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Exposure of eggs to solar UV-B leads to reduced hatching rates in two sparid fishes, red sea bream *Pagrus major* and black sea bream *Acanthopagrus schlegeli*

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Hatching success was examined under exposure to solar ultraviolet radiation (UVR) using filters to give three different light conditions [C1: UV-B, UV-A and photosynthetically active radiation (PAR), C2: UV-A and PAR, C3: PAR] in red *Pagrus major* and black *Acanthopagrus schlegeli* sea bream. Hatching rate of both species was reduced by an exposure over a 2 day period to UVR and was not significantly different between two species under the three light conditions.

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Ultraviolet (UV)-B radiation (280–320 nm) penetrating into the water column has increased due to the disruption of the ozone layer (Tedetti & Sempéré, 2006), and its detrimental effects can cause mortality during early life stages of fishes (Zagarese & Williamson, 2001). Egg stages are particularly vulnerable to UV radiation (UVR) because of their incapability of moving actively out of the reach of UVR during a period of accelerated cell division and differentiation. Furthermore, rapid cell division increases susceptibility to UVR due to a reduction in the time allowed to repair UV-induced damage. Recent evidence shows that in addition to increasing mortality rate (Béland *et al.*, 1999; Kouwenberg *et al.*, 1999; Steeger *et al.*, 1999), UV exposure magnifies the occurrence of malformation (Dong *et al.*, 2007) and DNA damage in fish embryos (Browman *et al.*, 2003). Despite these abundant reports on the harmful effects of UVR on eggs, few studies have focused on warm temperate marine fish species characterized by rapid developmental rate.

In order to evaluate the effects of increased UVR on fish eggs precisely, it would be better to use natural sunlight as a light source rather than artificial lights. Because the effect of light on fishes varies with the wavelength, it is not easy to duplicate diel changes of solar radiation at all wavelengths using lamps. Generally, shorter wavebands have a stronger biological effect per unit energy, and the total UV-induced

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DNA damage is considered to be determined by the balance between damage mainly caused by UV-B and its repair, one of the pathways which requires UV-A and blue light energy (385–450 nm) (Sancar & Sancar, 1988).

Red sea bream *Pagrus major* (Temminck & Schlegel) and black sea bream *Acanthopagrus schlegeli* (Bleeker) are distributed from Hokkaido to Kyushu in Japan (Masuda *et al.*, 1984) and are commercially important species. Both species spawn pelagic eggs in batches over a 2 month period from early spring to early summer, and adults show reproductive behaviour of swimming up to the surface from the sunset through the night to spawn (Ochiai & Tanaka, 1998). It takes *c.* 2 days to hatch under natural temperature conditions in both species. Although most eggs of the two species are distributed near the surface layer (Matsuda, 1969; Matsuura *et al.*, 1981), *A. schlegeli* lives in shallower areas after the larval stage than *P. major* (Kinoshita & Tanaka, 1990). In a previous study, it was shown that *A. schlegeli* larvae and juveniles have a stronger tolerance to UVR than *P. major* larvae and juveniles (Fukunishi *et al.*, 2006). Whether such a difference in UV tolerance also occurs in the egg stage will allow a better understanding of their ontogenetic adaptation to UVR. The aim of this study was to assess how the current level of UVR affects the egg stage mortality with particular interest on interspecific difference. Here, the effect of solar UVR on the hatching success of both species under three different light wavelength conditions in an outdoor aquarium was examined.

Naturally spawned and fertilized *P. major* and *A. schlegeli* eggs were provided by the Kyoto Prefectural Sea-Farming Center on 13 May 2005. Eggs were transferred to the Maizuru Fisheries Research Station (MFRS) of Kyoto University (35° 29' N; 135° 22' E) in light-shielded containers. Transportation took *c.* 1 h and the experiment was started after sunset on 13 May. Twenty eggs of both species were randomly selected from their stock tanks, fixed with 70% ethanol solution and their diameter was measured.

A glass aquarium (38 cm deep, 48 cm wide and 200 cm long) was set in the east-west direction on an open space in MFRS where there was no construction obstructing sunlight. Filtered sea water was circulated and well aerated in the aquarium. A chiller was installed at each end of the aquarium to prevent an increase in temperature. Solar irradiance was measured in the UV-B, UV-A (320–400 nm) and photosynthetically active radiation (PAR: 400–700 nm) waveband every 1 to 3 h above the surface of aquarium from sunrise to sunset using a portable photo-radiometer (UV: DO9721, Delta OHM; www.deltaohm.com. PAR: LUX meter LX-1332, Custom Co.; www.kk-custom.co.jp).

About 30 eggs (mean \pm s.e. 27.00 \pm 2.19 individuals) of each species were gently placed in 30 ml rectangular plastic containers using a glass pipette. These containers had a hole (diameter: 22 mm) at both sides, and the holes were sealed with 0.3 mm mesh net. This arrangement allowed water to circulate in the containers and provided sufficient oxygen to eggs during the experiment. Three light conditions were set by covering the container with one of the three different filters to manipulate wavelength bands of UVR (condition 1: UV-B, UV-A and PAR, condition 2: UV-A and PAR, and condition 3: PAR; see Fig. 1 for optical characteristics of the filters). Six replicates were prepared for each light condition in both species, and each container was distributed randomly and suspended at a depth of 3 cm in the aquarium. Eggs were incubated under exposure to solar radiation for 2 days (14–15 May 2005). On the third day, hatching was already completed early

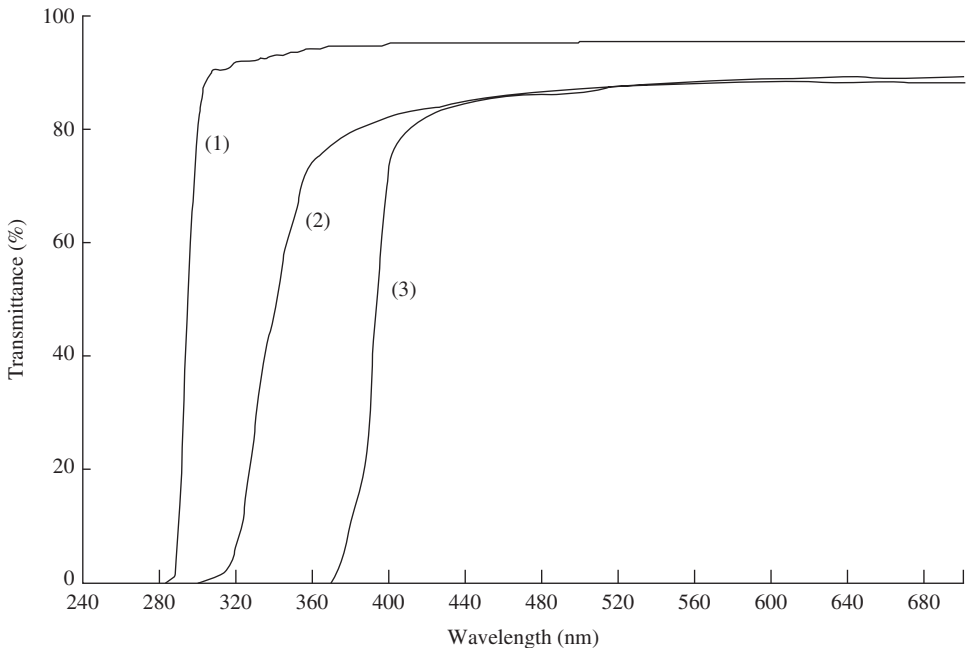


FIG. 1. Spectral transmittance of the three filters used in the experiments: (1) cellulose diacetate film (Clarifoil; www.clarifoil.com) for ultraviolet (UV)-B (280–320 nm) + UV-A (320–400 nm) + photosynthetically active radiation (PAR) (400–700 nm), (2) Teton G2 (Teijin DuPont films; www.tejindupontfilms.jp) for UV-A + PAR and (3) Teton HB for PAR.

in the morning, and hatching success was examined under a binocular microscope. Some of the containers had a defect in the connection between the container and mesh, where eggs drifted out through the interspaces, and were thus eliminated from analyses (8.3% of the total). Water temperature, salinity and dissolved oxygen measured using a portable multi-meter (YSI Model 85 SCOOT meter, YSI/Nanotech; www.nanotech.co.jp) ranged from 16.4–19.1°C, 32.9–33.0 and 7.09–8.06 mg l⁻¹, respectively, during the experiment.

A *t*-test was used to compare the diameter of eggs between *P. major* and *A. schlegeli*. Hatching rate was arcsin transformed and was analysed using two-way ANOVA with species and light treatment as independent factors, after verifying that variance was homogeneous (JMP Ver. 4.05.J, SAS Inst.; www.sas.com). After pooling data of both species together, Tukey–Kramer honestly significant difference (HSD) multiple comparisons test was performed to compare hatching rates under different light conditions.

The weather on 14 May 2005 was clear and sunny and that on 15 May was occasionally cloudy. The intensity of solar radiation reached a peak around noon and showed a parabolic change on the first day of the experiment, while it fluctuated substantially because of the cloud cover on the second day (Fig. 2). Changes in the intensity of each wavelength (UV-B, UV-A and PAR) were generally synchronized with each other on both days.

No significant difference in egg diameter was observed between *P. major* (mean ± S.D. 0.80 ± 0.04 mm) and *A. schlegeli* (0.80 ± 0.06 mm) (*t*-test, *n* = 20, *P* > 0.05).

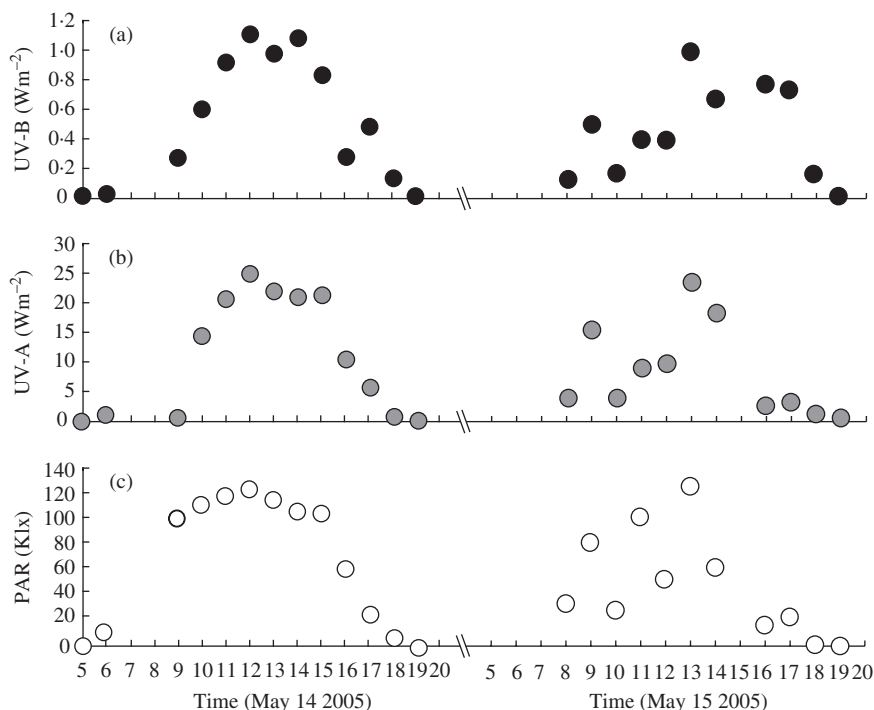


FIG. 2. Changes of (a) ultraviolet (UV)-B radiation, (b) UV-A radiation and (c) photosynthetically active radiation (PAR) on 14–15 March 2005, in Maizuru Fisheries Research Station, Kyoto, Japan.

Hatching rate did not differ between species (two-way ANOVA, F , d.f. = 1,26, $P > 0.05$; species \times light treatment interaction: F , d.f. = 2,26, $P > 0.05$) (Fig. 3). Exposure to UVR, however, significantly reduced hatching rate of both species (two-way ANOVA: F , d.f. = 2,26, $P < 0.05$) (Fig. 3); hatching success was significantly lower in light condition 1 relative to conditions 2 and 3 (Tukey–Kramer HSD test, $n = 32$, $P < 0.05$).

Hatching rate of both species was remarkably reduced by exposure to solar radiation under condition 1, where all wavelengths of solar radiation were allowed to penetrate (Fig. 3), indicating that current levels of solar UVR potentially inhibit hatching success. This trend is consistent with reports for other species such as Atlantic cod *Gadus morhua* L. (Béland *et al.*, 1999), yellow perch *Perca flavescens* (Mitchill) (Williamson *et al.*, 1997) and bluegill *Lepomis macrochirus* Rafinesque (Gutiérrez-Rodríguez & Williamson, 1999). Béland *et al.* (1999) exposed pelagic eggs of *G. morhua* to solar radiation for 10 days from 4 days after fertilization through to hatching, under different light conditions at two depth (3 and 50 cm) in outdoor chambers. They observed the lowest hatching rate in the quartz condition (UV-B, UV-A and PAR) at both depths (3 cm: 0%, 50 cm: mean \pm s.e. $1.13 \pm 0.45\%$ and $2.03 \pm 0.55\%$). One of the reasons why the hatching rate was higher in *P. major* and *A. schlegeli* compared with *G. morhua* at 3 cm depth may be that the exposure time to solar radiation was much shorter (2 days) in the present experiment than in the experiment for *G. morhua* (10 days).

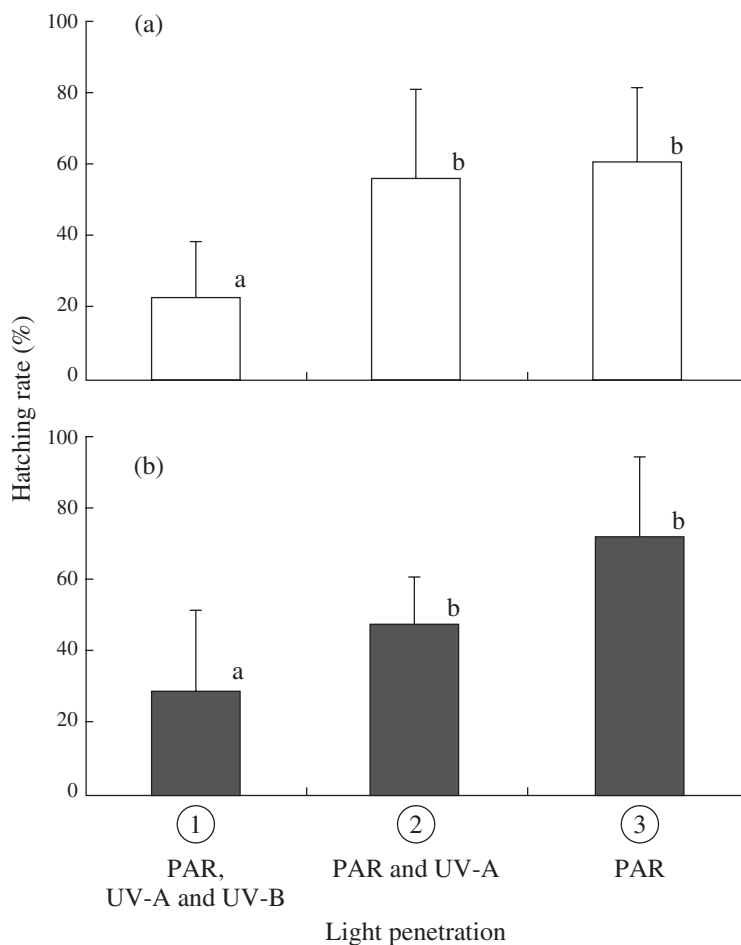


FIG. 3. Hatching rate of (a) *Pagrus major* and (b) *Acanthopagrus schlegeli* under different spectral light conditions (see Fig. 1). Different lowercase letters indicate significant difference among light conditions (Tukey–Kramer HSD test, $P < 0.05$).

Although negative effects of solar UVR have also been confirmed in *P. flavescens* and *L. macrochirus* (Williamson *et al.*, 1997; Gutiérrez-Rodríguez & Williamson, 1999), these species should be able to mitigate harmful UVR effects by selecting spawning sites. For instance, *P. flavescens* adults choose deeper spawning sites in lakes characterized by high UVR penetration (Huff *et al.*, 2004). Olson *et al.* (2006) reported that only 19% of *L. macrochirus* nests were predicted to have UV-induced mortality of $\geq 25\%$, as most of the nests were located at depths where much of the UVR had been attenuated and shallower nests were protected by overhanging trees or other submerged structures. In contrast, pelagic eggs of marine fishes are buoyant and drift near the sea surface without protection from sunlight. Therefore, they are potentially more vulnerable to UVR than the demersal eggs of freshwater fishes.

As the effect of UVR was compared from a wavelength viewpoint, hatching rate in condition 1 was significantly lower than in condition 3 (only PAR), and no significant

difference was found between conditions 2 and 3 in both species (Fig. 3). This indicates that exposure to both UV-B and UV-A leads to reduced hatching rate, whereas eggs were not affected by an exposure to UV-A wavelength alone in *P. major* and *A. schlegeli*. Similar results were obtained for *G. morhua* and *L. macrochirus* (Béland *et al.*, 1999; Gutiérrez-Rodríguez & Williamson, 1999). The UV-A waveband has been reported to photorepair DNA damage in goldfish *Carassius auratus auratus* (L.) cells (Mitani *et al.*, 1996). Dong *et al.* (2007) observed higher hatching rate and lower malformation rate when zebrafish *Danio rerio* (Hamilton) embryos were exposed to UV-A immediately after UV-B compared with those exposed to UV-B radiation alone. In contrast, Williamson *et al.* (1997) exposed *P. flavescens* eggs to solar radiation near the surface of a lake (0.8 m depth) and found that UV-A radiation reduced hatching rate. This suggests that sensitivity of fish embryos to UV-A wavelengths strongly varies between species.

No significant difference was observed in hatching rate between *P. major* and *A. schlegeli* under the three light conditions (Fig. 3). It may be due to their relatively similar morphology and pigmentation patterns (Mito, 1963) as well as identical egg developmental rates. Although egg size may vary depending on the broodstock condition, no difference was observed between the two species in the present experiment. In the sea, eggs of both species are distributed near the ocean surface in spring and thus undergo similar UVR levels, which may explain their identical UV tolerance. Marinaro & Bernard (1966) reported that eggs of the Mediterranean rainbow wrasse *Coris julis* (L.) spawned in the period of maximum sunlight showed higher tolerance to solar UVR than those of European pilchard *Sardina pilchardus* (Walbaum) spawned in winter. Therefore, interspecific differences in UV tolerance of fish eggs may reflect adaptation to the level of UVR encountered in the environment. A mechanism potentially contributing to interspecific differences in UV tolerance is the presence of UV-absorbing compounds such as mycosporine-like amino acids (MAAs). Such compounds are ubiquitous in marine organisms (Dunlap & Shick, 1998) and were found in eggs and larvae of corals, gastropods, urchins, ascidians and fishes (Karentz *et al.*, 1991; Gutiérrez-Rodríguez & Williamson, 1999; Karentz, 2001; Przeslawski *et al.*, 2005).

It was previously shown that *A. schlegeli* is characterized by higher UV tolerance than *P. major* during the larval and juvenile stages (Fukunishi *et al.*, 2006). Hence, interspecific differences in UV tolerance may occur through ontogeny, and the present results suggest they reflect adaptation to the UVR level encountered in the habitat of a given developmental stage.

This study showed that the current level of UVR may potentially hamper hatching success of *A. schlegeli* and *P. major* and account for a large fraction of egg stage mortality. As the period from fertilization to hatching is relatively short (*c.* 2 days) in both species, exposure to UVR in the field is expected to vary widely among batches depending on the weather conditions. The level of cloudiness and vertical mixing (Neale *et al.*, 1998) are two important factors determining the total amount of UVR a given batch of eggs will undergo. Variability in the mortality attributable to UVR during early life history of marine fishes would thus be maximal in fast-developing species.

Combined with the results of Fukunishi *et al.* (2006), UV tolerance may vary through the early ontogeny of a given species. This implies that estimating the effect of UVR on fish populations based on only one developmental stage is likely to

lead to spurious conclusions. It is necessary to examine the relationship between the potential cumulative UVR dose in the natural environment and the survival of each stage through ontogeny in order to assess how excess UVR may affect fish communities.

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