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Melatonin concentrations during larval and postlarval development of gilthead sea bream *Sparus auratus*: more than a time-keeping molecule?

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In this study, melatonin (MEL) and thyroxine (T_4) concentrations were measured during larval and postlarval development of gilthead sea bream *Sparus auratus* Hormones were measured in whole bodies of larvae or the head and trunk of postlarvae after 67 days of exposure to constant light, 24L:0D, constant darkness, 0L:24D or 12L:12D and in the plasma of 6 month juveniles kept under the 12L:12D, 0L:24D and 24L:0D regimes. High MEL concentrations in larvae suggested a distinct role of MEL in early organogenesis and development of *S. auratus*. In larvae, the gastro-intestinal tract seemed to be an important extrapineal and extraretinal source of MEL. No endogenous rhythm of MEL synthesis was demonstrated in 67 day larvae; however, in 6 month juveniles, it was evident. At early ontogenesis of *S. auratus*, the role of MEL is probably related mostly to the control of development and protection against free radicals, whereas its action as a time-keeping molecule develops later. The increase in T_4 concentration during the *S. auratus* larva–juvenile transition, *i.e.* between 50 and 70 days post-hatch, which was observed concurrently with the decrease of MEL concentration, may suggest an inverse relationship between T_4 and MEL.

Key words: development; fish; larvae; melatonin; rhythm; thyroxine.

INTRODUCTION

Fish development, from newly hatched larvae into juveniles, is associated with several morphological and physiological changes, which are controlled and co-ordinated by many endocrine factors. Melatonin (MEL; *N*-acetyl-5-methoxytryptamine) seems to be one of the hormones involved in the development of

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both animals and plants (Kvetnoy *et al*., 2002; Murch & Saxena, 2002). The influence of MEL on cell proliferation rate is well documented under *in vitro* conditions in transformed cells, fibroblasts and osteoblasts (Blask, 1993; Carossino *et al*., 1996; Roth *et al*., 1999), and in *in vitro* studies of chick embryo muscle cells MEL has been shown to be involved in growth and development processes, alone or together with growth factors (Lamošová *et al*., 1997). Moreover, MEL, acting through MT2 receptors, regulates cell proliferation rate in the zebrafish *Danio rerio* (Hamilton) embryo and accelerates the fish development (Danilova *et al*., 2004). In fish larvae and postlarvae, however, a clear-cut function of MEL is unknown.

In many vertebrate species, including fishes, MEL, which is synthesized in the pineal gland and retina, exhibits a diurnal rhythm with higher levels during dark periods (Falcón, 1999). MEL is thus an output signal of the circadian clock and is regarded as a time-keeping molecule. The lipophylic character and small size of MEL permits penetration and action in many peripheral tissues to synchronize and integrate functions that enable the organism to adapt to daily and seasonal changes in environment. The rhythm of MEL biosynthesis depends on the activity of the light-sensitive enzyme, arylalkylamine *N*-acetyltransferase (AA-NAT) (Klein *et al*., 1996; Falcón, 1999). The endogenous rhythms of AA-NAT messenger RNA (mRNA) expression, and consequently that of MEL production, have been demonstrated in retina and pineal organ of *D. rerio* and mummichog *Fundulus heteroclitus* (L.) embryos and in the pineal organ of *D. rerio* larvae (Gothilf *et al*., 1999; Kazimi & Cahill, 1999; Roberts *et al*., 2003; Ziv & Gothilf, 2006). As far as is known, however, there are no data about MEL rhythm in larvae and postlarvae of other fish species, including gilthead sea bream *Sparus auratus* L. In many adult vertebrates, the gastrointestinal tract (GIT), in addition to the pineal and retina, is an important all-day source of melatonin (Bubenik & Pang, 1997; Bubenik, 2002; Fernández-Durán *et al*., 2007; Konturek *et al*., 2007). So far, the contribution of GIT's melatonin to the overall pool of the hormone in fishes has not been assessed, and its distinct role remains unclear.

Thyroxine (T_4) is known as a primary hormone responsible for development and metamorphosis in lower vertebrates, especially in amphibians. The role of thyroid hormones (TH) during fish development seems to be important for both initial period of ontogenesis and larva–juvenile transition (Leatherland, 1994; Yamano, 2005). There is also some evidence for a relationship between TH and MEL in many vertebrates (Vriend, 1985; Nayak & Singh, 1987*a*, *b*; Haldar *et al*., 1992; Brzezińska-Ślebodzińska & Ślebodziński, 1993; Klencki *et al*., 1994; Wright *et al.,* 1997, 2003, 2004).

The aim of this research was to investigate the changes of MEL and T_4 during larval and postlarval development in *S. auratus*. A specific role of MEL during development is discussed.

MATERIALS AND METHODS

ANIMALS

Sparus auratus were reared in the Marine Station (University of Algarve, Faro, Portugal) or were supplied by the fish farm CUPIMAR (San Fernando, Cádiz, Spain). The larvae were kept in seawater tanks at 19–20° C, pH 7–8, salinity 32 and 90% oxygen saturation. The larvae were fed from day 3, twice a day with rotifers *Brachionus plicatilis* enriched with

the microalga *Nannochloropsis gaditana*, at a concentration of 6–10 rotifers ml^{-1}. From day 15, *Artemia* sp. nauplii enriched with Selco® (www.inve.com) were added to maintain *c*. 3 nauplii ml^{-1}. From day 20, *Artemia* sp. metanauplii were introduced into the diet. At this time, algae were progressively eliminated and replaced by gradually increasing the quantity of *Artemia* sp. metanauplii. After day 50, fish were fed with commercial dry pellets at a ration of 1% of body mass. The postlarvae in experiment III were fed once a day at *c*. 1100 hours with commercial dry pellets.

EXPERIMENTAL PROTOCOLS

Experiment I: whole-body MEL and T_4 concentration during development

Sparus auratus larvae were reared under the photoperiod of12L:12D; 0800 hours lights on, 2000 hours lights off. Larvae were collected daily from 1 to 10 days post-hatch (dph) and then every 10 days until 70 dph at 1200 hours. Samples were stored at -70° C prior to MEL, T_4 and protein analyses. Larvae collected from 1 to 70 dph were pooled before tissue processing.

Experiment II: head and trunk MEL concentration during development

Sparus auratus larvae were reared under12L:12D: (0800 hours lights on, 2000 hours lights off) and were taken at 30, 40, 50, 60 and 70 dph at 1200 hours. In each larva, the head was separated from trunk. All samples were stored at -70° C prior to MEL and protein analyses.

Experiment III: influence of different light regimes on head and trunk MEL concentration

Postlarvae of 67 days were reared in triplicate tanks exposed to different light regimes: a photoperiod of 12L:12D (0800 hours lights on, 2000 hours lights off), constant darkness, 0L:24D, and constant light, 24L:0D, for 2 weeks before experimentation. Samples were collected at 1200 hours and 2400 hours during 2 subsequent days. In each larva, the head was immediately separated from the trunk. All samples were stored at -70° C prior to MEL and protein analyses.

Experiment IV: influence of different light regimes on plasma MEL concentrationin juveniles

Juveniles *S. auratus* (40 g, 6 months old) were reared in triplicate tanks exposed to different light regimes: 12L:12D (0800 hours lights on, 2000 hours lights off), 0L:24D and 24L:0D for 1 week before experimentation. Blood samples were collected at 1200 and 2400 hours by needle puncture from fish anaesthetized in a seawater solution of MS-222 (0·1 $g l^{-1}$ 3-aminobenzoic acid ethyl ester methanesulphonate; Sigma-Aldrich; www.sigmaaldrich.com). After blood centrifugation at 10 000 **g** for 5 min, plasma was rapidly frozen and stored at -70° C prior to MEL analysis.

ANALYTICAL METHODS

Melatonin analyses

For MEL determination, tissue samples were sonicated in 0·05 M phosphate buffer (pH 7·4) containing 0·01% Thimerosal (Sigma-Aldrich) using Microson^{TM} XL (Misonix; www.misonix.com). After centrifugation of homogenates at 15 000 **g** for 20 min, supernatants were collected and assayed for MEL and protein.

Plasma and tissue MEL concentrations were assayed using total MEL kit (IBL; www.ibl-hamburg.com) with preceding specific extraction procedure (Kulczykowska & Iuvone, 1998). Solid phase extraction of MEL was carried out on Octadecyl C_{18} Speedisk Columns, 10 μm (J.T. Baker; www.mallbaker.com). The columns were conditioned with methanol followed by high performance liquid chromatography (HPLC)-grade water. Samples were applied on

the columns and then washed with 10% methanol. The elution was performed with methanol (2×300 µl). The eluate was collected, dried under vacuum and stored at -70° C prior to analysis. Before radioimmunoassay (RIA) procedure, dried samples were resuspended in Dulbecco's phosphate-buffered saline containing 0·01% Thimerosal. All samples in duplicate were counted in a Wallac Wizard γ-counter (www.perkinelmer.com). The labelled MEL with iodine-125 was used as a tracer for RIA. The detection limit was 2·5 pg ml^{-1} of plasma. The intra-assay coefficients of variation (c.v.) for plasma MEL were 8·0%. The interassay coefficient was not determined, because all samples were measured in the same assay. The plasma assay was validated by HPLC assay (Kulczykowska & Iuvone, 1998). Tissue extracts with known MEL concentration were enriched with increasing amounts of MEL standards (2·5–750 pg ml^{-1}) and analysed according to the assay procedure. The recovery of tissue assay was found in the range 86–112%. The binding curve of added MEL standards to tissue extract was linear and parallel to that of cold MEL standards over the range tested. The detection limit for tissue was 3·5 pg ml^{-1} of the extract. The intra and interassay c.v. for tissue MEL were 8·5 and 14·9%, respectively. Tissue MEL concentration was expressed as pg mg^{-1} protein.

T_4 analyses

For T_4 determination, tissue samples were sonicated in 0·05 M phosphate buffer (pH 7·4) containing 0·01% 6-propyl-2-thiouracil (PTU; Sigma-Aldrich) using Microson^{TM} XL (Misonix). After centrifugation of homogenates at 15 000 **g** for 20 min, supernatants were extracted according to Tagawa & Hirano (1987) method with the modification by Edeline *et al.* (2004). Samples were pooled and collected prior to T_4 and protein analyses. T_4 concentrations were detected by total thyroxine RIA-gnost kit (Schering; www.cisbiointernational.fr). The assay was performed using tubes coated with antibodies and 8-anilino-1-sulphonic acid (ANSA) as a displacement reagent. All samples in duplicate were counted in a Wallac Wizard γ-counter. The labelled T_4 with iodine-125 was used as a tracer for RIA. The detection limit was 2·5 ng mg^{-1} of the extract. The intra-assay c.v. was 5·9%. The interassay variation was not determined, because all samples were measured in the same assay. Tissue T_4 concentration was expressed as ng mg^{-1} of protein.

Protein analysis

Protein content was determined by the Lowry with Peterson's modification (Peterson, 1977) using a total protein kit (Sigma-Aldrich).

STATISTICAL ANALYSIS

Values are presented as means ± s.e. For multiple comparisons, ANOVA was used. *Post hoc* comparisons were made by the Tukey's test and the Spojtvoll–Stalin test. Significance was taken at $P < 0{\cdot}05$. Statistical analysis was performed using the STATISTICA programme (www.statsoft.com).

RESULTS

EXPERIMENT I: WHOLE-BODY MEL AND T_4 CONCENTRATION DURING DEVELOPMENT

MEL level increased until 10 dph with the highest MEL contents being observed between 6 and 10 dph. During the following days (up to 70 dph), MEL concentration diminished [Fig. 1(a)]. T_4 content was very low during the first 3 dph. Then a transient increase was observed until 9 dph. From 10 to 40 dph, however, T_4 content decreased and from 50 to 70 dph, T_4 concentration steadily increased [Fig. 1(b)].

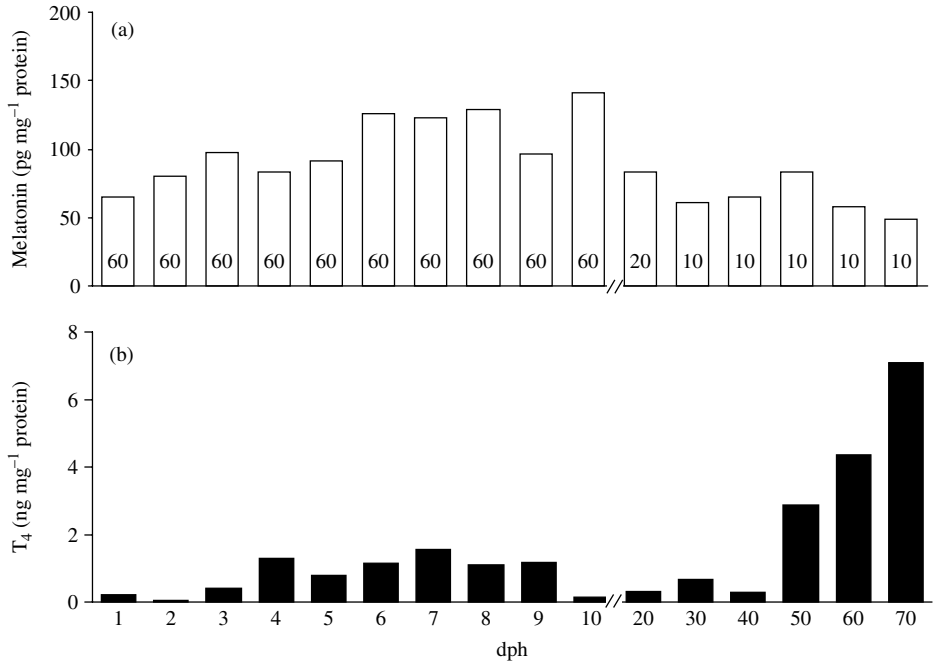

FIG. 1. Whole-body (a) melatonin and (b) thyroxine (T_4) concentrations in *Sparus auratus* from 1 to 70 days post-hatch (dph). Samples were taken at 1200 hours. Numbers of larvae pooled in each sample are given in (a).

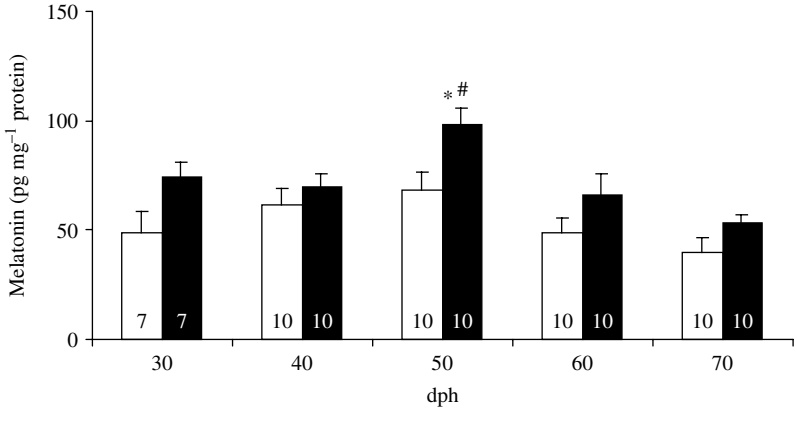

FIG. 2. Mean + S.E. head (□) and trunk (■) melatonin concentrations in postlarvae of *Sparus auratus* in relation to days post-hatch (dph). Samples were taken at 1200 hours. Numbers of samples are given. $^{*}P < 0{\cdot}05$ *v*. trunk of 30, 40, 60 and 70 dph; $^{\#}P < 0{\cdot}05$ *v*. head.

EXPERIMENT II: HEAD AND TRUNK MEL CONCENTRATION DURING DEVELOPMENT

Significant head–trunk difference in MEL level was observed only at 50 dph with trunk MEL content being higher than head levels (Fig. 2).

EXPERIMENT III: INFLUENCE OF DIFFERENT LIGHT REGIMES ON HEAD AND TRUNK MEL CONCENTRATION

In 67 day postlarvae under 12L:12D condition, distinct day and night differences in head MEL concentrations were observed, which was not evident in the trunk [Fig. 3(a)]. The diurnal changes in MEL contents were not observed in the head or trunk under DD [Fig. 3(b)] or LL regimes [Fig. 3(c)]. Head and trunk MEL concentrations decreased under both 0L:24D and 24L:0D conditions as compared with 12L:12D, and head–trunk differences completely disappeared under 24L:0D.

EXPERIMENT IV: INFLUENCE OF DIFFERENT LIGHT REGIMES ON PLASMA MEL CONCENTRATION IN JUVENILES

Plasma MEL concentrations in juveniles *S. auratus* plasma at 2400 hours were significantly higher then those at 1200 hours in fish kept under 12L:12D and 0L:24D conditions (Fig. 4). Under 24L:0D, however, MEL content at 2400 hours was below that at 1200 hours (Fig. 4). MEL concentrations at 1200 hours were similar under all experimental conditions, whereas these at 2400 hours differed significantly (Fig. 4).

DISCUSSION

In this study, for the first time, MEL and T_4 concentrations were studied during larval and postlarval development in *S. auratus*. Although in many studies MEL and enzymes involved in its synthesis have been identified at the very early stages of fish ontogenesis (Gothilf *et al*., 1999; Kazimi & Cahill, 1999; Roberts *et al*., 2003), a distinct role of MEL during development is still not clear. In the present studies, high levels of whole-body MEL were observed at the beginning of *S. auratus* post-embryonic life. Probably, at that time, there were three sources of the hormone, the yolk, pineal organ and retina, which contributed to the pool of MEL in the organism. MEL synthesis in the yolk has been recently postulated in avian eggs and early embryos (Olszańska *et al*., 2007). Moreover, it has been demonstrated that yolk of birds, reptiles and fishes is rich in MEL (Blount *et al.,* 2000; Yamada *et al.,* 2002; Olszańska *et al*., 2007). In fishes, however, synthesis of MEL in the pineal organ and retina starts during embryogenesis (Gothilf *et al*., 1999; Kazimi & Cahill, 1999; Roberts *et al.,* 2003). In the majority of cases, plasma MEL in adults reflects mainly the pineal production, whereas retinal MEL is a paracrine signal (Falcón *et al*., 2007). In fish larvae, however, eyes are large with respect to body size and thus the retina may make a quantitatively important contribution to the whole-body MEL.

The increase in whole-body MEL content observed in *S. auratus* larvae between days 6 and 10 coincides with the 'transition phase' described by Tanaka *et al*. (1995) in marine fish larvae. At this time, in *S. auratus* levels of hormones involved in growth, metabolism and development, *i.e*. growth hormone (GH) and prolactin (PRL), are very low (Herrero-Turrión *et al*., 2003*a*, *b*). It has been demonstrated that the number of GH cells and GH mRNA expression does not increase until 30 dph

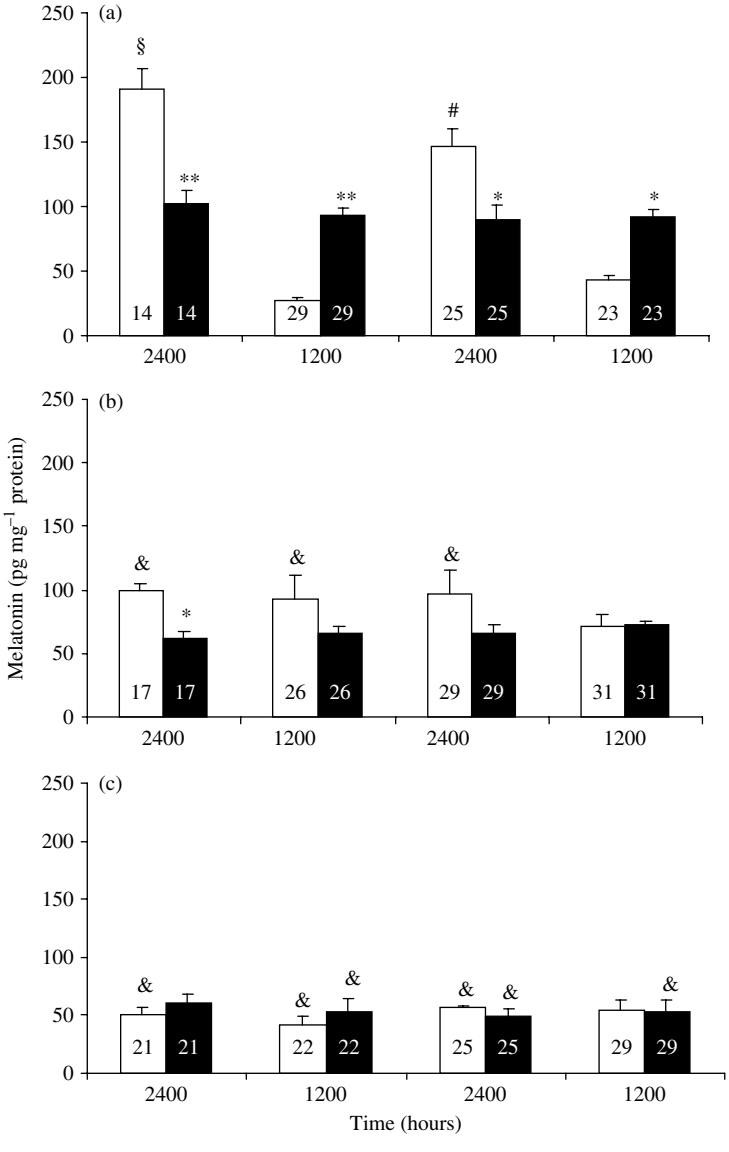

FIG. 3. Mean + S.E. head (□) and trunk (■) melatonin concentrations in 67 day postlarvae of *Sparus auratus* maintained under different lighting regimes:(a) 12L:12D, (b) 0L:24D and (c) 24L:0D. Samples were taken twice a day at 1200 hours and 2400 hours. Numbers of samples are given.
(a) Trunk values were significantly different from head values at all time ($*P < 0{\cdot}05$, $**P < 0{\cdot}01$). Head values were significantly different between 2400 and 1200 hours ($\S P < 0{\cdot}001$; $\#P < 0{\cdot}01$). (b) Trunk value was significantly different from head value at 2400 hours ($*P < 0{\cdot}05$). Head values at 2400 hours under 0L:24D were significantly different from those at 2400 hours under 12L:12D. Head value at 1200 hours under 0L:24D was significantly different from that at 1200 hours under 12L:12D ($P < 0{\cdot}05$). (c) Head values at 2400 hours under 24L:0D were significantly different from those at 2400 hours under 12L:12D ($P < 0{\cdot}05$). Head values at 1200 hours under 24L:0D and 0L:24D were significantly different ($P < 0{\cdot}05$). Trunk values at 1200 hours under 24L:0D and 12L:12D were significantly different ($P < 0{\cdot}05$). Trunk value at 2400 hours under 24L:0D was significantly different from that at 2400 hours under 12L:12D ($P < 0{\cdot}05$).

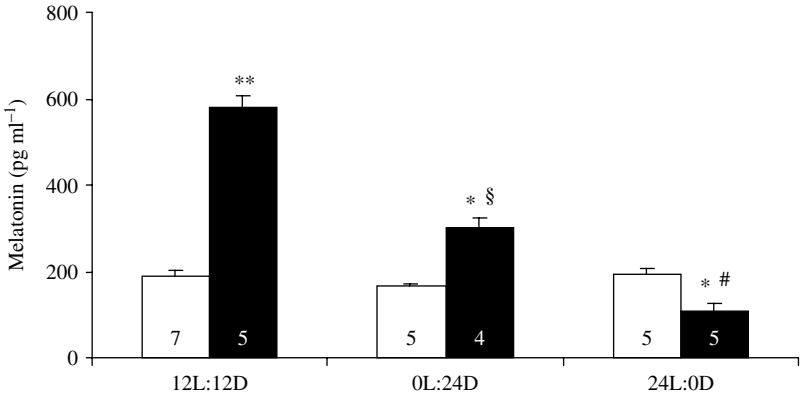

FIG. 4. Mean + S.E. plasma melatonin concentrations in samples collected at 1200 hours (□) and 2400 hours (■) from *Sparus auratus* juveniles exposed to different lighting regimes:12L:12D, 0L:24D and 24L:0D. Numbers of fish are given. Plasma values were significantly different between 1200 and 2400 hours at each light regime (*$P < 0{\cdot}05$; **$P < 0{\cdot}01$) and between different light regimes at 2400 hours; #$P < 0{\cdot}01$, 24L:0D *v*. value at 2400 hours under 12L:12D; §$P < 0{\cdot}05$, 0L:24D *v*. values at 2400 hours under 12L:12D and 24L:0D.

(Herrero-Turrión *et al*., 2003*b*). Similar observations were made in silver sea bream *Sparus sarba* (Forsskål), Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel), red sea bream *Pagrus major* (Temminck & Schlegel), black sea bream *Acanthopagrus schlegeli* (Bleeker) and temperate sea bass *Lateolabrax japonicus* (Cuvier) (Tanaka *et al*., 1995; Deane *et al*., 2003). In quickly developing larvae, when GH and PRL supply is still insufficient, MEL may play a role in stimulation of cell proliferation and differentiation processes, as it has been postulated in *D. rerio* (Danilova *et al*., 2004). Further studies, however, are needed to verify this hypothesis.

Vigorous cell proliferation and metabolic processes at early stage of post-embryonic development, however, are important sources of free radicals. The antioxidant enzymes such as glutatione peroxidase (GPX), superoxide dismutases (SOD) and catalase (KAT) play an important role in protection from oxidative stress during larval development and metamorphosis. Many fish species show significant changes in activity of antioxidant enzymes when their larvae change from endo- to exogenous feeding (Kalaimani *et al*., 2008). Moreover the altered diet, *i.e*. from live to inert food, enhances antioxidant defences in Senegalese sole *Solea senegalensis* Kaup larvae (Fernández-Díaz *et al*., 2006). MEL, which is present in high concentrations in larvae, is known as an effective free-radical scavenger in vertebrates (Reiter *et al*., 2000). In birds and mammals, MEL is shown to stimulate the activity of the antioxidant enzymes GPX, SOD and KAT, and the expression of their genes (Rodriguez *et al*., 2004). Therefore, MEL may be involved in defence against free radicals as an efficient oxidant scavenger and a regulator of antioxidant enzymes in *S. auratus* larvae.

In adult fishes, besides two main sources of MEL, *i.e*. pineal organ and retina, the GIT is considered a site of MEL production (Bubenik & Pang, 1997; Fernández-Durán *et al*., 2007). There is no information, however, when MEL synthesis appears in the intestine during fish development. In mammals, it has been shown that the main sites for MEL synthesis in the intestine are the enterochromaffin cells

of the mucosal epithelium, where the hydroxyindole-O-methyltransferase (HIOMT) and AA-NAT-activities are shown (Bubenik & Pang, 1997; Kvetnoy, 1999). In the intestine of fishes, MEL synthesis is postulated in both mucosal and non-mucosal tissues (Fernández-Durán *et al*., 2007). In *S. auratus*, at the lecitoexotrophic period (4 dph), when the mouth is open and endo and exogenous feeding coexist, the enterocytes are morphologically similar to those of adults and thus may produce MEL (Sarasquete *et al*., 1995; Calzada *et al*., 1998; Elbal *et al*., 2004). In mammals, MEL that is produced in the GIT contributes significantly to the hormone concentration in circulation (Bubenik, 2002). The high MEL levels measured in the trunk of larvae and postlarvae in this study support the evidence of MEL production in the GIT of fishes. Moreover, higher MEL content in the trunk than in head during the day suggests the synthesis of MEL in the GIT is not influenced by light. A role of MEL in the GIT of mammals seems to be associated with regulation of food intake and digestion (Bubenik, 2002; Konturek *et al.,* 2007), but in fishes this has yet to be elucidated (Fernández-Durán *et al*., 2007). A very high MEL level, however, was observed in the trunk of 50 dph postlarvae after initiation of feeding with commercial pellets. At the same time, and according to Yúfera *et al*. (2004), HCl is also secreted into the stomach after feeding in this species. Therefore, it is presumed that MEL intense production in GIT may be linked to control of the bicarbonate secretion in the fish intestine, similarly to that in humans and rat (Sjöblom, 2005). Trunk MEL values at 0L:24D and 24L:0D are significantly lower than those at 12L:12D. Assuming that GIT is a main source of trunk MEL, the lower trunk MEL concentration at 0L:24D than that at 12L:12D may be an effect of limited food intake by fish of diurnal activity while kept in continuous darkness. The lower trunk MEL concentration at 24L:0D is in accordance with the observation of the reduced food intake by day-active goldfish *Carassius auratus* (L.) kept in constant light (López-Olmeda *et al*., 2006).

Most adult fishes, including *S. auratus*, possess intrapineal circadian clocks driving the rhythm of MEL production (Bolliet *et al*., 1996; Molina-Borja *et al*., 1996; Coon *et al*., 1998). In *D. rerio* and *F. heteroclitus*, circadian rhythms of AA-NAT mRNA and MEL are present as early as before hatching (Gothilf *et al*., 1999; Kazimi & Cahill, 1999; Roberts *et al*., 2003). As far as is known, however, there is no information on when MEL rhythms first appear in *S. auratus*. In the present studies of 67 days *S. auratus*, a distinct day–night difference in MEL concentration in the head was observed, but only in the 12L:12D condition, and thus an endogenous rhythm of MEL synthesis in 67 day *S. auratus* was not demonstrated. In juveniles (*c*. 6 months old), however, the distinct day–night MEL variations observed at 12L:12D and 0L:24D conditions indicated the presence of an intrinsic rhythm of MEL production. Thus, the data suggest that at early ontogenesis of *S. auratus* MEL role is related mostly to the control of development and protection against free radicals, whereas its action as a time-keeping molecule developed later.

The results of the studies in *D. rerio*, grouper *Epinephelus coioides* (Hamilton), Japanese eel *Anguilla japonica* Temminck & Schlegel, *S. senegalensis*, chum salmon *Oncorhynchus keta* (Walbaum) and Pacific bluefin tuna *Thunnus orientalis* (Temminck & Schlegel) demonstrate an essential role of TH during the transition from larva to juvenile (Tagawa & Hirano, 1989; Brown, 1997; de Jesus *et al*., 1998; Yamano *et al*., 2007; Klaren *et al.,* 2008). The TH, which are required for post-embryonic development, organogenesis and metabolism, have been shown to be present in the eggs, yolk sac, embryos and larvae of many teleost species

(Power *et al*., 2001; Deane & Woo, 2003;Yamano, 2005). The pattern for TH changes may reflect the distinct phases of larva development. The very low contents of T_4 observed in *S. auratus* larvae during the first 3 days in the present study agree with those reported previously for this species and for *S. sarba* (Deane & Woo, 2003; Szisch *et al*., 2005). It is probably an effect of ongoing yolk-sac absorption with no production of the hormone (Tagawa & Hirano, 1989). The slight increase in T_4 content observed in larvae from 4 to 9 dph is probably linked to the onset of hormone production in thyroid gland in *S. auratus* (Tanaka *et al*., 1995; García Ayala *et al*., 2003). The decrease in T_4 concentration until 40 dph is probably an effect of T_4 conversion to T_3 by deiodinases, which are shown to be active at that time (Szisch *et al*., 2005). The increase in T_4 concentration during *S. auratus* larva-juvenile transition, *i.e*. between 50 and 70 dph, is consistent with earlier reported increases of both T_4 and T_3 concentrations in *S. auratus* and *S. sarba* (Deane & Woo, 2003; Szisch *et al*., 2005).

In *S. auratus*, during transition phase, increased T_4 was observed concurrently with decreased MEL concentration. Thus, it may suggest an inverse relationship between T_4 and MEL. During gonadal development of walking catfish *Clarias batrachus* (L.), TH levels in thyroid gland are higher after pinealectomy (Nayak & Singh, 1987*b*). In the same species, during the prespawning phase, MEL administration has an inhibitory effect on TH (Nayak & Singh, 1987*a*). During metamorphosis of American bullfrog *Rana catesbeiana* (Shaw), the rise of plasma T_4 was observed simultaneously with the decrease in plasma MEL concentration (Wright *et al*., 2003, 2004). Administration of MEL alone or with thyroid stimulating hormone (TSH) diminished T_4 secretion from tadpole thyroids *in vitro* in *R. catesbeiana*, northern leopard frog *Rana pipiens* (Schreber) and green frog *Rana clamitans* (Latreille) (Wright *et al.,* 1996, 1997). Moreover, MEL was shown to decrease thyrocytes morphometry and thyroid mass in tadpoles of *R. pipiens* and *R. catesbeiana* species as well as in mammals, *i.e*. rat *Rattus norvegicus* (Berkenhout) and Indian palm squirrel *Funambulus pennanti* L. (Haldar *et al*., 1992; Klencki *et al*., 1994; Wright *et al*., 1996). An inhibitory effect of MEL on T_4 production and release was observed in *F. pennanti* and Syrian hamster *Mesocricetus auratus* (Waterhouse) (Vriend, 1985; Haldar *et al*., 1992). Further work, however, is needed to prove that there is a functional interaction between MEL and TH in fish larvae.

Taken together, the high concentrations of MEL in larvae at early stage of development suggest a distinct role of the hormone in early organogenesis and development of *S. auratus*, when supply of GH and PRL is insufficient. GIT is probably an important source of extrapineal and extraretinal MEL in larvae. The MEL time-keeping signal does not appear before 67 days, although it is evident in 6 month juveniles. Thus, it is tempting to speculate that at early ontogenesis of *S. auratus* MEL role is related mostly to control of development and protection against free radicals, whereas its time-keeping role develops later. Further work is required to determine a distinct role of MEL during larval development.

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