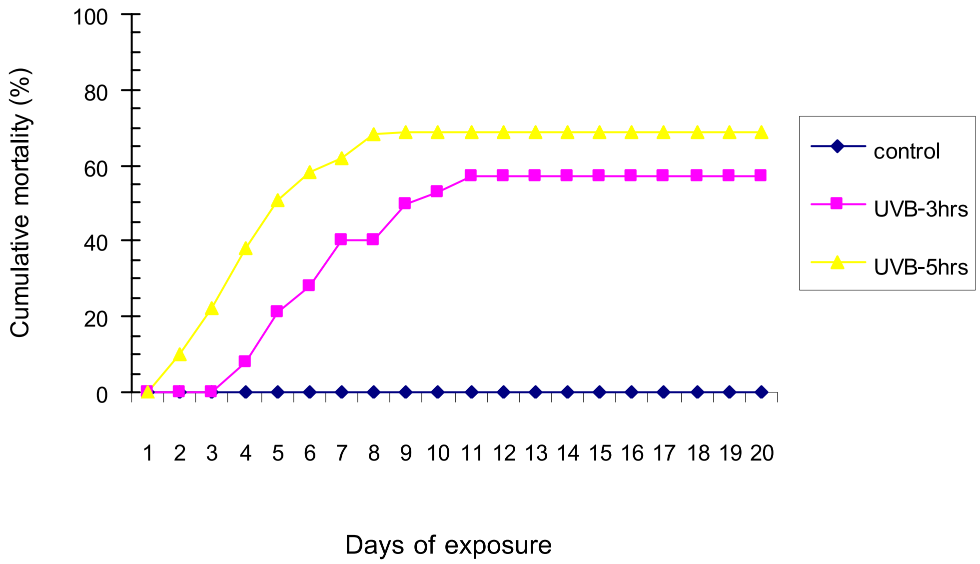

Fig 1. Cumulative mortality in early developmental stages of *O. niloticus* exposed to UV-B radiation as compared to controls

Histology of the skin

Larvae exposed to UV-B radiation at 3 and 5 hrs/day showed severe skin damages indicating by the epidermal changes. Their epidermis was significantly (*P*<0.05) thicker than that of controls (Fig 2, 3). Epidermal necrosis and sloughing were observed in some regions (Fig 4). Moreover, the skin melanocytes at the head area were shrunken and deformed (Fig 5).

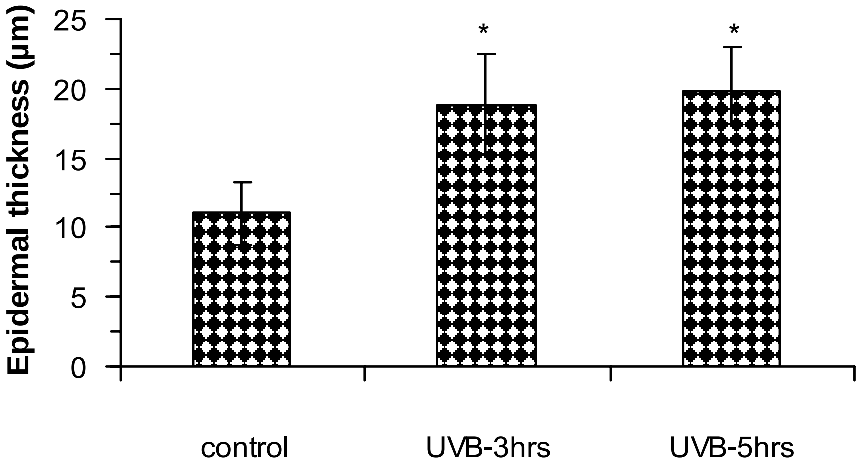

Fig 2. Epidermal thickness of skin of *O. niloticus* larvae exposed to UV-B radiation at 3 and 5 hrs/day as compared to controls

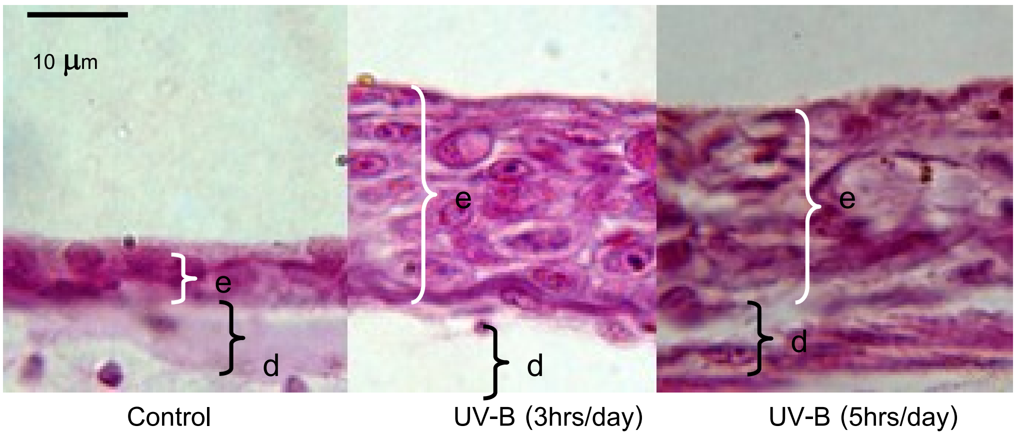

Fig 3. Histology of larval skin of *O. niloticus* exposed to UV-B radiation at 3 and 5 hrs/day as compared to controls. Notice the thickness of UV-B exposed larvae, e: epidermis, d:dermis (100x)

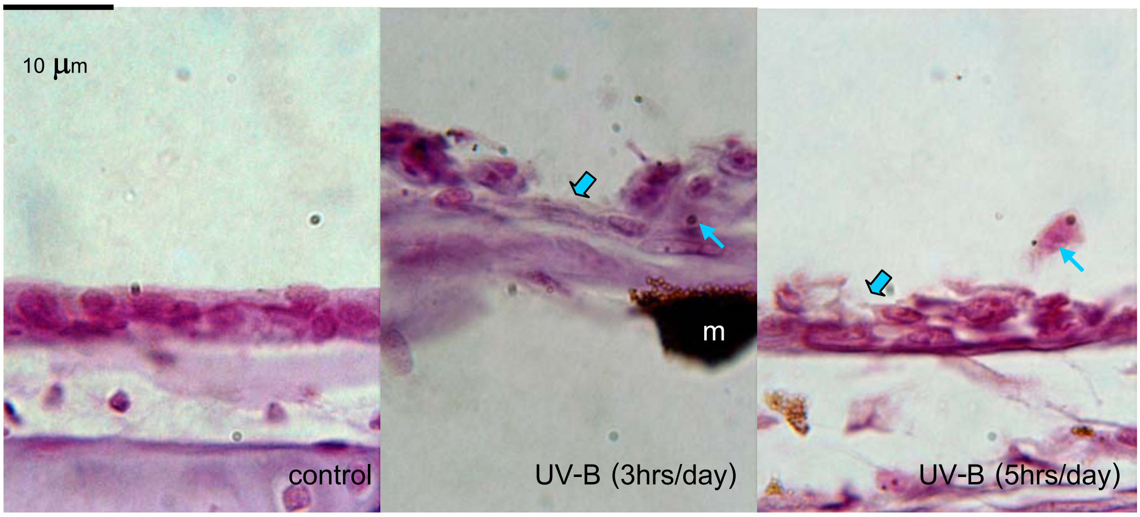

Fig 4. Sloughing of skin epidermis (big arrow) and necrotic cell (small arrow) in *O. niloticus* larvae exposed to UV-B radiation at 3 and 5 hrs/day as compared to controls. m: dermal melanocyte (100x)

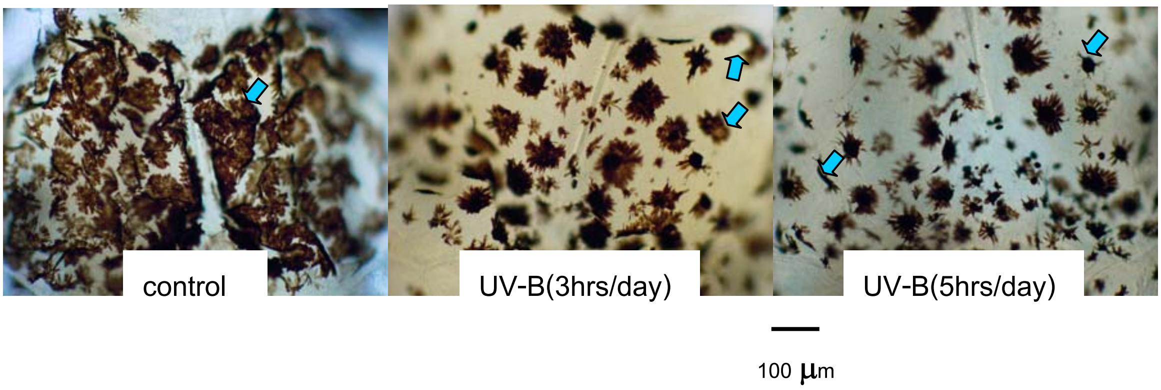

Fig 5. Skin melanocytes (arrow) at the head area of *O. niloticus* larvae. Notice the shrinkage and deformity of melanocytes in UV-B exposed larvae (10x).

Discussion

The epidermis of fish is a delicate tissue and very sensitive to physical, chemical and biotic impacts. In contrast to higher vertebrates the outermost epithelia of fish consists of living cells lacking pigmentation. This makes the fish skin highly vulnerable and UV-radiation can lead to sunburn cells and focal necrosis. Since epidermis of *O. niloticus* larvae consisted of 2 transparent layers of epidermal cells (Fig 4.), modest UV-B mediated cell damage like scattered necrotic cells and sloughing was not surprising. The penetration of UV-B radiation into central parts of their body may damage also internal organs which consequently affected homeostasis and finally led to death. Larvae hatched from UV exposed eggs of anchovy (*Engraulis mordax*) showed lesions of eyes, brain and olfactory bulbs and most of them could not complete the development (Hunter *et al*., 1981). The inhibition of embryonic cell movement caused by UV-B radiation has been reported in zebra fish with suggestion that the microtubules were destroyed by UV-B radiation (Strahle and Jesuthasan, 1993). Adverse environmental conditions including acid rain, dissolved toxicants, salinity changes, temperature stress or handling usually result in a thickening of the epidermal and mucus layers (van Oosten, 1967; Pickering and Macey, 1977; Muniz and Leivestad, 1980; Eddy and Fraser, 1982; Bolognani-Fantin and Trevisan, 1984; Bucher and Hofer, 1993). The elevated number of goblet cells in fry of *Phoxinus phoxinus* exposed to UV-B radiation was reported previously (Kaweewat and Hofer, 1997). It was suggested that this evidence was related to the need of protection against abrasion during the period of residence in the gravel of the spawning site (Blackstock and Pickering, 1982). The thickening of epidermis in *O. niloticu*s larvae (Fig 3.) may involve the protective role from UV-B radiation as well. *O. niloticu*s larvae are highly transparent and melanocytes scattered on dorsal and lateral sides of their body can not prevent sunburn as they are located in the dermis of the skin. Nevertheless, they expanded to shield a considerable area of the brain as seen in control fish. In UV-B radiated larvae, however, melanocytes were shrunken and deformed (Fig 5). In contrast, melanosome dispersion and increased melanization caused by UV-B radiation was reported in anchovy and mackerel larvae, rainbow trout, hammer shark and minnow (Hunter *et al*., 1979; Fabacher *et al*., 1994; Lowe and Lowe; 1996; Kaweewat and Hofer, 2001). This contradicting result may reflect the less efficiency of sunscreen mechanisms by melanocytes in this species. Although the considerable sensitivity of *O. niloticu*s larvae is of only minor ecological importance since they are hidden in the parent's mouth for a period of time, the climate conditions during the recent years are likely to make fish complete their development in a short time. For this reason, *O. niloticu*s larvae may be the high risk species to natural UV-radiation in a near future.

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