Measurement of Melatonin in Pearl mullets, an Endemic Species Growing in Lake Van, and Trouts

Van Yöresinde Yetişen Van Gölü Balığı (İnci Kefali) ve Alabalıkta Melatonin Hormonun Ölçülmesi

Research Article

Tülay Gürsoy[1], Nurhayat Atasoy[1,*], İhsan Alacabey[2]

[1]Yüzüncü Yıl University, Faculty of Science and Letters, Department of Chemistry, Van, Turkey

[2]Yüzüncü Yıl University, Van Health Services Junior Technical College, Van, Turkey

ABSTRACT

In the present study, the melatonin levels were measured in pearl mullets (also known as Van fish) and trouts. Pearl mullets grow naturally in Lake Van in Turkey, and trouts, raised by private fish farms, were used as study materials. These fish are known to secrete melatonin, a hormone associated with development and stress, as occurs in human beings. Melatonin was measured in the plasma taken from living pearl mullets and trouts by an ELISA method using commercial kits. The study was conducted in accordance with the ELISA kit procedure. In the spring, the mean melatonin levels of trouts were 160.8 ± 4.090 pg/mL in light and 198.3 ± 3.694 pg/mL in dark, whereas in winter 86.69 ± 3.203 pg/mL and 128.9 ± 1.999 pg/mL in light and dark, respectively. In pearl mullets, the mean melatonin levels were 84.73 ± 2.281 pg/mL in light and 118.1 ± 2.560 pg/mL in dark in spring, and in winter 36.32 ± 1.627 pg/mL in light and 70.53 ± 2.919 pg/mL in darks ($p < 0.001$).The results emphasized the significance of the photoperiod.

Key words

Trout, ELISA, Hormone, Melatonin, Pearl mullet

ÖZET

Bu çalışmada, Van Yöresinde yetişen Van Gölü balığı (inci kefali) ve alabalıkta melatonin hormonu seviyesi ölçüldü. Araştırma materyali olarak da Van Gölü'nde yetişen Van Gölü balığı (inci kefali) ve özel tesislerde yetiştirilen alabalık kullanıldı. Araştırma, insanlar gibi bu hormonun salgılandığı hayvanlar olan balıklarda; gelişim ve stres hormonu olan melatoninin bu özelliği göz önünde bulundurarak yürütülmüştür. (Metod; melatonin hormonu, canlılardan (Van Gölü balığı ve alabalıkta) alınan kanda, özel kitlerle elisa cihazı ile ölçüldü. Araştırma, alabalık ve Van Gölü balığının plazmasında melatonin ölçülmesinden sonra, elisa parelelizmi standartlarına uygun olarak yapıldı. Alabalıkta yapılan çalışmada ortalama değer; 160.8 ± 4.090pg/ml (aydınlık), 198.3 ± 3.694 pg/ml. (karanlık) olarak ilkbaharda bulunmuştur. Kış mevsimi ölçümlerimizde ortalama değer ise 86.69 ± 3.203 pg/ml (aydınlık) 128.9 ± 1.999 pg/ml (karanlık) olarak bulunmuştur. Van Gölü balığında ise ortalama 84.73 ± 2.281 pg/ml. (aydınlık), 118.1 ± 2.560 pg/ml. (karanlık) olarak ilkbahar ölçümleri bulunmuştur. Kış mevsimi ölçümlerimizde ise ortalama 36.32 ± 1.627 pg/ml (aydınlık), 70.53 ± 2.919 pg/ml (karanlık) değeri bulunmuştur.

Anahtar Kelimeler

Alabalık, Elisa, Hormon, Melatonin, Van Gölü balığı.

Article History: Received December 28, 2010; Revised October 22, 2011; Accepted November 12, 2011; Avaliable Online: December 02, 2011.

Correspondence to: Nurhayat Atasoy, Yüzüncü Yıl University, Faculty of Science and Letters, Department of Chemistry, Van, Turkey

Tel: +90 432 225 1256 Fax: +90 432 225 1256 E-Mail: nurhayatatasoy@ymail.com

INRODUCTION

Hormones are chemical messengers which play role in the regulation of several reactions after being secreted from endocrine glands and transported by the circulation to target organs. Hormonal imbalance occurs as a result of excessive or inadequate release of one or more hormones, which causes abnormalities in growth and metabolism [1]. Melanocyte-stimulating hormone (MSH) is a hormone secreted primarily by the cells in the intermediate lobe of the pituitary gland; it is also present in the posterior lobe. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone found in all living organisms at levels varying in a daily cycle. In highly developed animals, melatonin is synthesized in the pineal gland from tryptophan and serotonin by the enzyme, 5-hydroxyindole-O-methyl transferase, and is released into the circulation and acts as an endocrine hormone. MSH effects the distribution of pigment granules within the pigment cells of fish, amphibians, and reptiles. Thus, the skin color of these animals changes according to the environment [2].

MSH secretion is often induced by light stimulation. The synthesis and release of MSH is reduced as the amount of light increases. Consequently, pigment granules accumulate around the nucleus of the cell and the skin becomes fair-colored. In the dark, pigment granules spread throughout the cell as a result of the increase in MSH secretion [3].

Melatonin secretion also exhibits seasonal variations. While MSH is secreted later during the day in summer, it begins to be secreted earlier in winter. Long-term melatonin secretion is observed in short days, while short-term melatonin secretion occurs in longer days. The length of the daylight and response to light stimulation are associated with the physiology of the living organism. In animals, a period of long daytime before a short-daytime melatonin signal induces the reproductive cycle [4]. Most of the biological effects of melatonin are produced through the activation of melatonin receptors and also melatonin, as a potent antioxidant, plays a significant role especially in the protection of mitochondrial DNA and nucleus [5]. In animal studies, it has been demonstrated that melatonin inhibits the mechanism of cancer and is effective in protecting against DNA damage caused by a variety of carcinogens [6]. In the studies including mice, it has been noted that melatonin may increase the life span by 20% [7-9], and helps to fight against many diseases by fortifying the immune system [10]. Melatonin has an important role in seasonal affective disorders (SADs) and modulating the effects of drugs, such as cocaine which causes addiction [11]. It has been observed that melatonin improves insomnia and regulates thyroid function and gonadotropin levels in postmenopausal women, and helps to manage menopause depression as well as regulates reproductive function. Moreover, it has been reported in several clinical studies that melatonin is effective in the treatment of migraines [12,13]. Melatonin also has been shown in several studies to prevent cancer, HIV, and some viral infections [3]. Furthermore, it has been indicated in other studies that melatonin, when combined with calcium, may be effective in the treatment of obesity and the management of low body weight [3].

In the present study, considering the significance of melatonin, melatonin levels were measured in pearl mullets (also known as Van fish), grow naturally in Lake Van, and trouts, which are raised by private fish farms, under dark and light conditions in summer and winter which have not been investigated earlier with concern to melatonin.

MATERIALS AND METHODS

The present study included 15 pearl mullets and 15 trouts, all grown around the Lake Van region. Measurements of melatonin levels were performed in the light and dark conditions. In the present study, in order to measure the melatonin levels, ELISA method was used.The chemical substances used in this study were as follows; P-Nitrophenyl phosphate, 0.1% sodium azide, 1% albumin, ELISA melatonin kit (IBL, Hamburg, Germany), 2 mL of phosphate-buffer saline (PBS), NaCl, C_{18} phase extraction column (Waters, MA, USA), 1 N NaOH, 0.25 M EDTA and TRIS buffer. Blood collection was performed via a fine needle at an angle of 70-80° from the middle of an imaginary line connecting the base of the first pectoral fin ray, and 2-5 mL of blood samples were taken from the heart of the fish then transferred to a tube containing anticoagulant. Then blood samples were centrifuged at 3000 rpm for 10 minutes at

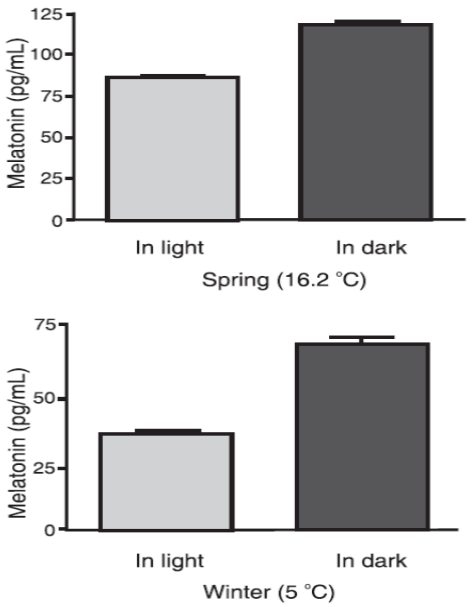

Figure 1. Melatonin levels in pearl mullets in light and dark conditions in winter and spring.

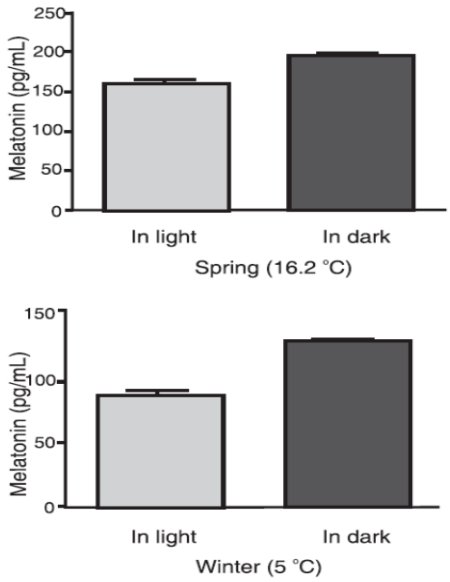

Figure 2. Melatonin levels in trouts in light and dark conditions in winter and spring.

4°C. An enzyme immunoassay method was used for the measurement of plasma melatonin. Melanonin was purified by extraction column from plasma.The plasma samples were homogenized one-by-one in 10 mM phosphate buffer containing 2 mL phosphate-buffered saline (PBS) and 140 mL NaCl, and as well as in 0.1% sodium azide and 1% albumin (pH 7.5) at 0°C. Samples were then purified again by an extraction column. Then, 50 μL of each sample was placed into ELISA plates and incubated by a melanin-biotin and antiserum solution at 4°C for 15 hours. After the samples were rinsed, first 50 μL of 1N NaOH, and then 0.5 M ethylene diamine tetraacetic acid (EDTA) solutions were added and the samples were incubated with p-nitrophenyl phosphate (p-NPP) solution for 30 minutes.Then absorbances of the samples were measured at 405 nm [14].

RESULTS

Melatonin levels of pearl mullets and trouts in light and dark conditions, and as well as, in winter and spring are presented in Table 1. There were a significant difference between pearl mullets and trouts in terms of melatonin levels in light and dark conditions, as well as in winter and spring ($p < 0.0001$, Figures 1,2). In trouts, the mean melatonin level was 198.3 ± 3.694 pg/mL in the spring and 128.9 ± 1.999 pg/mL in the winter under dark conditions, and 160.8 ± 4.050 pg/ml in the spring and 86.69 ± 3.203 pg/mL in the winter under light conditions ($p < 0.001$). In pearl mullets, the mean melatonin level was 118.1 ± 2.560 pg/mL in the spring and 70.53 ± 2.918 pg/mL in the winter under dark conditions, and 84.73 ± 2.281 pg/mL in the spring and 36.32 ± 1.627 pg/mL in the winter under light conditions ($p < 0.001$). The difference between the melatonin levels was statistically significant.

DISCUSSION

The aim of the present study was to determine melatonin levels in pearl mullets and trouts grown in the Lake Van region by ELISA method which has not been investigated before.

Following the measurement of plasma melatonin concentrations, a significant discrepancy was found in terms of existence of pearl mullets and trouts under dark and light conditions. The plasma melatonin concentration of fish under dark conditions was found to be very high as compared to the plasma melatonin concentration of fish under light conditions. This difference is due to the fact that melatonin synthesis is low during dayligth and high during night. The nerve impulses occurred by the light are transmitted to the superior cervical ganglion in the spinal cord via the retina,

Table 1. Melatonin levels (pg/mL) in pearl mullets and trouts in light and dark conditions and in winter and spring (Mean ± SD)

		Winter	Spring
Pearl mullet (n=15)	In light	36.32 ± 1.627	84.73 ± 2.281
	In dark	70.53 ± 2.919	118.1 ± 2.560
Trout (n=15)	In light	86.69 ± 3.203	160.8 ± 4.090
	In dark	128.9 ± 1.999	198.3 ± 3.694

the hypothalamic nuclei (suprachiasmatic and paraventricular nuclei), medial forebrain bundle (MFB), and the reticular formation. Sympathetic impulses from the superior cervical ganglion are transmitted to the pineal gland and lead to alternations in the amount of melatonin secreted, depending on the amount and duration of light stimulation. Melatonin regulates the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which in turn regulates the release of gonodotropic hormones from the pituitary gland. Consequently, the mating behavior and fertilization pattern of the animals are regulated according to seasonal changes [15]. Numerous animal species living in warm and cold climates exhibit seasonal fertilization patterns throughout the world.The stages of the seasonal mating are arranged in such a way that the birth of the offspring is adjusted to occur in the most appropriate season for its survival, development, and growth. As in many other species, fish also have photoperiodic behavior to monitor changes in the external environment. Pituitary gland activities are inhibited or activated based on photoperiod.

In the present study, it was shown that the melatonin levels of the fish during both in the spring and nighttime were higher as compared to those in the winter and daytime. Therefore, our results emphasized the significance of the photoperiod. In accordance with the present study, previous studies have also been revealed significant differences between night and day, winter and spring, and summer and fall in the melatonin levels [14,16–34]. Indeed, melatonin levels have been found to be higher during the night and lower during the daytime in these studies.

The results of the present study revealed that photosensory organs, such as the pineal gland or retina, were controlled by the photoperiod in trouts and pearl mullets.

There are also several factors affecting melatonin levels. These factors show their influences at different levels.

The melatonin level also varies according to seasonal periods [35-37]. The type of nutrition and environmental factors also affect the amount of melatonin secretion in fish [38]. Melatonin level also depends on the physical size and biological characteristics of the fish, such as amount of plasma [39]. Larson et al. [40], İnvestigated the effects of an increase or decrease in circulating melatonin levels on social stress in trouts and observed that plasma melatonin levels were low in blood samples obtained during the daytime, while it was high at night. Lepage et al. [41], and Heinzeller et al. [42], found that the effect of an increase or decrease in plasma melatonin levels on social stress was related to melatonin metabolism.In addition to seasonal photoperiod, water temperature also affects melatonin secretion. The cause of the seasonal difference between melatonin levels (winter-spring) is temperature. Therefore, many animal species living in warm and cold climate zones exhibit seasonal patterns of mating and non-mating throughout the world. This period is adjusted to the most appropriate season [16]. Melatonin synthesis in fish has also been associated with some environmental conditions [43,44]. These environmental factors include rain, change in water level, nutrition, and natural conditions [45]. Previous studies, as well as the current study, all demonstrate that melatonin levels vary with season and amount of light during the night according to fish physiology.

Environmental conditions, nutritional status, and physiology all have an effect on melatonin secretion. There are several parameters which have an effect on the amount of melatonin secretion in fish. However, there is no proportional effect with respect to the type and amount of these parameters. This has not been thoroughly clarified in previous studies. Melatonin is present in all living organisms, and the significance of this hormone has been consistently emphasized in all studies.

In conclusion, it has been found that season (spring-winter), light conditions (dark-light), and species have an effect on melatonin levels in pearl mullets and trouts grown in the Lake Van.

REFERENCES

1. A. Baysal, Nutrition. 9th Edn., Hatioğlu of Publication, Ankara (2002).
2. J. Arendt, The Pineal Gland, Basic Physiology and Clinical Implications. In, Endocrinology, L. J. DeGroot, (Eds.), 3rd Edn., Philadelphia, W.B. Saunders, (1995) 433.
3. J. Barrenetxe, P. Delagrange, J. A. Martínez, Physio–logical and metabolic functions of melatonin, J. Physiol Biochem., 60 (2004) 61., DOI: doi:10.1007/BF03168221; PMID 15352385.
4. A. Çam, M. F. Erdoğan, Melatonin, Ankara University, J. Medic., 56 (2003) 103.
5. J. A. Boutin, V. Audinot, G. Ferry, P. Delagrange, Molecular tools to study melatonin pathways and actions, Trends Pharmacol Sci., 26 (2005) 412. DOI: 10.1016/J.tips.2005.06.006; PMID: 15992934.
6. M. Karbownik, R. J. Reiter, J. Cabrera, J. J. Garcia, Comparison of the protective effect of melatonin with other antioxidants in the hamster kidney model of estradiol–induced DNA damage, Mutat Res., 474 (2001) 87. DOI: 10.1016/S0027–5107(00)00164–0; PMID 11239965.
7. V. N. Anisimov, I. N. Alimova, D. A. Baturin, I. G. Popovich, M. A. Zabezhinski, S. V. Rosenfeld, K. G. Manton, A. V. Semenchenko, A. I. Yashin, Dose–dependent effect of melatoninmon life span and spontaneous tumor incidence in female SHR mice, Exp Gerontol., 38 (2003) 449, DOI: 10.1016/S0531–5565(02)00240–1; PMID 12471612.
8. W. Dean, J. Margehattaller, S. W. Fowkes, Smart Drugs II: The Next generation: New drugs and nutriends to improve your memory and increase your intelligent, Smart Drug Series V. 2., Smart Publications ISBN 9627418(0), 7 (1993) 6.
9. S. Oaknin–Bendahan, Y. Anis, I. Nir, N. Zisapel, Effects of long–term administration of melatonin and a putative antagonist on the ageing rat, Neuroreport., 6 (1995) 785, DOI:10.1097/00001756–199503270–00020; PMID 7605949.
10. E. B. Arushanian, E. V. Beĭer, Immunotropic properties of pineal melatonin, Eksp Klin Farmakol., 65 (2002) 73, PMID 12596522.
11. A. J. Lewy, R. L. Sack, L. S. Miller, T. M. Hoban, Antidepressant and circadian phase shifting effects of light, Science, 235 (1987) 352, DOI: 10.1126/Science, 3798117; PMID 3798117.
12. L. Volicer, D. G. Harper, B. C. Manning, R. Goldstein, A. Satlin, Sundowning and circadian rhythms in Alzheimer's disease, Am. J. Psychiatry., 158 (2001) 704, DOI: 10.1176/appi.aj.p.158.5.704; PMID: 11329390.
13. W. Randolp, M. D. Evans, R. Frederick, M. D. Taylor, "Natural" or Alternative Medications for Migrane Prevention, HEADACHE, 46 (6) (2006) 1012.
14. M. J. Bayarri, J. A. Madrid, F. J. Sánchez–Vázquez, Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin, J. Pineal Res., 32 (2002) 34, DOI: 10.1034/J.1600–079x.2002.10806.x; PMID 11841598.
15. A. Noyan, Fizyoloji Ders Kitabı. 5th Edn., Meteksan Ltd. Şti., Ankara (1988).
16. R. García–Allegue, J. A. Madrid, F. J. Sánchez–Vázquez, Melatonin rhythms in European sea bass plasma and eye: influence of seasonal photoperiod and water temperature, J. Pineal Res., 31 (2001) 68, DOI:10.1034/J.1600–079X.2001.310110.x; PMID 11485007.
17. M. S. Rahman, B. H. Kim, A. Takemura, C. B. Park, Y D. Lee, Effects of moonlight exposure on plasma melatonin rhythms in the seagrass rabbitfish, Siganus canaliculatus, J. Biol Rhythms., 19 (2004) 325, DOI: 10.1177/0748730404266712; PMID 15245651.
18. A. Takemura, S. Ueda, N. Hiyakawa, Y. Nikaido, A direct influence of moonlightintensity on changes in melatonin production by cultured pineal glands of the golden rabbitfish, Siganus guttatus, J. Pineal Res., 40 (2006) 236,DOI:10.1111/J.1600–079X.2005.00306.x; PMID 16499560.
19. C. Pape, K. Lüning, Quantification of melatonin in phototrophic organisms, J. Pineal Res., 41 (2006) 157, DOI: 10.1111/J.1600–079X.2006.00348.x; PMID: 16879322.
20. N. W. Pankhurst, M. J. R. Porter, Cold and dark or warm and light: variations on the theme of environmental control of reproduction, Fish Physiol Biochem., 28 (2003) 385, DOI: 10.1023/B:FISH.0000030602.51939.50.
21. S. Sahu, M. Shedpure, Air–breathing rhythm in Clarias batrachus (Linn.): modulatoryrole of eyes, pineal and exogenous melatonin, Indian J. Exp Biol., 44 (2006) 55, PMID 16430092.
22. J. Ghosh, P. Nath, Seasonal effects of melatonin on ovary and plasma gonadotropin and vitellogenin levels in intact and pinealectomized catfish, Clarias batrachus (Linn). Indian J. Exp. Biol., 43 (2005) 224, PMID 15816408.
23. A. C. Roberts, N D. Martensz, M. H. Hastings, J. Herbert, Changes in photoperiod alter the daily rhythms of pineal melatonin content and hypothalamic beta–endorphin content and the luteinizing hormone response to naloxone in the male Syrian hamster, Endocrinology, 117 (1985) 141, PMID 3159563.
24. D. A. Guerrero–Tortolero, M. J. R. Porter, N. R. Bromage, The effects of different daytime light intensities on the diel patterns of plasma melatonin in fingerling and broodstock rainbow trout (Oncorhynchus mykiss), Fish Physiol. Biochem., 28 (2003) 415, DOI: 10.1023/B:FISH.0000030610.93492.

25. R. Chaube, K. P. Joy, Effects of altered photoperiod and temperature, serotonin affecting drugs, and melatonin on brain tyrosine hydroxylase activity in female catfish, Heteropneustes fossilis: a study correlating ovarian activity changes, J. Exp Zool., 293 (2002) 585, DOI: 10.1002/J.ez.10185; PMID 12410607.
26. J. Falcón, Y. Gothilf, S. L. Coon, G. Boeuf, D. C. Klein, Genetic, temporal and developmental differences between melatonin rhythm generating systems in the teleost fish pineal organ and retina, J. Neuroendocrinol., 15 (2003) 378, DOI: 10.1046/J.1365, 2826.2003. 00993.x; PMID 12622837.
27. H. Yamada, H. Chiba, M. Amano, M. Ligo, M. Iwata, Rainbow trout eyed–stage embryos demonstrate melatonin rhythms under light–dark conditions as measured by a newly developed time–resolved fluoroimmunoassay, Gen Comp. Endocrinol., 125 (2002) 41(b). DOI:10.1006/gcen.2001.7731; PMID 11825033.
28. T. Masuda, M. Ligo, K. Mizusawa, M. Naruse, T. Oishi, K. Aida, M. Tabata, Variations in plasma melatonin levels of the rainbow trout (Oncorhynchus mykiss) under various light and temperature conditions, Zoolog Sci., 20 (2003) 1011.
29. H. Migaud, J. F. Taylor, G. L. Taranger, A. Davie, J. M. Cerdá–Reverter, M. Carrillo, T. Hansen, N. R. Bromage, A comparative ex vivo and in vivo study of day and night perception in teleosts species using the melatonin rhythm., J. Pineal Res., 41 (2006) 42, DOI: 10.1111/J.1 600079X.2006.00330.x; PMID 16842540.
30. M. Pavlidis, L. Greenwood, M. Paalavuo, H. Mölsä, J. T. Laitinen, The effect of photoperiod on diel rhythms in serum melatonin, cortisol, glucose, and electrolytes in the common, Dentex, Gen Comp. Endocrinol., 113 (1999) 240, DOI 10.1006/gcen.1998.7190; PMID 10082626.
31. C. F. Randall, N. R. Bromage, J. E. Thorpe, M. S. Miles, J. S. Muir, Melatonin rhythms in Atlantic salmon (Salmo salar) maintained under natural and out–of–phase photoperiods, Gen Comp Endocrinol., 98 (1995) 73, DOI: 10.1006/gcen.1995.1045; PMID 7781966.
32. R. M. Ceinos, S. Rábade, J. L. Soengas, J. M. Míguez, Indoleamines and 5–methoxyindoles in trout pineal organ in vivo: daily changes and influence of photoperiod, Gen Comp. Endocrinol., 144 (2005) 67.
33. M. Amano, M. Ligo, S. Kitamura, N. Amiya, K. Yamamori, Changes in melatonin binding sites under artificial light dark, constant light and constant dark conditions in the masusalmon brain, Comp. Biochem.Physiol. A Mol. Integr. Physiol., 144 (2006) 509, DOI:10.1016/J. cbpa.2006.04.018; PMID 16759892.
34. N. Bromage, M. Porter, C. Randall, The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin, Aquaculture, 197 (2000) 63, DOI: 10.1016/S0044–8486(01)00583–X.
35. E. Sokolowska, H. Kalamarz, E. Kulczykowska, Seasonal changes in brain melatonin concentration in the three–spined stickleback (Gasterosteus aculeatus): towards an endocrinecalendar, Comp. Biochem. Physiol. A Mol. Integr. Physiol., 139 (2004) 365, PMID 15556393.
36. R. J. Reiter, B. Poeggeler, D. Tan, Antioxidant Capacity of Melatonin: A Novel Action not Requiring a Receptor, Neuroendocrinol Lett., 15 (1993) 103.
37. J. Duston, N. Bromage, Photoperiodic mechanisms and rhythms of reproduction in the female rainbow trout, Fish Physiol Biochem., 2 (1986) 35, DOI: 10.1007/ BF02264072.
38. J. A. Madrid, L. M. Vera, M. J. Sanchenz–Muros, N. De Petro, E. Gomez– Milan, F. J. Sanchenz–Vazguez, M. J. Delkado, Feding entrainment of lokomotor activitiy rhythmus, digestive enzymes and neuroendocrine factors in goldfishes, Physiol. Behar, 5 (1) (1006) 130.
39. M. Ligo, M. Hara, R. O. Kaneko, K. Hirata, M. Tabata, K. Aida, Photic and circadian regulations of melatonin rhythms in fishes, Biol. Signals., 6 (1997) 225, DOI: 10.1159/000109132; PMID 9500660.
40. E. T. Larson, S. Winberg, I. Mayer, O. Lepage, C. H. Summers, Ø. Øverli, Social stress affects circulating melatonin levels in rainbow trout, Gen Comp. Endocrinol., 136 (2004) 322, DOI: 10.1016/J.ygcen.2004.01.005; PMID 15081831.
41. O. Lepage, E. T. Larson, I. Mayer, S. Winberg, Tryptophan affects both gastrointestinal melatonin production and interrenal activity in stressed and nonstressed rainbow trout, J. Pineal Res., 38 (2005) 264, DOI: 10.1111/J.1600–079X.2004.00201.x; PMID 15813903.
42. T. Heinzeller, B. N. Joshi, F. Nürnberger, R. J. Reiter, Effects of aggressive encounters on pineal melatonin formation in male gerbils (Meriones unguiculatus, Cricetidae), J. Comp Physiol A., 164 (1988) 91, DOI: 10.1007/BF00612722; MID 3236263.
43. T. J. Lam, A. D. Munro, Environmental control of reproduc–tion in teleosts: Anoverview. In: Reproductive Physiology of Fish, D. R. Idler, L. W. Crim and J. M. Walsh, (Eds.). Canada, St. J.ohn's, Newfoundland (1987).
44. W. A. Tyler III, F. Stanton, Potential Influence of Food Abundance on Spawning Patterns in a Damselfish, Abudefduf Abdominalis, Bull. Mar. Sci., 57 (1995) 610.
45. M. J. R. Porter, S. O. Stefansson, G. Nyhammer, Ø. Karlsen, B. Norberg, N. R. Bromage, Environmental influences on melatonin secretion in Atlantic cod (*Gadus morhua L.*) and their relevance to commercial culture, Fish Physiol Biochem., 23 (2000) 191, DOI: 10.1023/A:1007816426509.