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SHORT PAPER

Melatonin Implantation in Preovulatory Rainbow Trout (*Oncorhynchus mykiss*) under Short Photoperiod Regime Reduces Egg Quality

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Abstract

As in other vertebrates, the pineal organ in salmonids receives photic information directly through specialized photoreceptor cells to synchronize both daily and seasonal behavioral and physiological events, including the reproductive. In this study, the effect of a melatonin implantation before the preovulatory period on egg quality was investigated in rainbow trout (*Oncorhynchus mykiss*) adults. Fish were exposed to two different lighting regimes [(constant short photoperiod (SP) of 6h light/18h dark) or natural photoperiod (NP) of 9h light/ 15h dark) with sub groups receiving a slow-release melatonin implant ((containing 9 mg melatonin hormone (MLT)) or sham implant. Melatonin implanted fish showed supra-physiological plasma MLT concentration during the 45 days after implantation. The spawning period started at the same time in all groups after implantation, although eggs were obtained from females exposed to SP lighting regimes only or SP lighting regimes plus implanted MLT exhibited significantly lower quality characterized by lower hatching rate and survival to first feeding than that of females expose to NP. In conclusion, it was determined that implantation of MLT in preovulatory rainbow trout females causes a decrease in egg quality. However, future investigations should be conducted to ascertain the MLT dose required for and time of implantation in rainbow trout.

Keywords: Trout, melatonin implantation, short photoperiod regimes, hatching rate.

Kısa Fotoperiyot Rejimi Altında Ovulasyon Öncesi Gökkuşağı Alabalıklarına (*Oncorhynchus mykiss*) Yapılan Melatonin Implantasyonu Yumurta Kalitesini Düşürür

Özet

Diğer tüm omurgalılarda olduğu gibi, alabalıklarda da pineal organ; üremeyi de içeren günlük ve mevsimsel davranışsal ve fiziksel olayları düzenleyen özelleşmiş foto-reseptör hücreler vasıtasıyla ışığa ait bilgiyi doğrudan alır. Bu çalışmada, ovulasyon öncesi melatonin implantasyonunun (yavaş salınan melatonin implant, 9 mg melatonin hormonu (MH) içeren ve canlı ağırlığa 3-4 mg/kg gelecek şekilde veya melatonin içermeyen (sahte) implant) farklı ışıklandırma rejimi (sürekli kısa fotoperiyot (KF) 6 saat ışık/18 saat karanlık, ya da normal fotoperiyot 9 saat gündüz/15 saat gece (NF)) uygulanan ergin gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) yumurta kalitesi üzerine etkileri araştırılmıştır. Melatonin implanti yapılan balıklar, uygulama sonrası 45 gün boyunca yüksek plazma MH konsantrasyonuna sahiptiler. Tüm gruplarda yumurta dökme periyodu aynı zamanda başlamasına rağmen NF grubu ile karşılaştırıldığında sadece KF ışıklandırma rejime tabi tutulan ya da KF ve melatonin implantı uygulanan gruplarda; düşük kuluçka oranı ve ilk yem alımına kadar geçen süredeki yaşama oranı ile karakterize edilen düşük kuluçka kalitesi gözlemlenmiştir. Sonuç olarak; ovulasyon öncesi gökkuşağı alabalık dişilerine uygulanan melatonin implantasyonunun yumurta kalitesini düşürdüğü belirlenmiştir. Fakat bununla birlikte, gökkuşağı alabalıklarına uygulanması gereken en uygun MH konsantrasyonunun ve implant uygulama zamanının belirlenmesi ile ilgili araştırmalar yapılmalıdır.

Anahtar Kelimeler: Alabalık, melatonin implantasyonu, kısa fotoperiyot rejimleri, kuluçka oranı.

Introduction

Developmental and maturational events in animal are dominated and coordinated by seasonal changes in photoperiod, temperature, food supplies, rainfall and so on (Porter *et al*., 1999; Sánchez–Vázquez *et al*., 2000). The photoperiod is considered as the most important factor that entrains animal rhythms, including the reproductive cycle. In most seasonally breeding fish, breeding takes place in

spring or summer and can be stimulated by long photoperiods. There are, however, also fishes, notably salmonids, that spawn in autumn or winter under stimulation by short or declining photoperiods (Bromage *et al*., 1993; Randal *et al*., 1995; Porter *et al*., 1999).

Photoperiodic information is transmitted via several neural pathways from the retina to the pineal gland, where the hormone melatonin (N-acetyl-5-methoxytryptamine, MLT) is produced and released as a direct response to the light signal (Ekstrom and Meissl, 1997). MLT rhythms provide the animal with information about the time of day and also the time of year, synthesizing and releasing high levels into the bloodstream during the dark period (Reiter, 1993). Although melatonin is synthesized in several tissues (Cassone, 1990), its rhythmic synthesis is primarily localized in the pineal organ and the retina (Cassone, 1998). Changes in the night/day MLT concentrations have been described in the rainbow trout (*Oncorhynchus mykiss*, Walbaum) (Alvariño *et al*., 1993), turbot (*Scophtalmus maximus* L.) (Rebollar *et al*., 1999), and other fish species.

MLT is not only involved in seasonal reproduction but also sleep/wake cycles (Reiter, 1991), osmoregulation and/or stress adaptation in seawater (Folmar and Dickhoff, 1981). Moreover, it is considered as a powerful free radical scavenger and likely to be a general promoter of anti-oxidative mechanisms and a potential antioxidant in vitro and in vivo (Konar *et al*., 2000). Some of its interactions with other hormones such as cortisol and arginine vasotocin (hormones closely associated with electrolyte balance) have been reported in teleosts (Delahunty *et al*., 1977; Kulczykowska, 1995).

Photoperiod and hormone implants are commonly used to reduce the protracted spawning period, and synchronize spawning events of fish for commercial production. However, considering the lack of knowledge regarding the possible combined effects of MLT implantation and photoperiod application on fish reproduction, this study aimed to investigate the in vivo effect of MLT implantation on the plasma level of MLT, the time of spawning and egg quality of rainbow trout females exposed to constant short photoperiod (SP) or natural photoperiod (NP).

Materials and Methods

Broodfish Husbandry and Maintance

The study was conducted in the Central Laboratory at the Fish Rearing Facility of the Marine Science and Technology Faculty at Çanakkale 18 Mart University with rainbow trout obtained from a commercial trout farm. A total of seventy-two females (mean weight 2450±170) and thirty-six males (mean weight 1980±200) were used in this trial. Fish were sorted into twelve 5000-l circular fiberglass tanks with constant renewal water and airlift type aeration at a density of 9 fish per tank (6 females and 3 males). One tank was assigned to one of four treatments (4 treatments; 3 replicates). The treatments used were: 1) NP (exposing natural photoperiod of 9h light/ 15h dark + sham implant); 2) NP+ MLT (exposing natural photoperiod + 3mg/kg melatonin implantation); 3) SP (exposing constant short photoperiod of 6h light/18h dark + sham implant); 4) SP+MLT (exposing constant short photoperiod + 3mg/kg melatonin implantation). Fish were acclimated for one week at 12 h light:12 h dark. Then the photoperiods were switched to natural or continuous darkness at a constant water temperature of 9°C. The fish were kept at those experimental photoperiod regimes and fed a commercial fishmeal-based extruded rainbow trout diet (50% crude protein; 18% crude lipid; 4800 cal/g diet gross energy) at 1% of their live body weight during the photophase (12:00 h) as a daily ration for 45 days.

Implant Application

Implants were administered on November 15th, approximately one month before the winter solstice trying to mimic an advanced short-day signal. Fish with sham implants were considered as control groups. All fish from each tank assigned to that application were removed, anesthetized (100mg/L tricaine methane sulfonate) and injected with an implant prepared according to Lee *et al*. (1986). The implants were injected under skin dorsal of the lateral line under the dorsal fin. The fish were then returned to the tank to recover. All fish were examined every week to visually grade into mature and immature fish depending on external morphological features, i.e., skin color, the presence of a kyped jaw, fin and body shape. The spawning time of each fish was recorded during this period.

Egg Incubation, Hatching Rate and Larvae Survival

Eggs taken from the each spawning event was placed into a hatching tray. At the eye stage, the exact number of eggs in each tray was counted. After that the trays were observed daily. Dead eggs and deformed and dead fry were recorded and discarded. Survival rates to the first feeding stage were determined by counting the fry remaining in each tray (Aras Hisar *et al*., 2003). Fertilization rate, hatching rate and survival to first feeding were determined by the following formulas:

Fertilization rate (%)=(number of fertilized eggs x100)/(total number of incubated eggs);

Hatching rate (%)= (number of hatchlings x100)/(total number of fertilized eggs);

Survival to first feeding (%)= (number of swim-up fry x100)/(total number of incubated eggs).

Blood Sampling and MLT Analysis

Every two weeks, the fish were captured, anesthetized in MS-222 (200mg/l) during the scotophase, and individually weighed. At the same time, blood samples were obtained from the caudal vasculature of each fish with a heparinized syringe. The blood samples were kept on ice for up to 30 min until the plasma was separated by centrifugation. Plasma samples were stored at –80°C until analysis. Plasma melatonin levels were measured using HPLC adapted from Kulczykowska *et al*. (2001).

Statistical Analysis

All data were subjected to a one-way analysis of variance followed by Duncan's multiple-range test to determine significant differences among the regimes at the 0.05 level.

Results

No broodstock mortalities occurred in any treatment during the study. Fish were not visible altered in body morphology and no behavioral changes were observed after implantation. The effects of two different photoperiod regimes and MLT implants on plasma MLT concentration of fish were shown in Figure 1. Plasma was taken from 6 fish (4 females and 2 males) at each experimental group and pooled together, as it was reported that sex-related differences were not detected (Bromage *et al*., 2001). There was a significant difference among the groups ($P<0.05$). Plasma MLT levels were significantly higher in the MLT implanted groups than that of the sham implanted groups whether exposed to the short photoperiod or natural photoperiod ($P<0.05$). The spawning period started at a similar time in 16 out of 18 control and 14 out of 18 implanted females. No statistical differences were found in egg production, incubation period and rate of egg fertilization. However, the rate of hatching and survival to first feeding was significantly less from fish in the SP or SP+MLT treatments than the other groups (Table 1).

Discussion

Although photoperiod requirement is extremely variable and is related to environmental adaptation, species and age specific (Britz and Piennaar, 1992; Silva-Garcial, 1996), the use of photoperiod manipulation to alter the incidence of sexual maturation and the time of spawning has been reported for different species (Duston and Bromage,

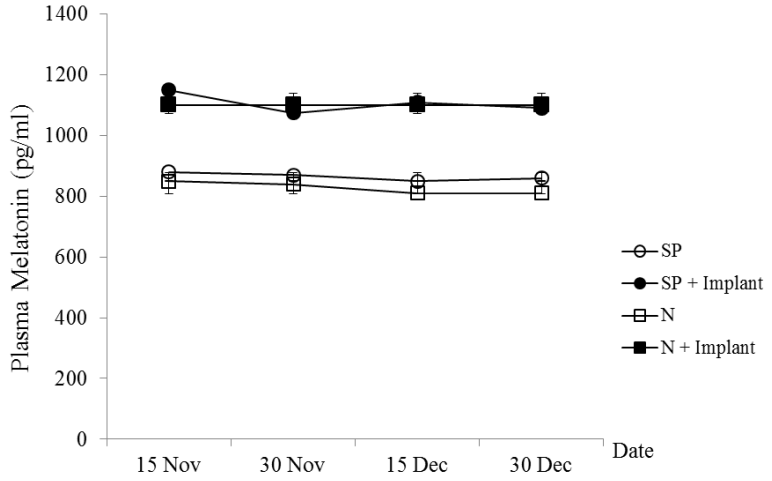

Figure 1. Plasma melatonin levels of the broodfish in four different treatments during 15 November-15 December (n=6; 4 females and 2 males); Experimental groups are represented as follows SP: (exposing constant short photoperiod + sham implant), SP+MLT (exposing constant short photoperiod+3mg/kg melatonin implantation), NP (exposing natural photoperiod of 9h light/ 15h dark + sham implant), NP+ MLT (exposing natural photoperiod + 3mg/kg melatonin implantation)

Table 1. Egg and larval production of rainbow trout subjected to MLT implants and/or continuous short photoperiod (SP) or natural photoperiod (NP). Means in rows with different superscript letters are statistically different. (n=18)

Experimental groups	NP	NP+MLT	SP	SP+MLT
Initial egg counts	3408±173	3325±200	3050±142	2880±151
Incubation periods (days)	30-32	29-33	28-36	28-35
Egg fertilization rate (%)	97.3±1.3	95.6±1.8	96.1±2.5	92.5±4.2
Hatching rate (%)	94.4±1.7 [a]	91.1±1.2 [a]	81.1±0.4[b]	61.2±10.5[c]
Survival to first feeding (%)	89.6±3.2[a]	86.9±2.9 [a]	68.9±3.6[b]	58.5±4.1 [c]

1988; Randall *et al*., 1995; Duston *et al*., 2003; Imsland *et al*., 2003). However, no differences were detected in terms of egg production and egg fertilization between females expose to SP and NP in this study. Many fish use the increasing and decreasing components of day length, transducing this information into a suitable message for integration by the neuroendocrine cascade which initiates and then modulates reproductive development (Bromage *et al*., 1994). In fact a reduction from long to short photoperiod is able to advance trout spawning (Randall *et al*., 1995). On the contrary, the short photoperiod regime negatively affected the rate of hatching and survival of larval fish in the present study. Lower survival in the larval fish was obtained from the females expose to SP. Although no information is available on the effects of photoperiod regime exposed to fish just one month before the winter solstice, the effectiveness of constant SP is thought not to adequate for trigger a appropriate gonadal development.

The fish pineal organ acts as a phototransducer, translating photoperiod information into a chemical signal via the release of indoleamines, primarily melatonin (Falcon *et al*., 1992). Salmonids are found to have a passive response to the environmental LD signal where the elevated melatonin levels accurately reflect the length of the dark phase (Randall *et al*., 1995). As regards to plasma melatonin concentration measured in broodfish, our results were within the range previously reported in other fish: common carp (*Cyprinus carpio*), brook trout (*Salvelinus fontinalis* Mitchill), Atlantik salmon (*Salmo salar*) and common dentex (*Dentex dentex*) (Kezuka *et al*., 1988; Randal *et al*., 1995; Zachmann *et al*., 1992; Pavlidis *et al*., 1999). However, MLT implantation resulted in high levels of melatonin concentration in plasma during the scotophase period. Moreover, it had a detractive effect on egg quality compare to that of control groups. Similarly, Stacey and Goetz (1982) reported that addition of melatonin to incubation medium both inhibited ovulation and decreased prostaglandin E and F synthesis in which appear to be involved in ovulation (follicular rupture) and female sexual behavior, and possibly in gonadotropin (GtH) secretion in yellow perch. Taken account these results, the photoperiod regimes could alter the effects of MLT implants, as it has been found in the stickleback *Gasterosteus aculeatus* (Borg and Ekstroem, 1981). In our previous study we also reported that excessive melatonin concentration on carbonic anhydrase enzyme activity in rainbow trout erythrocytes was determined as *in vitro* and *in vivo* and it was found that melatonin significantly inhibited the rainbow trout erythrocyte carbonic anhydrase enzyme activity after its injection in vivo (Hisar *et al*., 2005) Furthermore, it was documented that melatonin had effect of on glucose-6-phospate dehydrogenase from rainbow trout erythrocytes. *In vivo* studies showed that though initial glucose-6-phospate dehydrogenase activity was 8.33 EU/gHb, these values fell after injection of pharmacological dose of melatonin (Beydemir *et al*., 2005).

In conclusion, our study showed that MLT implantation had a negative effect on the rate of hatching and survival to first feeding on rainbow trout held under constant short photoperiod. However, these results should be confirmed by varying the MLT dosage, the timing of MLT implantation, and/or long photoperiod regimes.

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