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Light–dark variations in plasma melatonin concentrations in the pot-bellied seahorse *Hippocampus abdominalis* Lesson, 1827

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A diel plasma melatonin profile measured in adult pot-bellied seahorses *Hippocampus abdominalis* showed that this species produces elevated levels of melatonin during the scotophase, returning to basal levels during the photophase.

Key words: diel profile; *Hippocampus abdominalis*; melatonin; radioimmunoassay.

In teleosts, locomotor and physiological activities are controlled by biological synchronizers which can be triggered by endogenous or exogenous cues; the light–dark cycle is one of the most common synchronizers (Vera *et al.*, 2006). The pineal organ processes changes in the light related hormonal signals such as melatonin which has been associated with the mediation of physiological processes (Pavlidis *et al.*, 1999) and the transduction of light information to reproductive and growth processes in fishes (Bayarri *et al.*, 2004). In many vertebrate species blood melatonin concentration is low during the photophase (light phase) and elevated during the scotophase (dark phase). Reiter (1989) defined the three main melatonin synthesis profiles as A, B and C; Type C is characterized by a prolonged peak during most of the scotophase and is the most common melatonin production pattern in fishes. No studies on melatonin levels in seahorses have been reported in the literature. It is known, however, that seahorses (*Hippocampus* sp.) display a clear diurnal activity pattern (Ouyang, 2005; Karina *et al.*, 2006; Sheng *et al.*, 2006). The aims of this study on adult pot-bellied seahorses *Hippocampus abdominalis* Lesson, 1827, were to

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validate a melatonin radioimmunoassay for plasma samples from seahorses to demonstrate confidently that the technique was measuring melatonin and to compile a diel profile of melatonin levels.

Adult *H. abdominalis*, of mixed sex were maintained from birth in the marine hatchery at the School of Aquaculture, University of Tasmania, Launceston. The fish were maintained in a 1 m^3 tank in a re-circulating system at 17° C until sampling occurred. They were exposed to a 12 L:12 D photoperiod (lights on at 0900 hours, lights off at 2100 hours) at least 3 weeks before sampling. Illumination was provided by a timer controlled cool white light 35 W (General Electric Company) producing an intensity of 4·8 μE s^{-1} m^{-2} at the water surface. Pot-bellied seahorses were fed one to two meals daily at *ad hoc* times during the photophase with enriched *Artemia* sp. metanauplii in the first 4 months after birth, then transferred to frozen mysids until sampling.

The adult fish were anaesthetized by immersion in a solution of benzocaine (7·5 mg l^{-1}) in sea water. As soon as the fish lost equilibrium, they were removed and their total length (L_T) and mass were recorded. The L_T (distance between the tip of the coronet to the tip of the uncurled tail) was measured by placing the fish on a submerged plastic-covered 1 mm scaled sheet. Wet mass (M_W) of individuals were measured on an electronic balance and recorded to the nearest 0·1 g. Blood samples were taken (100–150 μl per fish) *via* the caudal aorta by 1 ml heparinized syringes and then pooled to give *c.* 600 μl aliquots. Fish were not resampled within the experiment. Blood was separated by centrifugation at 1811 *g* for 10 min at 4° C and the supernatant was collected and pipetted into 1·5 ml Eppendorf tubes prior to storage at −20° C until analysis. During the dark phase sampling under a dim red light was used to assist the operator but to avoid light-disturbance of the fish. The head of the fish was also covered by a damp cloth during blood withdrawal to minimize exposure to any light. There were a limited number of adult pot-bellied seahorses in the marine hatchery at the School of Aquaculture (University of Tasmania, Launceston). Therefore, statistical analysis was not conducted for the 24 h melatonin profile, as the number of aliquots obtained did not meet replication requirements.

All plasma samples were analysed for melatonin by direct radioimmunoassay (RIA) as described in Randall *et al.* (1995). The assay utilized sheep melatonin antiserum, (Batch 60, Stockgrand Ltd, Guilford, Surrey, U.K.) and [O-*methyl*-^{3}H] melatonin, sp act 70–85 Ci/mmol (Amersham International Ltd, Amersham, Bucks, U.K.). In order to determine the presence or absence of melatonin concentrations in plasma of pot-bellied seahorses, an inhibition curve was obtained from a serial dilution (1:2) of pooled fish plasma collected from six adult pot-bellied seahorses (mean ± S.E. $L_T = 187 \pm 2$ mm and $M_W = 21{\cdot}4 \pm 1{\cdot}3$ g) at mid-dark (0300 hours) as elevated levels of plasma melatonin are produced mostly during the scotophase (Reiter, 1989). The curve showed good parallelism with the standard curve (3·9–500 pg $tube^{-1}$) of the assay (Fig. 1), which was transformed from a sigmoidal curve to a linear curve and the slope compared with the serial dilution of the pot-bellied seahorse plasma. No statistical difference in the slopes was found (ANCOVA, $F_{1,6} < 0{\cdot}001$, $P > 0{\cdot}05$; using a significance level of $P < 0{\cdot}05$, SPSS 11.5; Fig. 1). Assumptions of normality and homogeneity were satisfied as determined by Levene's test and residual plots.

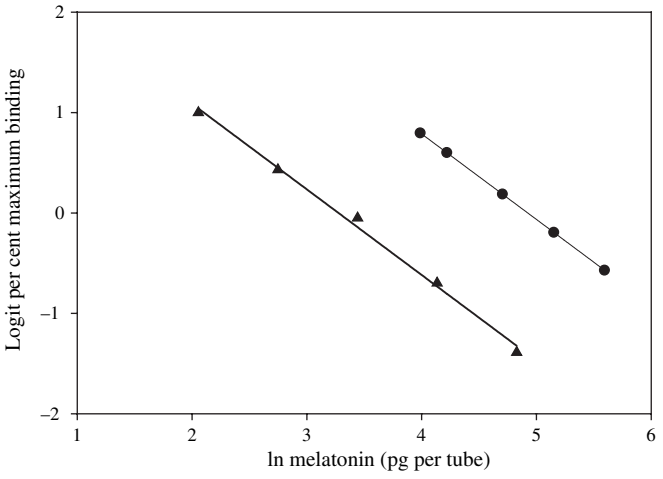

FIG. 1. Plot of a serial dilution of melatonin concentration in pot-bellied seahorse plasma pooled from six fish (●; $y = -0{\cdot}8518x + 4{\cdot}1947$) against a standard melatonin concentration curve (means of duplicate samples) (▲; $y = -0{\cdot}8518x + 2{\cdot}7903$) showing no significant difference in slope ($P > 0{\cdot}05$).

Duplicate blood samples were collected (following the protocol previously described for blood sampling) at eight time points over 24 h to describe a diel melatonin profile. Each duplicate sample comprised pooled blood from four to five fish (eight to 10 fish per sample point) with *c.* 100–250 μl of blood extracted from each pot-bellied seahorse.

Hippocampus abdominalis exhibited a clear diel melatonin profile (Fig. 2) with higher plasma levels during the dark phase reaching a maximum mean of 471 pg ml^{-1} at 2300–0000 hours, 2 h after the onset of darkness. Melatonin levels decreased in subsequent samples, reaching their lowest level just before the onset of the light phase (0800–0900 hours).

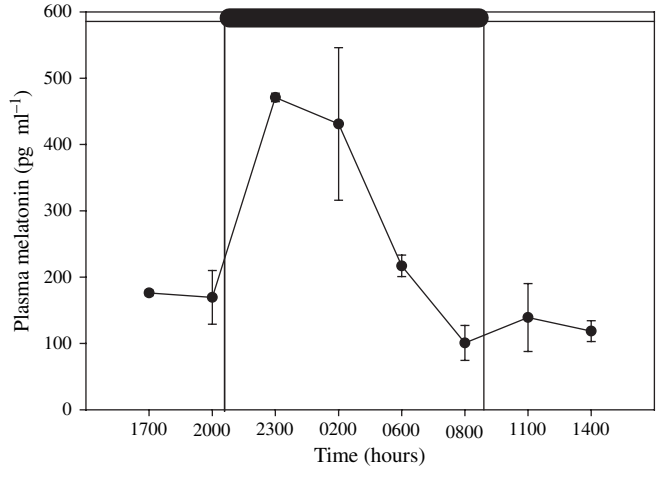

FIG. 2. Diel profile of pot-bellied seahorse mean ± S.E. ($n = 2$ replicates of pooled samples).

This work demonstrates for the first time in any seahorse species that plasma melatonin is present and furthermore that pot-bellied seahorses may exhibit a diel profile of melatonin over a 12L:12D period. Pot-bellied seahorses in the present study displayed a melatonin profile similar to that of river lamprey *Lampetra fluviatilis* (L., 1758) (Mayer *et al.*, 1998) and common carp *Cyprinus carpio* L., 1758 (Kezuka *et al.*, 1988). Of the three main melatonin production patterns described by Reiter (1989) it most closely resembles type B, where the highest level of melatonin occurs at the middle of the scotophase followed by a gradual decrease until the photoperiod restarts. The precise pattern of the profile, however, can only be determined by further repetition of the analysis.

The results of the present study suggest that the melatonin profile displayed could, like in other teleosts, be associated with the reduction of physiological processes such as locomotor activity during the dark phase (Pavlidis *et al.*, 1999).

The present results suggest that the radioimmunoassay technique detects melatonin in seahorse plasma and that *H. abdominalis* appears to display a diel fluctuation in melatonin concentration associated with the light and dark cycle, with the highest concentrations during the scotophase. Further research is needed to better describe the melatonin profile in relation to the photoperiod.

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