

Seasonal changes in brain melatonin concentration in the three-spined stickleback (*Gasterosteus aculeatus*): towards an endocrine calendar

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Abstract

Pineal organ and its hormone melatonin (*N*-acetyl-5-methoxytryptamine) is likely involved in timing and synchronisation of many internal processes, such as reproduction, with annual changes in environmental cues, i.e., photoperiod and water temperature. The seasonal changes in melatonin profile in stickleback brains related to the following reproductive phases were examined, and the link between melatonin concentrations and the stages of spawning cycle was analysed. Two wild populations of sticklebacks were exposed to annual environmental changes in their natural habitats. Brains, gonads, kidneys and livers were collected over 2 years. Melatonin was measured using RIA and the indices, gonadosomatic (GSI), nephrosomatic (NSI) and hepatosomatic (HSI), were calculated. The role of melatonin, as a component of internal calendar engaged in the control of seasonal breeding in this species, is discussed. The extremely high melatonin levels observed in early spring (March) and autumn (October) seem to mark out a time frame for spawning in sticklebacks. The seasonal pattern of melatonin production and identified development stages of gonads suggests the potential inhibitory effect of the hormone on stickleback reproduction in shortening photoperiod and stimulatory effect in lengthening photoperiod.

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1. Introduction

Most animals living in temperate regions, including fish species, have developed adaptation strategies to synchronise reproduction with annual changes in environmental cues (temperature, day length). Seasonally breeding fish have optimal time for spawning, different for any species, determined mainly by the combination of the environmental factors. Generally, the pineal, which is a component of the circadian system, is influenced by the light/dark cycle and is involved in the control of circadian and circannual rhythms in vertebrates (Reiter, 1993; Wehr, 1997; Hofman, 2004). Melatonin (*N*-acetyl-5-methoxytryptamine) is produced and secreted from

pineal exclusively at night (Binkley, 1988). The duration of nocturnal melatonin synthesis depends on the length of the night; that is, in winter, the intervals of melatonin synthesis are the longest. In the case of ectothermic vertebrates, such as fish, both photoperiodic and temperature signals are transduced by the pineal into a pattern of melatonin synthesis (Falcon et al., 1994; Garcia-Allegue et al., 2001; Masuda et al., 2003). Thus melatonin is recognised as a key component of an internal endocrine calendar to the organism (Reiter, 1993; Wehr, 1997; Hofman, 2004). It has been shown in fish that this hormone, acting on different elements of the hypothalamic–pituitary–gonadal axis, provides a crucial endocrine signal to influence reproduction activity. The final effect, however, varies between species (Borg and Ekström, 1981; Nayak and Singh, 1987; Joy and Agha, 1991; Joy and Khan, 1991; Popek et al., 1991; Amano et al., 2000; Bromage et al., 2001; Chaube and Joy, 2002;

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Bayarri et al., 2004). Often, the similar changes in melatonin pattern can trigger opposite processes: gonadal stimulation and breeding or gonadal regression and cessation of breeding in species which spawn in various seasons.

Three-spined stickleback is a long-day breeder in which photoperiodic information seems to be an important signal for spawning (Borg, 1982; Baggerman, 1985, 1989). Data suggest that melatonin influences the control of development, sexual maturation and seasonal reproductive cycle of this species (Borg and Ekström, 1981).

The aim of this work was to examine the seasonal changes in melatonin profile in stickleback brains related to the following reproductive phases. The role of melatonin, as a component of internal calendar engaged in the control of seasonal breeding in this species, was considered. Two wild populations of sticklebacks were exposed to annual environmental changes in their natural habitats, and the link between melatonin concentrations in the brain and the stages of spawning cycle was analysed.

2. Materials and methods

2.1. Animals and sampling

Adult three-spined sticklebacks (*Gasterosteus aculeatus*) of both sexes used in this study were caught in Vistula River (5–6‰ of salinity) and Oliva's stream (fresh water) 2 h after sunset, one, two or three times per month from May 1998 to August 2001. Water temperature was measured during all experimental periods. Fish were kept outdoors in a covered small tank containing the water from river or stream, respectively, until dissection. The brains ($n=1166$) were removed after decapitation under red light, immediately frozen and stored at -70°C until radioimmunoassay (RIA). Dissections of brains have been completed within 1–2 h. Whole brains were taken. To confirm that all pineal organ was present in brain tissue, the melatonin content in collected skull roofs was measured. All values were lower than the detection limit. Testes, ovaries, kidneys and livers were collected and the indices, gonadosomatic (GSI), nephrosomatic (NSI) and hepatosomatic (HSI), were calculated as follows: (gonad weight/body weight) $\times 100$, (kidney weight/body weight) $\times 100$ and (liver weight/body weight) $\times 100$, respectively. The fish and organs were weighed to the nearest 0.001 g.

2.2. Brain analysis

The brain samples collected each month were pooled and sonicated in 60% solution of methanol using Microson™ XL 2000. Homogenates were centrifuged at 15,000 g for 15 min at 4°C . Melatonin was extracted from supernatants by

solid phase extraction (SPE) method using C18 Bakerbond cartridges (J.T. Baker, USA). The brain samples were eluted with methanol according to previous procedure described for melatonin extraction (Kulczykowska and Iuvone, 1998). Total melatonin content was assayed in brain extracts by RIA method using IBL-Hamburg kit. The assay procedure was performed with modifications required for tissue samples without enzymatic pretreatment. All tubes were counted in a γ -counter (Beckman) for 1 min. The lowest detectable level of melatonin was 2.8 pg/g wet tissue. The intra-assay coefficient of variation was 8.0%. The interassay variation was not determined because all samples were measured in the same assay. RIA has been validated for tissue samples by HPLC assay (Kulczykowska and Iuvone, 1998). Melatonin concentration was expressed as a pg/g wet tissue.

2.3. Statistical analysis

Values are presented as means \pm standard error of the mean (S.E.M.). As the values for melatonin were not normally distributed, they were compared statistically using the nonparametric Kruskal–Wallis's range test followed by Mann–Whitney's U -test.

3. Results

The phases of annual reproductive cycle were distinguished by GSI, NSI and HSI. The calculated indices were similar in both fish populations in respective seasons. In males, the highest GSI value (0.97 ± 0.07) was observed in September and indicated the active spermatogenesis which prepared males for the next breeding season. In other months, GSI did not exceed value of 0.65. The NSI reached the highest value in May (2.87 ± 0.28) as a result of kidney hypertrophy in the phase of nest building. In general, the NSI value was noteworthy higher in summer (2.58 ± 0.44 , mean value for June, July and August) than in winter (0.95 ± 0.07 , mean value for December, January and February).

In females, elevated GSI and HSI values observed in April (10.49 ± 1.66 and 5.53 ± 0.26 , respectively) indicated the advance phase of the ovarian development. An increase of the GSI value in May (17.93 ± 1.74) was a consequence of rising diameter of the oocytes. A decrease of both indices, GSI and HSI, on the turn of June and July was related to the ovarian regression (10.70 ± 2.17 and 4.41 ± 0.48 , respectively).

Brain melatonin concentrations were presented for fish from Vistula River and Oliva's stream (Fig. 1A, B). Extremely high melatonin levels were detected for both groups during spring (March, April, May) and autumn (September, October, November). Mean water temperature

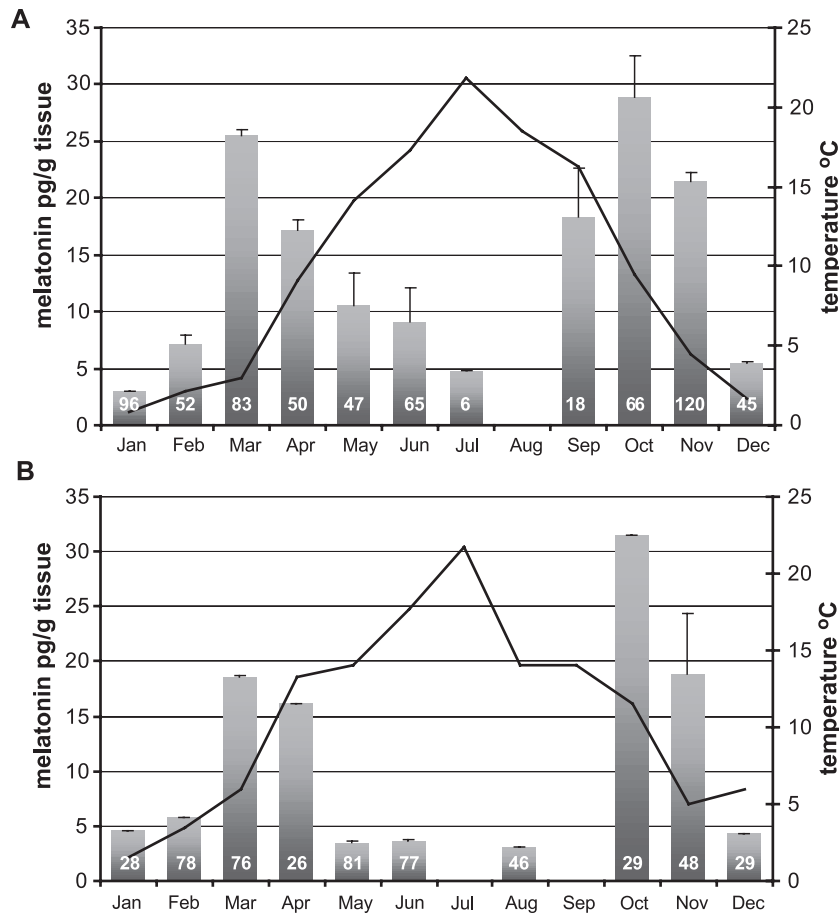


Fig. 1. The brain melatonin concentration in three-spined stickleback obtained from Vistula River (A) and Oliva's stream (B) during annual cycle. Values are means \pm S.E.M. Seasonal effect on stickleback's brain melatonin concentration is statistically significant ([A] $p < 0.01$, Kruskal–Wallis's range test). Mann–Whitney's *U*-test showed significant differences for melatonin level in spring (March) and autumn (November or October) vs. summer (May, June or July; $p < 0.05$) and winter (January; $p < 0.05$). Number of brains examined is given in bars. Line indicates mean water temperature for sampling periods.

for Vistula River and Oliva's stream in sampling periods was shown in Fig. 1A, B.

4. Discussion

The results presented in this paper show for the first time the seasonal changes in night melatonin concentration in the brain of free living stickleback. The highest night melatonin values are clearly demonstrated in early spring (March) and in autumn (October), when day and night lengths are of almost equal duration. The annual cycle observed in two populations seems to represent a general pattern of melatonin synthesis in this species. In our study, brains were collected only once during the night, and melatonin was analysed without sex separation, owing to the technical problems during outdoor collection of the samples. However, it is well established that the synthesis of melatonin in the pineal organ in fish represents a model with a rapid increase at the beginning of scotophase. The high melatonin production remains stable till the end of darkness, regardless of a lighting regime or season

(Kezuka et al., 1988; Masuda et al., 2003; Mayer et al., 1997; Randall et al., 1995). Moreover, no sexual difference in melatonin levels has been shown in fish (Kezuka et al., 1988; Iseki et al., 2000).

Studies on the effects of natural or experimental photoperiodic changes and/or melatonin level manipulations have suggested that melatonin is a component of endocrine calendar in this species (Ekström and Meissl, 1997). To explain the character of melatonin message as a component of internal calendar, the duration, the amplitude and the internal coincidence hypotheses are proposed (Reiter, 1993). In higher vertebrates, the experimental studies with melatonin infusion reproducing the effect of either a short or a long photoperiod on the sexual activity suggest the prominent role of the duration of elevated melatonin (Reiter, 1993; Wehr, 1997; Hofman, 2004). Our studies indicate that the amplitude of the nocturnal rise in melatonin is important, at least in stickleback. The extremely high night melatonin levels observed in early spring and autumn seem to mark out a time frame for spawning in sticklebacks from Vistula River and Oliva's stream. Moreover, the effect of melatonin on reproductive system may occur only when high melatonin

level is coincident with a sensitivity period to melatonin, as was shown in fish (Fenwick, 1970). The last may vary with seasons. This may be a case here in early spring and autumn, when extremely high melatonin levels occurred.

Generally, the annual pattern of melatonin synthesis in fish comprises the changes in the duration of elevated melatonin, which vary with changing night lengths, and the amplitude of the nocturnal peak of hormone, which probably depends on water temperature and physiological status of the organism. But the message encoded in the melatonin rhythm may be interpreted differently by organism at different physiological condition and at different time. Therefore, the similar signal of high melatonin synthesis capacity can be read differently at different phases of reproductive cycle and can result in gonadal stimulation or gonadal regression in stickleback. It should be stressed that in fish as poikilothermic organisms, the seasonal melatonin synthesis could be strongly affected by water temperature. There is strong evidence that the responses of the pineal neurons of teleosts to light depend on temperature, with an optimal temperature range between 10 and 20 °C (Tabata and Meissl, 1993). In studied fish, melatonin synthesis increases with increasing temperature to an optimum level, beyond which it declines (Max and Menaker, 1992; Zachmann et al., 1992; Iigo and Aida, 1995). It may be assumed that temperature influences the enzyme kinetics in the melatonin biosynthetic pathway, with enzymes HIOMT and AA-NAT as the most important (Morton and Forbes, 1989; Falcon et al., 1996).

The role of the pineal organ and its hormone melatonin in the control of reproduction is extensively studied in many fish species (Borg and Ekström, 1981; Nayak and Singh, 1987; Joy and Agha, 1991; Joy and Khan, 1991; Popek et al., 1991; for Review: Ekström and Meissl, 1997; Amano et al., 2000; Bromage et al., 2001; Chaube and Joy, 2002; Bayarri et al., 2004). However, direct experimental data are often confusing because the effects of pinealectomy and/or melatonin administration, i.e., procedures most often used, have been shown to vary with gender, lighting regimes and reproductive phase. Nevertheless, most of observations indicate that melatonin is involved in the timing of fish reproduction. Bromage et al. (2001) refer to many evidences for a melatonin's role in the transduction of information on day length to the reproductive axis. In the three-spined stickleback, Borg and Ekström (1981) have presented antigonadal effects of melatonin injections during winter. Popek et al. (1991) have demonstrated stimulation of gonads by a spring surge of melatonin in carp, the other "long-day breeder". In our study, the seasonal pattern of melatonin production and identified development stages of gonads suggests the potential inhibitory effect of the hormone on stickleback reproduction in shortening photoperiod and stimulatory effect in lengthening photoperiod. However, Mayer et al. (1997) and Bornestaf et al. (2001) failed to find any effects of melatonin on reproduction in stickleback. According to Ekström and Meissl (1997), the role of the pineal organ in

reproductive responses to photoperiod is unconvincing. However, it should be stressed that the sticklebacks were studied experimentally in the artificial environment of the laboratory in selected seasons, and melatonin was administered in doses far from being physiological.

In conclusion, our studies performed in two free living populations of stickleback show that the brain melatonin concentration changes over the year. The results point out the melatonin as a component of internal calendar, which probably controls seasonal breeding in this species. The extremely high melatonin levels observed in early spring and autumn seem to mark out a time frame for spawning in sticklebacks. However, future studies are required to elucidate the character of a seasonal message the pineal organ has sent directly or indirectly to reproductive system.

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