STUDY OF THYROID-PINEAL INTERRELATIONSHIP IN THE FISH, *Clarias gariepinus*

BY

YUMKHAIBAM PREMABATI
DEPARTMENT OF ZOOLOGY

SUBMITTED

IN

PARTIAL FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

OF

NORTH-EASTERN HILL UNIVERSITY
SHILLONG - 793 022

DECLARATION

NORTH-EASTERN HILL UNIVERSITY

SHILLONG - 793 022

MARCH, 2005

I, Ms. Yumkhaibam Premabati, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for award of the degree of Doctor of Philosophy in Zoology.

Y. Premabati

(Candidate)

B. B. P. Gupta

(Head)

Head
Department of Zoology
North Eastern Hill University
Shillong

B. B. P. Gupta

(Supervisor)

Prof. B. B. P. Gupta
Department Of Zoology
North-Eastern Hill University
Shillong—793022

CONTENTS

ACKNOWLEDGEMENT

I express my sincere gratitude to Professor B. B. P. Gupta, F. N. A. Sc., Department of Zoology, North-Eastern Hill University, Shillong for his benign guidance, encouragement and invaluable help throughout the execution of this work, without which this work would not have been completed.

I express my heartfelt gratitude to the Head of the Department of Zoology, Prof. B. B. P. Gupta, and former Head, Prof. K. Chatterjee for providing me the necessary infrastructure laboratory facilities to carry out my research work. I am thankful to the Dean of School of Life Sciences for the administrative help, and to all the faculty members and the non-teaching staff of the Department of Zoology for their kind help and co-operation.

I am thankful to the Head of the Department of Biochemistry, Prof. A. N. Rai and the former Head of the department, Prof. R. Sharma for allowing me to use the Liquid Scintillation counter. I am also thankful to the former Head of the Department of Botany, Prof. A. K. Mishra for allowing me to use the B. O. D. incubators.

I express my deep gratitude to all my laboratory colleagues and my friends for their kind help and encouragement. I express my sincere thanks to Mr. P. Munindro Singh for his help, encouragement and support in every possible way.

My sincere thanks also go to my parents and relatives for their care and blessings, constant unfailing assistance, moral support and encouragement.

Financial assistance provided by Department of Zoology, N. E. H. U under DRS- III (SAP) programme as a Project fellow and by CSIR, New Delhi in the form of a Senior research fellowship is gratefully acknowledged.

Y. Premabati

(Yumkhaibam Premabati)

Department of Zoology
North-Eastern Hill University
Shillong 793 022

PREFACE

Fishes perceive environmental light regimes with the help of eyes and the pineal complex. The pineal complex of the fish consists of a pineal gland and the parapineal (Vollrath, 1981). However, melatonin is produced only by the pineal and not by the parapineal. The fish pineal contains photoreceptor cells, supporting cells and glial cells (Vollrath, 1981). The photoreceptor cells possess a complete melatonin rhythm generating system incorporating a photodetector, a circadian clock and melatonin synthesizing machinery. As a result, the fish pineal has developed capabilities to modulate melatonin synthesis by responding directly to environmental light and indirectly to changes in environmental temperature via the central nervous system to ensure successful adaptation under the ever-changing aquatic environment. Unlike in homeotherms, melatonin secretion in fishes also seems to be influenced by environmental temperature (Zachmann *et al.*, 1992; Tabata and Meissl, 1993; Samejima *et al.*, 2000). The regulation of melatonin synthesis in fish pineal by light and its modulation by temperature seems to suggest that the fish pineal might be acting as a phototransducer as well as a thermotransducer organ (Matty, 1985b; Meissl, 1986). In mammals, the pineal cannot respond directly to light, and photic signals are received through lateral eyes and transmitted through suprachiasmatic nucleus of the hypothalamus to the pinealocytes (Vollrath, 1981; Korf *et al.*, 1998). The pineal gland acts as an interface between the environmental photoperiod and internal milieu, and translates photoperiodic information into a hormonal messenger. Therefore, melatonin is known as the chemical expression of darkness (Reiter, 1993a).

Melatonin acts as a pineal hormone and regulates/modulates a wide range of vertebrate physiology. In mammals, melatonin is involved in regulation of circadian, and circannual rhythms, reproductive cycles, immune system, metabolism etc. (Vollrath, 1981; Reiter and Maestroni, 1999; Pevet, 2000). In birds, pineal showed cyclical patterns relative to environmental factors, which are correlated with plasma melatonin. Changes in environmental factors promote annual variations in adrenal and gonadal activity in birds, probably by modulating the pineal gland (Sudhakumari *et al.*, 2001). Melatonin is considered to be the most physiologically active indole derivative in fishes, and reportedly produces prominent effects on pigmentation, reproductive system (Garg, 1989), locomotor activity (Garg and Sundararaj, 1986; Morita *et al.*, 1989), growth, metamorphosis, pituitary, interrenal function (Vollrath, 1981), thyroid (Nayak and Singh, 1987a,b), adrenal (Agha and Joy, 1989) etc.

As in other vertebrates, the fish thyroid also secretes thyroxine (T_4) and triiodothyronine (T_3) as major hormones. Other iodothyrhyronines like monoiodo-thyronine and diiodothyronine are also synthesised by the thyroid, and released in to blood (Melamed *et al.*, 1995; Mylonas *et al.*, 1997). In fishes, the thyroid hormones are actively involved in the regulation of the intermediary (Plisetskaya *et al.*, 1983), the oxidative metabolism (Gupta and Thapliyal, 1991; Lynshiang and Gupta, 2000), reproduction (Bhattacharya, 1987), growth and development (Power *et al.*, 2001), breeding cycle (Volkoff *et al.*, 1999), sexual maturation (Ueda *et al.*, 1984; Matty,

1985a; Cyr *et al.*, 1998; Pavlidis *et al.*, 2000), migration (Ueda *et al.*, 1984; Matty, 1985a), electrolyte and water metabolism (Peter *et al.*, 2000) etc.

There is a large body of information on seasonal variations in thyroid activity and the circulating levels of thyroid hormones in mammals (Singh *et al.*, 2002; Ben Saad and Maurel, 2004), birds (Dawson and Thapliyal, 2001), reptiles (Licht *et al.*, 1985a,b; Naulleau *et al.*, 1987; Kohel *et al.*, 2001) and amphibians (Kuhn *et al.*, 1983; Gancedo *et al.*, 1996). But there is scarcity of information on seasonal variations in the levels of thyroid hormones and melatonin in fish species (Iigo *et al.*, 1997; Samejima *et al.*, 1997). Both circadian and circannual rhythms in the circulating levels of T_4 and T_3 in fish species are reportedly influenced by fluctuations in the ambient temperature, physical activity, photoperiod, feeding etc. (Matty, 1985a; Cyr *et al.*, 1988; Leiner *et al.*, 2000; Pavlidis *et al.*, 2000), and seem to be closely associated with circannual variations in ambient temperature, daylengths and gonadal steriods (Pavlidis *et al.*, 2000). The seasonal fluctuations in photoperiods and temperature have also been reported to regulate the circadian and circannual rhythms of melatonin production in fishes (Randall *et al.*, 1995; Pavlidis *et al.*, 1999). Thyroid hormone production is maximum during summer when daylengths are long and pineal activity/melatonin production is minimum (Cyr *et al.*, 1988, 1998; Leiner and MacKenzie, 2001). Low levels of thyroid hormones are found in fish during winter (Cyr *et al.*, 1998; Pavlidis *et al.*, 2000) when daylengths are short and pineal activity is high (Zachmann *et al.*, 1992; Tabata and Meissl, 1993; Samijima *et al.*, 2000). Melatonin has been reported to

influence thyroid activity in mammals (Vriend, 1983a,b; Rom-Bugoslavaskaia and Shchervakova, 1985; Shchervakova and Rom-Bugoslavaskaia, 1988; Wajs and Lewinski, 1992; Ozturk *et al.*, 2000) and birds (Singh and Turner, 1972; John *et al.*, 1990; Prakash *et al.*, 1998), and pineal activity is reportedly influenced by thyroid hormones in mammals (Karasek and Stepien, 1980; Bondarenko, 1991). The levels of thyroid hormones are inhibited by administration of melatonin and increased after pinealectomy in mammals (Vaughan *et al.*, 1982; Vriend *et al.*, 1982; Vriend, 1983a,b, 1984; Vaughan and Priutt, 1985; Wajs and Lewiski, 1992; Ozturk *et al.*, 2000) and birds (Sharp *et al.*, 1984; John *et al.*, 1990; Prakash *et al.*, 1998). Pinealectomy in the fish *Clarias batrachus* during developmental and maturation phase has been reported to stimulate thyroid hormones (Nayak and Singh, 1987b). However, unlike in mammals, there is scarcity of information on the effects of melatonin on the circulating levels of thyroid hormones in any fish species. Though melatonin and thyroid hormones are involved in the regulation of a wide range of physiology as well as the gonadal cycles of fishes, so far no attempt has been made to investigate the interrelationship between pineal and thyroid in a fish with special reference to its annual gonadal cycles, temperature and photoperiod. Therefore, keeping in mind the scarcity of information and importance of melatonin, thyroid hormones, temperature and photoperiod in fish physiology, it was thought worthwhile to undertake a comprehensive study to explore the nature of interrelationship, if any, between the thyroid and the pineal gland in an air-breathing exotic cat fish, *Clarias gariepinus*.

The present Ph. D dissertation has been divided into six chapters. An introduction of the chapters is given in the following sections.

Chapter - 1: Materials and methods

This chapter deals with the details of materials and methods used for this Ph. D dissertation. It incorporates description of the experimental animals, mode of treatment, methods used for measuring the pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity, and plasma concentrations of T_4 and T_3, and the statistical and computational techniques used for analysing the data.

Chapter - 2: Studies on the interrelationship between the circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity

This chapter deals with the study of circadian rhythms in the plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity in the fish maintained under natural climatic conditions during quiescent, progressive, breeding and regressive phases of the annual breeding cycle.

Chapter - 3: Effects of photoperiods and simulated temperatures on circadian rhythms of plasma levels of thyroid hormones (T_4 and T_3) and pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity

This chapter deals with the effects of long photoperiod (LD 15:9), LD 12:12 and short photoperiod (LD 9:15) at a constant temperature ($25^{\circ} \pm 2^{\circ}$ C) on the circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during winter and summer seasons. In addition, it also deals with the effects of simulated temperatures (i.e., $15^{\circ} \pm 2^{\circ}$ C, $25^{\circ} \pm 2^{\circ}$ C and $35^{\circ} \pm 2^{\circ}$ C) under LD 12:12 photoperiod on plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during winter and summer seasons.

Chapter-4: *In vivo* effects of melatonin on plasma levels of thyroxine (T_4) and triiodothyronine (T_3)

This chapter deals with the *in vivo* effects of different doses of melatonin on plasma levels of T_4 and T_3 during quiescent, progressive, breeding and regressive phases of the annual breeding cycle.

Chapter - 5: Effects of thyroid hormones and propylthiouracil on the circadian rhythms in pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity

This chapter deals with the effects of immersion in solutions of thyroid hormones (T_4 and T_3) and propylthiouracil on circadian rhythms in pineal AA-NAT activity during winter and summer seasons.

Chapter - 6: Summary and conclusions

This chapter incorporates the summary of major findings presented in the above mentioned chapters and conclusions derived from the findings of the Ph. D dissertation.

Findings of this dissertation suggest that there is an inverse interrelationship between the AA-NAT activity and thyroid hormones in the fish, *Clarias gariepinus*. The opposite effects of temperature and photoperiods on the plasma levels of thyroid hormones and AA-NAT activity seem to regulate the inverse relationship between thyroid hormones and AA-NAT activity. Increased levels of thyroid hormones and low levels of melatonin due to decreased AA-NAT activity during summer are closely associated with the breeding phase. In contrast, decreased levels of thyroid hormones and increased melatonin synthesis due to increased AA-NAT activity during the winter seem to be responsible for inactivation of gonads and cessation of breeding during the regressive and quiescent phase.

INTRODUCTION

Animals have evolved strategies in order to time their reproductive activity at a particular time of the year when maximal survival of the offspring is ensured. The vertebrates programme the exact time of their breeding with the help of the circannual changes in the environmental factors such as photoperiod, temperature, rainfall, humidity, salinity etc. (Wilson and Donham, 1988; Spieler, 1990). Among the climatic factors, changes in daylength play a major role in synchronizing the breeding phase, and exert a variety of influence on the physiology and behavior of a large number of vertebrates (Binkley, 1993). The circannual variation in daylength serves as a calendar, allowing organisms to determine the time of the year, and thereby predict changes in the environment (Reiter, 1993a). Thus, the annual changes in the duration of the solar day has been proved to be the primary and regular variable that individually, or in combination with other environmental component(s), impel the 'driving function' in determining the physiological response in most animal species (Nicholls *et al.*, 1988; Wilson and Donham, 1988). Accordingly, most of the living organisms have developed adaptive mechanisms allowing anticipation and perception of changes in daylength. Lateral eyes and the pineal play a major role in detection of the daylength. The photoperiod influences pineal activity in mammals through the lateral eyes. Unlike in mammals, light is perceived directly by the non-mammalian photoreceptive pineal. The pineal gland exhibits circadian and circannual rhythms, which are a fundamental feature of all living organisms. The functional mechanism involved is built around internal biological clock(s), and the hormone melatonin is one of its critical components.

Numerous sources of melatonin have been identified in vertebrates, but melatonin is primarily produced by the pineal gland during the dark period of the light-dark cycle. This rhythm of melatonin is generated by a circadian clock. The periodic secretion of melatonin acts as a circadian mediator of a system that can read the message. The duration of the nocturnal melatonin production is directly proportional to the length of the dark phase of a photoperiodic regime. Thus, melatonin rhythm appears to be an endocrine code of the environmental light-dark cycle conveying photic information that is used by vertebrates for both circadian and seasonal temporal organization.

The thyroid gland and its hormones are found in all the vertebrates. The thyroid hormones perform diverse functions. Although the functions of the thyroid hormones are diverse, their major underlying role may be to support energy-demanding activities undertaken in favorable environment (Eales, 1979). It would be expected that annual cycles in thyroid hormones production increased during discrete annual periods of increased metabolic activity, and decreased during periods of metabolic quiescence, such as winter months. The environmental seasonal changes of photoperiod, temperature and rainfall seem to provide predictive information as indicators of seasons (Pavlidis *et al.*, 2000) and influence the activity of the neuroendocrine system of fish (Yokota and Oishi, 1992), which ultimately regulates the activities of various endocrine glands (Verma *et al.*, 1996). Increase in temperature has been reported to accelerate the synthesis and turnover of thyroid hormones and to increase the physical activity and metabolic rate of teleosts (Leloup and de-Luze, 1985; Nayak and Singh, 1991). It has

been reported that the circulating thyroid hormones exhibit distinct circadian and circannual rhythms in fish species (Bau and Parent, 2000; Pavlidis *et al.*, 2000; Leiner and Mackenzie, 2001), which are regulated mainly by ambient temperature and photoperiod (Grau *et al*, 1985; Cyr *et al.*, 1988,1998; Cerda-Reverter *et al.*, 1996). It has also been reported that T_3 controls mainly the metabolism, while T_4 is involved in the regulation of growth, development, sexual maturation and reproduction (Eales, 1979; Gupta and Thapliyal, 1991; Pavlidis *et al.*, 2000; Power *et al.*, 2001).

A large body of available information suggests that both thyroid hormones and melatonin play a major role in the regulation of annual breeding cycle of vertebrates (Malpaux *et al.*, 1999; Volkoff *et al.*, 1999; Joshi and Udaykumar, 2000; Lincoln *et al.*, 2003c) and migration (Ueda *et al.*, 1984; Matty, 1985a). As mentioned earlier, the circadian and circannual changes in environmental factors like daylength and temperature shape the circadian and circannual rhythms of thyroid and pineal activity, and hence that of the thyroid hormones and melatonin. It seems that the thyroid gland and pineal, while responding to the environmental factors, also interact with each other through their hormones for an integrated coordination of bodily functions in vertebrates. In mammals and birds, melatonin has been reported to influence thyroid activity (John *et al.*, 1990; Ozturk *et al.*, 2000), while pineal activity is influenced by thyroid hormones (Karasek and Stepien, 1980; Vriend, 1983b; Bondarenko, 1991). In the fish, thyroid hormone production is maximum during summer when daylenghts are long and pineal activity/melatonin production is minimum. In contrast, low levels of thyroid

hormones are found in fish species during winter when daylengths are short and pineal activity is high (Vivien-Roel and Arendt, 1981; Zachmann *et al.*, 1992; Tabata and Meissl., 1993; Samejima *et al.*, 2000).

Thyroid hormones play a major role in the regulation of the oxidative metabolism in all groups of vertebrates (Barre and Rouanet, 1983; Nelson *et al.*, 1984; Gupta and Thapliyal, 1991). There are also several reports on the involvement of the pineal complex in regulation of body temperature in mammals (Heldmaier *et al.*, 1989; Larkin *et al.*, 2001), birds (Lee *et al.*, 1990; Wolfe and Zatz, 1994; Zatz *et al.*, 1994; Barrett and Takahashi, 1995; Murakami *et al.*, 2001) and reptiles (Vivien-Roels *et al.*, 1988; Skene *et al.*, 1989; Moyer *et al.*, 1997; Ohshima *et al.*, 1999; Lutterschmidth *et al.*, 2002). Thus, there is a strong probability that, as important components of the neuroendocrine system of vertebrates, thyroid and pineal might be interacting and coordinating with each other in order to regulate the energy metabolism of the animals in the most efficient ways.

The neuroendocrine system acts as an interface between the environment and the vertebrates, and plays a major role in adaptation against ever changing environment. In general, the changes in environment alter the synthesis and release of a number of hormones. Therefore, there might be a definite interrelationship between the environmental factors and endocrine glands leading to active interactions among different endocrine glands with overlapping functions. Since both thyroid and pineal are

reportedly involved in the regulation of breeding cycle as well as the energy metabolism of vertebrates, attempts have been made to study the effects of photoperiod and temperature on thyroid activity and melatonin synthesis as well as the interrelationship of thyroid and pineal gland in higher vertebrates. A brief review of information available on impact of environmental factors on activities of thyroid and pineal as well as on the interrelationship between the two glands in different groups of vertebrates is given below.

Mammals

There are several reports on molecular components of adrenergic signal transduction and melatonin synthesis in mammalian pineal (Maronde *et al.*, 1999; Guillaumond *et al.*, 2000; Gupta *et al.*, 2001). In the mammals, light information is received through the lateral eyes and conveyed through the suprachiasmatic nucleus (SCN) and mammals cannot respond directly to light to control pineal melatonin synthesis (Vollrath, 1981; Korf *et al.*, 1998). The photic signal stimulates retinal cells and generates neural impulse. The neural impulse from retina is transmitted through the optic nerve via optic chiasma, supra chiasmatic nucleus (SCN) and via different parts of the brain. It reaches finally to superior cervical ganglion (SCG) via brain stem. Post synaptic sympathetic nerve fibers originate from SCG and form a nerve commonly called as nervi conarii. The nerve endings get hyperpolarized due to impulse generated by the presence of light. Due to hyperpolarization, the release of nor-epinephrine (NE) in the pineal gland is inhibited during the light phase. In the absence of NE, there is no

induction of the rate-limiting enzyme arylalkylamine N-acetyltransferase (AA-NAT). With the onset of dark phase, the generation of impulse in the retina is inhibited which leads to depolarization of the sympathetic nerve fibers terminating in the pineal gland. The depolarization in the nerve terminus leads to increased release of norepinephrine. The NE released from the adrenergic nerve terminal acts on the pinealocytes and stimulates the rate of melatonin synthesis by inducing AA-NAT, which acts as the rate-limiting enzyme in the melatonin biosynthetic pathway (Bronstein *et al.*, 1990; Privat *et al.*, 1999). Thus, the pineal gland translates the photoperiodic visual messages into chemical signal (melatonin). The pineal gland is the intermediary between the external photoperiod and internal milieu. It is the site at which light and dark information is translated, or transduced into a chemical messenger. Melatonin is thus known as the chemical expression of darkness (Reiter, 1993a), and has been described as a neuroendocrine transducer, which converts light-induced neural signal to a hormonal signal (Reiter *et al.*, 1983).

It appears that the photo-neuroendocrine mechanism is not fundamentally different in vertebrates as far as the role of melatonin is concerned. In mammals, melatonin is involved in regulation of circadian and circannual rhythms, reproductive cycles, immune system, metabolism etc. (Vollrath, 1981; Reiter and Maestroni, 1999; Pevet, 2000). Circadian and circannual melatonin or AA-NAT activity rhythms in mammals are influenced both by photoperiod and temperature (Larkin *et al.*, 2001). The circadian rhythm of melatonin is synchronized by the changes in photoperiod.

Melatonin synthesis shows a diurnal rhythm as well as seasonal modifications of the nocturnal peak (Steinlechner *et al.*, 1987; Reiter, 1993b). The daily rhythm is thought to be involved in the synchronization of various circadian functions (Redman *et al.*, 1983; Mc Arthur *et al.*, 1991), whereas the lengthening of the nocturnal melatonin peak (endogenously or exogenously) mimics the effects of short photoperiod on reproductive functions (Carter and Goldman, 1983; Bartness *et al.*, 1993). The precision with which photoperiodic vertebrates are able to discriminate daylength is remarkable. It has been reported that information about the time of year could be encoded in the total amount of melatonin synthesized per day, where the duration of elevated melatonin synthesis closely reflects the duration of the scotophase throughtout the seasons (Steinlechner *et al.*, 1987). The photoperiod appears to be the main factor in the regulation of the diurnal rhythm of melatonin (Skene *et al.*, 1987; Vivien-Roels *et al.*, 1997). It has been reported that pineal AA-NAT activity is suppressed by a short light pulse (e.g., 1 min of light exposure) and ultra-short light pulse (e.g., 1 milli second of light exposure) during the dark phase of the light-dark cycle (Maitra *et al.*, 1986; Vollrath *et al.*, 1989; Bronstein *et al.*, 1990). But in poikilotherms external temperature, in addition to photoperiod, also seems to play an important role in controlling melatonin rhythms (Vivien-Roels and Arendt, 1983; Underwood and Calaban, 1987; Vivien-Roels *et al.*, 1988).

Melatonin rhythms have frequently been related to seasonal cycles of reproduction in photoperiodic mammals (Reiter, 1980; Stetson and Watson-Whitmyre, 1986). Syrian hamsters exposed to 12.5 h of light/day (or more) maintain a functional

reproductive system, whereas those exposed to 12 h of light/day (or less) undergo complete gonadal regression (Karsch *et al.*, 1991). It has been reported that circadian clocks are involved in this photoperiodic time measuring capability. Use of several experimental lighting paradigms in Syrian hamster has revealed that reproductive state depends on the circadian timing of light presentation rather than on the total amount of light or the light/dark ratio. When the circadian system of Syrian hamsters was disrupted by SCN lesions, these animals no longer showed reproductive responses to changing photoperiod (Karsch *et al.*, 1991). Syrian hamster undergo gonadal regression after exposure to short days in the autumn, but gonadal activity resumes in the spring even under continued exposure to short photoperiod. Thus, in autumn the hamster is said to be photosensitive, while in spring it becomes photorefractory (Goldman and Darrow, 1983). Hamsters raised in a long day laboratory environment showed gonadal regression within 6-10 weeks following transfer to short days, regardless of the time of year at which short day exposure begins. After 20-24 weeks in short days, the hamsters become photosensitive and the gonads undergo complete recrudescence (Klemcke *et al.*, 1981). After exposure to long days for approximately 10 weeks, the hamsters are once again capable of undergoing gonadal regression in response to short days (Stetson *et al.*, 1977). Unlike Syrian hamster, the domestic pig has been found to exhibit a biphasic pattern of seasonal reproduction with reproductive performance being better during either spring or fall than during the summer and winter (Claus and Weiler, 1985; Armstrong *et al.*, 1986).

When placed as a subcutaneous depot, melatonin prevents gonadal regression due to exposure to darkness or evening melatonin injections in Syrian hamster and Djungarian hamster (Reiter, 1980). It has been reported that the mammalian pineal gland has receptors for various hormones such as estradiol, testosterone, 5α-dihydrotestosterone, progesterone, prolactin, corticosterone and somatostatin (Kellner *et al.*, 1997; Champier *et al.*, 2003). Melatonin has been found to exert its anti-reproductive effects in hamsters by decreasing estrogen receptor (ER) levels in neurons of the medial preoptic area, bed nucleus of the stria terminalis and ER mRNA of the hypothalamus in both intact and ovariectomized animals, thereby influencing steroid feedback mechanisms (Hill *et al.*, 1996). Oral melatonin administered 3 h before lights-off effectively inhibited endogenous ovarian activity and reversibly suppressed estrous elevations in fecal estrogens in domestic cat (Graham *et al.*, 2004). Melatonin administration also significantly decreased the levels of LH, estrogen and progesterone in women (Voordouw *et al.*, 1992). It has been reported that photoperiodic regulation of prolactin secretion occurs via melatonin-mediated changes (Johnston, 2004) and displays circannual rhythmicity under constant photoperiod (Lincoln *et al.*, 2003a) in the secretion of a prolactin from the pituitary pars tuberalis (Lincoln *et al.*, 2003b; Johnston, 2004). Melatonin injection given during the late light phase decreased reproductive organ weights and levels of serum and pituitary prolactin and serum T_4 (Hoover *et al.*, 1992).

It appears that two different types of time measurement mechanisms are used to shape the annual reproductive cycle in photoperiodic mammals. In one case, the animal is capable of monitoring daylength i.e., photoperiodic time measurement. In the second case, an endogenous mechanism appears to be used to measure long periods of time in the absence of changing environmental cues. This endogenous mechanism is responsible for deciding the time at which an animal switches from the photosensitive to the photorefractory state (Klemcke *et al.*, 1981). A diurnal rhythm of pineal melatonin production in most of the mammals (Klein *et al.*, 1983) is driven by the rhythm in the activity of the enzyme arylalkylamine-N-acetyltransferase (Illnerova *et al.*, 1983; Reiter *et al.*, 1983). The AA-NAT activity and melatonin rhythms are truly circadian as they persist in continuous darkness (Klein *et al.*, 1983) as well as in constant light with attenuated amplitude (White *et al.*, 1984), and are controlled by a pacemaker located in SCN of the hypothalamus (Klein *et al.*, 1983). Following sudden exposure of rats to light at night, the pineal serotonin content increases (Illnerova *et al.*, 1979), while AA-NAT activity (Deguchi and Axelrod, 1972) and melatonin (Illnerova *et al.*, 1979) decrease precipitously. AA-NAT activity declines with the same half-life of 3.8 minute as after the administration of beta-blockers (Vanecek and Illnerova, 1979). This indicates that light blocks the transmission of neural signals to the pineal gland and beta-adrenergic receptors cease to be activated. Even after a brief light pulse lasting for 1 minute, AA-NAT and melatonin levels have been reported to decline rapidly in the continuous darkness (Illnerova *et al.*, 1979, Illnerova and Vanecek, 1979). It has also been reported that increase in photoperiod reduced the time to the AA-NAT peak

(White *et al.*, 1984). The duration of photoperiod affects AA-NAT activity accompanied by parallel changes in melatonin rhythms (Vanecek and Illnerova, 1982b; Charron *et al.*, 1991; Ribelayga *et al.*, 1999). In male Badger, AA-NAT activity and melatonin exhibited diurnal rhythm during both January and June with similar peak amplitudes under natural daylight where the duration of melatonin levels have been reported to be modified by the photoperiod with a nighttime elevation and a low level during the day (Charron *et al.*, 1991). During long daylength, either artificial (LD 16:8) or natural (June), AA-NAT and melatonin are elevated for only 5 h at night both in rats (Vanecek and Illnerova, 1982b) and in Djungarian hamsters (Hoffmann *et al.*, 1981; Illnerova *et al.*, 1984). Light exposure in the late evening and early morning hours compresses rhythms of AA-NAT activity and melatonin (Vanecek and Illnerova, 1982a, b). A long photoperiod compresses the AA-NAT rhythm waveform. The magnitude of phase shifts of AA-NAT rhythm as well as their direction depend on a previous photoperiod (Illnerova and Sumova, 1997). Decompression of the rhythms occurs under short days. Under LD 8:16 or in natural daylight (December), the period of elevated AA-NAT and melatonin levels at night lasts for 9 to 12 hours. The period seems to be very important as it provides the animals with the necessary information on daylength (Vanecek and Illnerova, 1982a,b). During short days, the extension of the rhythm proceeds into the morning hours. It seems that during long days the pineal gland are synchronized by both the evening and the morning light, however, during short days only by the morning light (Illnerova and Vanecek, 1985). It has been reported that resetting of the circadian clock after shifts in the LD cycle depends on the photoperiod. Further, re-entrainment to an 8

h delay in the LD cycle took more than 3 days in rats maintained under a regime with 18 h of light and 6 h of darkness per day (LD 18:6) whereas it was completely within 3 days in those maintained under LD 12:12. Re-entrainment to an advance in the LD cycle proceeded through a transient diminution or almost disappearance of the AA-NAT rhythm amplitude following a 5 h, 3 h and even a mere 2 h advance shift under LD 18:6, whereas no such diminution occurred under LD 12:12 even after a 5 h advance shift (Humlova and Illnerova, 1992).

It has been reported that exposure of hamsters to low temperature (0° C) reduced the sensitivity of the pineal gland to light at night and prevented inactivation of arylalkylamine-N-acetyltransferase (Stieglitz *et al.*, 1991). It has also been reported that in male Syrian hamsters exposed to low temperature (5° C) for 24 h under LD 8:16, both AA-NAT activity and melatonin showed a clear diel rhythm with a moderate but significant increase late in the dark period where the nighttime peak levels of AA-NAT activity and serum melatonin were reported to be significantly higher in the animals that had been exposed to cold than in those remaining in warm (22° C) conditions (Stokkan *et al.*, 1991). However, acute cold exposure for 6 hours did not change either AA-NAT activity or the melatonin content of the gland in rat (Guerrero *et al.*, 1990b). It has also been reported that AA-NAT activity was depressed under long-day in the white-footed mice (*Peromyscus leucopus*) exposed to cold soon after the onset of darkness, whereas mice exposed to cold later at night had slightly elevated enzyme activity. AA-NAT activity in short-day mice exposed to cold soon after lights off did not have significant

effect. However, there was no rise of melatonin, which suggested that acute exposure may modulate AA-NAT activity, which is reportedly controlled primarily by the LD cycle (Tannenbaum *et al*, 1990). It has been reported that temperature induced fluctuations in AA-NAT activity in 4-, 12- and 20-day old rats placed for 4 h in 23° C or 34° C environments with increased enzyme activity in ectothermic 4- and 12-day old animals exposed to the 23° C environment, but not in endothermic 20-day old rats (Torres *et al.*, 1989). Further, the elevation in daytime AA-NAT activity seen in cold-exposed animals has been reported to be absent in rats previously subjected to chemical sympathectomy induced by 6-hydroxydopamine, or in animals treated with the beta-noradrenoceptors antagonist drug propranolol. Catecholaminergic nerves and beta-adrenoceptors have been reported to be important for light-induced changes in mammalian pineal gland biochemistry, and appear to be essential for environmental temperature-dependent elevations in neonatal AA-NAT activity (Torres *et al.*, 1989). It has been reported that adult male rats subjected to 30, 60, or 120 min of cold exposure (2° C) during the day elicited no significant changes in pineal indoleamine metabolism, while exposure to cold for 1 h during the second hour after 'lights off' has been reported to increase pineal melatonin content slightly without a concomitant change in AA-NAT activity. Rats exposed to 2 h of cold beginning 2 h after 'lights off' has been reported to display a 50% reduction in AA-NAT activity, whereas pineal melatonin content remained unchanged, which indicated that the paradoxical response of AA-NAT activity and melatonin are not common when rats are exposed to adverse stimuli (Tannenbaum *et al.*, 1988).

The plasma levels of thyroid hormones (T_4 and T_3) exhibit a circadian rhythm in the hamster (Vriend, 1984). Under a LD 14:10 cycle, the lowest levels of these hormones were detected late in the dark phase, while the concentrations of both hormones increased during the light phase of the daily cycle. The observed parallel changes in T_4 and T_3 during the day suggest that the circadian variations were the result of variations in their secretions from the thyroid (Vriend, 1984). When Syrian hamster were exposed to a short photoperiod (less than 12 hours light per day) for 2-3 months or were subjected to bilateral orbital enucleation, their circulating total T_4 concentration decreased signifi-cantly. The reduction in T_4 concentration in these animals was prevented by pinealectomy. These findings suggested that the inhibitory effects of short daylength and orbital enucleation were mediated by the pineal gland (Vriend, 1983a, b). It has also been reported that free T_4 concentration is depressed by blinding, and that this effect appears to be mediated by the pineal gland (Vaughan and Pruitt, 1985). Blinding-induced suppression of the reproductive system and prevention of this effect by pinealectomy indicated that the animals exhibited pineal-mediated reproductive responses to photoperiod (Reiter, 1980). There seem to be parallel changes between the reproductive system and circulating T_4, and the changes in T_4 may passively result from pineal-induced hypogonadism and the possible resultant effects on T_4 serum binding protein (Vaughan *et al.*, 1982).

In mammals when the environment, particularly ambient temperature, is constant, circulating thyroid hormone levels are maintained within a narrow range by a negative feedback mechanism involving the hypothalamo-hypophyeal-thyroid axis.

However, in contrast to the small fluctuation in plasma T_4 and T_3, a diurnal variation in circulating levels of TSH has been demonstrated under constant temperature (Rookh *et al.*, 1979; Ottenweller and Hedge, 1982a,b). The peak in TSH is followed by a slight increase in T_3 and T_4 concentration 3-4 h later suggesting that the variations in T_4 and T_3 were driven by CNS-induced changes in TSH secretion (Ottenweller and Hedge, 1982a,b). Circulating thyroid hormones exert a negative feedback inhibition on pituitary TSH release (Larsen *et al.*, 2003). Pretreatment with T_4 or T_3 prevents the increase in plasma TSH following TRH administration (Snyder and Utiger, 1972), indicating that the site of inhibition is at the pituitary. Further, both TRH-stimulated state and normal resting TSH levels are affected by thyroid hormones. Irrespective of the method of hormone administration, T_4 reduces plasma TSH concentrations as well as the responsiveness of pituitary to TRH stimulation (Rookh *et al.*, 1979). It has been reported that in hibernating mammals plasma T_4 levels drop about 8-folds from summer to winter (Demeneix and Henderson, 1978; Augee *et al.*, 1979) and the activity was also reduced prior to hibernation (Hulbert *et al.*, 1985).

Levels of thyroid hormones are reportedly inhibited by administration of melatonin and increased after pinealectomy in mammals (Vaughan *et al.*, 1982; Vriend *et al.*, 1982; Vriend, 1983a,b, 1984; Vaughan and Priutt, 1985; Wajs and Lewiski, 1992; Ozturk *et al.*, 2000). Sight deprivation of hamsters is followed by several endocrine changes including a depression in circulating levels of total and free thyroxine (Vaughan *et al.*, 1982). Evidence that the pineal gland was involved in this phenomenon

derived from studies showing that blinded and pinealectomized hamsters did not have significantly different circulating levels of thyroxine (Vriend and Reiter, 1977; Vaughan *et al.*, 1982). Further, support for the view that the pineal gland was involved in regulation of the neuroenocrine-thyroid axis came from studies on melatonin administration. Administration of melatonin resulted in reduced circulating thyroxine levels (Vriend and Reiter, 1977). In male hamsters the inhibitory effect of melatonin was found to be more effective if given towards the end of the daily photoperiod than if given in the morning near the beginning of the daily photoperiod. In ovariectomized female, only evening injections of melatonin were effective in inhibiting thyroxine levels (Vriend *et al.*, 1982). In rats, melatonin has been reported to inhibit both thyroid iodine uptake and secretion (Singh and Turner, 1972; Shchervakova and Rombugoslavskaia, 1988). The pineal gland has an inhibitory influence on circulating total as well as free thyroxine in the hamster (Vaughan *et al.*, 1982; Vriend, 1984), which vary depending on the time of blood collection (Vriend, 1984). The maximum difference between thyroxine concentra-tions and free thyroiodonines (TIs) of blinded and control hamsters occurs in the evening at or near the end of the light period, with the least difference in the morning at or near the end of the dark period (Vriend, 1984). It has been found that both the pineal gland and circadian rhythms influence thyrotropin-releasing hormone (TRH)-induced TSH release in the hamster (Vriend, 1984). TRH content of the medial basal hypothalamus was increased in blinded female hamsters (Vriend and Wilber, 1983). This increase was prevented by pinealectomy, suggesting that an active pineal gland inhibits TRH release in blinded hamsters (Vriend,

1984). Daily melatonin injections reduced reproductive activity and thyroid hormones in male Syrian hamsters (Champney, 2001). Chronic administration of melatonin to rats leads to an increase of whole cytoplasm stained cells (WC) in number but a decrease of spot-like stained cells (SC). On the other hand, the intensity of TSH-immunoreactivity and the number of rat pars tuberalis PT-TSH cells have been reported to decrease significantly after pinealectomy and recovered following melatonin administration (Sakamoto *et al.*, 2000). Melatonin levels showed a diurnal rhythm, and melatonin administration reportedly decreased TSH, T_3 and T_4 levels in rats and increased the activity of an antioxidant enzyme superoxide dismutase (SOD) (Ozturk *et al.*, 2000).

In tropical seasonally breeding Indian palm squirrel, *Funambulus pennanti* during its reproductive phase, continuous light (LL) had no effect on pineal, thyroid and ovarian functions as judged by thyroid weight and plasma levels of T_4 and estradiol (E2). However, continuous darkness (DD) reduced significantly thyroid and ovarian weight as well as plasma T_4 and E2 levels (Shavali and Haldar, 1998). Further, pinealectomy (Px) significantly increased thyroid and ovarian weights with no observable changes in their hormonal levels under normal photoperiod and under LL, while Px under DD condition prevented the reduction in thyroid and ovarian weights but the hormonal levels were increased. These results suggested that DD could be an effective stimulator for the pineal function and increased pineal activity in this mammal. Melatonin injection has been reported to reduce T_3 in pituitary-grafted rats and to decrease T_4 concentration in normal rats (Esquifino *et al.*, 1997). 5-Methoxytryptophol,

like melatonin, exerted the inhibitory influence on the mitotic activity; however, it did not affect thyroid weight (Wajs and Lewinski, 1992). Further, it has also been reported that melatonin administered in late afternoon decreased, while PX increased indices of thyroid growth *in vivo*. However, administration of melatonin as subcutaneous implanted pellets reversed the inhibitory effect of melatonin injections (Lewinski *et al.*, 1992; Wajs and Lewinski, 1992).

In mammals, several attempts have been made to study thyroid-pineal interrelations *in vivo* (Vriend, 1983a,b, Lewinski, 1986; Ruzsas and Mess, 1987). It has been found that hypo-thyroidism in rats increased melatonin levels in the pineal, hypothalamus and serum at an early age (25 days), but in adults melatonin levels were lowered in the hypothalamus and serum yet unaltered in the pineal (Catala *et al.*, 1987). It has been reported that T_3 can have direct effects on short-term (6 h) synthesis and release of melatonin in rat pineal *in vitro*. These effects depend upon the age of the donor animal, ambient photic environment (with temperature constant), and concentration of T_3 (Catala *et al.*, 1988). It has been reported that both hypo-thyroidism and hyper-thyroidism did not alter pineal melatonin content during the day or night. However, the nocturnal increase of melatonin has been phase-advanced in the hypothyroid group (Bauer *et al.*, 1989). In women, melatonin concentrations were found to be positively correlated with TSH levels in hypo-thyroidism, and negatively correlated with T_3 in hyper-thyroidism (Soszynski *et al.*, 1988).

Melatonin has been reported to act as a scavenger and an antioxidant (Reiter *et al.*, 1995; Karbownik and Lewinski, 2003a,b; Kukner *et al.*, 2004; Liang *et al.*, 2004; Sayan *et al.*, 2004). Melatonin has been found to be highly protective against damage to macromolecules resulting from oxygen and nitrogen-based reactants (Reiter *et al.*, 2003, 2004). The reported higher incidences of neurodegenerative diseases like Parkinsonism, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, epileptic seizures, and stroke in elderly persons are attributable to a decrease in the levels of melatonin with age (Gupta *et al.*, 2003). Melatonin has been shown to either stimulate gene expression for the antioxidant enzymes {superoxide dismutase (SOD, a principal antioxidant enzyme levels), catalase, glutathione peroxidase, glutathione reductase} or to increase their activity, and also neutralizes hydoxyl radical, superoxide radical, peroxyl radical, peroxynitrite anion, singlet oxygen, hydrogen peroxide, nitric oxide, and hypochlorous acid which damage cells (Reiter *et al.*, 1995). In rats, it has been reported that ischemia-reperfusion (I/R) produced free radicals leading to lipid peroxidation and damage of the nervous tissue, while where melatonin treatment reversed the I/R-induced increase and decrease in malondialdehyde (MDA, an oxidative stress marker) and SOD, respectively (Sayan *et al.*, 2004). Unlike other antioxidants, it has been reported that melatonin can easily cross all morphophysiological barriers, e.g., the blood brain barrier, and enters cells and subcellular compartments in human (Gupta *et al.*, 2003).

Melatonin with its antioxidant effects has been reported to protect the erythrocytes from impaired deformability in sodium nitroprusside (SNP)-induced oxidative stress in human (Aydogan *et al.*, 2004), and protect against oedema formation in guinea pig (Kukner *et al.*, 2004) and rats (Sayan *et al.*, 2004), plasma membrane fluidity and free radical production in guinea pig (Kukner *et al.*, 2004), the myelin sheaths, axons and the sciatic nerve from I/R injury in rats (Sayan *et al.*, 2004), and testicular damage in rats (Onur *et al.*, 2004). Melatonin supplementations have been reported to strengthen the antioxidant defence system because of decreased reactive oxygen substances (ROS), and play a role in treating uveitis in guinea pig (Kukner *et al.*, 2004). Futher, melatonin has been reported to help in limiting cytokine production and inflammatory processes (actions that would also lower toxic reactant generation) (Reiter *et al.*, 2004), and protects against the overproduction of reactive oxygen species produced by hyperbaric oxygen (HBO) in rat lung tissue (Topal *et al.*, 2004). Melatonin produces a positive effect on both angiogenesis and wound healing in Wistar albino rats (Soybir *et al.*, 2003). In human, melatonin can protect retinal pigment epithelial (RPE) cells against hydrogen peroxide (H_2O_2)-induced oxidative stress and cell death (Liang *et al.*, 2004). Melatonin also protects rat brain cells from oxidative damage induced by ionizing radiation (Undeger *et al.*, 2004). Antioxidant potential of melatonin has been reported on hyper-ammonemia (induced by ammonium acetate treatment) in rats (Lena and Subramanian, 2004). Antioxidant enzymes (e.g., SOD, catalase and glutathione peroxidase) and non-enzymatic antioxidants (e.g., reduced glutathione) in brain tissues have been reported to be significantly decreased in ammonium acetate-treated rats and

to be increased significantly in rats treated with a combination of melatonin and ammonium acetate. Further, daily administration of a high dose of melatonin significantly attenuated the increase in hepatic thiobarbituric acid reactive substances (TBARS) and reduced GSH levels and myeloperoxidase (MPO) activity after bile duct ligation (BDL), indicating that melatonin administered orally at pharmacological doses exerted a therapeutic effect on cholestatic liver injury in rats with BDL possibly through its antioxidant and anti-inflammatory actions (Ohta *et al.*, 2003). These biochemical alterations could be due to the ability of melatonin to (i) scavenge a variety of radicals and reactive oxygen species, (ii) induce antioxidative enzymes which reduce steady state levels of reactive oxygen species, and (iii) stabilize cell membranes which assist them in reducing oxidative damage and, thus, could prevent oxidative stress in rats (Lena and Subramanian, 2004).

Oxidative stress plays an important role in hyper-thyroidism-induced tissue damage as well as in the development of autoimmune disorders in human (Bednarek *et al.*, 2004). Further, oxidative stress is also involved in pathomechanism of thyroid disease, e.g., Graves' disease, goiter formation or thyroid cancer (Karbownik and Lewinski, 2003a,b). In rat, treatment with T_3 has been found to be associated with increased oxidative capacity, lipid peroxidase activity in liver (Venditti *et al.*, 1997, 1999) and heart (Venditti *et al.*, 1997), susceptibility to oxidative stress and decreased antioxidant levels (Venditti *et al.*, 1999). It has been reported that administration of T_3 or diiodothyronine (T_2) to hypothyroid teleosts (*Anabas testudineus*) increased lipid

peroxidation rate (Varghese *et al.*, 2001). Supplementation of 6-n propyl 2-thiouracil (PTU) through drinking water in rabbit has been found to have potent antioxidant and immunosuppressive effects, and PTU reportedly plays a role in the prevention of atherosclerosis by inhibiting vascular smooth muscle cell proliferation and migration (Chen *et al.*, 2004). Due to its antiproliferative and immunomodulatory effects, antioxidant potential and low toxicity, PTU has been reported as an effective agent in the treatment of psoriasis in human (Kose *et al.*, 2001). Hypo-thyroidism induced by PTU and methimazole (MMI) in rabbits resulted in a significant decrease in serum T_4 and T_3 concentration and also decreased the serum concentration of the lipid peroxidase (Brzezinska-Slebodzinska, 2003). Further, erythrocytes of hypothyroid animals exhibited higher resistance to oxidative stress when exposed to free radicals generator 2, 2′-azo-bis (2-amidinopropane) hydrochloride *in vitro*. The plasma level of hypothyroid animal showed about 20% higher ability to protect against iron-binding organic radicals (Brzezinska-Slebodzinska, 2003). MMI significantly scavenged H_2O_2 and decreased the amount of H_2O_2 generated by the glucose-glucose oxidase system. Further, both PTU and MMI can impair thyroid H_2O_2 generation in addition to their potent thyroperoxidase inhibitory effects (Ferreira *et al.*, 2003). Under physiological conditions melatonin and, possibly, other antioxidants regulate ROS generation for thyroid hormone synthesis (Karbownik and Lewinski, 2003a). Further, melatonin might protect against extensive oxidative damage in the course of certain thyroid disorders, cancer initiation or in case of a harmful action of some external factors on the thyroid (Karbownik and Lewinski, 2003a,b).

Thus, a large body of available information suggests that both melatonin and thyroid hormones are involved in regulation of the oxidative stress, and seem to produce opposite effects. While hyper-thyroidism increases the oxidative stress-induced tissue damage, hypo-thyroidism and melatonin produce antioxidant effects and protect the tissues from the ill effects of the oxidative stress. Since melatonin and thyroid hormones are actively involved in the regulation of the oxidative stress, there is a possibility that pineal and thyroid interact with each other through their hormones in order to provide an optimal neuroendocrine management of the oxidative metabolism as well as the oxidative stress.

Melatonin has been reported to regulate the release of several hormones. It has been reported that melatonin inhibited hypothalamic prostaglandin synthesis (Cardinali *et al.*, 1980). It has been reported that melatonin has a suprapituitary site of action to inhibit naloxone-induced LH release. It has been suggested that melatonin inhibits the activity of the hypothalamic LHRH pulse generator, either directly or indirectly, in female rats (Akema *et al.*, 1997). Melatonin has been found to inhibit LH and FSH response to LHRH in rats and dogs (Reiter *et al.*, 1983). However, administration of melatonin either decreased or did not affect LH secretion in man (Weinberg *et al.*, 1980; Reiter *et al.*, 1983). Melatonin has been reported to inhibit hCG-induced stimulation of steroid synthesis in ovarian follicles of rabbits and to delay the appearance or to abolish luteal progesterone secretion in monkey (Reiter *et al.*, 1983). Melatonin has also been reported to increase prolactin (PRL) in cultured pituitary cells from fetal rats and to

counteract the effects of pinealectomy on LH and PRL release during late pregnancy (Hanew *et al.*, 1980). However, chronic daily adminis-tration of melatonin to anosmic rats has been reported to inhibit PRL release (Blask and Nodelman, 1980). In Syrian hamster, injected at the end of the light or dark phase of daily photoperiod, melatonin induces gonadal involution and depression of PRL, LH and FSH secretion in males and acyclicity and afternoon surges of LH and FSH in females (Reiter *et al.*, 1983). In sheep, castration-induced increase in plasma LH was blocked by melatonin (Roche *et al.*, 1970). Melatonin reportedly decreases plasma testosterone, steroidogenesis *in vitro* (Relkin, 1983) and androgenic effects of testosterone (Reiter *et al.*, 1983). Melatonin has been reported to increase vasopressin release from neurohypophysis (Lemay *et al.*, 1979).

Melatonin administration has been reported to decrease plasma corticosterone levels (Yamada, 1990) and adrenal corticosterone production *in vitro* (Ng, 1987). In rats, melatonin blocked insulin release *in vitro* (Relkin, 1983). Melatonin also enhances pineal argentine-vasotosin release in cat and inhibits T_4 uptake by bovine pineal slices *in vitro* (Relkin, 1983). Further, melatonin injection also reduced the high serum prolactin and T_3 levels found in pituitary-grafted rats, and decreased T_4 concentration in control rats. However, high concentration of prolactin was found to depress melatonin content (Cardinali *et al.*, 1987). It has been reported that neither melatonin nor pituitary grafts modified serum TSH concentration (Esquifino *et al.*, 1997). Acute infusion of melatonin to pregnant and non-pregnant ewes did not change the plasma arginine-

vasopressin and arginine-vasotocin levels (Ross *et al.*, 1985). However, melatonin produced a significant increase in plasma oxytocin in the non-pregnant and not the pregnant ewes, suggesting that there is an interaction between melatonin and oxytocin in the integration of mammalian reproductive cycles (Ross *et al.*, 1985). Exogenous melatonin as subcutaneous implants has also been reported to elevate plasma leptin and thyroid hormone levels in mink, *Mustela vison* (Mustonen *et al.*, 2000).

Melatonin synthesis and secretion also seem to be regulated by a number of hormones. Thyroid stimulating hormone (TSH), GH and LH augment and FSH decreases melatonin content in rat pineal significantly (Cardinali *et al.*, 1987). Pineal AA-NAT activity is significantly increased following administration of 5α-dihydro-testosterone (5α-DHT) in castrated male and intact female rats (Menendez-Pelaez *et al.*, 1990). Testosterone reportedly down-regulates pineal melatonin synthesis in rat (Cardinali *et al.*, 1987) and men (Luboshitzky *et al.*, 1996a). Further, gonadotropins and other gonadal steroids have also been reported to modulate melatonin secretion in humans (Luboshitzky *et al.*, 1996a,b). In mice, testosterone has been found to be closely associated with the secretory process of pinealocytes, and plays an inhibitory role in the regulation of activity of the pineal gland (Redins *et al.*, 1999). Dehydroepiandroste-rone-sulfate has a direct effect on adrenergically stimulated melatonin release in the rat pineal (Martin and Touitou, 2000). Estradiol-17β and estradiol benzoate have been reported to inhibit melatonin synthesis in the peripubertal female rats (Okatani *et al.*, 1997; Hayashi and Okatani, 1999). The inhibitory effects of estrogens on melatonin

synthesis might be due to changes in NE-induced stimulation of pineal adenylate cyclase activity (Hayashi and Okatani, 1999). The inhibitory effect of estrogens on melatonin also seems to involve modulation of AA-NAT activity (Okatani *et al.*, 1999). Estrogens have also been reported to modulate melatonin secretion in perimenopausal women (Okatani *et al.*, 2000). It has been found that estrogens, but not progesterone, can modulate nocturnal pineal melatonin synthesis in female rats (Cardinali *et al.*, 1987; Okatani *et al.*, 1997). Ovarian steroids reportedly regulate interaction between α_1- and β-adrenergic receptors, and thereby modulate pineal melatonin biosynthesis in female rats (Alonso *et al.*, 1995; Hernandez-Diaz *et al.*, 2001). It has been reported that luteinizing hormone stimulates pineal melatonin synthesis and release mainly by increasing AA-NAT activity (Hosaka *et al.*, 2002). In rat pinealocytes, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP) and peptide histidine isoleucine stimulated melatonin biosynthesis and caused cAMP efflux and phosphorylation of the cyclic AMP response element binding protein (CREB) (Rekasi and Czompoly, 2002). Many peptidergic fibers containing peptides such as vasopressin and oxytocin have also been found in the rat pineal gland. Oxytocin reportedly has no effect on pineal metabolism (Barassin *et al.*, 2000) and it is not involved in the regulation of pineal gland function in rat (Reuss *et al.*, 1993). However, vasopressin has been found to modulate melatonin synthesis (Barassin *et al.*, 2000). Corticosteroids also seem to influence AA-NAT activity and melatonin synthesis. Administration of hydrocortisone acetate at birth retarded the developmental decline in daytime serotonin-N-acetyltransferase activity (Yuwiler and Brammer, 1981). Corticosterone injection in

hypophysectomized rats has been reported to depress AA-NAT activity in rats (Troiani *et al.*, 1988). Corticotropin releasing hormone has also been reported to inhibit the pineal secretion of melatonin in normal men (Kellner *et al.*, 1997).

It is, thus, clear that melatonin affects physiology of several endocrine glands, while its own synthesis and release is also influenced by a large number of hormones. The reported interactions between pineal and other neuroendocrine tissues might be essential for the regulation of normal physiology of mammalian species.

The mammalian clock, located in the hypothalamic SCN, is important in the coordination of a broad spectrum of physiological, endocrine and behavioral circadian rhythms as well as in the regulation of pineal gland metabolism and melatonin secretion (Challet *et al.*, 2003; Tournier *et al.*, 2003; Hofman, 2004; Simonneaux *et al.*, 2004). The endogenous rhythm of the SCN is influenced by photoperiod (Sumova *et al.*, 2003). A combination of a constant stimulatory output (both during day and night time) and a rhythmic inhibitory output of SCN controls the rhythm of melatonin synthesis in the mammalian pineal (Perreau-Lenz *et al.*, 2003). The endogenous circadian rhythmic oscillations in SCN rely upon genetic mechanisms involving clock genes coding for transcription factors working in negative and positive feedback loops (Reppert and Weaver, 2002; Tournier *et al.*, 2003; Simonneaux *et al.*, 2004). The basic helix-loop-helix PAS (Period-arylhydrocarbon receptor-singleminded) transcription factors CLOCK (Circadian locomotor output cycles kaput) and BMAL1 (Brain and muscle

arylhydrocarbon receptor nuclear translocator-like protein 1) have been reported to act as positive regulators, and three period proteins (PER1, PER2 and PER3) and two cryptochrome proteins (CRY1 and CRY2) have been reported to operate as negative regulators (King and Takahashi, 2000; Reppert and Weaver, 2002). The autoregulatory feedback loop begins with the heterodimer CLOCK/BMAL1 binding to the E-box of the *Per1* gene promoter thereby initiating the transcription of the gene (Reppert and Weaver, 2002). This leads to rise in the levels of the PER1 protein in the cytoplasm. Soon after that, PER3 and later PER2 are also accumulated, with each of these proteins reaching peak levels at different circadian times (Shearman and Weaver, 1999; Jung *et al.*, 2003). CLOCK/BMAL1 also acts as a transcriptional activator of the two mammalian *cryptochrome* genes (Gekakis *et al.*, 1998; Shearman and Weaver, 1999; Jung *et al.*, 2003). Once the PER proteins (PER1, PER2 AND PER3) and the CRY proteins (CRY1 and CRY2) synthesized in the cytoplasm have reached determined levels, they form heterodimers with each of the PER proteins. Then the heterodimer gets translocated to the nucleus (Barnes *et al.*, 2003). Having reached the nucleus, the PER/CRY heterodimers inhibit the transcription of their own genes through inactivation of the activating CLOCK/BMAL1 complex (Barnes *et al.*, 2003). At this point the levels of the PER1, PER2, PER3, CRY1 and CRY2 proteins in the cytoplasm begins to decrease, leading to a parallel decrease in the formation of the inhibiting heterodimers. Ultimately, the level of heterodimers has been found to be insufficient to inhibit the transcription of the *Per* and *Cry* genes, which will once again fall under the positive control of the CLOCK/BMAL1 complex (Cahill, 2002; Barnes *et al.*, 2003). The

rhythmic transcription of clock genes, regulated by their own gene products, provides the basis for self-sustaining circadian clockworks (Karolczak *et al.*, 2004). The mammalian homolog of Drosophila clock protein timeless (TIM) has also been reported to play a role in the clock oscillation by interacting with PER (Barnes *et al.*, 2003). In addition, basic helix-loop-helix transcription factors DEC1 and DEC2 inhibit the CLOCK/BMAL1 function, and the expression of *Dec1* gene has been found to be controlled by CLOCK/BMAL1, suggesting that DEC proteins act as additional negative regulators in the feedback loop (Grechez-Cassiau *et al.*, 2004; Kawamoto *et al.*, 2004). On the other hand, the transcription of positive regulator gene *Bmal1* has been found to be repressed by an orphan nuclear receptor REV-ERBα, whose mRNA expression is activated by CLOCK/BMAL1 (Preitner *et al.*, 2002; Ueda *et al.*, 2002). This regulation results in circadian oscillation of *Bmal1* expression in antiphase with the rhythm of *Per* expression (Yamaguchi *et al.*, 2000; Mitsui *et al.*, 2001). These two loops of negative and positive regulators are tightly coupled with each other and constitute the core of the circadian oscillator (Yamuguchi *et al.*, 2000; Mitsui *et al.*, 2001). It has also been reported that the cone rod homeobox (*Crx*) gene, a member of the orthodenticle homeobox (*Otx*) family, also regulates pineal circadian activity in mouse (Gamse *et al.*, 2002). Inactivation of *Crx* in mouse has been reported to cause a reduction in pineal gene expression and attenuate entrainment to light/dark cycles, while in the zebra fish pineal complex, *Otx5* rather than *Crx* has been reported to regulate genes that show circadian expression (Gamse *et al.*, 2002).

In the rat pineal gland, *Per1*, *Per3*, *Cry1* and *Cry2* clock genes are also expressed with increased transcription during night (Simonneaux *et al.*, 2004). Further, the expression of *Per1* and *Cry2* in the rat pineal gland is regulated by the clock-driven changes in NE, in a similar manner to the melatonin rhythm-generating enzyme, AA-NAT. However, the expression of *Per3* and *Cry1* displayed a daily rhythm not regulated by NE, suggesting the involvement of another day/night regulated transmitter(s). Day and night mRNA levels of Per3, Per2 and Cry1 have been reported to be unresponsive to adrenergic ligands (Simonneaux *et al.*, 2004). An endogenous clock located in the pineal photoreceptor cells in lower vertebrates, particularly in most teleosts and pike, drives the daily rhythm in pineal melatonin synthesis and release *in vitro* (Bernard *et al.*, 1997; Klein *et al.*, 1997; Begay *et al.*, 1998; Gothilf *et al.*, 1999), with an exception in the trout in which the pineal melatonin rhythm is a direct response to darkness (Bolliet *et al.*, 1997; Coon *et al.*, 1998). In rainbow trout, the melatonin rhythms are reset daily by the ambient light:dark cycle (Bolliet *et al.*, 1997; Okimoto and Stetson, 1999). These features require the expression of an array of specific genes in the pineal gland that are involved in photoreception, photo-transduction, clock function, and melatonin production (Appelbaum *et al.*, 2004).

As in mammals, CLOCK and BMAL1 are the central components of the molecular circadian oscillator in the photoreceptive pineal of fish, which form CLOCK/BMAL1 heterodimer and binds to the promoters of negatively acting clock genes (Ishikawa *et al.*, 2002). The CLOCK/BMAL1 heterodimers activate transcription

and induce expression of the circadian clock genes *Per*, *Cry* and *Tim* genes in a circadian feedback loop (Hogenesch *et al.*, 1997; Sangoram *et al.*, 1998; Ishikawa *et al.*, 2002). CRY has been reported to inhibit transcriptional activity of the CLOCK/BMAL1 heterodimer by generating a negative-feedback loop (Ishikawa *et al.*, 2002). In contrast to that of mammals with only one *Bmal* gene (Hogenesch *et al.*, 1997; Ikeda and Nomura, 1997), it has been reported that Zebra fish has two *Bmal* (*Bmal1* and *Bmal2*) genes, which are rhythmic in both central and peripheral clocks (Whitmore *et al.*, 1998; Cermakian *et al.*, 2000). Although *Clock* expression is constant in mammals (Shearman and Weaver 1999; Oishi *et al.*, 1998), it oscillates in fish with a pronounced circadian rhythm in the brain, retina, pineal gland, in *ex vivo* kidney and heart in culture dishes (Whitmore *et al.*, 1998; Cahill, 2002) as well as in a zebrafish-derived cell line, where the circadian clock are directly entrained by light (Whitmore *et al.*, 2000). Expression of *Bmal1* in rodents and fish is almost antiphasic, for which the reason is still not clear (Cermakian *et al.*, 2000). In zebrafish *Clock* and *Bmal1* genes oscillate in a rhythmic manner, whereas in other animal systems rhythmicity has been observed in either *Clock* or *Bmal1* expression, but not both, and the abundance of all three transcripts has been reported to peak in the late day or early night in zebrafish (Cahill, 2002). Further, two differences from the mammalian system lie in the regulation of *Per2* and in the number of *Cry* genes. However, there is no information on clock protein expression pattern in Zebrafish *in vivo*, or on post-transcriptional and post-translational mechanisms that are likely to play important roles in rhythm generation (Cahill, 2002).

In addition, in several fishes including that of Zebrafish, *aanat* gene possesses E-box in the 5'-regulatory region which controls the rhythmic expression of the gene (Gothilf *et al.*, 2002). Vertebrate *aanat* gene is recognized as a clock-controlled gene that serves as a link between the circadian clock and the output signal-melatonin (Appelbaum *et al.*, 2004). The expression of *aanat-1* in retina and *aanat-2* in the pineal gland of fish (Coon *et al.*, 1999; Falcon *et al.*, 2003) is determined by distinct, but very closely related, regulatory mechanism (Appelbaum *et al.*, 2004). The number of photoreceptors releasing melatonin was higher during the subjective dark than during the subjective light under both light: dark and constant darkness in culture (Bolliet *et al.*, 1997). It has been reported that *Clock* controls expression of both AA-NAT and tryptophan hydroxylase (TPH) mRNAs in various teleost pineal except in trout, in which the levels of these mRNAs are tonically elevated (Begay *et al.*, 1998; Coon *et al.*, 1998).

The endogenous hypothalamic clock has been suggested to be involved in the integration of seasonal information (Hastings and Follett, 2001). Consistent with its role in circadian timing, it has been suggested that the neuronal changes and adaptations are driven by this clock located in the SCN, and that this neural calendar is reset by the seasonal fluctuations in photoperiod enabling organisms to anticipate seasonal environmental changes (Hofman, 2004). It has been reported that melatonin plays only a minor role in SCN function by supplementing the information about light and darkness from the RHT (Stehle *et al.*, 2003). Melatonin exerts its synchronizing

properties indirectly on clock inputs or clock outputs, or directly on the clock via high-affinity melatonin receptors (MEL-R) located on cells of the pituitary pars tuberalis and SCN (Song *et al.*, 1999; von Gall *et al.*, 2002) or other binding sites (Pevet *et al.*, 2002), and regulates seasonal reproduction (von Gall *et al.*, 2002).

Photoperiod has been reported to affect the expression of *Bmal1*, *Clock*, *Period* (*Per1, Per2, Per3*) and *Cryptochrome* (*Cry1, Cry2*) genes differentially in the SCN of Syrian hamsters when kept under long and short photoperiods (Tournier *et al.*, 2003; Sumova *et al.*, 2003). The CRE-mediated induction of *Per1* gene through phosphorylation of CREB at Ser142 has been reported to play a pivotal role in photic resetting of the central clock (Oster *et al.*, 2003). Antisense oligonucleotides against *Per1* inhibit phase-dependent phase shifts of the clock by light, i.e., phase delay in early night (Akiyama *et al.*, 1999) and phase advance in late night (Tischkau *et al.*, 2003a). In mice, prolonged light exposure during daytime and exposure to light pulses at night positively modulates daily levels of Per1 and Per2 mRNA in the SCN of mice (Challet *et al.*, 2003). However, the light induction of *Per2* but not *Per1* is strongly suppressed in mice lacking cGMP-dependent protein kinase II (PKG II) (Oster *et al.*, 2003). In the mutant mice, only the light induced phase delay is inhibited moderately, suggesting that PKG II is required for the photic induction of *Per2* that delays the phase of the clock (Gillette and Mitchell, 2002; Tischkau *et al.*, 2003b). However, the precise mechanism generating the phase shift in the opposite direction (delay or advance) is still unknown (Hirota and Fukada, 2004). *C-fos* (Vuillez *et al.*, 1996; Jacob *et al.*, 1997) and *Per1*

gene expression in the SCN have been found to display MEL-independent photoperiodic variations (Messager *et al.*, 2000; Nuesslein-Hildesheim *et al.*, 2000). The integration of the photoperiod by the SCN has been proposed to involve two components (one recognizing variations of the dawn and the other of the dusk) with the increase (evening) and the decrease (morning) of melatonin synthesis being regulated separately during photoperiodic changes (Illnerova and Vanecek, 1985). Further, the phase relationship between these two oscillator components determined the duration of the nocturnal melatonin peak. By affecting the daily profile of the light-sensitive *Per* expression, photoperiod in turn affected the kinetics of the expression of the clock proteins and consequently the expression of all the clock-regulated genes (Tischkau *et al.*, 2003b). The whole complex molecular clockwork in the rat SCN has been reported to be photoperiod-dependent, which differs according to the season of the year (Sumova *et al.*, 2003).

Birds

Birds use the annual photoperiodic cycle to ensure that seasonal events such as breeding, molt and song production take place at the most appropriate time of year (Dawson *et al.*, 2001). Increasing daylength, ambient temperature, and rainfall are inversely correlated with pineal gland activity and the avian pineal shows cyclical patterns relative to environmental factors which are correlated with plasma melatonin, and the pineal gland modulates annual variations in adrenal and gonadal activity (Sudhakumari *et al.*, 2001). Unlike in mammalian pineal, the avian pineal gland has

been reported to retain a photoreceptive capability and the gland is a photosensor, which possess a photic input pathway to the oscillator, a self sustained oscillator/circadian clock in the pinealocytes and melatonin synthesizing capability, and expresses circadian oscillation of melatonin release (Underwood *et al.*, 2001; Natesan *et al.*, 2002). It has been reported that light affects melatonin synthesis through two mechanisms, i) by entraining the clock, and ii) by suppression of melatonin synthesis (Bernard *et al.*, 1997; Zatz *et al.*, 2000). The SCN of the hypothalamus is essential for the persistence of free running circadian activity rhythms in house sparrows (Takahashi and Menaker, 1982). Lesion in SCN has been found to abolish circadian locomotory rhythms in constant darkness (Takahashi and Menaker, 1982), and arrhythmicity in house sparrows and Japanese quail (Underwood *et al.*, 2001). Light plays an important role in the regulation of the amplitude, period, phase of the melatonin circadian rhythm (Zatz, *et al.*, 1994), migratory activity (Deguchi, 1981; Underwood and Siopes, 1985; Pohl, 2000), daily and seasonal changes of behavior, and reproduction (Brandstatter, 2003). It has been reported that melatonin acts at various target sites including a complex hypothalamic oscillator that, unlike in mammals, is not confined to a single cell group in house sparrow (Brandstatter, 2003).

There are only few reports regarding involvement of pineal in regulation of gonadal activity or gonadal cycles of tropical and sub-tropical birds (Thapliyal, 1981; Vivien-Roels, 1981). Pinealectomy induced early and complete gonadal development in weaver bird even under non-stimulatory short daylength which has been reported to

increase hypothalamic LHRH and plasma LH, and stimulated unseasonal testicular development in the weaver bird (Saxena *et al.*, 1979; Narula and Saxena, 1981). In the red headed bunting, pinealectomy has been reported to depend on the phase when the birds were pinealectomized, where pinealectomy during the quiescent phase (Nov and Jan) accelerated testicular growth during spring (Lal, 1987). In several avian species (e.g., house and Java sparrows, European starling, domestic pigeon), melatonin has been reported to regulate circadian rhythms of locomotor activity, feeding, and body temperature (Chabot and Menaker, 1992; Gwinner, 1989). In songbirds, pinealectomy resulted in the abolition or destabilization of overt circadian rhythms such as the rhythm of locomotor activity, feeding, or body temperature which were restored either by re-implanting a pineal organ, by periodic injections or infusions of melatonin, or by applying melatonin rhythmically through drinking water (Gwinner *et al.*, 1997). AA-NAT activity has been reported to depend on the season, sex and age in chicken (Markowska *et al.*, 2000).

As in mammals, in birds also AA-NAT activity (Klein and Voisin, 1999) and plasma melatonin concentrations (Gwinner *et al.*, 1997; Dawson and Van't, 2002) have been reported to exhibit seasonal variations and day/night rhythm with peak activity occurring in the dark phase and low during day or light phase (Siguenza *et al.*, 1988; Binkley *et al.*, 1989; Rudeen *et al.*, 1990). The mechanism controlling the nighttime increase and the daytime decrease in AA-NAT activity has been reported to be regulated differently in rats and chickens (Binkley, 1981). Both light and NE have been

found to inhibit nocturnal AA-NAT activity in cultured chicken pineal glands (Deguchi, 1979; Wainwright and Wainwright, 1980). As in mammals, in birds also, the rhythmic changes in melatonin content of the pineal gland has been reported to be regulated primarily by changes in activity of the enzyme AA-NAT (Klein and Weller, 1970; Binkley *et al.*, 1973). It has been found that the daily changes in melatonin content and AA-NAT activity in the pineal gland are manifestations of true circadian rhythms (Binkley *et al.*, 1973; Binkley and Geller, 1975). The shape (phase, amplitude and duration) of the chick AA-NAT activity rhythm has been found to change after exposure to light-dark cycles of short or long photoperiod, and constant light *in vivo* (Binkley and Moser, 1984; Binkley *et al.*, 1985). It has been reported that the circadian pacemaker of avian pineal gland oscillates both *in vivo* and *in vitro* (Wainwright, 1980; Binkley and Moser, 1984), where light exposure suppresses the night-time increase in AA-NAT activity (Deguchi, 1981; Hamm *et al.*, 1983). The melatonin circadian rhythm in chick pineal cells culture persisted in continuous darkness for at least 5 cycles with the dampened amplitude, while high amplitude melatonin rhythm was found when maintained in light/dark cycle and constant light exposure lengthened the free-running period of the melatonin rhythm (Robertson and Takahashi, 1988). However, the melatonin levels in pineal and blood in quail showed a daily rhythm with depressed amplitude and lengthened duration under LD 6:18 as compared to LD 18:6 (Underwood and Siopes, 1985). Diurnal rhythm in AA-NAT activity was not found either in LL, DD or LD 12:12 in 14-day old chick embryos but it was present in 18-day old chick

embryos incubated under LD 12:12 and LD 16:8 but not under DD or LL (Zeman and Illnerova, 1990).

Nocturnal AA-NAT activity has been found to be increased in 21-day old chicks than those of 4-days old chicks (Rudeen *et al.*, 1990). Light caused a significant decrease in AA-NAT activity in explanted chick pineal glands (Rudeen *et al.*, 1990) as well as in static organ culture, and inhibited melatonin release in flow-through organ culture, which was reportedly increased when lights were turned off (Hamm *et al.*, 1983). Light pulse has been reported to reset the phase of circadian rhythm (Binkley *et al.*, 1980). Further, light pulses have also been reported to delay the rhythm when they were imposed in the early subjective dark time, advanced the rhythm when they were imposed in the late subjective dark time, and the phase of the rhythm was found to be insensitive in the subjective light time (Binkley *et al.*, 1980). Light pulse applied to cultured chick pineal cells maintained in constant darkness or red light has been reported to phase shift the rhythm of melatonin release (Zatz *et al.*, 1988; Takahashi *et al.*, 1989). A 3 h light pulse to pineal cells in constant red light, at a certain phase has been reported to cause both an acute decrease in melatonin output and a phase advance in the clock generating the rhythm of melatonin release (Zatz *et al.*, 1988).

A typical nighttime rise in serum melatonin levels was exhibited in hen subjected to different cycles of photoperiods such as LD 12:12, LD 16:8 and LD 9:15, and the duration of rise was found to be directly proportional to the hours of darkness

during each light/dark regime (Siguenza *et al.*, 1988). In the house sparrow, time of the year is encoded in a particular melatonin signal, being short in duration and high in amplitude in long photoperiods, and long in duration and low in amplitude in short photoperiods, independent of whether the light zietgeber is natural or artificial or varies in strength (Brandstatter, 2003). Both direct photoreception (Binkley *et al.*, 1981, 1983) and neurotransmitters (Cassone *et al.*, 1983) have been reported to affect AA-NAT activity and melatonin synthesis. Whether entrained or free running, rhythmicity in AA-NAT has been found to produce identical oscillations in melatonin production by superfused chicken pineal organs (Takahashi *et al.*, 1980). Under DD and both *in vivo* and *in vitro*, the genes are reportedly controlled by the circadian oscillator of the pineal (Thomas *et al.*, 1998; Klein and Voisin, 1999). It has been reported that the sensitivity of AA-NAT activity to light was increased in the second 8 h under LD 8:16 and sensitivity to dark was increased in the first 8 h under LD 16:8. The transition from LD16:8 to LD 8:16 prolonged the AA-NAT sensitivity during the dark period (Binkley *et al.*, 1989). In LD 16:8, the period of elevated nocturnal activity has been reported to last for 7 hours, while under LD 8:16 the evening AA-NAT rise was advanced by almost 3 hours relative to the rise in LD 16:8 and occurred at the same time as during the 3rd, 7th, and 14th day after the transition (Binkley *et al.*, 1989). It has been reported that the decline in morning AA-NAT did not shift during the first long night under LD 8:16, but during the third night it was delayed relative to the decline in LD 16:8 by more than two hours and occurred at the same time as during the 7th and 14th night following the LD 16:8 to LD 8:16 transition (Zeman and Illnerova, 1988). 3rd, 7th and 14th days

after transition, the period of elevated AA-NAT activity was found to last for 12 hours, which suggested that extension of the AA-NAT rhythm preceded first into the evening and then only into the morning hours, and accomplished within 2 to 3 days (Zeman and Illnerova, 1988). It has been reported that under LL, AA-NAT activity in the pineal gland of 16-day-old chicks was reduced to about a half of the usual nocturnal level only in the first day, but no suppressive effect of light was observed throughout the following two days. However, under DD, AA-NAT circadian rhythm persisted for three consecutive days (Doi *et al.*, 1983). Under LL, the phase of AA-NAT rhythm in chick pineal delayed remarkably for three consecutive days, while the phase delayed in a lesser degree in DD (Doi *et al.*, 1983). However, constant light had no adverse effect on pinealectomized chicks and indicated that the effect of constant light was mediated by the pineal gland (Osei *et al.*, 1989). The rhythm of AA-NAT activity in the pineal gland of 13-day-old chicks was entrainable to a short LD 8:8 and a long LD18:18 photoperiod as in a LD 12:12 photoperiod, but a shorter photoperiod such as a LD 3:3 photoperiod failed to change the pre-established LD 12:12 rhythm (Doi *et al.*, 1983).

It has been reported that the expression of AA-NAT mRNA in the pineal gland exhibited circadian rhythm with peak levels at night (Bernard *et al.*, 1997; Kato *et al.*, 1999) and darkness during the day did not increase the pineal AANAT mRNA levels (Kato *et al.*, 1999). However, exposure to light for 2 hours just after lights-off blocked the increase in AA-NAT mRNA and decreased AA-NAT mRNA by 50% at midnight (Kato *et al.*, 1999). In the chicken pineal gland, AA-NAT mRNA rhythm persisted in

DD and LL where the amplitude of the rhythm was not decreased in LL (Bernard *et al.*, 1997). Further, light influenced the phase of the clock driving the pineal AA-NAT mRNA rhythm, and the rhythmic changes in pineal AA-NAT activity reflected clock generated changes in mRNA levels. In contrast, changes in mRNA content are not involved in the rapid light-induced decrease in AA-NAT activity (Bernard *et al.*, 1997).

Under polar day, the melatonin rhythms in penguins (*Pygoscelis* spp, Cockrem, 1991) and Weddell seals (*Leptomychotes weddelii*, Barrell and Montgomery, 1989) were completely abolished except in high arctic *Lapland Longspurs* where the amplitude of plasma melatonin concentrations were strongly suppressed but showed a significant diel rhythm with elevated plasma melatonin concentrations during the night (Hau *et al.*, 2002). It has been found that the diel rhythm in this species is due to lower ambient light intensity and the rhythm might have been due to masking effect rather than a circadian rhythm, or rhythmic alteration between higher and lower intensities might have acted as a zietgeber, entraining the circadian system of the bird (Wever, 1980). Other zietgeber such as the azimuth of the Sun, light intensity, and spectral composition of the light (Pohl, 1999), non-photic zietgeber such as availability of food or social cues might have kept the circadian system entrained (Reierth and Stokkan, 1998).

In avian species, temperature has been reported to play an important role in entrainment of the circadian clock and circadian melatonin rhythm *in vitro*, and directly

influenced the synthesis and release of melatonin (Barrett and Takahashi, 1995). Cold stress during scotophase significantly decreased melatonin levels in pineal glands and serum in quails (Lee *et al.*, 1990). It has been reported that changes in temperature probably act directly on all processes, which have been reported to affect membrane properties, ion homeostasis, calcium influx and other signal cascades (cAMP, cGMP, protein kinase A and protein kinase C), and influence protein phosphorylation process of the clock mechanism (Rensing and Ruoff, 2002). Further, the effects of temperature resemble, to some degree, to those induced by light or by light-transducing neurons and their transmitters (Rensing and Ruoff, 2002). In addition to photoperiod, it has been found that temperature is also one of the major regulators of amplitude, period, and phase of the melatonin circadian rhythm in the cultured chick pineal (Zatz *et al.*, 1994; Barrett and Takahashi, 1997). Melatonin synthesis and its rhythm have been reported to be sensitive to changes in temperature where several heat shock proteins (e.g., hsp25, hsp70 and hsp90 etc.) have been reported to be synthesized under changing temperature conditions, which also caused changes in melatonin production and melatonin rhythms in the avian pineal (Wolfe and Zatz, 1994). It has been found that chick pineal cells maintained at 40.0° - 43.3° C instead of 36.7° C (Zatz *et al.*, 1994) or exposed to 40° C instead of 37° C (Barrett and Takahashi, 1997) increased the amplitude of the melatonin circadian rhythm. In contrast, the amplitude was reduced by about half at lower temperature of 33.3° C, and melatonin production was stopped within few hours at further higher temperature of 46.7° C (Zatz *et al.* 1994). It has also been reported that melatonin rhythm persisted longer in constant conditions at 40° C than at 37° C (Barrett

and Takahashi, 1997). The phase response curve to low-intensity light pulses of 6 h duration has been reported to have higher amplitude at 37° C than at 40° C, which suggested an increase in sensitivity to phase-shifting stimuli as temperature decreased (Barrett and Takahashi, 1997).

Thyroid function in birds is also controlled by many environmental and physiological factors (Sharp and Klandorf, 1985). Thyroxine production has been reported to be directly controlled by the hypothalamus through thyrotropin releasing hormone (TRH) and TSH. Further, the secretion of TRH is reportedly controlled by the circadian system and directly responsive to environmental stress, daylength and temperature (Kanematsu and Mikami, 1970; Sharp and Klandorf, 1985). It has been found that electrolytic lesions in the anterior hypothalamus resulted in the atrophy of the thyroid gland (Kanematsu and Mikami, 1970). Similarly, in the quails, plasma thyroid hormone levels were depressed by hypothalamic lesions and elevated after localized hypothalamic electrical stimulation (Sharp and Klandorf, 1985). TRH in chicken (Klandorf *et al.*, 1978; Kuhn and Nouwen, 1978) and quail (Kamis and Robinson, 1978) resulted in increased levels of plasma T_4 and T_3. Photoperiod has been shown to be the prime regulator of breeding cycles in birds (Chakravorty *et al.*, 1985). Increase in daylength leads to the development of the gonads and deposition of fat in the bodies of birds (Thapliyal and Gupta, 1989). Seasonal changes in the plasma levels of T_4 have been reported in male ring dove, *Streptopelia risoria* (Lea *et al.*, 1986). In young chick and duck continuous light treatment stimulated the thyroid (Renden *et al.*, 1994; Kuhn

et al., 1996). A similar daily T_4 rhythm also occured in the Japanese quail (Herbute *et al.*, 1981). The concentration of plasma T_4 showed no rhythmicity in chickens exposed to LD 8:8 and plasma levels of T_3 were found to be depressed during the dark periods and elevated during the light periods, and responded directly to changes in food intake (Klandorf *et al.*, 1978). Daily rhythms in plasma T_4 and T_3 disappeared in chicken exposed to LL or DD (King *et al.*, 1977; Klandorf *et al.*, 1981). However, a low amplitude circadian rhythm in plasma T_4 levels persisted after transfer from LD 14:10 to LL or DD (Klandorf *et al.*, 1981).

The daily changes in levels of plasma T_3 in the chicken are inversely related to changes in levels of plasma T_4 in immature and adult chickens (Klandorf *et al.*, 1978, 1981; Herbute *et al.*, 1981). Circadian rhythm was exhibited in the levels of circulating thyroid hormones (T_4 and T_3) in the domestic chicken (Klandorf, *et al.*, 1978) and the duck (Harvey, *et al.*, 1980). Constant light significantly depressed plasma concentrations of T_4 and T_3 in chicks (Osei *et al.*, 1989). In birds also, the production of T_3 has also been found to be regulated peripherally by the rate of monoiodination of T_4, and the conversion of T_4 to T_3 is dependent on energy intake and influenced by circulating levels of other hormones including prolactin (Sharp and Klandorf, 1985). A direct evidence that the daily rhythm in the level of plasma T_4 and T_3 depends on the pattern of food intake and the metabolizable energy of the diet suggested that energy intake is the main factor regulating plasma T_3 levels and, presumably, the conversion of T_4 to T_3 (Harvey and Klandorf, 1983; Sharp and Klandorf, 1985). It has been reported

that in any situation in which energy intake changes, there is a corresponding change in plasma T_3 levels in birds (Alshaikh *et al.*, 1997). The level of plasma T_3 is inversely related to the ambient temperature in birds with free access to food. At a high ambient temperature (30° C), the concentrations of plasma T_3 were depressed in sexually immature (Cogburn and Harrison, 1980) and laying hens, where the depression in plasma T_3 levels was found to be associated with a reduction in food intake (Klandorf *et al.*, 1981). Concentrations of plasma T_3 increased in sexually immature chickens when the temperature fell from 27.5 to 17.5 ° C (Kuhn and Nouwen, 1978) and in quails transferred from 22-23° C to 10° C (Oishi and Konishi, 1978). In contrast, plasma levels of T_3 have been reported to be increased in quails after transfer from 22° C to 35° C (Bobek *et al.*, 1980). T_4 levels in chicken were not affected by high ambient temperatures (Klandorf *et al.*, 1981; Harvey and Klandorf, 1983), while T_4 levels were reported to be depressed in quail (Bobek *et al.*, 1980). In chickens (Kuhn and Nouwen, 1978) and quails (Bobek *et al.*, 1980; Herbute *et al.*, 1981), levels of plasma T_4 increased with decrease in the ambient temperature. In quail, the increase was found to occur within 30 minutes of transfer from 26° C to 4° C (Herbute *et al.*, 1981), which has been reported to be mediated by a direct effect on the secretion of TRH rather than by alterations in food intake (Sharp and Klandorf, 1985).

The levels of thyroid hormones are inhibited by administration of melatonin and increased after pinealectomy in birds (Sharp *et al.*, 1984; John *et al.*, 1990; Prakash *et al.*, 1998). However, pinealectomy did not have any effect on the plasma levels of

thyroid hormones in cockerels exposed to LD 14:10 or to DD (Osol *et al.*, 1980) or to different ambient temperatures (Cogburn and Harrison, 1980). Estrogen and testosterone have been reported to depress thyroid function in birds (Sharp and Klandorf, 1985). Subcutaneous implantation of melatonin pellets in pigeon has been reported to decrease plasma levels of thyroxin with no significant effect on plasma levels of T_3, while T_3/T_4 ratio was found to be higher (John *et al.*, 1990). However, regardless of the photoperiod, no clear functional relationship was found between the avian pineal gland and thyroid function, although a transitory increase in T_4 levels has been reported in both pinealectomized (PX) and sham-operated birds (Rintamaki *et al.*, 1985). It has been reported that PX-cockerels had smaller thyroids at 23° C and larger thyroid at 37° C. The concentration of T_3 was found to be higher in PX-cockerels than the intact ones (Cogburn and Harrison, 1980). High environmental temperature has been reported to depress serum T_3 concentration independent of surgical treatment (Cogburn and Harrison, 1980).

Reptiles

In reptiles also daily and seasonal variations in hormone levels influence the complex behavior and physiology (Lutterschmidt *et al.*, 2002). Pineal organ in ecto-therms has been reported to be a part of a circadian pacemaker system, transducing photo-thermal environmental information into a neuro-chemical signal (Mahapatra *et al.*, 1988; Firth and Kennaway, 1989), where photoperiod and thermoperiod have been reported to influence melatonin rhythms and the timing of annual physiological cycles

(Firth *et al.*, 1991). It has been reported that both light and temperature are important modulators of pineal function in gecko (*Christinus marmoratus*), although their combined effects on pineal melatonin production is complex and unclear (Moyer *et al.*, 1997). Both light and temperature reportedly control the pineal rhythm of melatonin synthesis and secretion in the lizard, *Anolis carolinensis* (Underwood and Calaban, 1987). It has been reported that photoperiod and temperature interact with each other to influence serum melatonin in *Nerodia rhombifera*, with photoperiod affecting the phase and temperature affecting the amplitude of the diel melatonin cycle (Tilden and Hutchinson, 1993). The lizard pineal can act as a photo- and thermo-endocrine transducer translating light and temperature information into an internal cue in the form of pineal melatonin rhythm, which in turn may control the phase and period of the circadian clocks located elsewhere insuring that the right internal events occur at the right time of the day (Underwood and Calaban, 1987).

Seasonal variations in circadian rhythms of plasma melatonin has been reported in ruin lizard (*Podorcis sicula*) during spring, summer and autumn under a LD cycle, and concomitant responses when transferred to DD (Bertolucci *et al.*, 2000). Further, melatonin levels have been reported to remain high under DD in spring, unchanged in summer, low in fall and exhibited circadian rhythm only in summer (Bertolucci *et al.*, 2000). Pineal melatonin content in *Testudo hermanni* in natural lighting has been reported to show peak night-time melatonin levels during summer (May to September) at the period of greatest activity, while melatonin levels were reported to be very low

with no circadian rhythm during hibernation (Vivien-Roels and Arendt, 1979). Plasma melatonin levels in intact green inguana, *Inguana inguana* were found to be high during the night and low during the day (Tosini and Menaker, 1996). PX-lizards have been reported to show low levels of plasma melatonin during both the day and night (Tosini and Menaker, 1996). Illumination for 2 h did not suppress the nocturnal melatonin peak in the *Anolis carolinensis* pineal (Underwood and Calaban, 1987). In *Terrapene Carolina triunguis*, no clear cut melatonin rhythm was observed under short photo-period LD 8:16 (Skene *et al.*, 1989). In male lizard, *Anolis carolinénsis* photoperiod at a constant temperature had a profound effect on the duration, amplitude, and phase of the pineal melatonin rhythm (Underwood and calaban, 1987). Further, both daily light and temperature cycles have been reported to entrain the pineal melatonin rhythm, where melatonin levels peaked during the dark phase of a LD cycle or during the cool phase of a temperature cycle. The pineal melatonin rhythm under cyclic temperature has been found to exhibit a peak during the cool (20° C) phase of the cycle regardless of whether or not the cool phase occurred during the light or dark phase under LD 12:12 and DD. However, the amplitude of the rhythm under DD was been found to be depressed as compared to that observed under LD 12:12 (Underwood and Calaban, 1987). Temperature cycles as low as 2° C entrained the pineal melatonin rhythm in lizards held in LL or DD. The lengths of the photoperiod and thermoperiod have been found to affect the phase, amplitude, or duration of the pineal melatonin rhythm (Underwood and Calaban, 1987; Vivien-Roels *et al.*, 1988). The effects of light and temperature cycles on the pineal melatonin rhythm depended on the phase relationship between the light

and temperature cycles, as well as on the strength of the entraining stimuli, such as the amplitude of the temperature cycle (Underwood and Calaban, 1987; Vivien-Roels *et al.*, 1988).

Melatonin has been reported to decrease mean preferred body temperature in nocturnal snake, *L. fuliginosus* (Lutterschmidt *et al.*, 2002). Seasonal variation in accordance with changes in the environmental temperature in serotonin immuno-reactivity and ultrastructure of the secretory rudimentary photoreceptor cells has been reported in the pineal organ of the Japanese grass lizard, *Takydromus tachydromoides* (Ohshima *et al.*, 1999). Temperature acclimation of adult turtles has been reported to induce changes in metabolic physiology (O'steen and Janzen, 1999). Ambient temperature has also been found to alter serotonin, NE and epinephrine content in the pineal-parapineal complex of turtles (*Lissemys punctata punctata*) (Mahapatra *et al.*, 1989). Diurnal melatonin concentrations has been found to rise linearly with the increase in ambient temperatures (5, 15, 20 and 27° C) with no day/night differences suggesting that pineal melatonin in the box turtle, *Terrapene Carolina triunguis* is modified by the photoperiod and to a lesser extent by temperature (Skene *et al.*, 1989). Environmental temperature was found to affect the amplitude of the day-night rhythm of melatonin production in turtle (Vivien-Roels *et al.*, 1988). Exposure of *Anolis carolinensis* to a constant cold temperature of 10° C eliminated the pineal melatonin rhythm, yet a rhythm was expressed under 24 h temperature cycle 32° C:10° C, and the rhythm peaked during the 10° C phase of the cycle (Underwood and Calaban, 1987).

The pineal organ of gecko, *Christinus marmolatus* maintained *in vitro* at 30° C: 15° C thermocycle has been found to elicit a rhythm of melatonin production under LD 12:12, LL or DD (Moyer *et al.*, 1997). When the cryophase coincided with the dark phase of the photocycle or with the subjective night, then the thermocycle was 180° out of phase with the photoperiod and the rhythm of melatonin production was found to be disrupted suggesting a differential pattern of sensitivity to photothermal stimuli (Moyer *et al.*, 1997). Further, in snakes under LD 12:12, a diel cycle of serum melatonin concentrations at 25° C was elevated during scotophase which was higher than those exposed to 35° C, where serum melatonin concentrations during photophase at 10° C was lower than that at 25° C with no increase during scotophase (Tilden and Hutchinson, 1993).

Seasonal variations in thyroid hormones have been reported in several reptilian species. In the male aspic viper (*Vipera aspic*), the plasma thyroxine was at maximum concentration from February to March, after which the levels decreased markedly reaching to a minimum in November-December (Naulleau *et al.*, 1987). The seasonal profile of thyroxine has been reported to be clearly marked during the period of hibernation (Naulleau *et al.*, 1987). The plasma levels of T_4 remains uniform throughout the year in male green sea turtle, *Chelonia mydas* (Licht *et al.*, 1985b). There is a significant secretion of thyroid hormones in reptiles when kept at 30° C than that of 20° C (Hulbert and Williams, 1988). The temperature dependence of thyroid hormone action has been reported in several lizard species (Thapliyal, 1980; Gupta and Thapliyal,

1991). The secretion rates of thyroid hormones in reptiles (at their preferred temperature) are less than those in mammals indicating that thyroid gland activity is less in reptiles than in mammals (Hulbert and Williams, 1988). Further, plasma T_4 concentrations are also less in reptiles than in mammals. In the cobra (*Naja naja*) and lizards, T_4 levels were found to be low during most part of the year, and were high only during summer (Bona-Gallo *et al.*, 1980; John-Alder and Bennett, 1987). Although the seasonal peaks are found to be high during summer for reptiles, they were found to be lower than that of the mammals (Bona-Gallo *et al.*, 1980; John-Alder and Bennett, 1987). In the turtle, *Pseudemys scripta* the thyroid gland was relatively insensitive to temperature and responded to TSH between 12° C and 32° C with no difference between 20° C and 28° C (Licht *et al.*, 1989). An increase in plasma T_4 levels has been reported in *Vipera aspis* kept at 5° C or exposed to natural conditions (Fleury and Naulleau, 1987).

Amphibians

Day-night variations in pineal AA-NAT activity and melatonin contents in pineal organ and serum have been reported in the frog, *Rana esculenta,* which seems to be affected by photoperiod (d'Istria *et al.*, 1994). Plasma melatonin level in the neotenic tiger salamander (*Ambystoma tigrinum*) was affected by photoperiod and pinealectomy (Gern and Norris, 1979). No melatonin rhythm was observed in pineal and plasma of the frog, *Rana perezi* kept under short photoperiod and low temperature in February (Delgado and Vivien-Roels, 1989). Further, the duration or intensity of light exposure

was not able to inhibit nocturnal AA-NAT activity in 1 month and 6 months old frog, *Rana tigrina* (Lee *et al.*, 1997). Plasma melatonin rhythms has been reported to be entrained by the LD cycle and affected by temperature (Wright *et al.*, 2003). Consequently, melatonin has been reported to transduce environ-mental information to regulate endocrine periodicity and larval circadian organization, and influences metamorphic rate (Wright *et al.*, 2003). In *Rana perezi*, increase in environmental photoperiod and temperature induced a day-night rhythm of melatonin levels in the plasma, while a decrease in environmental temperature abolished the melatonin rhythm (Delgado and Vivien-Roels, 1989). In cultured pineal of the green frog (*Rana perezi*) AA-NAT activity and melatonin rhythms were similar to that observed *in vivo* under natural environmental conditions, where forskolin increased AA-NAT activity up to 2-fold and melatonin production upto 4-fold irrespective of lighting conditions. The addition of cycloheximide *in vitro* significantly reduced both nocturnal AA-NAT activity and melatonin release (Alonso-Gomez *et al.*, 2000). Melatonin plays a role in metamorphosis in anuran amphibians as a thyroid antagonist, whose level falls at the metamorphic climax when the thyroid hormones reach a peak (Rose and Rose, 1998; Wright and Alves, 2001; Wright, 2002).

The endogenous secretion in T_4 has been found to exhibit rhythmicity in the tadpoles of *Rana pipiens* (Wright *et al.*, 1986). Metamorphic progress has been found to be affected by T_4-melatonin interactions at the tissue level (Wright, 2002). In anuran tadpoles under LD 12:12, it has been reported that at Stage XX, both follicle cell height

and cell division were rhythmic while lumen diameter, showed no significant daily changes. Thyroids from *Rana catesbeiana* larvae at Stages XVII to XVIII had a significant circadian rhythm of T_4 secretion *in vitro* (Wright *et al.*, 1995). It has been reported that the daily sequence of thyroid structure and function in preclimax Rana tadpoles included larger follicle lumina early in the light and maximum cell division, T_4 secretion, and follicle cell height within a 4 h interval beginning around the onset of the dark phase (Wright *et al.*, 1995). In *Rana catesbeiana* tadpoles during premetamorphosis, circulating levels of both T_4 and T_3 were below the limits of detection and a gradual rise in plasma of total as well as free T_3 and T_4 became apparent during prometamorphosis. At the onset of the metamorphic climax, the levels of both T_3 and T_4 increased sharply (Regard *et al.*, 1978). Further, the peak levels for both hormones were observed in the middle of metamorphic climax (stage XXIII) where circulating T_3 level reached a mean peak at least 15 times greater, and T_4 level exhibited about a 10-fold increase over the premetamorphosis level (Regard *et al.*, 1978).

The rhythms of T_4 and the corticosteroids are reportedly entrained to the LD cycle, and these rhythms and those of melatonin exhibit changes during development in a specific way under each LD cycle. Differences in the direction and magnitude of phase shifts during development has been reported to place the peaks of thyroid modulators, such as the corticosteroids and melatonin, in different relationships to the T_4 peaks suggesting an important aspect of the hormonal regulation of metamorphosis (Wright, 2002). In the tadpoles of *Rana catesbeiana* under LD 6:18, no LD cycle-

specific diel fluctuations was recorded in froglet plasma T_4 at prometamorphosis while under LD 12:12 plasma T_4 peaked during the scotophase at prometamorphosis and early climax. Under both LD cycles, the 24 h mean of plasma T_4 was found to increase at climax and and then fall (Wright *et al.*, 2003). An annual variation in the T_4 contents of the thyroids in females of the giant swamp frog, *Dicroglossus occipitalis* has been reported to be positively correlated with gonado-somatic index (GSI) and egg size, and plasma T_4 concentrations negatively correlated with GSI. Changes in thyroidal and plasma T_4 may be the result of an activation of thyroid function by the peripheral conversion of T_4 into T_3 (Kuhn *et al.*, 1987). In adult *Rana perezi*, significant daily changes in plasma T_3 levels were present during spring, summer and autumn but not in winter, the lowest values being observed during the scotophase (Gancedo *et al.*, 1996). In contrast, only plasma T_4 showed significant changes in spring, following a similar pattern as that of T_3. Thyroid fT_3 content has been reported to exhibit day/night significant changes only in spring showing high contents at early scotophase. Further, the existence of seasonally changing daily fluctuations in thyroid activity in *Rana perezi* has been suggested, and an interaction between photoperiod and temperature is supposed to plays a role in the regulation of these daily changes (Gancedo *et al.*, 1996). Thyroid hormones exhibited circadian rhythmicity in plasma and the thyroid gland of male *Rana ridibunda* before and during hibernation, while hibernating frogs in January had a lower T_3 and T_4 content of their thyroid gland. Plasma levels of T_3 were maintained and of T_4 increased compared to fed September or October frogs. Increased photoperiod in January was supposed to be responsible for the increased T_4 secretion,

since in December there was no influence of low temperature on circulating T_3 or T_4 levels (Kuhn *et al.*, 1983).

Plasma melatonin levels were found to decrease in tadpoles of the bullfrog (*Rana catesbeiana*) at the climax of metamorphosis when the T_4 level exhibited peaks, and melatonin was found to inhibit thyroid function *in vitro* (Wright and Alves, 2001). Daily injection of melatonin to tadpoles kept at 22° C under an LD 18:6 cycle had no effect on metamorphic progress, or on plasma T_4 or melatonin levels, except for a transient rise in melatonin just after the injection (Wright and Alves, 2001). Further, melatonin accelerated metamorphosis which have been reported to occur through alterations in the concentration of prolactin and impaired body size in *Xenopus laevis* (Rose and Rose, 1998) and *Rana catesbeiana*, and decreased plasma melatonin had no effect on plasma T_4 (Wright and Alves, 2001). Administration of T_4 accelerated metamorphosis more than by immersion in melatonin solution, raised plasma T_4 to climax levels, and induced a decrease in plasma melatonin (Wright and Alves, 2001).

Fishes

Fishes perceive environmental light regimes through eyes and the pineal complex. The pineal complex of fish consists of pineal gland and the parapineal (Vollrath, 1981). However, the pineal hormone is produced only by the pineal and not by the parapineal. The fish pineal contains photoreceptor cells, which perceive light directly and synthesize and release melatonin in absence of light (Falcon *et al.*, 1989).

The fish pineal has developed mechanisms to modulate melatonin synthesis by responding directly to environmental light and indirectly to environmental temperature via the central nervous system to ensure survival under the ever-changing aquatic environment. The regulation of melatonin synthesis in fish pineal by light and/or temperature (Gern and Greenhouse, 1988; Falcon *et al.*, 1989; Kezuka *et al.*, 1989; Zachmann *et al.*, 1992; Bolliet *et al.*, 1993) seems to suggest that the fish pineal might be acting as a photo-transducer as well as a thermo-transducer organ and mediates influence of lighting conditions on fish physiology (Matty, 1985b; Meissl, 1986; Falcon *et al.*, 1989; Underwood, 1989; Zachmann *et al.*, 1992). It has been reported that diurnal cycle of melatonin in blood is the result of rhythmic melatonin production in the pineal organ (Kezuka *et al.*, 1989). In fishes also AA-NAT is the rate-limiting enzyme in the melatonin biosynthetic pathway (Coon *et al.*, 1999), which controls the circadian pattern of melatonin synthesis (Kezuka *et al.*, 1988; Coon *et al.*, 1999). Although a single AA-NAT gene has been found in mammals and chicken, two *aa-nat* genes in fishes, which are designated as *aa-nat-1* and *aa-nat-2*, have been identified in pike and trout (Coon *et al.*, 1999; Mizusawa *et al.*, 2000). In pike it has been reported that AA-NAT-1 is exclusively expressed in the retina and AA-NAT-2 in the pineal gland (Coon *et al.*, 1999; Gothilf *et al.*, 1999). Rhythmic changes in melatonin synthesis has been found to be regulated by changes in AA-NAT activity. The rhythm in AA-NAT activity in fishes, as in most vertebrates, is driven by circadian clocks, and photoperiod resets and entrains the clocks (Kezuka *et al.*, 1988; Coon *et al.*, 1999). The cyclic appearance of melatonin in relation to photoperiod has been described in other vertebrates as well

(Gern and Norris, 1979; Maeda *et al.*, 1984; Underwood *et al.*, 1984; Siguenza *et al.*, 1988). As in mammals, plasma melatonin levels in fishes also fluctuate in a rhythmic manner with high levels during the dark phase and low levels during the light phase (Gern and Greenhouse, 1988; Kezuka *et al.*, 1988, 1989; Falcon *et al.*, 1989; Zachmann *et al.*, 1992). The daily cyclic of plasma melatonin levels in gold fish was abolished following pinealectomy (Kezuka *et al.*, 1988). Diel melatonin secretion profile has been reported to reflect the illumination regimen *in vitro* with light or photophase associated with low melatonin titer in the perfusate and darkness or scotophase associated with high titer in *Salmo gairdneri* (Gern and Greenhouse, 1988). Light acts independently via downstream mechanism to turn off AA-NAT activity by initiating proteasomal proteolysis of AA-NAT protein (Gastel *et al.*, 1998). Organ culture studies with trout and seabream also indicate that the light-induced decrease of AA-NAT-2 activity is prevented when proteasomal proteolysis is blocked (Falcon *et al.*, 2001). It has been reported that light pulses during the normal scotophase resulted in a depression in melatonin secretion regardless of whether it was given early or late in the dark period, while pulses of darkness given early or late in a normal photophase has been reported to increase melatonin secretion (Gern and Greenhouse, 1988). An endogenous rhythm in melatonin synthesis under DD was not detectable in trout pineal, whether the pineals were first entrained to LD prior to DD or exposed to DD at the initiation of the superfusion. Exposure to a LD 4:4 cycle after DD has been reported to result in a decreased melatonin secretion under light and increased under darkness (Gern and Greenhouse, 1988). Further, melatonin secretion in fishes also seem to be influenced by

environmental temperature (Zachmann *et al*, 1992; Tabata and Meissl, 1993; Samejima *et al.* 2000). The seasonal fluctuations in photoperiods and temperature have also been reported to regulate the circadian and circannual rhythms of melatonin production in fish (Randall *et al.*, 1995; Pavlidis *et al.*, 1999).

Temperature is directly involved in the control of the fish pineal melatonin production *in vitro*. No significant endogenous rhythm in the release of melatonin was found at low temperature (10° C) by pineals of lamprey (*Petromyzon marinus*) cultured under DD (Bolliet *et al.*, 1993) and white sucker, *Catostomus commersoni* (Zachmann *et al.*, 1992) and in superfused trout (*Oncorhynchus mykiss*) pineals at 9° C (Gern and Greenhouse, 1988). However, endogenous rhythmicity of melatonin production was reported at higher temperature (20° - 27° C) under DD in the pineal of *L. marinus* and *Carassius auratus* (Iigo *et al.*, 1991), in white sucker (*Catostomus commersoni*) pineal maintained *in vitro* (Zachmann *et al.*, 1992), in statically cultured pineal at 27° C of pike (*Esox licius*) (Falcon *et al.*, 1989), and in static gold fish pineal organ culture at 25° C (Kezuka *et al.*, 1989). In contrast to these species, it has been reported that the pineal organ of the rainbow trout did not contain any endogenous oscillator entraining the rhythm of melatonin secretion (Bolliet *et al.*, 1997; Coon *et al.*, 1998). Temperature has been reported to have a strong influence on the amplitude of melatonin rhythm in *Petromyzon marimus*, where the peak melatonin titers were decreased at 10° C when compared with those at 20° C (Zachmann *et al.*, 1992). At 10° C, a low plasma melatonin rhythm amplitude has been reported in *P. marimus* maintained under LD

12:12 (Bolliet, *et al.*, 1993), whereas pineal of animals reared at 20° C and cultured at the same temperature released constant levels of melatonin during the night (Zachmann *et al.*, 1992).

In fishes, melatonin is considered to be the most physiologically active indole derivative (Zachmann *et al.*, 1992). Pineal plays an important role in controlling gonadal maturation. Melatonin may either stimulate or inhibit gonadal maturation depending on the time of the year and stage of the reproductive cycle and also on the photoperiod-temperature regime to which the fishes are exposed (Garg, 1989). It has been reported in lamprey that light information perceived by the pineal is transmitted to the central nervous system and contributes to the control of the locomotor activity rhythm (Garg and Sundararaj, 1986; Morita *et al.*, 1989). The pineal gland in fish has been reported to play an important role in the appearance of a circadian rhythm in the daily shifts of a fish's activity as analysed by behavior analysis (Kavaliers, 1981; Tabata *et al.*, 1988). Melatonin reportedly produces prominent effects on growth and metamorphosis (Eddy, 1969), pituitary (Vollrath, 1981), thyroid (Nayak and Singh, 1987a,b), adrenal (Agha and Joy, 1989), interrenal function (Vollrath, 1981) etc. As in mammals, melatonin appears to exert its effects via the hypothalamo-hypophyseal-gonadal axis (Vollrath, 1981).

Administration of melatonin increased the size of gonadrotropic cells in *Carassius auratus* and reduced the number of gonadotorphs in *Heteropneustes fossilis*

(Vollrath, 1981). An increase in the number of gonadotrophs in *Notemigonus* after pinealectomy points to an inhibitory influence of the pineal on the pituitary gland. Further, administration of melatonin has been reported to reduce pituitary prolactin levels in *Fundulus similis* (Vollrath, 1981). However, effects of pinealectomy on pituitary prolactin levels seem to depend on the photoperiodic regime (Vodicnik and de Vlaming, 1978). The pineal organ in fish exerts an inhibitory influence on the activity of the adrenal in the Indian catfish (Agha and Joy, 1989). However, there is paucity of information on the effects of melatonin on thyroid hormones in any fish in relation to temperature, seasons and photoperiod.

The fish thyroid gland secrets thyroxine (T_4) and triiodothyronine (T_3) as in other vertebrates. In teleosts, thyroid hormones exert effects on embryonic development, growth, function of the central nervous system, and activities of certain metabolic enzymes (Varghese *et al.*, 2001; Tripathi and Verma, 2003; Boiko *et al.*, 2004; Lema and Nevitt, 2004). In piscine species thyroid hormones are involved in the regulation of the oxidative and intermediary metabolism (Plisetskaya *et al.*, 1983; Gupta and Thapliyal, 1991; Lynshiang and Gupta, 2000), reproduction (Bhattacharya, 1987), growth and development (Power *et al.*, 2001), breeding cycle (Volkoff *et al.*, 1999), sexual maturation, behavior, migration (Ueda *et al.*, 1984; Matty, 1985a; Cyr *et al.*, 1998; Pavlidis *et al.*, 2000), electrolyte and water metabolism (Peter *et al.*, 2000) etc.

There is scarcity of information on seasonal variation in the levels of thyroid hormones and melatonin in fish species (Iigo *et al.*, 1997; Samejima *et al.*, 1997). Circadian variations in the circulating levels of thyroxine (T_4) and triiodothyronine (T_3) of fishes are reportedly controlled by diurnal fluctuations in the ambient temperature, physical activity, photoperiod, feeding etc. (Matty, 1985a; Cyr *et al.*, 1988; Leiner *et al.*, 2000). However, the circannual variations in these hormones seem to be closely associated with circannual variations in ambient temperature, daylengths and gonadal steriods (Pavlidis *et al.*, 2000). Acclimation to warm has been generally observed to stimulate thyroid activity in fish, although an inverse relationship between thyroid epithelial cell height and ambient temperature has been often reported (Eales, 1979). It has also been reported that plasma T_3 and T_4 were increased at low temperature, and positively correlated with thyroid epithelial cell hight (Eales *et al.*, 1982). In rainbow trout (*Salmo gairdneri*), there were no changes in plasma T_4 or T_3 levels as a function of temperature (Perrier *et al.*, 1979). A 3-fold increase in the secretion of hormonal iodine has been recorded in eels (*Anguilla anguilla*) at 25° C as compared to 6.5° C (Leloup and Fontaine, 1960). Further, differential effects of temperature on metabolism and plasma levels of T_4 and T_3 have also been reported in eels (Leloup and DeLuze, 1985).

Unlike in mammals, there is scarcity of information on the effects of melatonin on the circulating levels of thyroid hormones in any fish species. It also remains unknown whether hypo- and hyper-thyroidism influence AA-NAT activity in the fish pineal. Though melatonin and thyroid hormones are involved in the regulation

of a wide range of physiology as well as the gonadal cycles of fishes, so far no attempt has been made to investigate the interrelationship between pineal and thyroid in a fish with special reference to its annual gonadal cycles, temperature and photoperiod. Therefore, keeping in mind the scarcity of information and importance of melatonin, thyroid hormones, temperature and photoperiod in fish physiology, it was thought worthwhile to undertake a comprehensive study to explore the nature of interrelationship, if any, between the thyroid and the pineal gland in an air-breathing exotic cat fish, *Clarias gariepinus*.

CHAPTER - 1

MATERIALS AND METHODS

INTRODUCTION

Fishes are aquatic vertebrates that differ in many aspects of habits and habitats compared to the terrestrial vertebrates. During the course of evolution, they developed a special mechanism by which they could extract dissolved oxygen from water through their gills. The air breathing in fish is a common phenomenon, which can be found among a wide range of freshwater as well as marine species (Sayer and Davenport, 1991). The freshwater air breathing fishes supplement their aquatic respiration primarily by utilizing atmospheric oxygen (O_2) and release carbon dioxide (CO_2) aquatically (Hazel, 1993). However, marine air breathing fish species emerge out of water completely and are able to exchange O_2 as well as CO_2 in air (Bridges, 1993).

The bodily functions of the fish are directly or indirectly subjected to fluctuations in the environment. Changes in the environment lead to continuous adjustment in the physiological and morphological characteristics of the fish (Hazel, 1993). Fish body temperature is more or less parallel to water temperature, and increases/decreases with the rise/fall of the temperature. This change in body temperature has a pervasive effect on the biological processes and greatly accelerates metabolic pathways with increasing temperature (Cossins and Bowler, 1987). Unlike mammals and birds, fishes must cope with the effects of extreme and varying body temperature on their activity and behavior.

Fluctuations in environment exert a regulatory influence on the plasma levels of thyroid hormones and pineal arylalkylamine-N-acetyltransferase activity (AA-NAT) activity.

All the experiments of this Ph. D. dissertation were conducted on the air-breathing fish, *Clarias gariepinus* due to its easy availability, excellent survival under laboratory conditions and reasonable cost throughout the year at Shillong. A brief introduction of the fish, techniques used for the measurement of plasma thyroid hormone levels and AA-NAT activity, and the biostatistical and computational methods used for the analysis of the data is presented in the following sections.

***Clarias gariepinus*:**

Clarias gariepinus is a teleost, which is widely distributed all over India and other tropical countries. In nature it usually lives in shallow rivers, ponds and muddy places, and survives even in water with low oxygen content. It exhibits a bimodal (aquatic and atmospheric) breathing habit, and often comes to the surface of water to engulf atmospheric air. *Clarias gariepinus* breeds during monsoon (Yalcin *et al.*, 2001). The gonadal activity undergoes a cyclic change (both in morphology and histology) with the change in season so that spawning takes place in the most propitious time of the year ensuring maximum survival and faster growth of the young ones. At Shillong, gonadal activity remains minimum (quiescent phase) during the months of January and February, which increases gradually during the months of March to May (progressive phase). Breeding occurs from June to August (breeding phase). Then gonads undergo regression

during the months of November and December (regressive phase). This cyclic change in the gonadal activity is possibly cued by the external factors (e.g. water temperature, photoperiod etc.). It has been suggested that pituitary gland through the cyclic synthesis and release of gonadotropins regulates the cyclic activity of gonads resulting in synchronised gonadal maturation and spawning (Reiter *et al.*, 1983). *Clarias gariepinus* is commonly used for fish farming in different parts of India.

For this dissertation, adult male *Clarias gariepinus* (body weight: 90-100g; body length: 23-27 cm) were purchased from the local fish suppliers. Fishes were maintained in clear plastic tubs and acclimatized before starting any experiment at least for 15 days in the laboratory under natural climatic conditions at Shillong (Latitude 25°.30′ N, Longitude 91°.52′ E; Altitude 1450 ASL; Minimum water temperature: 4° C; maximum water temperature: 24.5° C). During acclimatization, the fishes were fed daily with minced earthworms and commercial fish food *ad libitum*. Water was changed everyday to avoid infections.

Chemicals:

All fine chemicals including hormones used in the experiments were purchased from Sigma-Aldrich, USA. ^{14}C-acetyl coenzyme A was purchased from Amersham Pharmacia Biotech (U. K). Radioimmunoassay kits were obtained from the Division of Radiopharmaceutical Operations, Board of Radiation and Isotope Technology (BRIT),

Mumbai. General chemicals were purchased from S. D. fine chemicals, India and E. Merck, India.

Collection of blood samples and pineals:

Fishes were decapitated and blood samples from the post-caudal region were collected in numbered heparinized centrifuge tubes, avoiding the body fluid. The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain plasma. The plasma samples were stored in the ice-chamber (-8 to -10° C) of a refrigerator for measuring the thyroid hormones.

Simultaneously, in order to collect pineal, the pineal window was exposed with the help of a sterilized surgical blade. During the dark phase, pineals were collected under dim red light. The collected pineals were placed in Eppendorf tubes and immediately stored in liquid nitrogen.

Measurement of plasma levels of total thyroxine (T_4) and triidothyronine (T_3):

The plasma levels of total T_3 and total T_4 were measured with the help of radioimmunoassay using kits (RIAK4/4A for T_3 and RIAK5/5A for T_4) procured from the Board of Radiation and Isotope Technology (BRIT), Mumbai (for details, please see Protocol 1 and Protocol 2). The RIA of T_3 and T_4 were conducted following manufacturer's protocol with slight modifications (Gupta and Premabati, 2002a). The hormone free fish plasma used in the assay was prepared by two cycles of addition of

dextran-coated charcoal to pooled fish plasma, continuous stirring for 6 hours and centrifugation. Polyethylene glycol (PEG) solutions (12% for T_3 and 22% for T_4) were used to separate the bound and free fractions of T_3 and T_4 in the respective RIA. The intra- and inter-assay variations in the assays were found to be on average less than 3.5% and 6.5% for T_3 and T_4, respectively. The radioactivity in the bound fraction was counted with the help of a well-type gamma counter (Electronic Corporation of India). The concentrations of total T_3 and total T_4 were expressed as ng/ml of plasma.

Measurement of pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity:

Arylalkylamine-N-acetyltransferase (AA-NAT) activity was measured with the help of radio-enzymatic assay as described by Deguchi and Axelrod (1972) with slight modifications (for details, please see Protocol 3). For the measurement of AA-NAT activity, the pineals were sonicated in 75 µl of homogenization buffer (phosphate buffer: pH 6.5 with 6 nM acetyl Co-enzyme A). The 15 µl samples (in duplicate) of the sonicated pineals were incubated for 20 min at 25° C with 5 µl of the reaction mixture (tryptamine hydrochloride solution, phosphate buffer, ^{14}C-acetyl Co-A). 100 µl of borate buffer was added to stop the reaction. In order to extract acetylated tryptamine, a mixture of isoamyl alcohol and toluene (3:97) was added to the tubes, and the tubes were rotated in a rotary mixer (Stuart Scientific, U. K) for 15 minutes, and centrifuged at 3000 rpm for 5 minutes. Two ml of the upper phase of the mixture was transferred to a scintillation vial containing 5 ml of scintillation fluid. The radioactivity in each sample was counted in terms of DPM with the help of a liquid scintillation counter

(Wallace). Vials (in duplicates) treated as 'Blanks' contained homogenization buffer, reaction mixture, extraction solution, and scintillation fluid only. After calculations, AA-NAT activity was expressed as nmol/pineal/hour.

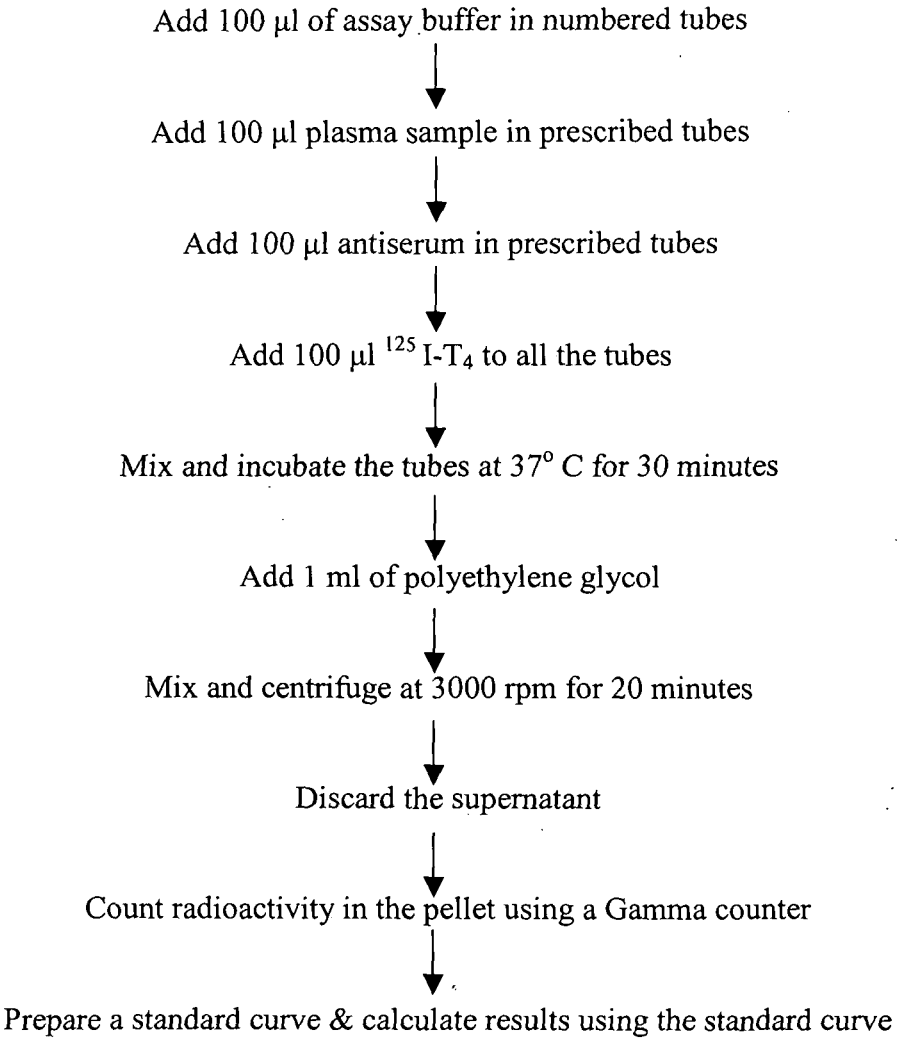
Analysis of data:

Statistical: The data were analyzed statistically with the help of Student's 't' test and regression analysis (Snedecor, 1961). A $p < 0.05$ was considered as significant.

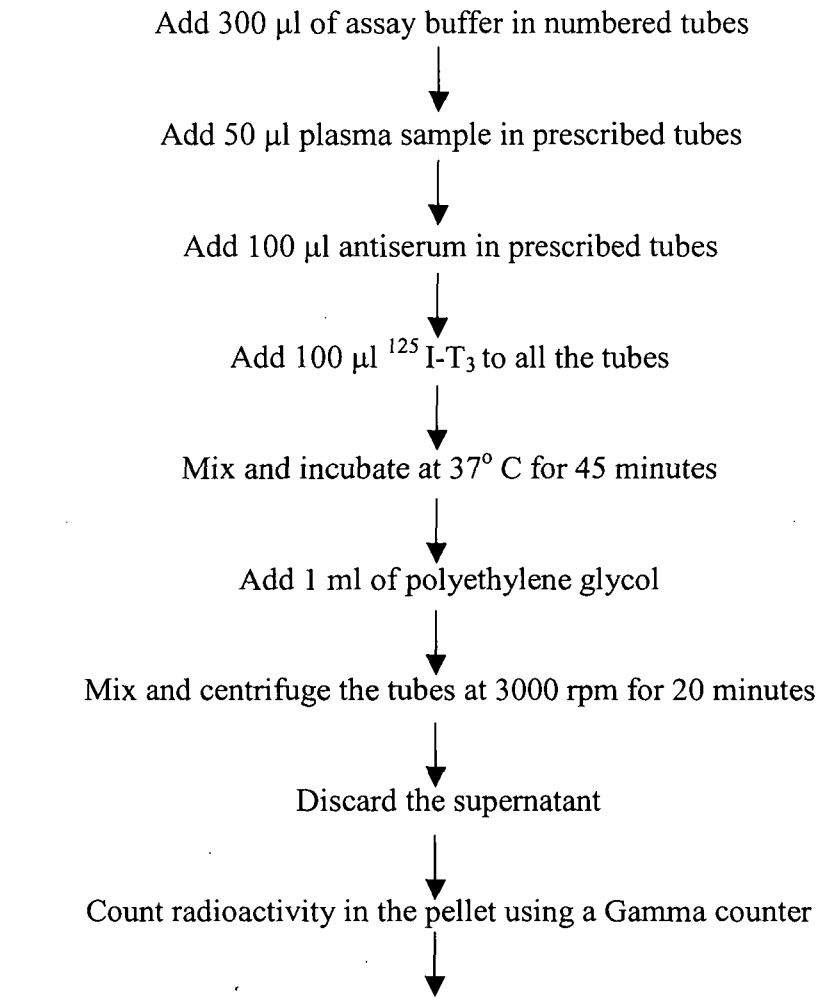
Computational methods: Acro program based on least square fitting of single cosine function (Nelson *et al.*, 1979) was used for the analysis of mesor (mean level), amplitude (half of the excursion range), acrophase (time of the peak of the rhythm), 95% confidence interval (C. I), goodness of fit and threshold of the circadian rhythm. A $p < 0.05$ was considered as significant.

These standard methods were followed in all experiments. The details of experimental protocols are described in the following chapters.

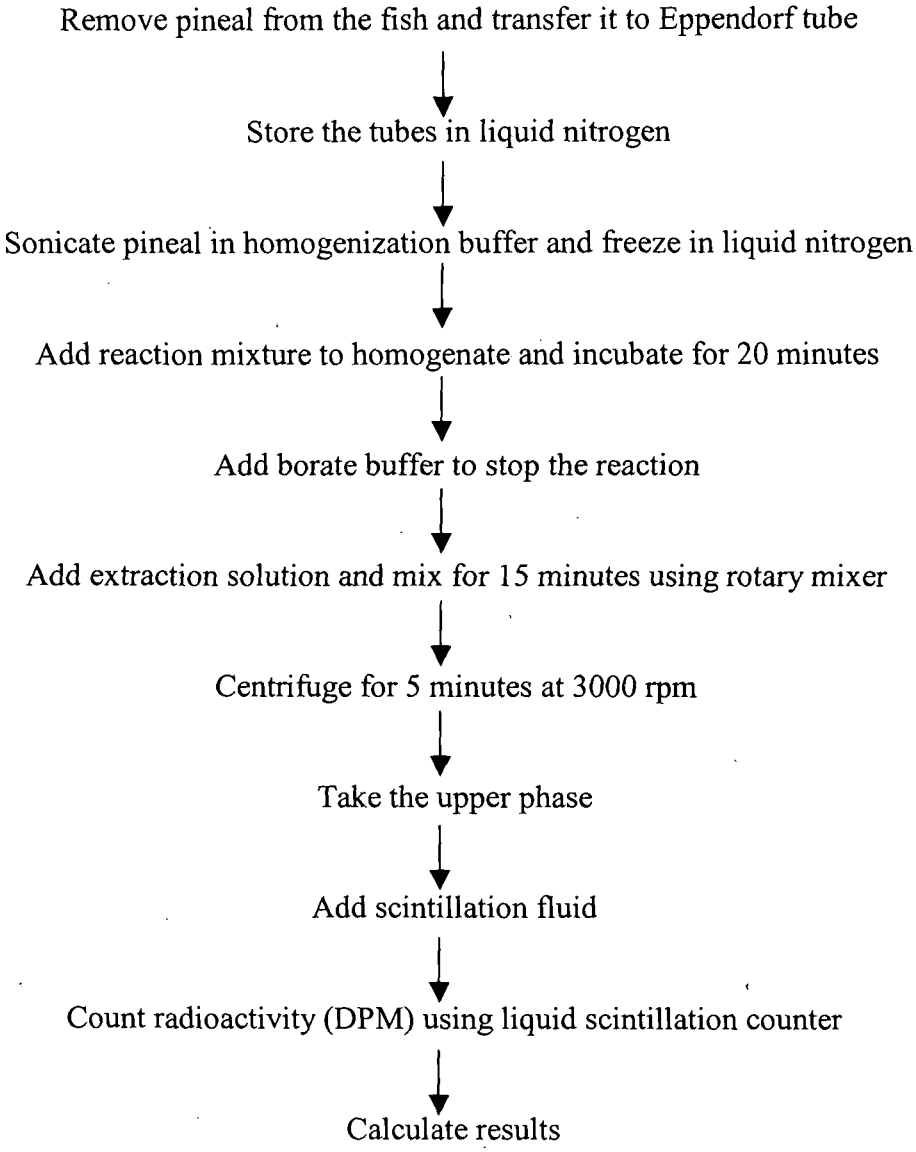
Protocol 1: Major steps in radioimmunoassay of total T_4



Protocol 2: Major steps in radioimmunoassay of total T_3



Protocol 3: Major steps in the measurement of AA-NAT activity



Calculation of AA-NAT activity:

AA-NAT activity was calculated using the following equation:

$$\text{AA-NAT activity} = (A-B) \times \frac{C \times E \times G}{D \times F \times H} \times \frac{60}{Z}$$

A= Radioactivity of probe/unknown (DPM); B= Radioactivity of blanks (DPM); C= Total acetyl CoA (nmol) per sample; D= Total radioactivity used (DPM)/sample; E= Total homogenate volume; F= pineal homogenate volume used; G= Volume of extraction material; H= Volume of extract used for counting; Z= Incubation period in minutes.

CHAPTER - 2

Studies on the interrelationship between the circadian rhythms in plasma levels of thyroid hormones and pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity

INTRODUCTION

In fish species, thyroid hormones play a major role in the regulation of the oxidative metabolism (Peter and Oomen, 1989; Gupta and Thapliyal, 1991), growth and development (Cyr *et al.*, 1998; Power *et al.*, 2001), sexual maturation (Ueda *et al.*, 1984; Cyr *et al.*, 1998; Pavlidis *et al.*, 2000), breeding cycle (Volkoff *et al.*, 1999), migration (Ueda *et al.*, 1984; Matty, 1985a), electrolyte and water metabolism (Peter *et al.*, 2000) etc. As a result thyroid hormones help the fish in continuous adjustments in physiological and morphological characteristics in relation to ever-changing aquatic environment (Matty, 1985a). There is a large body of information on seasonal variations in thyroid activity and the circulating levels of thyroid hormones in mammals (Singh *et al.*, 2002; Ben Saad and Maurel, 2004), birds (Silverin *et al.*, 1989; Dawson and Thapliyal, 2001), reptiles (Licht *et al.*, 1985a,b; Naulleau *et al.*, 1987; Kohel *et al.*, 2001) and amphibians (Kuhn *et al.*, 1983; Gancedo *et al.*, 1996). Since thyroid hormones play a major role in the regulation of energy metabolism and reproduction, production of thyroid hormones is increased during the months with increased gonadal activity associated with increased metabolic activity, and decreased during periods of reproductive and metabolic quiescence in winter months (Cyr *et al.*, 1988, 1998). The environmental cycles of photoperiod, temperature and rainfall seem to provide

predictive information as indicators of seasons in fishes (Yokota and Oishi, 1992; Pavlidis *et al.*, 2000). Thyroid activity and circulating levels of thyroid hormones are influenced significantly by the seasonal variations in daylength and ambient temperature, and exhibit prominent circannual rhythms closely associated with the annual breeding cycle in fishes (Cyr *et al.*, 1998; Leiner *et al.*, 2000; Pavlidis *et al.*, 2000; Leiner and MacKenzie, 2001). In fish species, circulating levels of thyroid hormones and/or thyroid activity are also influenced by glucocorticoids (Leatherland, 1987), catecholamines (Eales *et al.*, 1986), gonadal steroids (Chakraborti and Bhattacharya, 1984), which show circannual rhythms in their secretions closely related to the breeding cycle and seasonal variations in ambient temperature (Pavlidis *et al.*, 2000). In some species of teleosts, increase in temperature has been reported to accelerate the synthesis and turnover of thyroid hormones and to increase the physical activity and metabolic rate of fish species (Leloup and deLuze, 1985; Nayak and Singh, 1991). Both circadian and circannual rhythms in the circulating levels of thyroxine (T_4) and triiodothyronine (T_3) in fish species are reportedly influenced by fluctuations in the ambient temperature, physical activity, photoperiod, feeding etc. (Matty, 1985a; Cyr *et al.*, 1988; Leiner *et al.*, 2000; Pavlidis *et al.*, 2000).

As in the case of thyroid activity, pineal activity and melatonin synthesis are also influenced by photoperiod and/or temperature in mammals (Vivien-Roels *et al.*, 1997; Garcia *et al.*, 2003), birds (Sudhakumari *et al.*, 2001; Dawson and Van't, 2002), reptiles (Vivien-Roels and Arendt, 1983) and amphibians (Delgado and Vivien-Roels,

1989). There are reports that the pineal complex is actively involved in regulation of body temperature in mammals (Larkin *et al* 2001; Smith *et al.*, 2004), reptiles (Vivien-Roels *et al.*, 1988; Skene *et al.*, 1989; Moyer *et al.*, 1997; Ohshima *et al.*, 1999) and birds (Lee *et al.*, 1990; Wolfe and Zatz, 1994; Zatz *et al.*, 1994; Barrett and Takahashi, 1995; Murakami *et al.*, 2001). Therefore, there is a possibility that the pineal activity is influenced by circadian and circannual variations in environmental temperature in fish as well as in other groups of vertebrates. Seasonal changes in temperature and photoperiod have been reported to influence the activity of the neuroendocrine system of fish (Joy and Senthilkumar, 1998), which ultimately regulates the activities of various endocrine glands (Verma *et al.*, 1996). Since the circadian and circannual rhythms of thyroid activity and pineal activity are influenced by photoperiod and environmental temperature, there is a possibility that the circadian rhythms of these two important glands exhibit an interrelationship in relation to the annual breeding cycle.

Though melatonin and thyroid hormones are involved in the regulation of a wide range of physiology as well as the gonadal cycles of fishes, there is paucity of information on the interrelationship between the plasma levels of thyroid hormones and pineal AA-NAT activity and melatonin synthesis with special reference to environmental photoperiod and temperature in any fish species. Therefore, keeping in mind the scarcity of information and importance of melatonin and thyroid hormones in fish physiology, we investigated the nature of interrelationship between the circadian rhythms of thyroid hormones (T_3 and T_4) and the pineal AA-NAT activity in an air-

breathing exotic cat fish, *Clarias gariepinus* maintained under natural climatic conditions during quiescent, progressive, breeding and regressive phases of the annual breeding cycle.

MATERIAL AND METHODS

For this study, adult male *Clarias gariepinus* (body weight: 90-100g; body length: 23-27 cm) were purchased from the local fish suppliers. Fishes were maintained in clear plastic tubs and acclimatized at least for 15 days in the laboratory under natural climatic conditions at Shillong (Latitude 25°.30′ N, Longitude 91°.52′ E; Altitude 1450 ASL; minimum water temperature 4° C; maximum water temperature 24.5° C). During acclimatization, the fishes were fed daily with minced earthworms and commercial fish food *ad libitum*. Water was changed everyday to avoid infections.

After acclimatization, 5 fishes were decapitated at an interval of 3 hours at 8 time-points over a 24 h period, and blood samples and pineals were collected. The blood samples collected in heparinised tubes were centrifuged at 3000 rpm to obtain plasma. The plasma samples were stored at -8 to -10° C in a refrigerator for the measurement of T_4 and T_3. The removed pineals were immediately frozen in liquid nitrogen for the measurement of AA-NAT activity. The levels of thyroid hormones (total T_3 and total T_4) and pineal AA-NAT activity were measured following the procedures described in details in the Chapter 1. The data were analyzed statistically with the help of Student's 't' test,

regression analysis (Snedecor, 1961) and acro program based on least square fitting of single cosine function (Nelson *et al.*, 1979). A $p< 0.05$ was considered as significant.

The experimental protocol for the proposed study is given below:

EXPERIMENTAL PROTOCOL

Experiments	Time of Experiment	Sampling time points
Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity	(i) Quiescent phase (February) (ii) Progressive phase (April) (iii) Breeding phase (August) (iv) Regressive phase (November)	06.00 h, 09.00 h, 12.00 h, 15.00 h, 18.00 h, 21.00 h & 24.00 h; N = 5 samples at each time point.

RESULTS

Circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) during different phases of the breeding cycle:

The data are presented in Tables 2:1, 2:2, 2:3 and 2:4; Figs. 2:1, 2:2, 2:3 and 2:4. Prominent circadian rhythms were recorded in the plasma levels of both T_4 and T_3 during all the four phases of the annual breeding cycle. Irrespective of the phase of the breeding cycle, patterns of the circadian rhythms of T_4 and T_3 were found to be similar. However, the plasma levels of T_4 were always found to be higher than that of T_3 during

all the phases. Further, the plasma levels of both the hormones were found to be high during the daytime and low during the nighttime. The high T_3/T_4 ratio was recorded during the quiescent and regressive phases (pre-winter and winter), which declined during the progressive and breeding phases (summer/rainy seasons). Further, the maximum T_3/T_4 ratio was observed at mid-night during the progressive and breeding phases, while the maximum T_3/T_4 ratio was recorded at 3.00 h during the regressive phase and at 6.00 h during the quiescent phase. The minimum T_3/T_4 ratio was recorded around mid-day during the progressive and the breeding phases, at the evening during the regressive phase and at mid-night during the quiescent phase.

The results of acro analysis of the data are presented in Table 2:6. The mesor (24 h average) of T_4 circadian rhythm was found to be 6 to 7 fold higher than that of T_3 circadian rhythm (Tables 2:5 and 2:6). Further, minimum mesor of both T_3 and T_4 circadian rhythms was recorded during the regressive and the quiescent phases. The mesor of both the rhythms increased gradually during the progressive phase reaching to the maximum during the breeding phase.

The amplitude of the T_4 rhythm was maximum during the breeding phase (summer/rainy season) and minimum during the quiescent phase (winter). In contrast, the amplitude of the T_3 rhythm was maximum during the quiescent phase (winter) and minimum during the regressive phase (pre-winter). Further, the amplitude of T_4 circadian rhythm was found to be 6-, 9-, 13- and 21-fold higher than that of the T_3

circadian rhythm during quiescent, progressive, breeding and regressive phase, respectively.

The acrophase of both T_4 and T_3 rhythms was always recorded during the mid-day at 14.00 h except that of T_4 rhythm during the quiescent phase, when it was delayed by 3 hours and occurred in the evening at 17.00 h.

Regression analysis of the data (Table 2:7A) indicated a strong positive correlation between the plasma levels of T_4 & T_3 and water temperature during all the four phase of the breeding cycle.

Circadian rhythms in pineal AA-NAT activity during different phases of the breeding cycle:

The data are presented in Tables 2:1, 2:2, 2:3 and 2:4; Figs. 2:1, 2:2, 2:3 and 2:4. Prominent circadian rhythms were recorded in pineal AA-NAT activity under natural daylength during all the four phase of the annual breeding cycle. Irrespective of the phase, the pineal AA-NAT activity was found to be minimum/basal during the daytime and several fold higher during the nighttime. Though the AA-NAT activity increased immediately after the sunset during all the phase of the breeding cycle, the pattern of increase and decrease in the activity of AA-NAT was found to be phase-dependent. AA-NAT activity increased gradually after the sunset and reached to a peak at 3.00 h during all the phases except during the breeding phase when the AA-NAT activity

abruptly reached to a peak value at 21.00 h, where it was maintained for 6 h. The AA-NAT activity declined sharply to a basal level at 6.00 h during all the four phases.

The results of acro analysis of the data are presented in Table 2:6. The mesor (24 h average) of AA-NAT activity circadian rhythm was found to be high during the regressive and quiescent phases, which declined gradually during the progressive phase and reached to a minimum during the breeding phase (Table 2:5). Further, the amplitude of the AA-NAT activity rhythm was found to be maximum during the quiescent phase, minimum during the breeding phase and intermediate during the regressive and progressive phases. The acrophase of the AA-NAT circadian rhythm was invariably located during nighttime around the mid-night (23.00 h) except during the breeding phase when it was delayed by 3 hours and occurred at 02.00 h.

Regression analysis of the data (Table 2:7A) indicated a significant negative correlation between water temperature and the pineal AA-NAT activity during all the four phases of the breeding cycle. Further, a negative correlation was also recorded between AA-NAT activity vs T_4 as well as AA-NAT activity vs T_3 (Table 2:7B).

DISCUSSION

Attempts have been made to study circadian variations in the circulating levels of thyroid hormones in fish species with special reference to cortisol, growth hormone and metabolites (Laidley and Leatherland, 1988; Gomez *et al.*, 1997), feeding status

(Cerda-Reverter *et al.*, 1996; Leiner *et al.*, 2000), sex steroids and photoperiod (Cyr *et al.*, 1988; Leiner and Mackenzie, 2001). Similarly, there are reports on the circadian and circannual variations in pineal AA-NAT activity in mammals (Fowler, 1988) and birds (Binkley *et al.*, 1989). But so far no attempt has been made to study the circadian rhythm of circulating levels of thyroid hormones (T_3 and T_4) and pineal AA-NAT activity or melatonin during all the four phases of the annual breeding cycle of any poikilothermic vertebrates in general and any fish species in particular. The present study, to the best of our knowledge, might be the first of its kind in which the interrelationship between the circadian rhythms of thyroid hormones and pineal AA-NAT activity has been studied in a fish maintained under natural day length and temperature during all phases of the breeding cycle.

In the present study, prominent circadian rhythms in the plasma levels of thyroid hormones (T_4 and T_3) were observed during all the four phases of the breeding cycle (Tables 2:1, 2:2, 2:3 and 2:4; Figs. 2:1, 2:2, 2:3 and 2:4). Comparatively higher levels of plasma T_4 than those of T_3 during all the phases seem to suggest that the synthesis and secretion of T_4 is dominant over T_3 secretion in this species. Elevated levels of the thyroid hormones during the daytime irrespective of the phase as well as during progressive and breeding phases seem to be associated with increased water temperature as indicated by a positive correlation between temperature and the plasma levels of thyroid hormones (Table 2:7A). High T_3/T_4 ratio during the regressive and the quiescent phases (pre-winter and winter months) as well as the maximum T_3/T_4 ratio

during mid-night/early morning irrespective of the phase of the breeding cycle indicate that low water temperature favors increased peripheral conversion of T_4 to T_3 leading to higher T_3/T_4 ratio, which seems to help the fish in maintaining metabolic rate at low temperature. Whether the recorded shift in the maximum and the minimum T_3/T_4 ratio over the circadian scale can play a role in shaping the annual breeding cycle and/or reflects shifts in energy requirements in relation to activity of the fish remains to be investigated. Immersion of a teleosts fish (*Sciaenops ocellatus*) in T_3 has been reported to inhibit the hypothalamo-hypophyseal-thyroidal axis leading to inhibition of the peak and amplitude of T_4 circadian rhythm (Leiner and Mackenzie, 2003). Therefore, there is a possibility that suppression of T_4 by increasing level of T_3 can adversely affect the breeding cycle. Since *Clarias gariepinus* is a nocturnal species, the high T_3/T_4 ratio recorded at night during all the phases seems to be directly correlated with the increased activity of the fish during the nighttime. It, thus, seems that while the high T_3/T_4 ratio during winter months is essential for meeting the energy requirements during night, the temporal shift in the ratio during the dark phase in different phase of the cycle might be responsible for cyclical activation or inactivation of the hypothalamo-hypophyseal-testicular axis.

Significant increase in the mesor of T_4 and T_3 circadian rhythms (Table 2:6) during progressive and breeding phases (summer/rainy season) as compared to regressive and quiescent phases (pre-winter and winter months) indicates that the synthesis and release of the thyroid hormones is increased during the summer/rainy

seasons, and decreased during the pre-winter and winter months. The recorded increase in the mesor might be due to increased water temperature, increased food intake and increasing levels of other hormones, separately or jointly, as reported in other fish species (Laidley and Leatherland, 1988; Cerda-Reverter *et al.*, 1996; Gomez *et al.*, 1997; Leiner *et al.*, 2000; Pavlidis *et al.*, 2000). There are several reports that while T_4 plays a major role in regulation of growth, development, sexual maturation and reproduction (Cyr *et al.*, 1998; Pavlidis *et al.*, 2000; Power *et al.*, 2001), T_3 is involved mainly in regulation of the oxidative metabolism in fish species (Eales, 1979; Gupta and Thapliyal, 1991; Peter and Oommen, 1989). Thus, the high levels of both T_3 and T_4 during reproductively active seasons seem to be associated with increased breeding activity and energy metabolism of the fish, *Clarias gariepinus*.

Acro analysis of the data revealed that the amplitude of T_4 circadian rhythm was, depending on the phase/season, six to 21-folds higher than that of the T_3 rhythm (Table 2:6). The temporal difference between the amplitudes of T_4 and T_3 rhythms was found to be minimum during the quiescent phase (6 folds), increased during the progressive phase (9 folds) and breeding phase (13 folds), and became maximum during the regressive phase (21 folds). It has been reported in the red drum (*Sciaenopes ocellatus*) that while food deprivation is more effective in reducing the amplitude of T_4 rhythm, both feeding time and nutrient status significantly increase the amplitude of T_4 rhythm (Leiner *et al.*, 2000). In the present study we observed that the fishes consumed more food during the breeding phase as compared to that during the quiescent phase.

Therefore, the minimum and the maximum amplitude of the T_4 rhythm during quiescent (winter) and breeding (summer/rainy season) phases, respectively seem to be directly related to the feeding status of the fish.

As in mammals, the rate of melatonin in the fish pineal is also directly related to AA-NAT activity (Falcon *et al.*, 1989; Gupta and Premabati, 2002b). There are several reports on circadian variations in melatonin secretion from fish pineal *in situ* (Pavlidis *et al.*, 1999; Rensing and Ruoff, 2002; Masuda *et al.*, 2003; Bayarri *et al.*, 2004a, b; Saito *et al.*, 2004) as well as *in vitro* (Falcon *et al.*, 1989; Zachmann *et al.*, 1992; Iigo *et al.*, 1997; Samejima *et al.*, 2000; Iigo *et al.*, 2004). These studies were conducted on temperate zone fish species, and that too only during a particular phase of the annual breeding cycle. The circadian rhythm in melatonin levels has been studied during different phases of the breeding cycle only in the gold fish (*Carassius auratus*), the Atlantic salmon (*Salmo salar*) and the sea bass (*Dicentrarchus labrax*) (Randall *et al.*, 1995; Garcia-Allegua *et al.*, 2001). Unlike the circadian rhythm of melatonin, there is paucity of information on the circadian rhythm of the rate-limiting enzyme AA-NAT in the fish pineal. A marked diurnal fluctuation has been reported in the pineal AA-NAT activity of the rainbow trout, *Salmo gairdneri* during summer and winter (Morton and Forbes, 1988). This might be the first study in which circadian rhythm in pineal AA-NAT activity has been recorded in an air breathing tropical/subtropical fish during all the four phases of the annual breeding cycle. As in case of AA-NAT activity and

melatonin circadian rhythm reported earlier, AA-NAT activity was found to be minimum during the daytime and increased by several folds during the night.

Though the pineal of *Clarias gariepinus* exhibited a significant circadian rhythm in AA-NAT activity during all the phases of the breeding cycle, the mesor and the amplitude of the AA-NAT rhythm were found to be different during different phases (Table 2:6). The mesor (24 h average) of the AA-NAT activity rhythm was significantly higher during the regressive and the quiescent phases than that of the breeding phase (Tables 2:5 and 2:6). Thus, these findings seem to suggest that the 24 h average rate of melatonin synthesis was maximum during the regressive and the quiescent phases, and minimum during the breeding phase as reflected by the AA-NAT activity. On the basis of these findings, we suggest that the increased melatonin synthesis during the regressive and the quiescent phase (as indicated by the mesor of AA-NAT) is responsible for the suppression of the testicular activity, while decrease in melatonin synthesis during the progressive phase (as indicated by the mesor of AA-NAT) allows the activation of the hypothalamo-hypophyseal-testicular axis. Moreover, the minimum 24 h average rate of melatonin synthesis during the breeding phase seems to favor breeding of the fish. The amplitude of the AA-NAT rhythm was found to be maximum during the quiescent phase, which declined during the progressive phase, reached to a minimum during the breeding phase and then increased during the regressive phase. These phase-dependent changes in the amplitude of the AA-NAT rhythm seem to be closely associated with the testicular cycle of the fish. While higher amplitude of the

rhythm seems to suppress the testicular activity, lower amplitude of AA-NAT rhythm seems to favor testicular activation. On the basis of these findings, it may be suggested that the annual rhythmicity in the amplitude of melatonin synthesis, as reflected by the amplitude of AA-NAT activity, somehow regulates the annual breeding cycle of the fish. Unlike the amplitude, the acrophase of the thyroid hormones (T_3 and T_4) and pineal AA-NAT activity rhythms remained more or less constant indicating that the acrophase of the T_3, T_4 and AA-NAT rhythms in this species is not influenced by the phase of the breeding cycle and seasons. It remains to be investigated whether the acrophase of the rhythms are controlled by an endogenous "Zeitgeber".

In contrast to thyroid hormones, a negative correlation was found between AA-NAT activity and water temperature irrespective of phase of the breeding cycle (Table 2:7A). This indicates that low temperature stimulates and high temperature inhibits the fish pineal AA-NAT activity. Effect of temperature on pineal AA-NAT activity is not consistent in mammals. Depending on the mammalian species, temperature has been reported to stimulate (Stokkan *et al.*, 1991), inhibit (Tannenbaun *et al.*, 1988) and to have no effect on the AA-NAT activity (Guerrero *et al.*, 1990a).

In temperate fish species, temperature has been reported to determine the amplitude of the melatonin circadian rhythm of pineal *in situ* (Randall *et al.*, 1995; Garcia-Allegue *et al.*, 2001) as well as *in vitro* (Zachmann *et al.*, 1992), while the rhythm disappeared when the pineal was maintained *in vitro* at low water temperature

(Samejima *et al.*, 2000). A careful analysis of the available information indicates that there is a direct a relationship between the amplitude of the melatonin rhythm in temperate zone fish species. In contrast, the findings of the present study suggest an inverse correlation between the water temperature and pineal AA-NAT activity (Table 2:7A). It, thus, seems that water temperature produces differential effects on the amplitude of melatonin/AA-NAT activity rhythms in temperate and tropical/subtropical fish species.

It is amply clear from the findings of the present study that irrespective of the phase (i) while the acrophase (peak) of the circadian rhythm of thyroid hormones (both T_3 and T_4) occurs around midday, the acrophase of AA-NAT activity rhythm occurs during midnight, and (ii) a significant negative correlation exists between the plasma levels of thyroid hormones (T_3 and T_4) and the pineal AA-NAT activity in *Clarias gariepinus*. These findings, thus, seem to indicate an inverse interrelationship between the circulating levels of the thyroid hormones and AA-NAT activity/melatonin synthesis. A direct relationship between water temperature and plasma levels of thyroid hormones and an inverse relationship between water temperature and AA-NAT activity seem to be responsible for the observed inverse interrelationship between the plasma levels of thyroid hormones and AA-NAT activity. It seems that the long daylength and high ambient temperature during progressive and breeding phases (summer/rainy seasons) decrease AA-NAT activity/melatonin synthesis and increase plasma level of thyroid hormones resulting in increased testicular/breeding activity, while short

daylength and low ambient temperature during the regressive and the quiescent phases (winter months) stimulate AA-NAT activity/melatonin synthesis and inhibit synthesis of the thyroid hormones leading to testicular inactivation in the fish.

Table 2:1- Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity during quiescent phase.

Time (h)	Water temperature (° C)	Plasma levels of thyroid hormones			AA-NAT activity (nmol/pineal/h)
		T_4 (ng/ml)	T_3 (ng/ml)	T_3/T_4	
6.00	8.5	5.64 ± 0.34* [h]	1.32 ± 0.13 [f]	0.235	3.42 ± 0.12 [f, h]
9.00	10.5	7.72 ± 0.31 [b, g, l]	1.30 ± 0.12 [f]	0.168	2.80 ± 0.10 [c, h, i]
12.00	12.5	10.20 ± 0.72 [a, f]	1.71 ± 0.08 [a, f]	0.168	2.60 ± 0.09 [h]
15.00	23.0	10.30 ± 0.64 [f]	1.80 ± 0.08 [f]	0.174	2.44 ± 0.10 [h]
18.00	13.5	9.10 ± 0.45 [f]	1.66 ± 0.11 [k]	0.182	3.57 ± 0.15 [d, h]
21.00	12.0	7.92 ± 0.45 [f, k]	1.39 ± 0.12 [f, i]	0.175	6.22 ± 0.13 [d, h, l]
24.00	9.0	8.74 ± 0.57 [l]	1.02 ± 0.09 [a, h]	0.116	6.46 ± 0.10 [l]
3.00	5.5	5.18 ± 0.38 [c, h]	1.19 ± 0.06 [g]	0.230	7.27 ± 0.11 [c, l]
6.00	8.5	5.64 ± 0.34 [h]	1.32 ± 0.13 [f]	0.235	3.42 ± 0.12 [d, h, l]
r		T Vs T_4 = 0.82	T Vs T_3 = 0.64	-0.83	T Vs AA-NAT = -0.59

*All values are expressed as mean ± standard error (S. E); N = 5.

[a, b, c, d] Differ from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[f, g, h] Differ from the maximum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

[i, k, l] Differ from the minimum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

Table 2:2- Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity during progressive phase.

Time (h)	Water temperature (° C)	Plasma levels of thyroid hormones			AA-NAT activity
		T_4 (ng/ml)	T_3 (ng/ml)	T_3/T_4	(nmol/pineal/h)
6.00	10.0	10.60 ± 0.35* [h, k]	1.64 ± 0.11 [e]	0.155	2.53 ± 0.20 [k, h]
9.00	12.5	11.52 ± 0.79 [f, j]	1.70 ± 0.09 [e]	0.147	2.35 ± 0.25 [h]
12.00	18.5	15.50 ± 0.28 [b, j]	2.19 ± 0.18 [b, j]	0.141	1.64 ± 0.12 [a, h]
15.00	22.5	13.10 ± 0.62 [b, l]	2.07 ± 0.18 [i]	0.158	2.31 ± 0.12 [c, h]
18.00	20.0	13.60 ± 0.57 [b, f, l]	2.14 ± 0.17 [j]	0.157	2.66 ± 0.11 [h, l]
21.00	18.0	12.90 ± 0.55 [f, l]	1.85 ± 0.10 [i]	0.143	4.75 ± 0.10 [d, g, l]
24.00	15.0	8.52 ± 0.46 [g]	1.62 ± 0.10 [e]	0.190	6.09 ± 0.19 [d, l]
3.00	8.0	8.96 ± 0.36 [c, h]	1.42 ± 0.14 [f]	0.158	6.17 ± 0.30 [l]
6.00	10.0	10.60 ± 0.35 [b, h, k]	1.64 ± 0.11 [e]	0.155	2.53 ± 0.20 [d, h, k]
r		T Vs T_4 = 0.72	T Vs T_3 = 0.88		T Vs AA-NAT = -0.42

*All values are expressed as mean ± standard error (S. E); N = 5.

[a, b, c, d] Differ from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e, f, g, h] Differ from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[i, j, k, l] Differ from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

Table 2:3- Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity during breeding phase.

Time (h)	Water temperature (° C)	Plasma levels of thyroid hormones			AA-NAT activity (nmol/pineal/h)
		T_4 (ng/ml)	T_3 (ng/ml)	T_3/T_4	
6.00	20.0	13.18 ± 0.46* $^{h, j}$	1.96 ± 0.05 ^{g}	0.14	2.13 ± 0.15 $^{i, h}$
9.00	21.5	18.34 ± 0.75 $^{c, l}$	2.36 ± 0.18 ^{i}	0.12	1.72 ± 0.11 ^{h}
12.00	23.0	20.40 ± 0.55 $^{d, l}$	2.55 ± 0.16 ^{k}	0.12	1.49 ± 0.13 ^{h}
15.00	24.0	17.18 ± 0.64 $^{b, f, l}$	2.37 ± 0.10 ^{k}	0.13	1.31 ± 0.16 ^{h}
18.00	22.5	15.32 ± 0.63 $^{h, l}$	2.46 ± 0.10 ^{l}	0.16	1.87 ± 0.16 $^{a, h}$
21.00	21.5	12.58 ± 0.46 $^{b, h, k}$	2.33 ± 0.13 ^{j}	0.18	3.67 ± 0.26 $^{a, l}$
24.00	20.0	11.04 ± 0.55 ^{h}	2.18 ± 0.14 ^{i}	0.19	3.73 ± 0.10 ^{l}
3.00	16.0	10.48 ± 0.41 ^{h}	1.80 ± 0.08 $^{a, h}$	0.17	4.02 ± 0.11 ^{l}
6.00	20.0	13.18 ± 0.46 $^{b, h, j}$	1.96 ± 0.05 ^{g}	0.14	2.13 ± 0.15 $^{d, i, h}$
r		T Vs T_4 = 0.75	T Vs T_3 = 0.90		T Vs AA-NAT = -0.76

*All values are expressed as mean ± standard error (S. E); N = 5.

$^{a, b, c, d}$ Differ from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

$^{f, g, h}$ Differ from the maximum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

$^{i, j, k, l}$ Differ from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

Table 2:4- Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity during regressive phase.

Time (h)	Water temperature (° C)	Plasma levels of thyroid hormones			AA-NAT activity (nmol/pineal/h)
		T_4 (ng/ml)	T_3 (ng/ml)	T_3/T_4	
6.00	8.0	5.76 ± 0.26* ^{h}	1.15 ± 0.09 ^{g}	0.200	3.61 ± 0.12 ^{h}
9.00	10.0	8.78 ± 0.62 $^{b, h, l}$	1.36 ± 0.10	0.154	2.70 ± 0.07 $^{d, h}$
12.00	11.5	12.40 ± 0.80 $^{b, l}$	1.47 ± 0.08	0.119	3.02 ± 0.08 $^{a, h}$
15.00	21.5	13.20 ± 0.77 ^{l}	1.52 ± 0.04 ^{i}	0.115	3.39 ± 0.18 $^{h, k}$
18.00	13.5	14.04 ± 0.50 ^{l}	1.42 ± 0.14	0.101	4.05 ± 0.07 $^{b, h, l}$
21.00	13.0	9.24 ± 0.69 $^{c, g, l}$	1.32 ± 0.15	0.142	5.41 ± 0.09 $^{d, h, l}$
24.00	9.5	6.10 ± 0.56 $^{b, h}$	1.09 ± 0.15	0.179	6.38 ± 0.15 $^{d, l}$
3.00	6.0	5.02 ± 0.37 ^{h}	1.13 ± 0.06 $^{a, h}$	0.225	6.75 ± 0.11 ^{l}
6.00	8.0	5.76 ± 0.26 ^{h}	1.15 ± 0.09 ^{g}	0.200	3.61 ± 0.12 $^{d, h}$
r		T Vs T_4 = 0.78	T Vs T_3 = 0. 77		T Vs AA-NAT = - 0.40

*All values are expressed as mean ± standard error (S. E); N = 5.

$^{a, b, c, d}$ Differ from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

$^{g, h}$ Differ from the maximum value of the respective group: $p < 0.01$ and 0.001, respectively.

$^{i, k, l}$ Differ from the minimum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

Table 2:5- 24-hour average of plasma level of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during different phases of the breeding cycle under NDL.

Phase	Plasma levels of thyroid hormones		AA-NAT
	T_4 (ng/ml)	T_3 (ng/ml)	(nmol/pineal/h)
Quiescent phase	8.10 ± 0.33* [b, g]	1.42 ± 0.05 [b]	4.34 ± 0.29 [b]
Progressive phase	11.83 ± 0.40 [b]	1.82 ± 0.06 [a, d, e]	3.56 ± 0.27 [a, c]
Breeding phase	14.81 ± 0.56	2.25 ± 0.06	2.49 ± 0.17
Regressive phase	9.31 ± 0.53 [b, f, e]	1.30 ± 0.04 [b]	4.41 ± 0.23 [b]

*All values are expressed as mean ± standard error (S. E); N = 40.

[a, b] Differ significantly from the breeding phase: $p < 0.01$ and 0.001, respectively.

[c, d] Differ significantly from the regressive phase of the respective group: $p < 0.05$ and 0.001, respectively.

[e] Differs significantly from the quiescent phase of the respective group: $p < 0.01$.

[f, g] Differ significantly from the progressive phase of the respective group: $p < 0.01$ and 0.001, respectively.

Table 2:6- Acro analysis of the data on the circadian rhythms of thyroid hormones (T_4 and T_3) and pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity during different phases of the breeding cycle (Based on data presented in Tables 2:1, 2:2, 2:3 and 2:4).

Phase	Mesor			Amplitude			Acro phase (h)			95% Confidence Interval (C. I) (h)			Goodness of fit			Threshold (20%) (h)		
	T_4 (ng/ml)	T_3 (ng/ml)	AA-NAT activity (nmol/ pineal/h)	T_4 (ng/ml)	T_3 (ng/ml)	AA-NAT activity (nmol/ pineal/h)	T_4	T_3	AA-NAT acti-vity	T_4	T_3	AA-NAT activity	T_4	T_3	AA-NAT activity	T_4	T_3	AA-NAT acti-vity
Quiescent phase	8.10	1.42	4.34	2.56	0.39	2.41	17	14	23	15.81 to 18.19	13.18 to 14.82	22.06 to 23.94	0.1 $p < 0.05$	0.043 $p < 0.005$	0.066 $p < 0.02$	8.6	5.6	14.6
Progressive phase	11.83	1.82	3.56	3.49	0.38	2.26	14	14	23	12.98 to 15.02	13.10 to 14.90	22.02 to 23.98	0.065 $p < 0.02$	0.053 $p < 0.01$	0.071 $p < 0.02$	5.6	5.6	14.6
Breeding phase	14.81	2.25	2.49	4.96	0.37	1.35	14	14	2	13.11 to 14.89	12.79 to 15.21	1.05 to 2.95	0.052 $p < 0.01$	0.093 $p < 0.05$	0.068 $p < 0.02$	5.6	5.6	17.6
Regressive phase	9.31	1.30	4.41	4.51	0.21	2.02	14	14	23	13.21 to 14.79	13.56 to 14.44	22.11 to 23.89	0.044 $p < 0.005$	0.013 $p < 0.001$	0.055 $p < 0.01$	5.6	5.6	14.6

Table 2:7A- Correlation coefficient (r) between plasma levels of thyroid hormones (T_4 and T_3), pineal AA-NAT activity and water temperature during different phases of the breeding cycle.

Phase	Water temperature Vs T_4	Water temperature Vs T_3	Water temperature Vs AA-NAT activity
Quiescent phase	*0.82*	*0.64*	*-0.59*
Progressive phase	0.72	0.88	-0.42
Breeding phase	0.75	0.90	-0.76
Regressive phase	0.78	0.77	-0.40

Table 2:7B- Correlation coefficient (r) between pineal AA-NAT activity and plasma levels of thyroid hormones (T_4 and T_3) during different phases of the breeding cycle.

Phase	AA-NAT activity Vs T_4	AA-NAT activity Vs T_3
Quiescent phase	-0.50	-0.72
Progressive phase	-0.75	-0.70
Breeding phase	-0.88	-0.61
Regressive phase	-0.58	-0.70

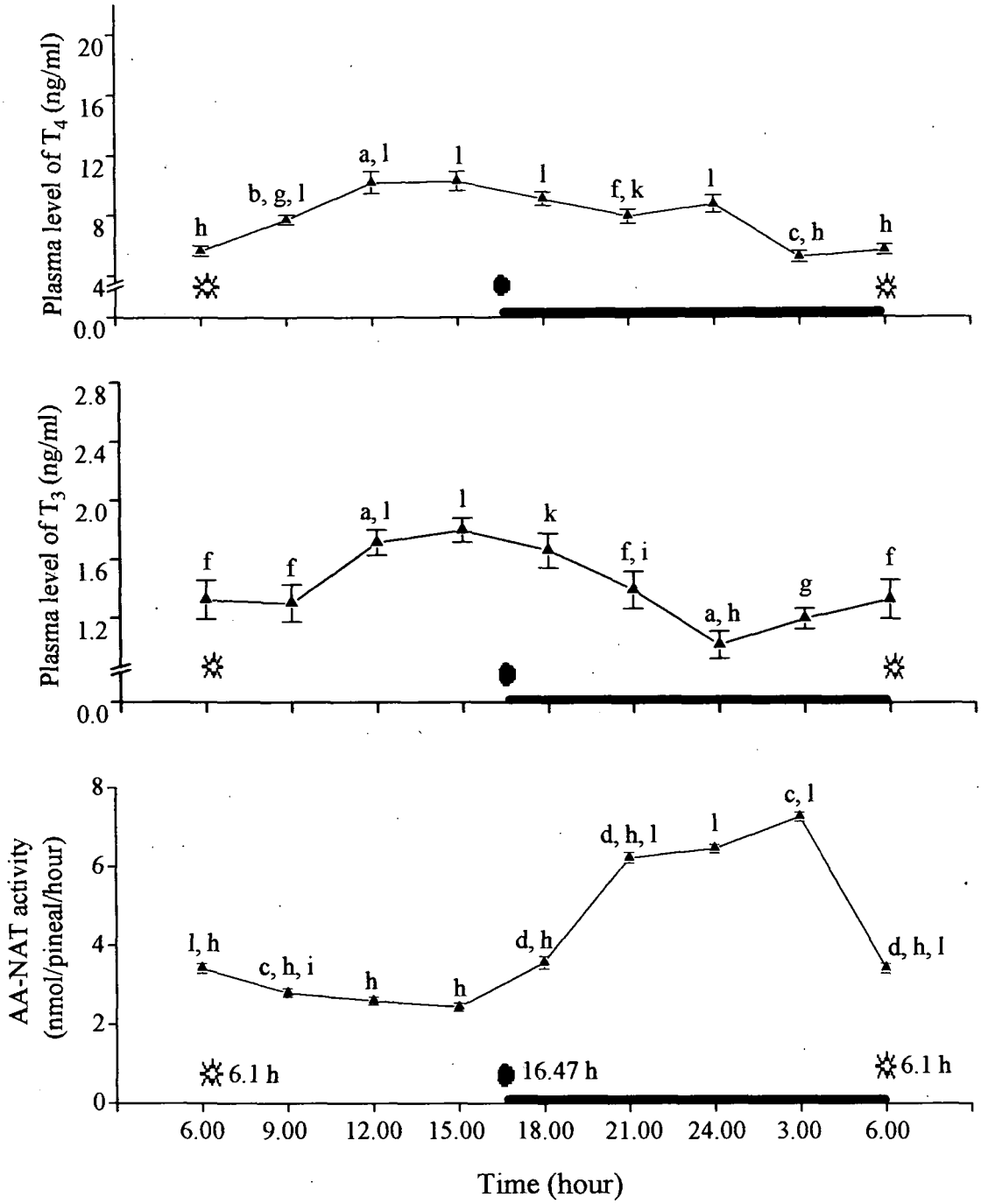


Fig. 2:1- Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity during quiescent phase. (☼= time of sunrise; ● = time of sunset).

All values are expressed as mean ± standard error (S. E); N = 5.

[a, b, c, d] Differ significantly from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[f, g, h] Differ significantly from the maximum value of the respective group: $p < 0.02$, 0.01and 0.001, respectively.

[i, k, l] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

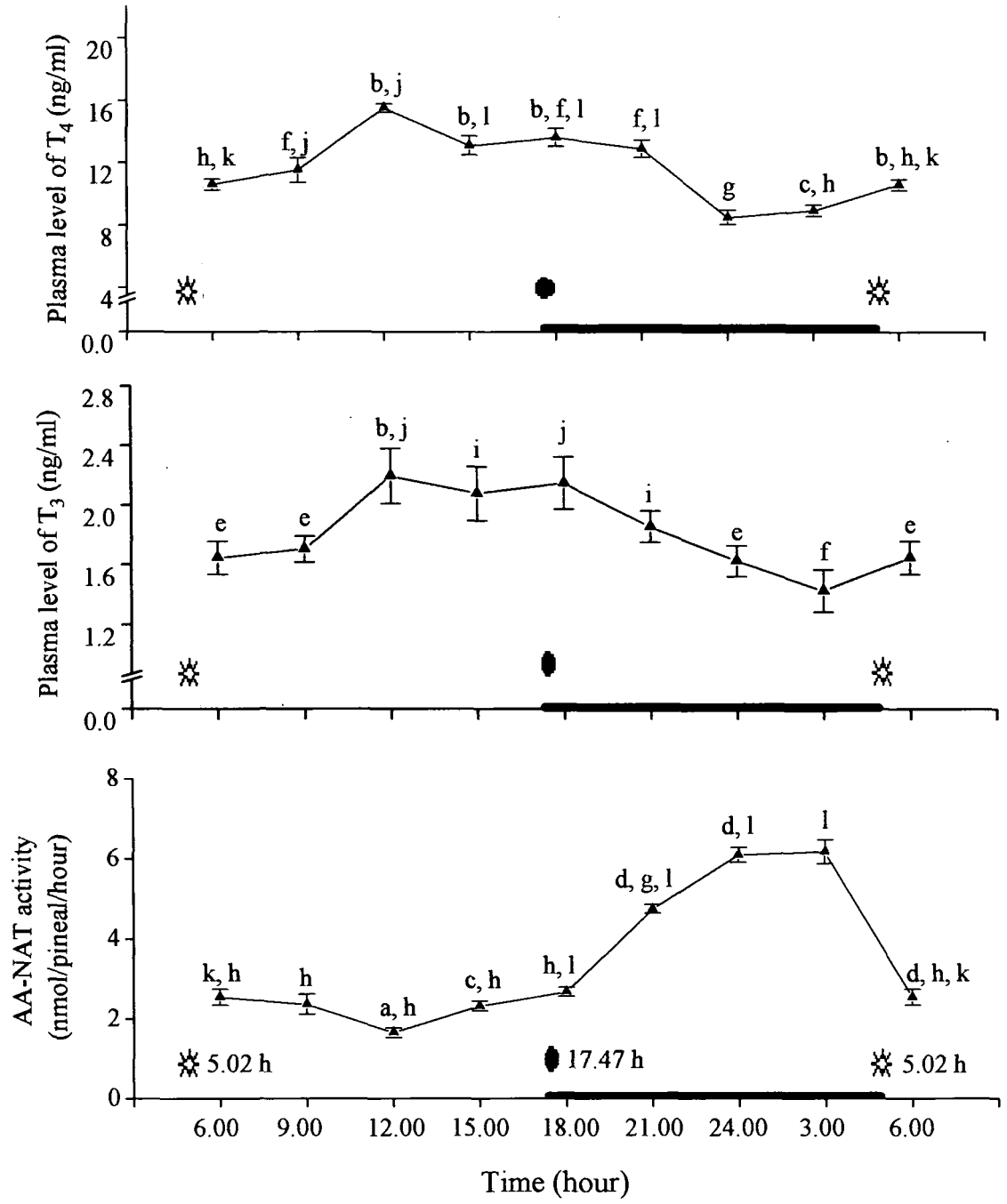


Fig. 2:2- Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity during progressive phase. (☼ = time of sunrise; ● = time of sunset).

All values are expressed as mean ± standard error (S. E); N = 5.

[a, b, c, d] Differ significantly from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e, f, g, h] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01and 0.001, respectively.

[i, j, k, l] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

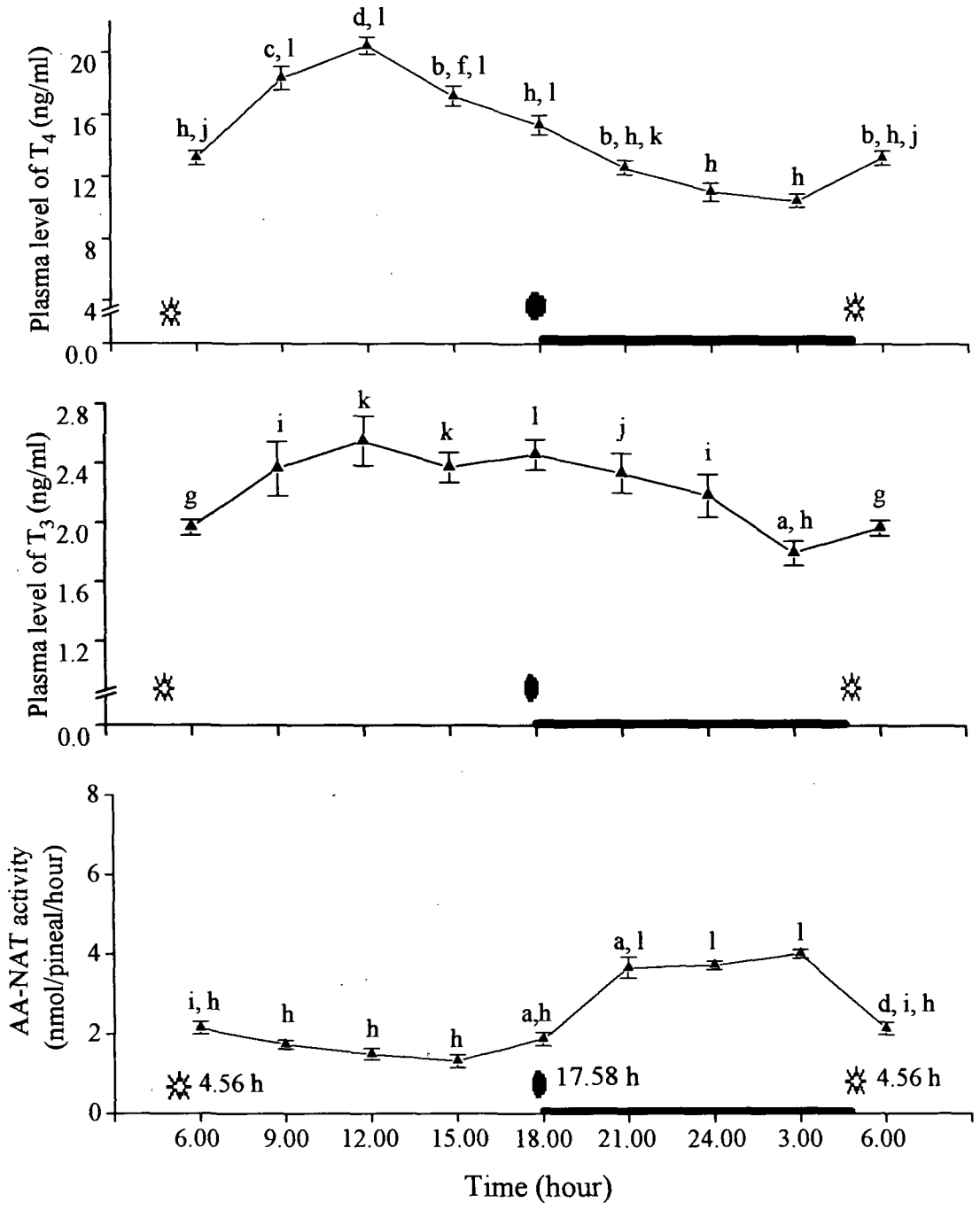


Fig. 2:3- Circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity during breeding phase. (☼ = Time of sunrise; ● = Time of sunset).

All values are expressed as mean ± standard error (S. E); N = 5.

[a, b, c, d] Differ significantly from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[f, g, h] Differ significantly from the maximum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

[i, j, k, l] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

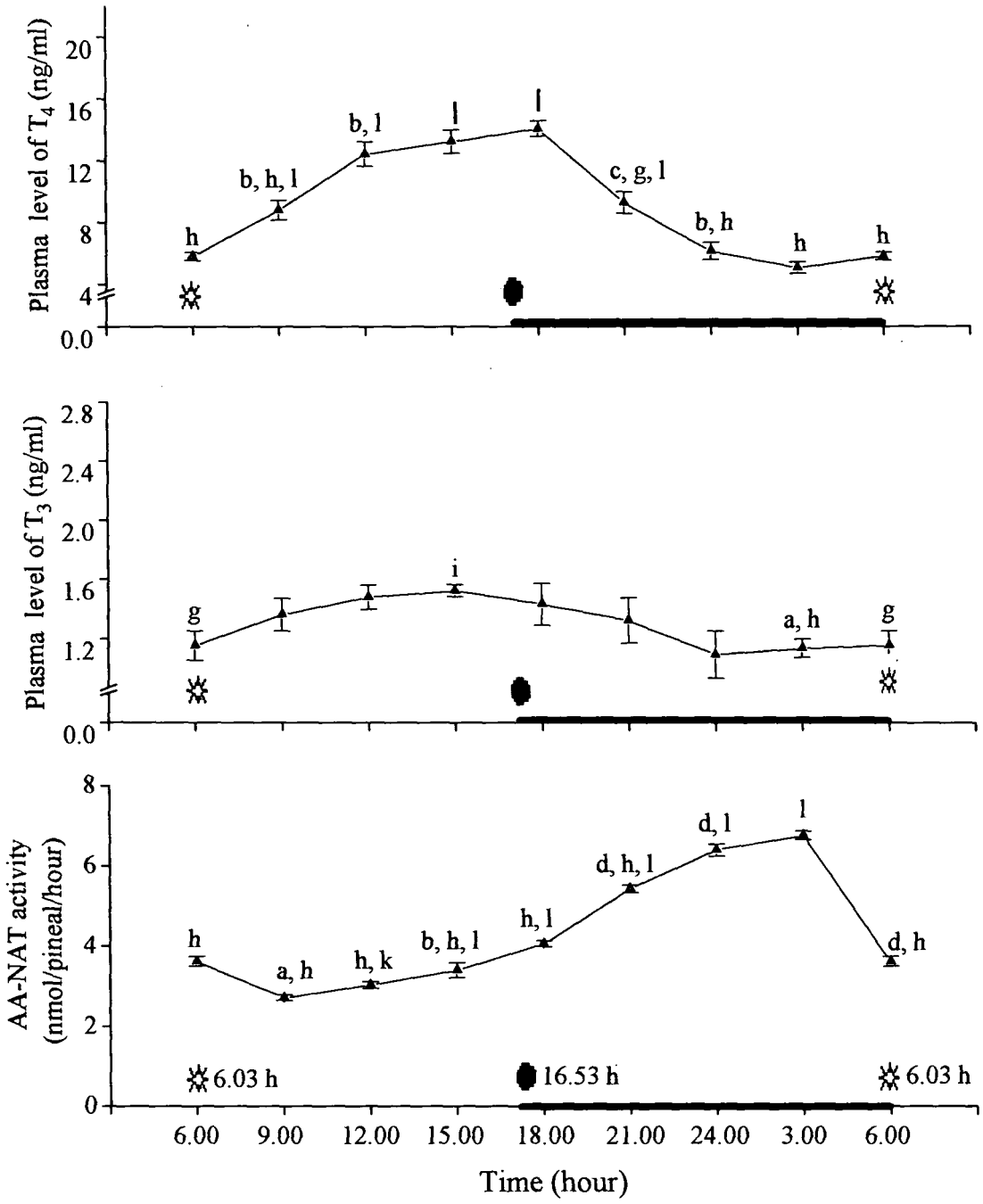


Fig. 2:4- Circadian rhythms in plasma levels of T_4 , T_3 and pineal AA-NAT activity during regressive phase. (☼ = Time of sunrise; ● = time of sunset).

All values are expressed as mean standard error (S. E); N = 5.

[a, b, c, d] Differ significantly from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[g, h] Differ significantly from the maximum value of the respective group: $p < 0.01$ and 0.001, respectively.

[i, k, l] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

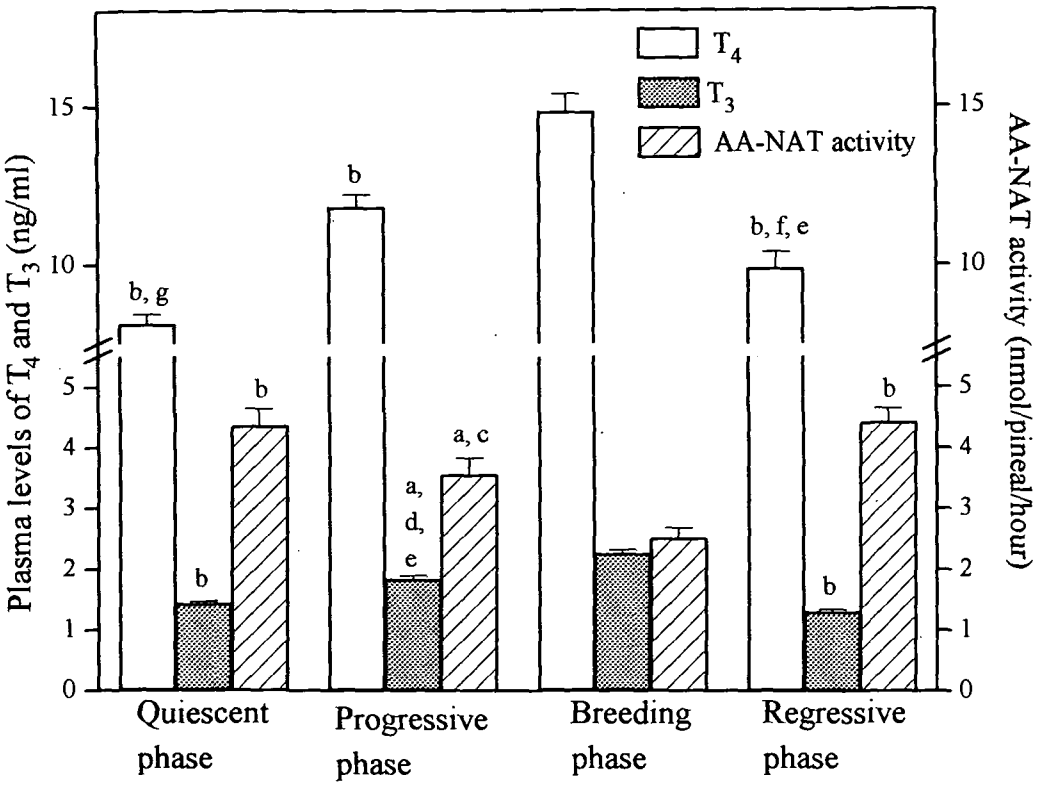


Fig. 2:5- 24-hour average of plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during different phases of the breeding cycle under NDL.

All values are expressed as mean ± standard error (S. E); N = 40.

[a, b] Differ significantly from the breeding phase: $p < 0.01$ and 0.001, respectively.

[c, d] Differ significantly from the regressive phase of the respective group: $p < 0.05$, 0.001, respectively.

[e] Differs significantly from the quiescent phase of the respective group: $p < 0.01$.

[f, g] Differ significantly from the progressive phase of the respective group: $p < 0.01$, 0.001, respectively.

CHAPTER - 3

Effects of photoperiods and simulated temperatures on circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity

INTRODUCTION

The circannual changes in the neuroendocrine system and adaptations are driven by the master clock located in the SCN, which is reset by the seasonal fluctuations in climatic factors like photoperiod, temperature etc., and enables vertebrates to anticipate seasonal environmental changes (Reiter, 1993a; Sumova *et al.*, 2003; Hofman, 2004). Most of the vertebrates use the annual changes in environmental variables such as photoperiod, temperature, rainfall or food supply to synchronize several physiological and endocrine functions associated with reproduction, hibernation, thermoregulation etc. (Vivien-Roels *et al.*, 1988). The exact mechanism(s) by which environmental information is integrated are not yet known, but it is well established in vertebrates that the pineal is involved in conveying the photoperiodic message via the daily pattern of melatonin secretion (Goldman and Darrow, 1983; Engel *et al.*, 2004a). Such a daily pattern of melatonin secretion in homeotherms is entrained by the light-dark cycle (Charron *et al.*, 1991; Humlova and Illnerova, 1992; Illnerova and Sumova, 1997). The pineal gland of mammals (Illnerova and Sumova, 1997; Engel *et al.*, 2004b) and birds (Binkley *et al.*, 1989; Bernard *et al.*, 1997; Natesan *et al.*, 2002) exhibits a circadian rhythm in activity of the rate-limiting enzyme, arylalkylamine-N-acetyltransferase (AA-

NAT) (Gupta *et al.*, 2005). Photoperiods and/or temperature influence pineal activity, melatonin synthesis and induce changes in the peak duration of nocturnal melatonin secretion and/or AA-NAT activity in mammals (Illnerova *et al.*, 1984; Skene *et al.*, 1987; Tannenbaum *et al.*, 1988; Charron *et al.*, 1991; Stokkan *et al.*, 1991; Humlova and Illnerova, 1992; Vivien-Roels *et al.*, 1997; Garcia *et al.*, 2003), birds (Underwood and Siopes, 1985; Lee *et al.*, 1990; Wolfe and Zatz, 1994; Zatz *et al.*, 1994; Barrett and Takahashi, 1995; Sudhakumari *et al.*, 2001; Natesan *et al.*, 2002; Holthues and Vollrath, 2004), reptiles (Vivien-Roels and Arendt, 1983; Underwood and Calaban, 1987; Vivien-Roels *et al.*, 1988; Firth and Kennaway, 1989; Firth *et al.*, 1991; Tilden and Hutchinson, 1993; Moyer *et al.*, 1997; Bertolucci *et al.*, 2003), and amphibians (Delgado and Vivien-Roels, 1989; Wright, 2002). In the fish pineal, melatonin synthesis and AA-NAT activity are reportedly influenced by photoperiods under both *in vivo* and *in vitro* conditions (Morton and Forbes, 1988; Zachmann *et al.*, 1992; Bolliet *et al.*, 1993; Randall *et al.*, 1995; Pavlidis *et al.*, 1999; Masuda *et al.*, 2003; Bayarri *et al.*, 2004a,b).

In the rat pineal, adrenergic induction of cAMP and subsequent phosphorylation of the transcription factor "cyclic AMP response element binding protein" (CREB) results in the nocturnal activation of several genes (Gupta *et al.*, 2005). Among these are genes that encode for the transcription factors "inducible cAMP early repressor" (ICER) (Stehle *et al.*, 1993) and "Fos-related antigen-2" (Fra-2) (Baler and Klein, 1995; Guillaumond *et al*, 2000; Spessert *et al.*, 2000). In the rat, photoperiodic adaptation of

pineal melatonin synthesis appears to be mediated by ICER which regulates the *aa-nat* gene (Foulkes *et al.*, 1996). The amount of ICER protein increases under short photoperiods and decreases under long photoperiods (Foulkes *et al.*, 1996) and is inversely correlated with the inducibility of the *aa-nat* gene (Engel *et al.*, 2004b). In addition to the role of ICER, Fra-2 also plays a role in the photoperiodic adaptation of pineal gene expression. Fra-2 expression has been reported to be photoperiod-dependent and involved in imprinting photoperiod on the pineal gland, transmits photoperiodic information and hence seasonal information to the Fra-2 target gene "iodothyronine deiodinase type II" (*dII*) (Engel *et al.*, 2005).

Acute exposure to light at night induces rapid fall in pineal melatonin levels and AA-NAT activity in mammals and birds (Binkley *et al.*, 1980; Hamm *et al.*, 1983; Maitra *et al.*, 1986; Vollrath *et al.*, 1989; Bronstein *et al.*, 1990; Holthues and Vollrath, 2004), and fishes (Masuda *et al.*, 2003; Bayarri *et al.*, 2004b). It is known that, in addition to photoperiod, environmental temperature is an important cue in regulating several seasonal physiological adaptations in poikilotherms (Underwood and Calaban, 1987; Vivien-Roels *et al.*, 1988; Randall *et al.*, 1995; Moyer *et al.*, 1997; Masuda *et al.*, 2003). Moreover, environmental temperature has also been shown to control pineal activity and circulating levels of melatonin in mammals (Tannenbaum *et al.*, 1988; Stokkan *et al.*, 1991), reptiles (Vivien-Roels and Arendt, 1981, 1983; Moyer *et al.*, 1997) and amphibians (Delgado and Vivien-Roels, 1989; Wright, 2002).

Plasma levels of thyroid hormones (T_4 and T_3) have also been reported to exhibit diurnal and circadian rhythms in human (Lakatua *et al.*, 1984; Wilson *et al.*, 1992), rats (Ottenweller and Hedge, 1982a), and hamsters (Vriend, 1984) with lowest levels during the dark phase, which increased during the light phase of the daily cycle. Photoperiods have been reported to influence plasma levels of thyroid hormones and thyroid activity in mammals (Vaughan *et al.*, 1982, 1994; Ben Saad and Maurel, 2004), birds (Kuhn and Nouwen, 1978; Renden *et al.*, 1994; Yoshimura *et al.*, 2003), reptiles (Gower *et al.*, 1996), amphibian (Kuhn *et al.*, 1983; Gancedo *et al.*, 1996) and fishes (Cerda-Reverter *et al.*, 1996; Leiner *et al.*, 2000; Leiner and MacKenzie, 2001). Besides photoperiods, thyroid hormones and thyroid activity are also influenced by temperature in mammals (Vaughan *et al.*, 1994; Ben Saad and Maurel, 2004), birds (Oishi and Konishi, 1978; Bobek *et al.*, 1980; Herbute *et al.*, 1981; Collin *et al.*, 2003), reptiles (Thapliyal, 1980; Fleury and Naulleau, 1987; Hulbert and Williams, 1988; Licht *et al.*, 1989), amphibian (Kuhn *et al.*, 1983; Gancedo *et al.*, 1996) and fishes (Eales *et al.*, 1982; Cyr *et al.*, 1998; McCormick *et al.*, 2000; Pavlidis *et al.*, 2000).

Since daylength and ambient temperature exhibit prominent circannual variations, and thyroid hormones and AA-NAT activity show opposite responses to the two climatic factors, there is a possibility that the temporal relationship between the levels of thyroid hormones and AA-NAT activity during different phases of the annual breeding cycle of the fish (Chapter - 2) are regulated by the seasonal changes in the two factors. Notwithstanding reports on effects of photoperiod and temperature on circula-

ting levels of thyroid hormones and melatonin in the fish, there is paucity of information on effects of photoperiod and temperature on the temporal relationship of thyroid hormones and AA-NAT activity/melatonin with special reference to the seasons. Therefore, it was decided to investigate effects of different photoperiods and simulated temperatures on the circadian rhythms of thyroid hormones (T_3 and T_4) and the pineal AA-NAT activity in the fish, *Clarias gariepinus* during winter and summer seasons.

MATERIAL AND METHODS

All experiments were conducted on adult male *Clarias gariepinus*. Fishes (body weight: 90-100g; body length: 23-27 cm) were purchased from the local fish suppliers, and maintained in clear plastic tubs and acclimatized at least for 15 days in the laboratory under natural climatic conditions at Shillong (Latitude 25°.30′ N, Longitude 91°.52′ E; Altitude 1450 ASL; minimum water temperature 4° C; maximum water temperature 24.5° C). During acclimatization, the fishes were fed daily with minced earthworms and commercial fish food *ad libitum*. Water was changed everyday to avoid infections.

After acclimatization, fishes were transferred to thermo- and photo-regulated chambers. In order to study the effects of photoperiod, the fishes were exposed to LD 9:15, LD 12:12 and LD 15:9 ("lights on" at 6.00 h) at a constant temperature (25 ± 2° C) for 30 days. To study the effects of simulated temperatures, the fishes were maintained at 15 ± 2° C, 25 ± 2° C and 35 ± 2° C under LD 12:12 for 30

days. The studies were conducted during both winter and summer seasons. At the end of the experiments, the fishes of each group were decapitated at 8-time points at an interval of 3 hours over a 24 h period, and blood and pineals samples were collected following the methods described in Chapter - 1. The blood samples were centrifuged at 3000 rpm to obtain plasma. The plasma samples were stored at -8 to -10° C in a refrigerator for the measurement of T_4 and T_3. The removed pineals were immediately frozen in liquid nitrogen for the measurement of AA-NAT activity. The levels of thyroid hormones (total T_3 and total T_4) and pineal AA-NAT activity were measured following the procedures described in details in the Chapter- 1. The data were analyzed statistically with the help of Student's 't' test and regression analysis (Snedecor, 1961). Acro program based on least square fitting of single cosine function was used for analyzing the circadian rhythm (Nelson *et al.*, 1979). A $p < 0.05$ was considered as significant.

The experimental protocol for the proposed study is given below:

EXPERIMENTAL PROTOCOL

Experiments	Photoperiod	Water Temperature	Time of Experiment	Duration	Sampling Time
1. Effects of different photoperiods on circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity	(i) LD 9:15 (ii) LD 12:12 (iii) LD 15:9	25 ± 2° C	(i) Winter (ii) Summer	30 days	8- time points at an interval of 3 h over a 24 h period
2. Effects of simulated temperatures on circadian rhythms in plasma levels of T_4, T_3 and pineal AA-NAT activity	LD 12:12	(i) 15 ± 2° C (ii) 25 ± 2° C (iii) 35 ± 2° C	(i) Winter (ii) Summer	30 days	8-time points at an interval of 3 h over a 24 h period

RESULTS

Effects of photoperiods on circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during winter and summer seasons:

The data are presented in Tables 3:1, 3:2 and 3:3; Figs. 3:1A, 3:1B, 3:2A, 3:2B, 3:3A and 3:3B. Plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT

activity exhibited prominent circadian rhythms under all photoperiodic regimes (i.e., LD 9:15, LD 12:12 and LD 15:9) during both winter and summer seasons. The maximum levels of thyroid hormones were recorded during the light phase of all the photoperiodic regimes irrespective of the seasons. In contrast, the maximum AA-NAT activity was always found during the dark phase irrespective of photoperiod and seasons. The patterns of diurnal increase and decrease in the levels of thyroid hormones were different under different photoperiods and seasons. Under all the three photoperiods during both winter and summer seasons, plasma levels of T_4 and T_3 increased after lights "on" at 6.00 h and remained high during the light phase. Depending on the photoperiod, the plasma T_4 and T_3 levels declined reaching to a low level at the onset or during early hour or in the middle of the dark phase.

During both the seasons, the average plasma levels of both T_4 and T_3 were significantly higher during the light phase than that of the dark phase under all the photoperiodic regimes except under LD 15:9 in winter when the average level of T_4 was found to be similar during light and dark phases (Table 3:7A).

The results of acro analysis of the data are presented in Tables 3:8A. The mesor (24 h average) of T_4 and T_3 circadian rhythms were found to be minimum in fishes under LD 9:15 and maximum in the fishes exposed to LD 15:9. The mesor of T_4 circadian rhythm was found to be 14 and 16-18 folds higher than that of T_3 during winter and summer seasons, respectively. Regression analysis of the data indicated a

positive correlation between the length of photoperiod and mesors of T_4 and T_3 during both winter and summer seasons (Table 3:8A).

The amplitude of T_4 circadian rhythm was higher under LD 9:15 and LD 15:9 during winter than the respective photoperiods during summer (Table 3:8A), while under LD 12:12 the amplitude of T_4 rhythm was higher during summer than during winter. In case of the T_3 rhythm, the amplitude was higher under LD 9:15 and LD 15:9 during summer as compared to winter, while LD 12:12 the amplitude was higher during winter (Table 3:8A). Regression analysis of the data indicated a negative correlation between the amplitude of T_4 rhythm and the length of photoperiod only during winter, while a positive correlation was found between the amplitude of T_3 rhythm and the length of photoperiod only during summer (Table 3:8A).

The acrophase of T_4 occurred between 8.00 and 11.00 h during winter and between 11.00 and 14.00 h during summer season. As compared to LD 12:12, the acrophase of T_4 was delayed under short photoperiod during summer, and was advanced under long photoperiod during winter (Table 3:8A). Unlike T_4, the acrophase of T_3 circadian rhythm invariably occurred at 14.00 h irrespective of photoperiods and seasons except under LD 9:15 during summer, when the acrophase was advanced by 3 h and occurred at 11.00 h.

The pineal AA-NAT activity exhibited prominent circadian rhythm under all the photoperiods during both the seasons. Further, irrespective of photoperiods and seasons, the diurnal rhythm of AA-NAT activity invariably exhibited a basal level of the enzyme during the light phase, which increased rapidly after the onset of dark phase, remained significantly higher during the dark phase and declined sharply at the onset of light phase (Table 3:3; Figs. 3:3A and 3:3B).

During both the seasons, the mesor (24 h average) of pineal AA-NAT activity (Tables 3:8A and 3:9C) was found to be significantly lower under LD 15:9 as compared to that under LD 12:12 and LD 9:15. Regression analysis of the data indicated a strong negative correlation between the length of photoperiod and the mesor of AA-NAT activity rhythm during both winter and summer seasons (Table 3:8A).

The amplitude of the AA-NAT activity circadian rhythm was found to be invariably higher during winter as compared to the respective photoperiods during summer. Irrespective of the seasons, the amplitude of pineal AA-NAT activity under LD 12:12 was found to be higher than that under LD 15:9 and lower as compared to that under LD 9:15 (Table 3:8A). Regression analysis of the data indicated a strong negative correlation between amplitude of pineal AA-NAT activity circadian rhythm and length of the photoperiod during both winter and summer seasons (Table 3:8A).

During summer, the acrophase of pineal AA-NAT activity circadian rhythm occurred at 23.00 h under all photoperiods. However, during winter the acrophase of the AA-NAT activity rhythm under LD 12:12 and LD 15:9 occurred at 02.00 h, while the acrophase was advanced by 3 hours under LD 9:15 and occurred at 23.00 h (Table 3:8A).

Effects of simulated temperatures on circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during winter and summer seasons:

The data are presented in Tables 3:4, 3:5 and 3:6; Figs. 3:4A, 3:4B, 3:5A, 3:5B, 3:6A and 3:6B. Plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity exhibited prominent circadian rhythm under all the simulated temperatures (15° C, 25° C and 35° C) during both winter and summer seasons.

Irrespective of the seasons, the maximum levels of thyroid hormones were recorded during the light phase under all the temperatures. However, the patterns of diurnal increase and decrease in the levels of thyroid hormones were different under different temperatures and seasons. Irrespective of temperatures and seasons, plasma levels of T_4 and T_3 increased after lights "on" at 6.00 h except at 15° C when the plasma level of T_3 increased at 12.00 h during both the seasons. Depending on the temperature, the plasma T_4 and T_3 levels declined reaching to a low level during the early-, mid-, or late-dark phase, or at the onset of light phase (Tables 3:4 and 3:5; Figs.

3:4A, 3:4B, 3:5A and 3:5B). During both the seasons, the average plasma levels of both T_4 and T_3 were significantly higher during the light phase than that of the dark phase under all three temperatures except at 15° C when the average level of T_3 was found to be similar during light and dark phases (Table 3:7B).

The results of acro analysis of the data are presented in Table 3:8B. During both the seasons, the mesor (24 h average) of T_4 and T_3 circadian rhythms were found to be highest in the fishes exposed to 35° C and lowest in the fishes at 15° C. Further, the mesors of both the rhythms at 25° C were significantly lower than that of 35° C and significantly higher than that of 15° C during both the seasons (Tables 3:9D and 3:9E). The mesor of T_4 circadian rhythm was found to be 13-17 and 12-14 folds higher than that of T_3 during winter and summer season, respectively. Regression analysis of the data indicated a strong positive correlation between water temperature and mesors of T_4 and T_3 rhythms during both winter and summer seasons (Table 3:8B).

During both the seasons, the amplitude of T_4 circadian rhythm was found to be maximum at 25° C, and was greater than those at 15 ° C and 35 ° C (Table 3:8B). In contrast, the amplitude of the T_3 rhythm increased with increase in water temperature. The amplitude of both T_4 and T_3 circadian rhythms were found to be lower at all three temperatures during winter as compared to that of the respective temperatures during the summer season. Regression analysis of the data indicated a positive correlation between the amplitude of T_3 rhythm and temperature during both winter and summer

seasons, while no significant correlation was found between water temperature and the amplitude of the T_4 rhythm (Table 3:8B).

The acrophase of T_4 circadian rhythm occurred between 11.00 and 14.00 h during both winter and summer seasons (Table 3:8B). As compared to 25° C, while the acrophase of T_4 was delayed by 3 hours at 35° C during winter, it was delayed by 3 hours at both 15° C and 35° C during summer season. The acrophase of the T_3 circadian rhythm occurred between 14.00 and 17.00 h during both winter and summer seasons. Further, as compared to 25° C the acrophase of the T_3 rhythm was delayed by 3 hours at 15° C during the winter season, while it was advanced by 3 hours at both 15° C and 35° C during the summer season.

In contrast to the plasma levels of thyroid hormones, pineal AA-NAT activity was always found to be low during the light phase. AA-NAT activity increased rapidly after the onset of the dark phase irrespective of temperatures and seasons, and remained high during the dark phase. However, the pattern of increase in AA-NAT activity during the first half and decrease during the second half of the dark phase was found to be different under different temperatures as well as during different seasons (Table 3:6; Figs. 3:6A and 3:6B).

During both the seasons, the mesor (24 h average) of pineal AA-NAT activity was found to be maximum at 15° C and decreased significantly at both 25° C and 35° C

(Table 3:9F). Regression analysis of the data indicated a strong negative correlation between water temperature and mesor of the AA-NAT activity rhythm during both the seasons (Table 3:8B).

The amplitude of the AA-NAT activity circadian rhythm was found to be higher at 15° C and 35° C during summer as compared to the respective temperatures during winter. However, at 25° C the amplitude was found to be higher during winter than during summer season. Irrespective of the seasons, the amplitude of pineal AA-NAT activity decreased with the increase in temperature. Regression analysis of the data indicated a strong negative correlation between the amplitude of pineal AA-NAT activity circadian rhythm and water temperature during both winter and summer seasons (Table 3:8B).

Irrespective of the simulated temperature, the acrophase of pineal AA-NAT activity circadian rhythm occurred at 23.00 h during summer season, while during winter it was delayed by three hours and occurred at 2.00 h (Table 3.8B).

DISCUSSION

Though there are several reports on the effects of photoperiods and temperature either on the circulating levels of thyroid hormones or on AA-NAT activity and melatonin levels (Ottenweller and Hedge, 1982a; Vivien-Roels and Arendt, 1983; Sharp *et al.*, 1984; Licht *et al.*, 1989; Stokkan *et al.*, 1991; Zatz *et al.*, 1994; Gower *et al.*,

1996; Moyer *et al.*, 1997; Pavlidis *et al.*, 2000; Leiner and MacKenzie, 2001; Rensing and Rouff, 2002; Wright, 2002; Collin *et al.*, 2003), there is practically no information on effects of photoperiod and/or temperature on the temporal relationship of thyroid hormones and AA-NAT activity/melatonin levels with special reference to the breeding cycle or seasons in poikilothermic vertebrates in general and in any fish species in particular. To the best of our knowledge, the present study seems be the first of its kind in which the interrelationship between the circadian rhythms of thyroid hormones and pineal AA-NAT activity have been studied in a fish maintained under different photoperiods and simulated temperatures during both winter and summer seasons.

In the present study, levels of thyroid hormones and AA-NAT activity exhibited prominent circadian rhythms under all regimes of photoperiods and simulated temperature. However, the thyroid hormones and AA-NAT activity responded differently to the photoperiods and temperatures. It is important to mention that the levels of thyroid hormones increased only after the onset of light, and in general the average levels of the thyroid hormones were found to be invariably and significantly higher during the light phase than that of the dark phase irrespective of photoperiods and seasons. Similarly, irrespective of seasons the average levels of the thyroid hormones were also high during the light phase than during the dark phase under all three simulated temperatures. In contrast to thyroid hormones AA-NAT activity increased only after the onset of dark phase and the average enzyme activity remained significantly high during the dark phase as compared to light phase irrespective of

photoperiods and seasons. Further, the findings of experiments conducted under different photoperiods and simulated temperature indicate a positive correlation between the length of photoperiod and the levels of thyroid hormones as well as a positive correlation between water temperature and the levels of thyroid hormones (Tables 3:9A, B, D and E). Unlike in the case of thyroid hormones, a negative correlation was found between the length of photoperiod and AA-NAT activity as well as between water temperature and AA-NAT activity (Tables 3:9C and 3:9F). These findings seem to suggest that both photoperiod and temperature produce opposite effects on the levels of thyroid hormones and AA-NAT activity, and hence melatonin synthesis. Increase in the length of photoperiod and water temperature seem to increase the circulating levels of thyroid hormones and simultaneously decrease pineal AA-NAT activity. On the other hand, decrease in the length of photoperiod (increased length of dark phase) and water temperature seem to increase AA-NAT activity/melatonin synthesis and simultaneously decrease the levels of thyroid hormones. It, thus, seems that a direct relationship between photoperiod as well as temperature and thyroid hormones, and an inverse relationship between photoperiod as well as temperature and AA-NAT activity jointly regulate the opposite/inverse temporal relationship between the circadian rhythms of thyroid hormones and AA-NAT activity (hence melatonin synthesis) in the fish during both winter and summer seasons.

As revealed by the Acro analysis, depending on the photoperiod, temperature and season, the amplitude of T_4 circadian rhythm was found to be 13-18 folds higher

than that of the T_3 rhythm (Tables 3:8A and 3:8B). Comparatively higher mesor (24 h average) of the T_4 rhythm than that of the T_3 rhythm under all photoperiods and simulated temperatures during both winter and summer seasons seems to suggest that the synthesis and secretion of T_4 predominates the formation of T_3 in this species. The observed positive correlations between the thyroid hormones and photoperiod as well as water temperature seem to suggest that there is a direct relationship between environmental temperature and photoperiod and the plasma levels of the thyroid hormones in *Clarias gariepinus*. In hibernating mammals, plasma T_4 levels drop by about 8-folds from summer to winter (Demeneix and Handerson, 1978; Augee *et al.*, 1979) and thyroid activity is also reduced prior to hibernation (Hulbert, 1985). In Syrian hamster, total T_4 and T_3 concentrations were decreased significantly under short photoperiod, and the decrease was mediated by the pineal gland (Vaughan *et al.*, 1982; Vriend, 1983a). When collared lemmings were transferred from LD 22:2 to LD 8:16; serum concentrations of thyroid hormones declined significantly (Nagy *et al.*, 1993, 1994). These reports and the findings of the present study seem to suggest that the stimulatory effects of long photoperiod on thyroid activity is conserved from the fish to mammals.

The amplitude of T_4 circadian rhythm was found to be higher under both long and short photoperiods and lower under LD 12:12 during winter, while the amplitude of the T_3 rhythm was higher under both long and short photoperiods and lower under LD 12:12 during summer. Thus, the photoperiods seem to produce season-dependent

differential effects on the amplitudes of T_4 and T_3 circadian rhythms. Similarly, water temperature also seems to produce differential effects on the amplitudes of the two hormones. During both the seasons, while the amplitude of the T_4 rhythm was maximum at 25° C (the preferred body temperature of the fish), the amplitude of T_3 rhythm increased with increase in water temperature. These observations seem to suggest that while the amplitude of T_4 rhythm is closely associated with the preferred body temperature, the amplitude of T_3 rhythm is directly controlled by water temperature. This suggestion is also supported by the observed positive correlation between water temperature and T_3 levels.

In general, while the acrophase of T_4 circadian rhythm was influenced by both seasons and photoperiods, the acrophase of the T_3 rhythm was not influenced by the photoperiods and seasons. However, water temperature, depending on the season, either advanced or delayed the acrophase of the circadian rhythms of both the thyroid hormones. It, thus, seems that the acrophase of the T_4 rhythm in the fish is regulated jointly by photoperiod and water temperature, the acrophase of the T_3 rhythm is regulated mainly by water temperature.

The mesor (24 h average) of the circadian rhythm of pineal AA-NAT activity was found to be significantly higher under short daylength as compared to long daylength during both winter and summer (Tables 3:8A and 3:9C), and a strong negative correlation was recorded between the length of the photoperiod and the mesor

as well as the amplitude of the AA-NAT activity rhythm (Table 3:8A). Further, the mesor of the rhythm was found to increase significantly with decrease in water temperature, and a strong negative correlation was found between water temperature and the mesor as well as the amplitude of AA-NAT activity rhythm (Tables 3:8B and 3:9F). These findings seem to suggest that long daylength and higher ambient temperature decrease AA-NAT activity/melatonin synthesis during summer months, when the fish breeds. In contrast, short daylength and low temperature stimulate AA-NAT activity/melatonin synthesis during winter months when the testis remains quiescent. Melatonin has been reported to produce anti-gonadal effects in other fish species (Borg and Ekstrom, 1981; Nayak and Singh, 1987a). It, thus, seems that the circannual changes in environmental photoperiod and temperature regulate the circannual changes in AA-NAT activity and hence melatonin synthesis, which in turn may be regulating the circannual rhythm of testicular activity of the fish. In mammals, short photoperiod has been reported to increase melatonin levels (Vivien-Roels *et al.*, 1997; Gutjahr *et al.*, 2004).

At a constant temperature (25° C), the acrophase of AA-NAT activity rhythm was not influenced by photoperiod during summer, but it was advanced by three hours only under LD 9:15 during winter. Further, the simulated temperatures had no effects on acrophase of AA-NAT activity rhythm under LD 12:12 during winter and summer. These findings seem to suggest that the acrophase of AA-NAT activity rhythm in the fish pineal might be controlled by an indigenous clock, which is not controlled either by

environmental temperature or photoperiod. *In vitro* studies on pineal AA-NAT activity in this fish have revealed that the circadian rhythm of AA-NAT activity persists under constant darkness and constant temperature (data not included) supporting the view that the circadian rhythm of AA-NAT activity is controlled by an indigenous clock. The presence of internal clock have also been reported in other temperate zone fish species like gold fish, *Carassius auratus* (Kezuka *et al.*, 1989), the pike, *Esox lucius* (Falcon *et al.*, 1989; Coon *et al.*, 1998), white sucker, *Catostomus commersoni* (Zachman *et al.*, 1992), lamprey, *Lampetra japonica* (Samejima *et al.*, 1997), zebra fish, *Danio rerio* (Begay *et al.*, 1998) and ayu, *Plecoglossus altivelis* (Iigo *et al.*, 2004).

Table 3:1- Effects of different photoperiods on circadian rhythms in plasma levels of T_4 during winter and summer seasons.

	PHOTOPERIOD		
	LD 9:15	LD 12:12	LD 15:9
	Plasma levels of T_4 (ng/ml)		
Time (h)	WINTER		
6.00	14.24 ± 0.43* [h]	17.50 ± 0.58 [h, l]	18.20 ± 0.60 [k]
9.00	19.20 ± 0.70 [d, l]	18.70 ± 0.52 [l]	19.54 ± 0.57 [l]
12.00	18.62 ± 0.57 [l]	17.80 ± 0.48 [l]	18.70 ± 0.59 [l]
15.00	15.50 ± 0.56 [c, g, j]	16.36 ± 0.55 [f, k]	16.66 ± 0.60 [a, g, i]
18.00	13.20 ± 0.52 [b, h]	14.20 ± 0.61 [a, h]	17.30 ± 0.59 [e, k]
21.00	16.35 ± 0.62 [c, f, k]	13.78 ± 0.41 [h]	14.32 ± 0.60 [c, h]
24.00	14.32 ± 0.56 [b, h]	15.90 ± 0.45 [c, g, k]	17.20 ± 0.60 [c, e, k]
3.00	13.50 ± 0.58 [h]	13.66 ± 0.39 [c, h]	17.50 ± 0.60 [e, k]
6.00	14.24 ± 0.43 [c, h]	17.50 ± 0.58 [d, h, l]	18.20 ± 0.60 [k]
	SUMMER		
6.00	14.72 ± 0.55 [h]	16.06 ± 0.45 [h]	18.70 ± 0.79 [f]
9.00	19.20 ± 0.55 [d, l]	21.54 ± 0.53 [d, l]	21.70 ± 0.52 [b, l]
12.00	17.20 ± 0.36 [b, f, k]	20.00 ± 0.58 [l]	20.70 ± 0.46 [k]
15.00	16.20 ± 0.64 [g]	18.80 ± 0.54 [g, l]	19.10 ± 0.63 [f, i]
18.00	16.56 ± 0.64 [f]	17.60 ± 0.43 [h, l]	19.60 ± 0.51 [e, j]
21.00	17.50 ± 0.37 [e, k]	15.76 ± 0.49 [a, h]	18.20 ± 0.57 [g]
24.00	15.30 ± 0.57 [b, g]	17.50 ± 0.42 [h, k]	17.10 ± 0.57 [h]
3.00	15.10 ± 0.45 [h]	15.20 ± 0.41 [c, h]	18.40 ± 0.66 [g]
6.00	14.72 ± 0.55 [h]	16.06 ± 0.45 [h]	18.70 ± 0.79 [f]

*All values are expressed as mean ± standard error; (N = 4 - 5).

[a, b, c, d] Differ from the preceding value: $p < 0.05$, 0.02, 0.01 and 0.001, respectively

[e, f, g, h] Differ from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[i, j, k, l] Differ from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

Table 3:2- Effects of different photoperiods on circadian rhythms in plasma levels of T_3 during winter and summer seasons.

	PHOTOPERIOD		
	LD 9:15	LD 12:12	LD 15:9
	Plasma levels of T_3 (ng/ml)		
Time (h)	WINTER		
6.00	0.84 ± 0.04* [f]	0.78 ± 0.03 [f]	1.02 ± 0.06 [e]
9.00	1.42 ± 0.06 [c,j]	1.25 ± 0.05 [c]	1.47 ± 0.07 [b,j]
12.00	1.24 ± 0.06 [j]	1.63 ± 0.05 [b]	1.52 ± 0.08 [j]
15.00	1.14 ± 0.07 [d,i]	1.30 ± 0.07 [b,j]	1.34 ± 0.05 [a]
18.00	1.32 ± 0.08 [a,j]	1.32 ± 0.05 [j]	1.26 ± 0.07 [i]
21.00	1.05 ± 0.04 [a,e,i]	0.88 ± 0.05 [c,f]	1.36 ± 0.05
24.00	0.93 ± 0.04 [f]	1.07 ± 0.07 [f,i]	0.96 ± 0.03 [c,f]
3.00	0.81 ± 0.05 [f]	0.79 ± 0.03 [b,f]	0.98 ± 0.07 [f]
6.00	0.84 ± 0.04 [f]	0.78 ± 0.03 [f]	1.02 ± 0.06 [e]
	SUMMER		
6.00	0.92 ± 0.05 [e]	0.91 ± 0.06 [e]	0.69± 0.06 [f]
9.00	1.38 ± 0.08 [b,j]	0.96 ± 0.06 [e]	1.25 ± 0.06 [d]
12.00	1.21 ± 0.07 [i]	1.36 ± 0.07 [b,j]	1.48 ± 0.05 [a,j]
15.00	1.15 ± 0.06 [a,i]	1.17 ± 0.06 [i]	1.37 ± 0.05 [a,j]
18.00	0.96 ± 0.03 [a,e,h]	1.23 ± 0.06 [j]	1.34 ± 0.06 [j]
21.00	0.94 ± 0.04 [e,g]	0.85 ± 0.05 [b,f]	1.11 ± 0.07 [d,i]
24.00	0.76 ± 0.05 [a,f]	0.82 ± 0.06 [f]	0.90 ± 0.05 [f,g]
3.00	0.78 ± 0.05 [f]	0.77 ± 0.06 [f]	0.87 ± 0.06 [f]
6.00	0.92 ± 0.05 [e]	0.91 ± 0.06 [e]	0.69± 0.06 [f]

*All values are expressed as mean ± standard error; (N = 4 - 5).

[a, b, c] Differ from the preceding value: $p < 0.05$, 0.01 and 0.001, respectively.

[d, e, f] Differ from the maximum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

[g, h, i, j] Differ from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

Table 3:3- Effects of different photoperiods on circadian rhythms in pineal arylalkyl-amine-N-acetyltransferase activity during winter and summer seasons.

	PHOTOPERIOD		
	LD 9:15	LD 12:12	LD 15:9
	AA-NAT activity (nmol/pineal/hour)		
Time (h)	WINTER		
6.00	2.43 ± 0.14* [f, g]	2.43 ± 0.12 [f, j]	2.02 ± 0.11 [f, i]
9.00	1.86 ± 0.11 [a, f]	2.02 ± 0.15 [f]	1.93 ± 0.12 [f, i]
12.00	2.19 ± 0.14 [f]	1.90 ± 0.04 [f]	1.58 ± 0.11 [f]
15.00	2.48 ± 0.11 [f, g]	1.83 ± 0.17 [f]	1.52 ± 0.09 [f]
18.00	3.31 ± 0.22 [a, f, i]	2.03 ± 0.13 [f]	1.65 ± 0.12 [f]
21.00	5.41 ± 0.07 [c, i]	2.94 ± 0.13 [b, e, h]	1.41 ± 0.12 [e, f]
24.00	4.43 ± 0.10 [b, f, i]	5.35 ± 0.33 [b, i]	2.80 ± 0.12 [e]
3.00	4.13 ± 0.156 [f, i]	3.93 ± 0.05 [b, e, i]	3.45 ± 0.13 [b, i]
6.00	2.43 ± 0.141 [c, f, g]	2.43 ± 0.12 [c, f, i]	2.02 ± 0.11 [c, f, i]
	SUMMER		
6.00	1.14 ± 0.04 [f, g]	1.17 ± 0.03 [f]	1.15 ± 0.04 [f]
9.00	1.27 ± 0.04 [f]	1.26 ± 0.04 [f]	1.08 ± 0.0 [f]
12.00	0.89 ± 0.06 [b, f]	1.23± 0.06 [f]	1.08 ± 0.03 [f]
15.00	1.20 ± 0.03 [b, f]	1.25 ± 0.04 [f]	1.08 ± 0.03 [f]
18.00	2.29 ± 0.08 [c, f, i]	1.26 ± 0.09 [f]	1.14 ± 0.03 [f]
21.00	3.72 ± 0.09 [c, i]	2.93 ± 0.13 [c, i]	2.24 ± 0.10 [c, e, i]
24.00	3.51 ± 0.02 [i]	3.32 ± 0.10 [i]	2.40 ± 0.13 [i]
3.00	3.16 ± 0.15 [d]	3.05 ± 0.11 [i]	2.64 ± 0.03 [i]
6.00	1.14 ± 0.04 [c ,,f, g]	1.17 ± 0.03 [c, f]	1.15 ± 0.04 [c, i]

*All values are expressed as mean ± standard error; (N = 4 - 5).

[a, b, c] Differ from the preceding value: $p < 0.02$, 0.01 and 0.001, respectively

[d, e, f] Differ from the maximum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

[g, h, i] Differ from the minimum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

Table 3:4- Effects of simulated temperatures on circadian rhythms in plasma levels of T_4 during winter and summer seasons.

	WATER TEMPERATURE		
	15° C	25° C	35° C
	Plasma levels of T_4 (ng/ml)		
Time (h)	WINTER		
6.00	13.60 ± 0.57 [i]	16.60 ± 0.53 [j]	18.40 ± 0.58 [i]
9.00	15.46 ± 0.68 [j]	20.70 ± 0.76 [c, j]	21.46 ± 0.71 [c, j]
12.00	14.86 ± 0.51 [j]	18.30 ± 0.52 [a, j]	19.80 ± 0.59 [a, j]
15.00	14.60 ± 0.62 [j]	18.50 ± 0.58 [j]	20.54 ± 0.47 [j]
18.00	12.82 ± 0.39 [a, f, i]	15.56 ± 0.62 [c, h]	20.44 ± 0.63 [c, j]
21.00	13.22 ± 0.57 [e, i]	12.98 ± 0.45 [b, h]	16.96 ± 0.61 [b, h]
24.00	12.70 ± 0.45 [g]	14.56 ± 0.66 [h]	17.30 ± 0.48 [h]
3.00	10.60 ± 0.43 [b, h]	13.88 ± 0.69 [h]	15.62 ± 0.52 [c, h]
6.00	13.60 ± 0.57 [c]	16.60 ± 0.53 [b, j]	18.40 ± 0.58 [c]
	SUMMER		
6.00	8.75 ± 0.61 [h]	16.26 ± 0.54 [i]	15.76 ± 0.53 [h]
9.00	15.26 ± 0.44 [d, j]	21.37 ± 0.63 [d, j]	21.30 ± 0.83 [d, j]
12.00	14.04 ± 0.48 [j]	20.10 ± 0.51 [j]	18.10 ± 0.68 [b, i]
15.00	15.30 ± 0.54 [j]	15.9 0± 0.55 [d, h, i]	19.40 ± 0.62 [j]
18.00	11.47 ± 0.58 [c, i]	14.08 ± 0.48 [a, h]	18.60 ± 0.55 [e, i]
21.00	9.32 ± 0.66 [a, h]	12.46 ± 0.64 [h]	15.80 ± 0.54 [c, h]
24.00	8.39 ± 0.79 [h]	16.20 ± 0.46 [c, h]	16.56 ± 0.70 [g]
3.00	8.17 ± 0.51 [h]	13.16 ± 0.51 [c, h, i]	15.30 ± 0.41 [h]
6.00	8.75 ± 0.61 [h]	16.26 ± 0.54 [c, i]	15.76 ± 0.53

All values are expressed as mean ± standard error; (N = 5).

[a, b, c, d] Differ from the preceding value: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e, f, g, h] Differ from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[i, j] Differ from the minimum value of the respective group: $p < 0.01$ and 0.001, respectively.

Table 3:5- Effects of simulated temperatures on circadian rhythms in plasma levels of T_3 during winter and summer seasons.

	WATER TEMPERATURE		
	15° C	25° C	35° C
	Plasma levels of T_3 (ng/ml)		
Time (h)	WINTER		
6.00	0.75 ± 0.04*	0.98 ± 0.05 [f]	1.26 ± 0.06 [e]
9.00	0.71 ± 0.05 [c]	1.12 ± 0.06	1.44 ± 0.05 [i]
12.00	0.86 ± 0.04	1.44 ± 0.06 [b, j]	1.62 ± 0.05 [j]
15.00	0.91 ± 0.06	1.37 ± 0.06 [i]	1.48 ± 0.05 [i]
18.00	0.89 ± 0.05	1.34 ± 0.06 [i]	1.51 ± 0.05 [j]
21.00	0.91 ± 0.05 [g]	1.18 ± 0.06 [d, g]	1.44 ± 0.06 [j]
24.00	0.75 ± 0.03 [a]	1.14 ± 0.07 [d]	1.25 ± 0.05 [e]
3.00	0.73 ± 0.05 [c]	0.97 ± 0.04 [f]	1.08 ± 0.05 [f]
6.00	0.75 ± 0.04	0.98 ± 0.05 [f]	1.26 ± 0.06 [e]
	SUMMER		
6.00	0.85 ± 0.05 [c]	0.91 ± 0.06 [f]	0.93 ± 0.08 [f]
9.00	0.90 ± 0.05	1.18 ± 0.06	1.54 ± 0.08 [b, j]
12.00	1.06 ± 0.08 [g]	1.42 ± 0.05 [a, j]	1.58 ± 0.07 [j]
15.00	0.96 ± 0.07	1.28 ± 0.06 [i]	1.46 ± 0.05 [j]
18.00	1.08 ± 0.07 [g]	1.32 ± 0.06 [i]	1.52 ± 0.05 [j]
21.00	0.84 ± 0.05 [a, c]	1.22 ± 0.07 [h]	1.32 ± 0.05 [d, i]
24.00	0.86 ± 0.06	1.13 ± 0.05 [e, h]	1.38 ± 0.04 [c, i]
3.00	0.82 ± 0.05 [c]	1.02 ± 0.06 [e]	1.28 ± 0.04 [e]
6.00	0.85 ± 0.05 [c]	0.91 ± 0.06 [f]	0.93 ± 0.08 [b, f]

*All values are expressed as mean ± standard error; (N = 5).

[a, b] Differ from the preceding value: $p < 0.05$ and 0.01, respectively.

[c, d, e, f] Differ from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[g, h, i, j] Differ from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

Table 3:6- Effects of simulated temperatures on circadian rhythms in pineal arylalkyl-amine-N-acetyltransferase activity during winter and summer seasons.

	WATER TEMPERATURE		
	15° C	25° C	35° C
	AA-NAT activity (nmol/pineal/h)		
Time (h)	WINTER		
6.00	5.08 ± 0.14* [e, h]	2.86 ± 0.10 [e, g]	2.54 ± 0.14 [e, g]
9.00	3.52 ± 0.33 [c, e]	2.75 ± 0.14 [e, g]	2.48 ± 0.04 [e, h]
12.00	2.90 ± 0.17 [e]	2.11 ± 0.14 [b, e]	1.88 ± 0.16 [b, e]
15.00	2.92 ± 0.14 [e]	1.66 ± 0.20 [e]	1.54 ± 0.09 [e]
18.00	3.17 ± 0.28 [e]	1.78 ± 0.23	2.46 ± 0.07 [d, e]
21.00	6.46 ± 0.34 [d, h]	3.38 ± 0.21 [c, g]	3.14 ± 0.22 [a, h]
24.00	7.88 ± 0.17 [c, h]	4.96 ± 0.20 [c, h]	3.73 ± 0.07 [a, h]
3.00	6.71 ± 0.38 [a, h]	4.50 ± 0.13 [h]	3.09 ± 0.11 [a, h]
6.00	5.08 ± 0.14 [c, e, h]	2.86 ± 0.10 [d, e, g]	2.54 ± 0.14 [b, e, g]
	SUMMER		
6.00	2.76 ± 0.09 [e]	1.27 ± 0.11 [e, f]	1.18 ± 0.10 [e]
9.00	2.66 ± 0.06 [e]	1.02 ± 0.04 [e]	1.04 ± 0.03 [e]
12.00	2.61 ± 0.09 [e]	0.82 ± 0.07 [e]	0.80 ± 0.80 [e]
15.00	2.83 ± 0.04 [e]	1.22 ± 0.06 [e]	0.95 ± 0.06 [e]
18.00	3.04 ± 0.08 [e, f]	1.32 ± 0.06 [h]	1.31 ± 0.02 [c]
21.00	3.98 ±0.02 [d, h]	3.17 ± 0.04 [d, h]	1.91 ± 0.08 [d]
24.00	7.76 ± 0.42 [d, h]	3.75 ± 0.10 [c, h]	3.11 ± 0.03 [d, h]
3.00	4.83 ± 0.17 [d, g]	3.51 ± 0.10 [h]	2.77 ± 0.07 [c, h]
6.00	2.76 ± 0.09 [d, e]	1.27 ± 0.11 [d, e, f]	1.18 ± 0.10 [d, e]

*All values are expressed as mean ± standard error; (N = 4 - 5).

[a, b, c, d] Differ from the preceding value: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e] Differs from the maximum value of the respective group: $p < 0.001$.

[f, g, h] Differ from the minimum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

Table 3:7A- Average plasma levels of thyroid hormones (T_4 and T_3) and average pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity during the light and dark phases under different photoperiods during winter and summer seasons.

	Plasma levels of T_4 (ng/ml)			
	Winter		Summer	
Photoperiod	Light phase	Dark phase	Light phase	Dark phase
LD 9:15	17.77 ± 0.61* [d]	14.32 ± 0.54	17.53 ± 0.51 [a]	15.83 ± 0.51
LD 12:12	16.76 ± 0.55 [a]	15.21 ± 0.46	19.48 ± 0.52 [d]	16.13 ± 0.44
LD 15:9	17.30 ± 0.59	17.63 ± 0.67	19.86 ± 0.53 [a]	18.06 ± 0.67

	Plasma levels of T_3 (ng/ml)			
	Winter		Summer	
Photoperiod	Light phase	Dark phase	Light phase	Dark phase
LD 9:15	1.26 ± 0.06 [c]	0.99 ± 0.05	1.24 ± 0.07 [d]	0.87 ± 0.04
LD 12:12	1.37 ± 0.05 [d]	0.88 ± 0.04	1.18 ± 0.06 [d]	0.83 ± 0.05
LD 15:9	1.39 ± 0.06 [b]	0.98 ± 0.05	1.31 ± 0.06 [d]	0.82 ± 0.05

	AA-NAT activity (nmol/pineal/hour)			
	Winter		Summer	
Photoperiod	Light phase	Dark phase	Light phase	Dark phase
LD 9:15	2.17 ± 0.13 [d]	3.15 ± 0.13	1.12 ± 0.04 [d]	2.76 ± 0.08
LD 12:12	1.94 ± 0.12 [d]	3.66 ± 0.14	1.25 ± 0.06 [d]	3.61 ± 0.10
LD 15:9	1.61 ± 0.10 [d]	2.75 ± 0.09	1.32 ± 0.06 [d]	2.06 ± 0.06

*All values are expressed as mean ± standard error (S. E).
i). LD 9:15: Light phase N = 13 - 15 and Dark phase N = 22 - 25;
ii). LD 12:12: Light phase N = 17 - 20 and Dark phase N = 17 - 20;
iii). LD 15:9: Light phase N = 22 - 25 and Dark phase N = 13 - 15.

[a, b, c, d] Differ from the dark phase of the respective group: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

Table 3:7B- Average plasma levels of thyroid hormones (T_4 and T_3) and average pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity during the light and dark phases under simulated temperatures during winter and summer seasons.

Water Temperature	Plasma levels of T_4 (ng/ml)			
	Winter		Summer	
	Light phase	Dark phase	Light phase	Dark phase
15° C	14.43 ± 0.55* [b]	12.53 ± 0.51	14.02 ± 0.51 [d]	8.72 ± 0.64
25° C	18.27 ± 0.62 [d]	14.49 ± 0.53	17.86 ± 0.54 [d]	14.52 ± 0.54
35 ° C	20.56 ± 0.60 [d]	17.07 ± 0.55	19.38 ± 0.66 [d]	15.85 ± 0.54

Water Temperature	Plasma levels of T_3 (ng/ml)			
	Winter		Summer	
	Light phase	Dark phase	Light phase	Dark phase
15° C	0.85 ± 0.05	0.78 ± 0.04	1.00 ± 0.07	0.84 ± 0.05
25° C	1.32 ± 0.05 [d]	1.06 ± 0.05	1.30 ± 0.05 [c]	1.07 ± 0.06
35 ° C	1.51 ± 0.05 [d]	1.25 ± 0.05	1.52 ± 0.06 [d]	1.22 ± 0.05

Water Temperature	AA-NAT activity (nmol/pineal/hour)			
	Winter		Summer	
	Light phase	Dark phase	Light phase	Dark phase
15° C	3.13 ± 0.23 [d]	6.54 ± 0.25	2.78 ± 0.06 [d]	4.83 ± 0.23
25° C	2.07 ± 0.18 [d]	3.92 ± 0.17	1.09 ± 0.05 [d]	2.93 ± 0.09
35 ° C	2.09 ± 0.09 [d]	3.13 ± 0.13	1.02 ± 0.04 [d]	2.24 ± 0.07

*All values are expressed as mean ± standard error (S. E).
i). LD 9:15: Light phase N = 13 - 15 and Dark phase N = 22 - 25;
ii). LD 12:12: Light phase N = 17 - 20 and Dark phase N = 17 - 20;
iii). LD 15:9: Light phase N = 22 - 25 and Dark phase N = 13 - 15.

[a, b, c, d] Differ from the dark phase of the respective group: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

Table 3:8A- Acro analysis of the data on the circadian rhythms of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity under different photoperiods during winter and summer seasons (Based on data presented in Tables 3:1, 3:2 and 3:3).

WINTER

	Mesor				Amplitude				Acrophase (h)			95% Confidence interval (C. I) (h)			Goodness of fit			Threshold (20%) (h)		
	LD 9:15	LD 12:12	LD 15:9	r	LD 9:15	LD 12:12	LD 15:9	r	LD 9:15	LD 12:12	LD 15:9	LD 9:15	LD 12:12	LD 15:9	LD 9:15	LD 12:12	LD 15:9	LD 9:15	LD 12:12	LD 15:9
T_4 (ng/ml)	15.61	15.98	17.42	0.98	3.00	2.52	2.61	-0.76	11	11	8	9.37 to 12.63	9.79 to 12.21	6.59 to 9.41	0.209 p > 0.05 N. S	0.108 p >0.05 N. S	0.111 p > 0.05 N. S	2.6	2.6	-
T_3 (ng/ml)	1.09	1.12	1.23	0.94	0.30	0.42	0.28	-0.14	14	14	14	12.67 to 15.33	12.86 to 15.14	12.88 to 15.12	0.127 p > 0.05 N. S	0.086 p < 0.05	0.094 p < 0.05	5.6	5.6	5.6
AA-NAT activity (nmol/ pineal/h)	3.28	2.80	2.04	- 0.99	1.77	1.76	1.02	-0.87	23	2	2	22.3 to 23.7	0.8 to 3.2	0.65 to 3.35	0.031 p < 0.001	0.96 p < 0.05	0.124 p > 0.05 N. S	14.6	17.6	17.6

SUMMER

	Mesor				Amplitude				Acrophase (h)			95% Confidence interval (C. I) (h)			Goodness of fit			Threshold (20%) (h)		
T_4 (ng/ml)	16.47	17.80	19.18	0.99	2.24	3.17	2.30	-0.05	14	11	11	11.93 to 16.07	9.57 to 12.43	9.8 to 12.2	0.322 p > 0.05 N. S	0.14 p > 0.05 N. S	0.085 p < 0.05	5.6	2.6	2.6
T_3 (ng/ml)	1.01	1.00	1.12	0.47	0.31	0.29	0.39	0.78	11	14	14	9.93 to 12.07	12.15 to 14.85	13.11 to 14.89	0.074 p < 0.02	0.047 p < 0.05	0.051 p < 0.01	2.6	5.6	5.6
AA-NAT activity (nmol/ pineal/h)	1.89	2.14	1.60	-0.97	1.41	1.07	0.78	-0.88	23	23	23	21.84 to 24.16	22.41 to 23.59	21.92 to 24.08	0.103 p < 0.05	0.025 p < 0.001	0.097 p < 0.05	14.6	14.6	14.6

Table 3:8B- Acro analysis of the data on the circadian rhythms of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity under simulated temperatures during winter and summer seasons. (Based on data presented in Tables 3:4, 3:5 and 3:6).

WINTER

	Mesor				Amplitude				Acrophase (h)			95% Confidence interval (C. I) (h)			Goodness of fit			Threshold (20%) (h)		
	15° C	25° C	35° C	r	15° C	25° C	35° C	r	15° C	25° C	35° C	15° C	25° C	35° C	15° C	25° C	35° C	15° C	25° C	35° C
T_4 (ng/ml)	13.48	16.38	18.81	0.99	2.43	3.86	2.92	0.33	11	11	14	9.49 to 12.51	10.16 to 11.84	12.83 to 15.17	0.14 $p > 0.05$ N. S.	0.043 $p < 0.005$	0.091 $p < 0.05$	2.6	2.6	5.6
T_3 (ng/ml)	0.81	1.19	1.38	0.98	0.1	0.23	0.27	0.94	17	14	14	16.21 to 17.79	12.99 to 15.01	13.0 to 15.0	0.048 $p < 0.005$	0.07 $p < 0.02$	0.058 $p < 0.01$	8.6	5.6	5.6
AA-NAT activity (nmol/ pineal/h)	4.83	3.0	2.60	-0.93	2.49	1.65	1.09	-0.99	2	2	2	1.26 to 2.74	1.19 to 2.81	0.93 to 3.07	0.039 $p < 0.005$	0.043 $p < 0.005$	0.067 $p < 0.02$	17.6	17.6	17.6

SUMMER

	Mesor				Amplitude				Acrophase (h)			95% Confidence interval (C. I) (h)			Goodness of fit			Threshold (20%) (h)		
T_4 (ng/ml)	11.33	16.19	17.60	0.95	3.56	4.45	3	-0.38	14	11	14	13.11 to 14.89	9.72 to 12.28	12.5 to 15.5	0.063 $p < 0.02$	0.11 $p > 0.05$ N. S.	0.158 $p > 0.05$ N. S.	5.6	2.6	5.6
T_3 (ng/ml)	0.92	1.18	1.37	0.99	0.13	0.25	0.32	0.98	14	17	14	12.83 to 15.17	15.69 to 18.31	12.14 to 15.86	0.101 $p < 0.05$	0.104 $p > 0.05$ N. S.	0.233 $p > 0 .05$ N. S.	5.6	8.6	5.6
AA-NAT activity (nmol/ pineal/h)	3.80	2.01	1.63	-0.93	2.57	1.46	1.15	0.95	23	23	23	21.58 to 24.42	22.13 to 23.87	21.92 to 24.08	0.135 $p > 0.05$ N. S.	0.058 $p < 0.01$	0.083 $p < 0.05$	14.6	14.6	14.6

Table 3:9A- Effects of different photoperiods on the 24 h average of plasma level of thyroxine (T_4).

	Plasma levels of T_4 (ng/ml)	
Photoperiod	WINTER	SUMMER
LD 9:15	15.61 ± 0.46*	16.47 ± 0.29
LD 12:12	15.98 ± 0.32	17.80 ± 0.36 [a]
LD 15:9	17.42 ± 0.31[c]	19.18 ± 0.30 [b, c]
r	0.98	0.99

*All values are expressed as mean ± standard error (S. E); N = 36 - 40.

[a, b] Differ significantly from LD 9:15 group of the respective group: $p < 0.05$ and 0.01, respectively.

[c] Differs significantly from LD 9:15 and LD 12:12 groups of the respective group: $p < 0.001$.

Table 3:9B- Effects of different photoperiods on the 24 h average of plasma level of triiodothyronine (T_3).

	Plasma levels of T_3 (ng/ml)	
Photoperiod	WINTER	SUMMER
LD 9:15	1.09 ± 0.03*	1.01 ± 0.03
LD 12:12	1.12 ± 0.04	0.99 ± 0.03
LD 15:9	1.23 ± 0.03 [a]	1.12 ± 0.04 [a]
r	0.94	0.47

*All values are expressed as mean ± standard error (S. E); N = 36 - 40.

[a] Differs significantly from LD 19:15 and LD 12:12 group of the respective group: $p < 0.01$.

Table 3:9C- Effects of different photoperiods on the 24 h average of pineal AA-NAT activity.

	AA-NAT activity (nmol/pineal/hour)	
Photoperiod	WINTER	SUMMER
LD 9:15	3.28 ± 0.19*	2.15 ± 0.17
LD 12:12	2.80 ± 0.19	1.93 ± 0.14
LD 15:9	2.05 ± 0.11 [a, b]	1.60 ± 0.09 [c, d]
r	-0.99	-0.97

*All values are expressed as mean ± Standard error (S. E); N = 36 - 40.

[a, b] Differ significantly from LD 9:15 and LD 12:12 group respectively during winter season: $p < 0.01$ and 0.001, respectively.

[c, d] Differ significantly from LD 12:12 and LD 9:15 group respectively during summer season: $p < 0.05$ and 0.01, respectively.

Table 3:9D- Effects of simulated temperatures on the 24 h average of plasma levels of thyroxine (T_4).

	Plasma levels of T_4 (ng/ml)	
Water temperature	WINTER	SUMMER
15° C	13.48 ± 0.29*	11.33 ± 0.50
25° C	16.38 ± 0.44 [a]	16.19 ± 0.50 [a]
35° C	18.81 ± 0.36 [b]	17.60 ± 0.37 [b]
r	0.99	0.95

*All values are expressed as mean ± standard error (S. E); N = 36 - 40.

[a] Differs significantly differs from 15° C of the respective group: $p < 0.001$.

[b] Differs significantly from 15° C and 25° C of the respective group: $p < 0.001$.

Table 3:9E- Effects of simulated temperatures on the 24 h average of plasma levels of triiodothyronine (T_3).

	Plasma levels of T_3 (ng/ml)	
Water temperature	WINTER	SUMMER
15° C	0.81 ± 0.02*	0.92 ± 0.02
25° C	1.19 ± 0.03 [a]	1.18 ± 0.03 [a]
35° C	1.38 ± 0.03 [b]	1.37 ± 0.03 [b]
r	0.98	0.99

*All values are expressed as mean ± standard error (S. E); N = 36 - 40.

[a] Differs significantly from 15° C of the respective group: $p < 0.001$.

[b] Differs significantly from 15° C and 25° C of the respective group: $p < 0.001$.

Table 3:9F- Effects of simulated temperatures on the 24 h average of pineal AA-NAT activity.

	AA-NAT activity (nmol/pineal/hour)	
Water temperature	WINTER	SUMMER
15° C	4.83 ± 0.3*	3.80 ± 0.25
25° C	3.00 ± 0.21 [a]	2.01 ± 0.18 [a]
35° C	2.61 ± 0.12 [b]	1.63 ± 0.13 [b]
r	-0.93	-0.93

*All values are expressed as mean ± standard error (S. E); N = 36 - 40.

[a, b] Differ significantly from 15° C of the respective group: $p < 0.01$ and 0.001, respectively.

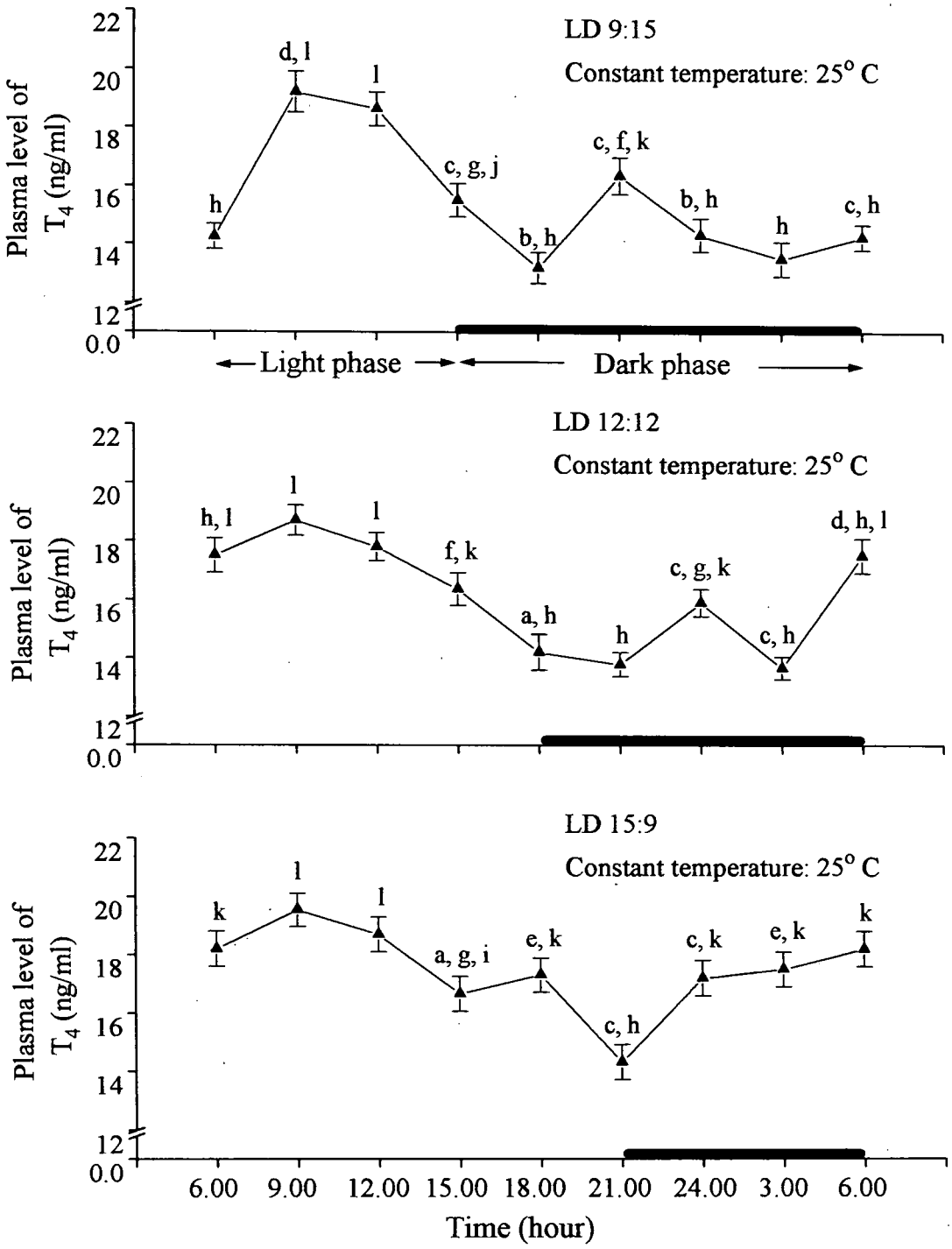


Fig. 3:1A- Effects of different photoperiods on circadian rhythm in plasma levels of T_4 during winter season at a constant temperature (25° C).

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[a, b, c, d] Differ significantly from the preceding value: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e, f, g, h] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[i, j, k, l] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

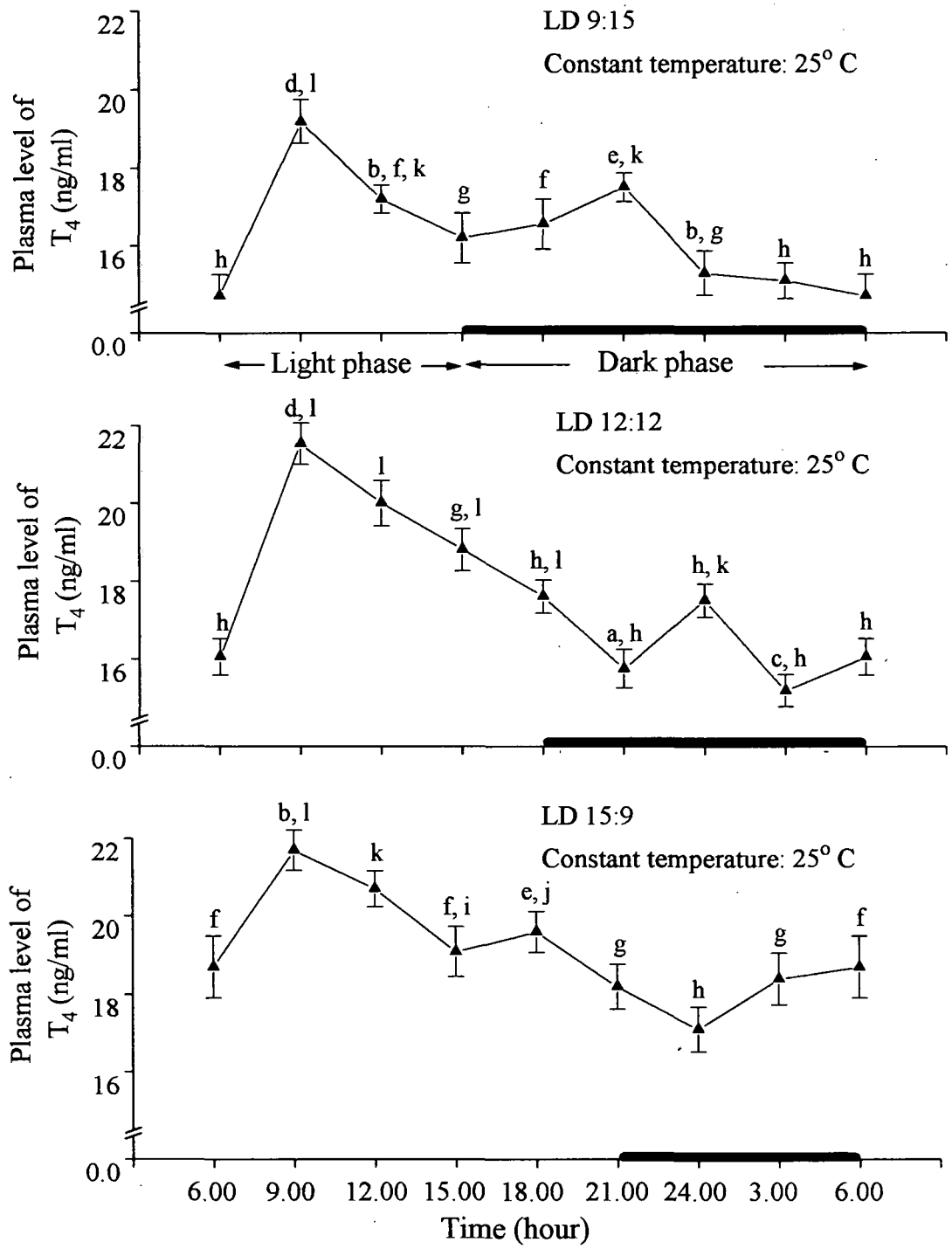


Fig. 3:1B- Effects of different photoperiods on circadian rhythm in plasma levels of T_4 during summer season at a constant temperature (25° C).

All values are expressed as mean ± standard Error (S. E); N = 4 - 5.

[a, b, c, d] Differ significantly from the preceding value: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e, f, g, h] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[i, j, k, l] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

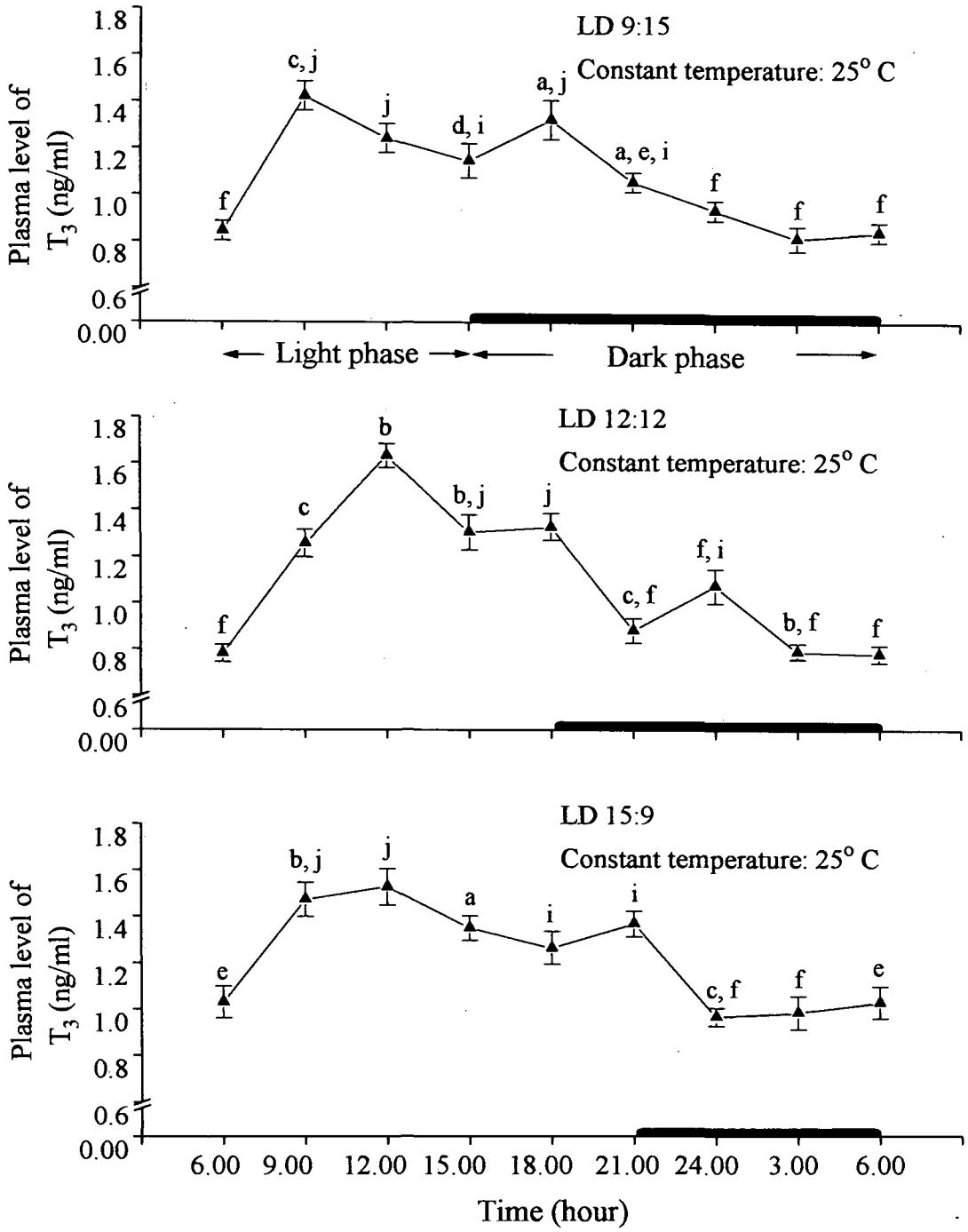


Fig. 3:2A- Effects of different photoperiods on circadian rhythm in plasma levels of T_3 during winter season at a constant temperature (25° C).

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[a, b, c] Differ significantly from the preceding value: $p < 0.05$, 0.01 and 0.001, respectively.

[d, e, f] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

[i, j] Differ significantly from the minimum value of the respective group: $p < 0.01$ and 0.001, respectively.

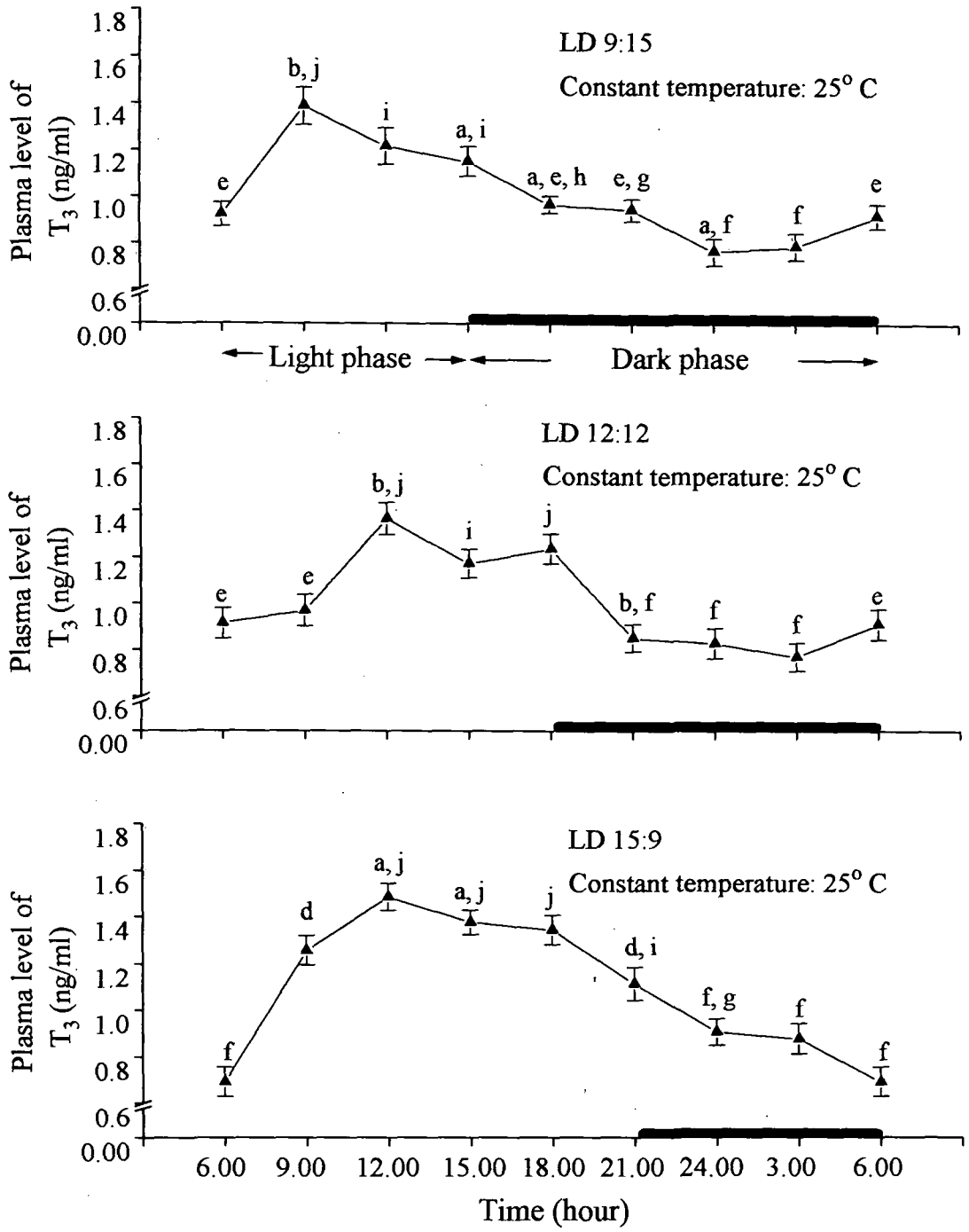


Fig. 3:2B- Effects of different photoperiods on circadian rhythm in plasma levels of T_3 during summer season at a constant temperature (25° C).

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[a, b] Differ significantly from the preceding value: $p < 0.05$ and 0.01, respectively.

[d, e, f] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

[g, h, i, j] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

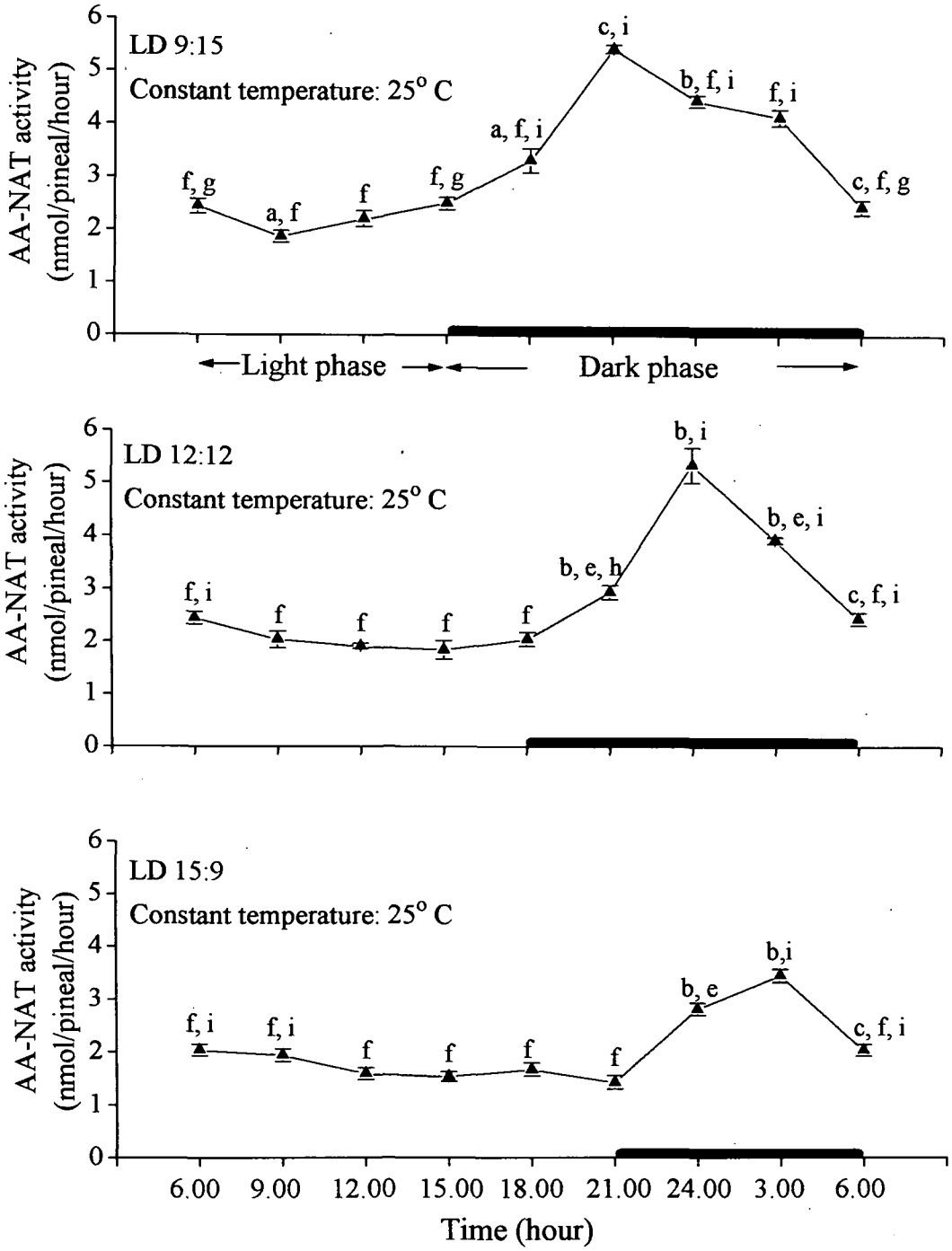


Fig. 3:3A- Effects of different photoperiods on circadian rhythm in pineal AA-N-acetyltransferase (AA-NAT) activity during winter season at a constant temperature (25° C).

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[a, b, c] Differ significantly from the preceding value: $p < 0.02$, 0.01 and 0.001, respectively.

[e, f] Differ significantly from the maximum value of the respective group: $p < 0.01$, and 0.001, respectively.

[g, h, i] Differ significantly from the minimum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

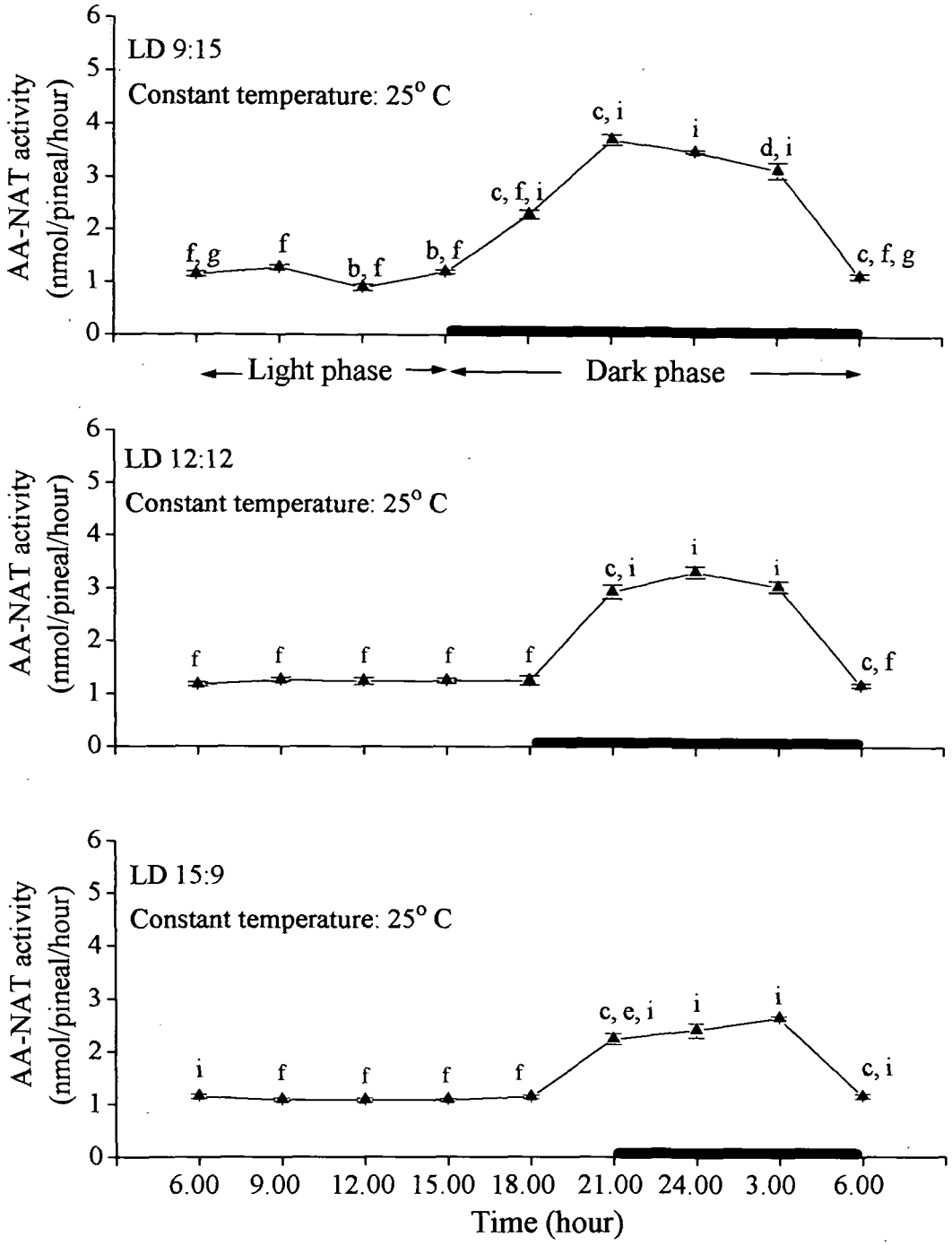


Fig. 3:3B- Effects of different photoperiods on circadian rhythm in pineal AA-N-acetyltransferase (AA-NAT) activity during summer season at a constant temperature (25° C).

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[b, c] Differ significantly from the preceding value: $p < 0.01$ and 0.001, respectively.

[d, e, f] Differ significantly from the maximum value of the respective group: $p < 0.02$, 0.01 and 0.001, respectively.

[g, i] Differ significantly from the minimum value of the respective group: $p < 0.02$, and 0.001, respectively.

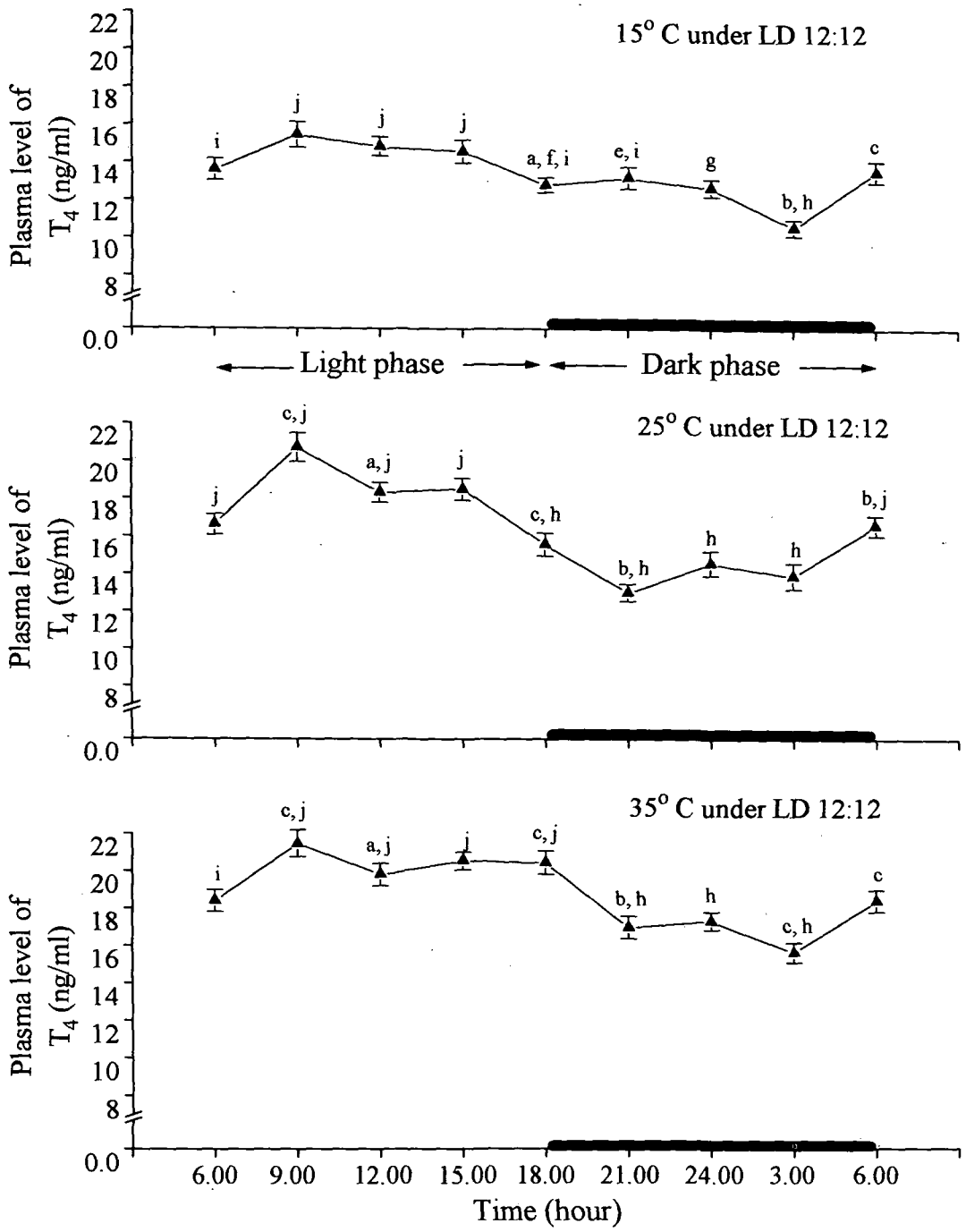


Fig. 3:4A- Effects of simulated temperatures on circadian rhythm in plasma levels of T_4 during winter season under LD 12:12.

All values are expressed as mean ± standard error (S. E); N = 5.

[a, b, c] Differ significantly from the preceding value: $p < 0.05$, 0.02 and 0.01, respectively.

[e, f, g, h] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[i, j] Differ significantly from the minimum value of the respective group: $p < 0.01$ and 0.001, respectively.

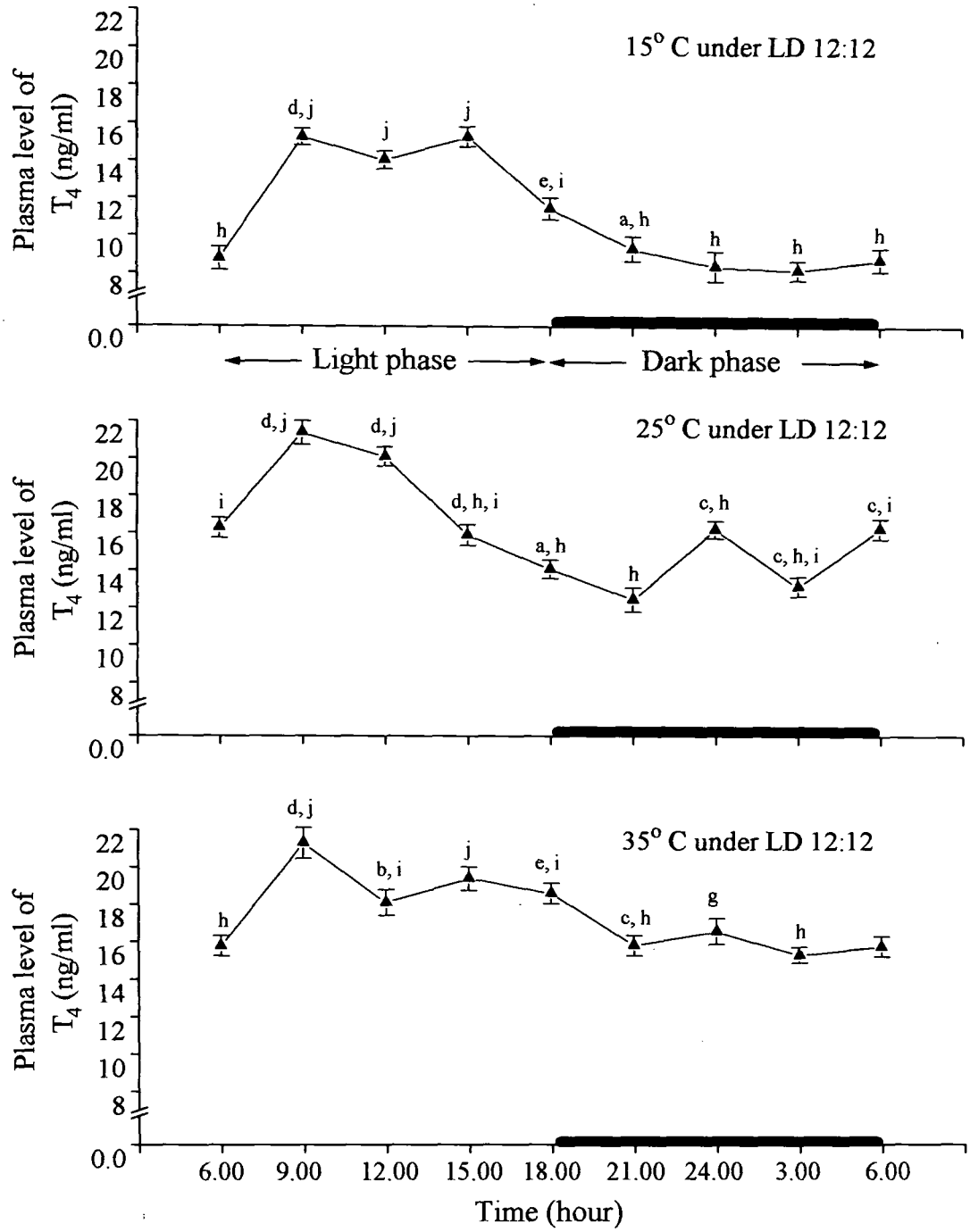


Fig. 3:4B- Effects of simulated temperatures on circadian rhythm in plasma levels of T_4 during summer season under LD 12:12.

All values are expressed as mean ± standard error (S. E); N = 5.

[a, b, c, d] Differ significantly from the preceding value: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e, g, h] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.01 and 0.001, respectively.

[i, j] Differ significantly from the minimum value of the respective group: $p < 0.01$ and 0.001, respectively.

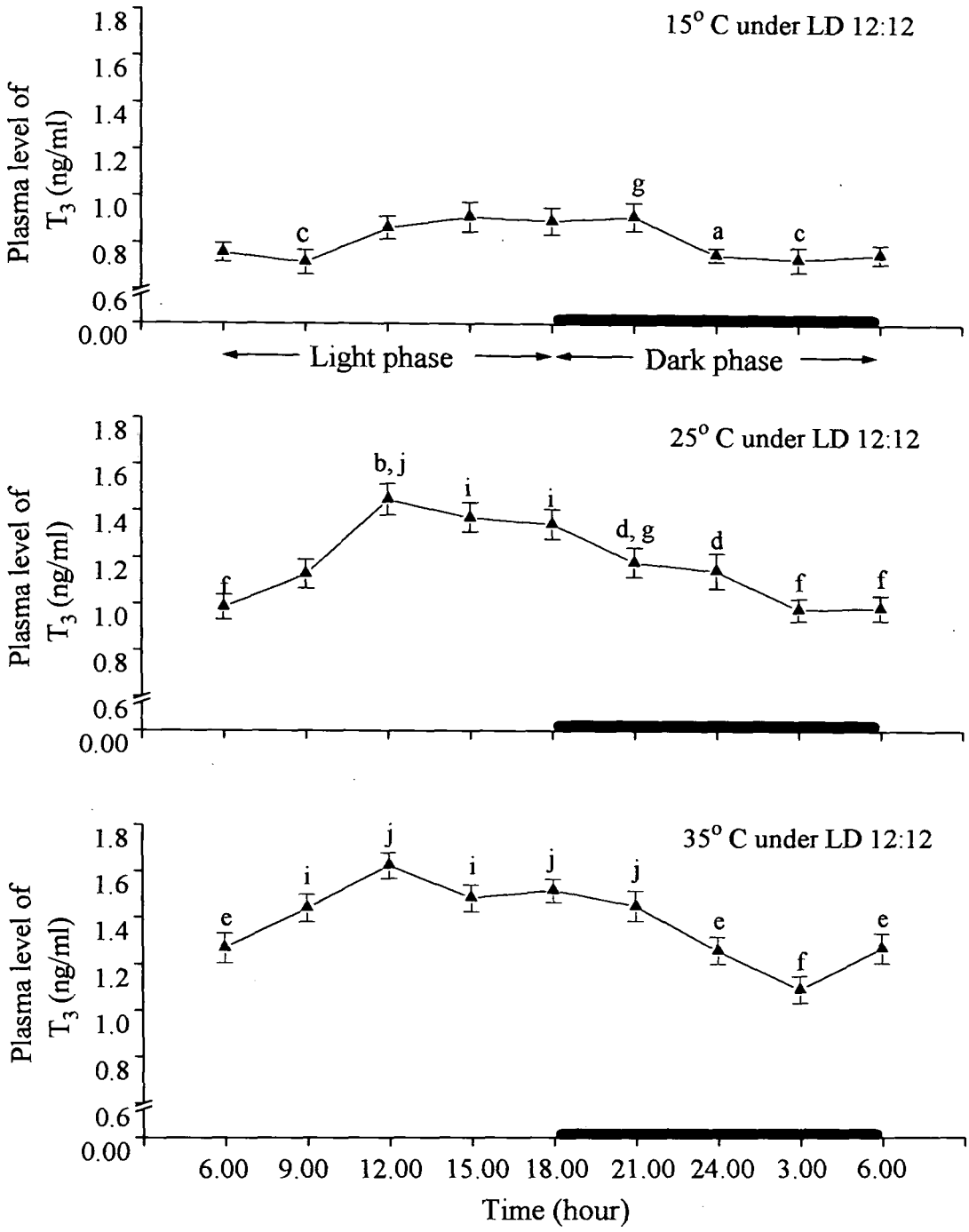


Fig. 3:5A- Effects of simulated temperatures on circadian rhythm in plasma levels of T_3 during winter season under LD 12:12.

All values are expressed as mean ± standard error (S. E); N = 5.

[a, b] Differ significantly from the preceding value: $p < 0.05$, and 0.001, respectively.

[c, d, e, f] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

[g, i, j] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.01, and 0.001, respectively.

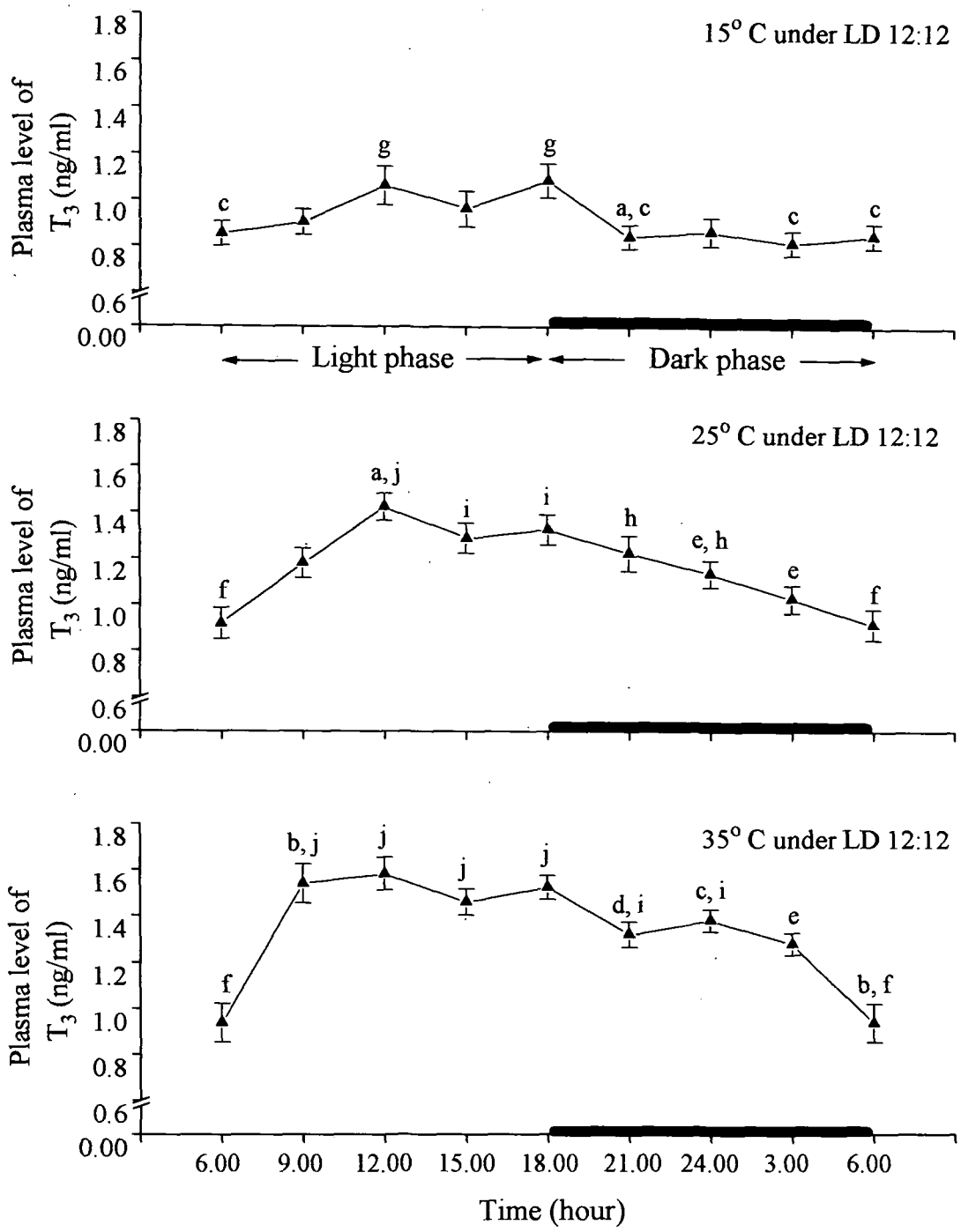


Fig. 3:5B- Effects of simulated temperatures on circadian rhythm in plasma levels of T_3 during summer season under LD 12:12.

All values are expressed as mean ± standard error (S. E); N = 5.

[a, b] Differ significantly from the preceding value: $p < 0.05$, and 0.001, respectively.

[c, d, e, f] Differ significantly from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

[g, h, i, j] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

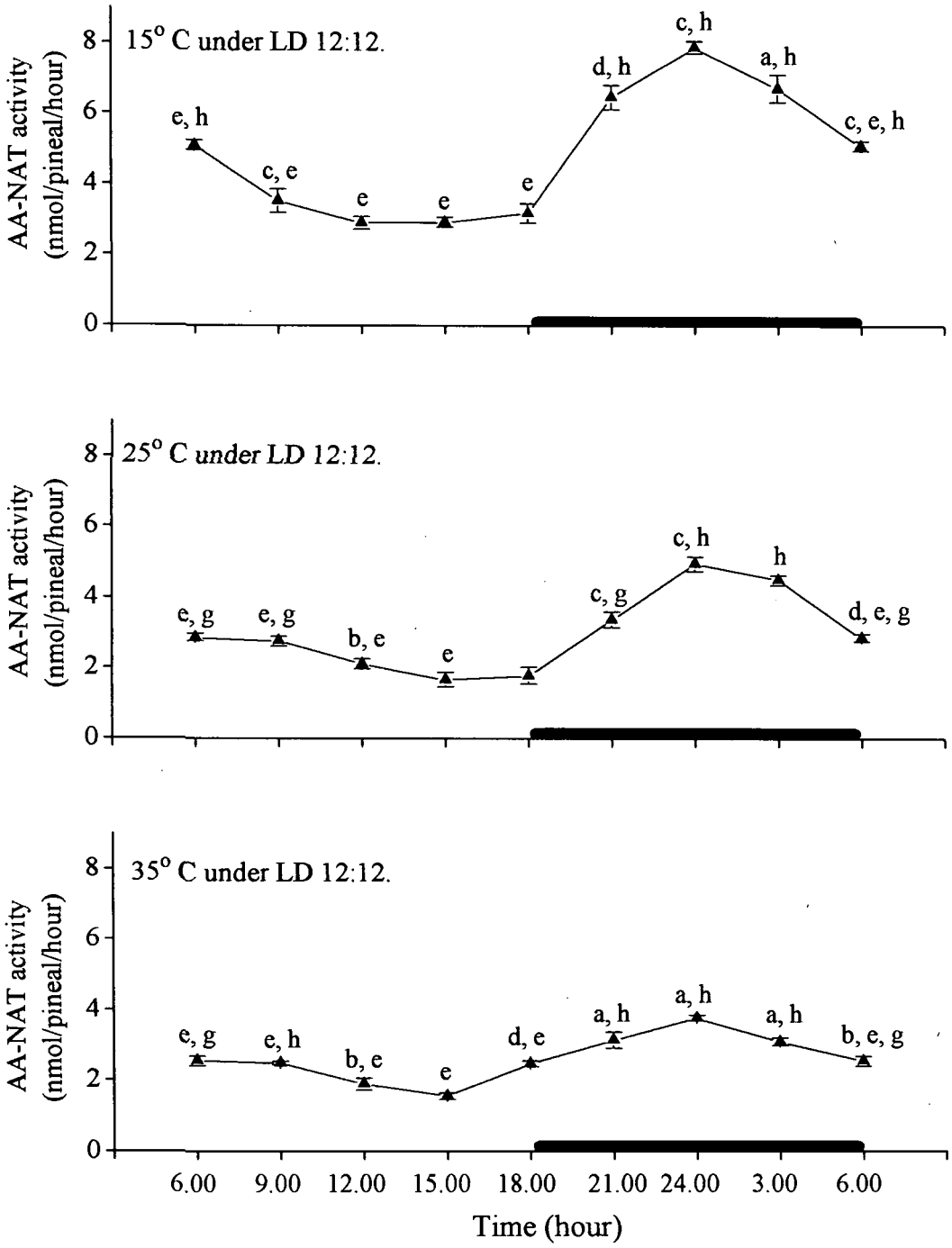


Fig. 3:6A- Effects of simulated temperatures on circadian rhythm in pineal AA-N-acetyltransferase (AA-NAT) activity during winter season under LD 12:12.

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[a, b, c, d] Differ significantly from the preceding value: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[e] Differs significantly from the maximum value: $p < 0.001$.

[g, h] Differ significantly from the minimum value of the respective group: $p < 0.01$ and 0.001, respectively.

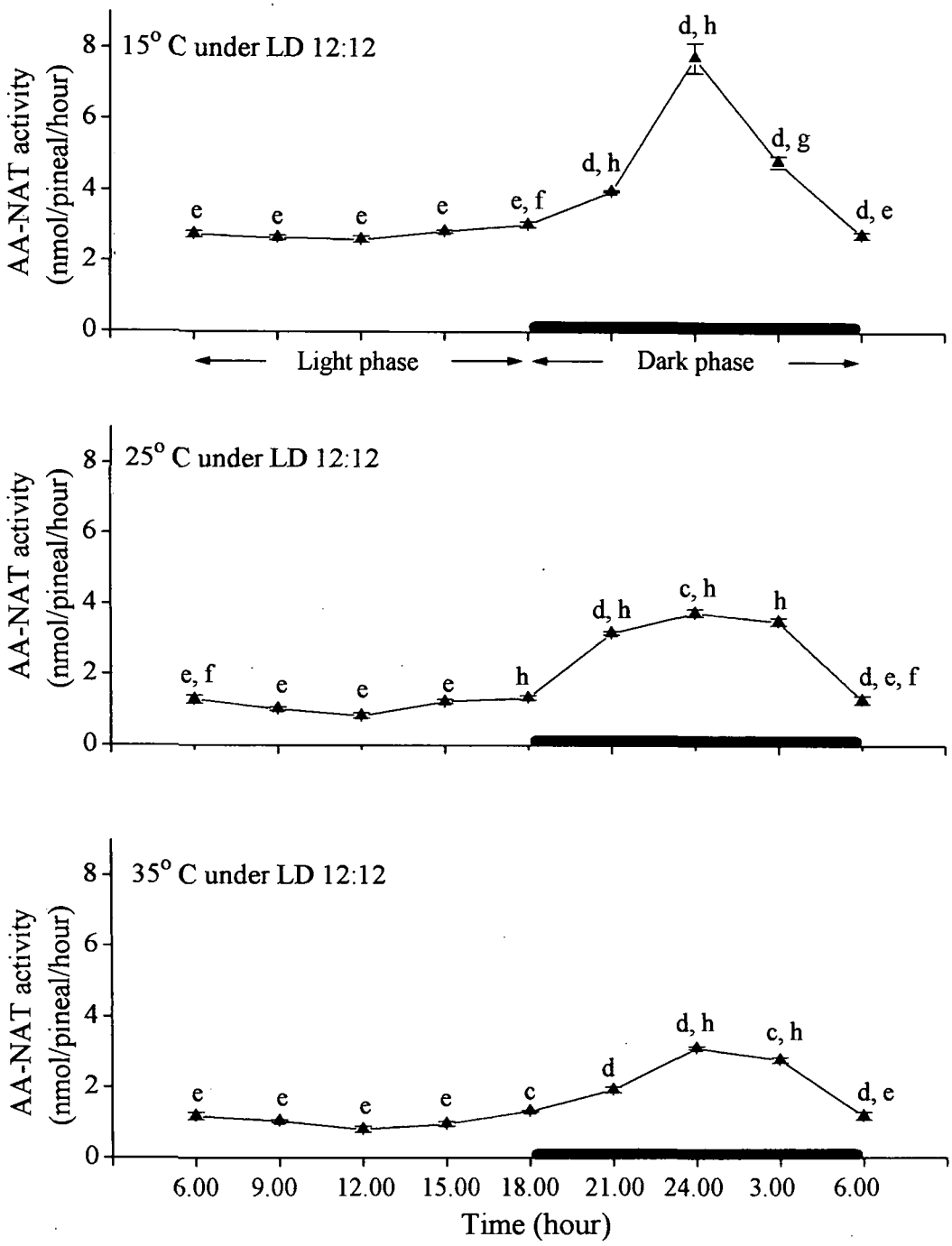


Fig. 3:6B- Effects of simulated temperatures on circadian rhythm in pineal AA-N-acetyltransferase (AA-NAT) activity during summer season under LD 12:12.

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[c, d] Differ significantly from the preceding value: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

[e] Differs significantly from the maximum value: $p < 0.001$.

[f, g, h] Differ significantly from the minimum value of the respective group: $p < 0.02$, 0.01, and 0.001, respectively.

CHAPTER - 4

In vivo effects of melatonin on plasma levels of thyroxine (T_4) and triiodothyronine (T_3)

INTRODUCTION

Thyroid hormones have been reported to play a major role in the regulation of metabolism (Peter and Oommen, 1989; Gupta and Thapliyal, 1991), growth and development (Cyr *et al.*, 1998; Power *et al.*, 2001), sexual maturation (Ueda *et al.*, 1984; Pavlidis *et al.*, 2000), breeding cycle (Volkoff *et al.*, 1999), migration (Ueda *et al.*, 1984; Matty, 1985a), electrolyte and water metabolism (Peter *et al.*, 2000) etc. of the fish, which experiences continuous adjustments in physiological and morphological characteristics in relation to ever-changing aquatic environment (Matty, 1985a). The circulating thyroid hormones exhibit distinct circadian and circannual rhythms in fish species (Cyr *et al.*, 1998; Leiner *et al.*, 2000; Pavlidis *et al.*, 2000; Leiner and MacKenzie, 2001). Variations in the circulating levels of thyroxine (T_4) and triiodothyronine (T_3) of fishes are reportedly controlled by fluctuations in ambient temperature, physical activity, photoperiod, feeding, etc. (Matty, 1985a; Cyr *et al.*, 1988; Leiner *et al.*, 2000). However, the circannual rhythms of these hormones seem to be closely associated with circannual variations in ambient temperature, daylength and gonadal steroids (Pavlidis *et al.*, 2000). Seasonal fluctuations in photoperiods and temperature have also been reported to regulate the circadian and circannual rhythms of melatonin production in some fish species (Randall *et al.*, 1995; Pavlidis *et al.*, 1999;

Samejima *et al.*, 2000; Garcia-Allegue *et al.*, 2001). Thyroid hormone production is maximum during summer when daylengths are long and pineal activity/melatonin production is minimum, and low levels of thyroid hormones are found in fish during winter when daylengths are short and pineal activity is high (Vivien-Roels and Arendt, 1981; Zachmann *et al.*, 1992; Samejima *et al.*, 2000). Levels of thyroid hormones are reportedly inhibited by melatonin administration and increased after pinealectomy in mammals (Vaughan *et al.*, 1982; Vriend *et al.*, 1982; Vriend, 1984; Vaughan and Pruitt, 1985; Wajs and Lewiski, 1992; Ozturk *et al.*, 2000; Champney, 2001; Baltaci *et al.*, 2003, 2004) and birds (Sharp *et al.*, 1984; John *et al.*, 1990; Prakash *et al.*, 1998). Pinealectomy in *Clarias batrachus* during development and maturation phase increased thyroid hormones (Nayak and Singh, 1987a). As in mammals, melatonin might also influence circulating levels of thyroid hormones in the fish. However, there is scarcity of information on the effects of melatonin on thyroid hormones in any fish species in relation to seasons/temperature. Therefore, it was thought worthwhile to investigate effects of exogenous melatonin on plasma levels of thyroid hormones in an air-breathing fish, *Clarias gariepinus* during the four phases of the breeding cycle.

MATERIALS AND METHODS

All the experiments were conducted on male *Clarias gariepinus* (body weight: 90-100g; body length: 23-27cm.) purchased from local fish suppliers. Fishes were maintained in 100 liter aquaria and acclimatized for 15 days in the laboratory under natural climatic conditions at Shillong (Latitude 25°.30′ N, Longitude 91°.52′ E,

Altitude 1450 ASL). The fishes were fed with minced earthworms *ad libitum* daily between 11.00 am and 11.30 am. Water was changed everyday to avoid infections. The experiments were done during quiescent phase (February; morning water temp: 12° - 13° C; evening water temp: 14° - 15° C), progressive phase (April; morning water temp: 12.40° - 13.20° C; evening water temp: 19.5° - 20° C), breeding phase (August; morning water temp: 19° - 20° C; evening water temp: 21° - 22° C) and regressive phase (November; morning water temp: 8.5° - 9.6° C; evening water temp: 15.8° - 16.5° C). In all the phases, the acclimatized fishes were divided into 8 groups of 5-7 fishes each (4 groups each for morning and evening experiments). Melatonin (Sigma Chemical Co., USA) was dissolved in few drops of ethyl alcohol and diluted in fish saline. Three doses of melatonin (0.5μg/g/day, 1μg/g/day and 2μg/g/day) were administered intramuscularly on the lateral side of the dorsal fin to separate groups at 8 am (2 hours after "lights on") and 4 pm (2 hours before "lights off") everyday for 8 days during all the four phases. The fishes of control groups were injected with fish-saline only. Twenty-four hours after the last injection, the fishes were decapitated and blood samples from the post-caudal region were collected in numbered heparinised centrifuge tubes. The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain plasma. The plasma samples were stored in the ice-chamber (-8°C to –10°C) of a refrigerator for measuring the thyroid hormones. The plasma levels of thyroid hormones (total T_3 and total T_4) were measured following the procedures described in detail in Chapter - 1. The data were analyzed statistically with the help of Student's 't' test and regression analysis (Snedecor, 1961). A $p < 0.05$ was considered as significant.

The experimental protocol for the proposed study is given below:

EXPERIMENTAL PROTOCOL

Experiments	Doses of Melatonin (μg/g body wt.)	Time of Experiment	Photo-period	Ambient Water Temperature	Time of Injections	Duration	Sampling Time
In vivo effects of melatonin injections on plasma levels of T_4 and T_3	i) 0.5 μg/g/day	i) Quiescent phase	LD 12:12	5.5 - 23.0 ° C	8.00 am & 4.00 pm	8 days	8.00 am & 4.00 pm
	ii) 1.0 μg/g/day	ii) Progressive phase	- do -	12.0 - 23.5 ° C	- do -		- do -
	iii) 2.0 μg/g/day	iii) Breeding phase	- do -	16.6 - 24.8 ° C	- do -		- do -
		iv) Regressive phase	- do -	6.0 - 22.5 ° C	- do -		- do -

RESULTS

The data are presented in Tables 4:1, 4:2, 4:3 and 4:4; Figs. 4:1, 4:2, 4:3 and 4:4. During the quiescent phase, there was no significant difference between the plasma T_4 levels of the morning and evening control groups. However, the plasma T_3 level was significantly higher in the evening control group as compared to that of the morning group. Morning injection of melatonin had no significant effect on plasma T_4 levels, except in 1μg/g melatonin injected group in which plasma T_4 levels were increased significantly. In the evening group, 2μg/g dose of melatonin induced significant

increase in plasma T_4 levels. However, unlike T_4, administration of melatonin during both morning and evening hour significantly suppressed T_3 levels in a dose-dependent manner (Table 4:1).

During the progressive phase, the T_4 level was found to be significantly higher in the evening control group as compared to that of the morning group. However, there was no significant difference in the plasma levels of T_3 between the evening and morning control groups. Administration of 2μg/g during morning and 1μg/g and 2μg/g dose of melatonin during evening significantly increased plasma T_4 levels. While administration of melatonin suppressed plasma T_3 level in a dose-dependent manner during morning, higher doses of melatonin (1μg/g and 2μg/g) suppressed it when administered in the evening (Table 4:2).

During the breeding phase, there was no significant difference in the plasma levels of both T_4 and T_3 in their respective morning and evening control groups. Administration of melatonin during morning had no significant effect on the plasma T_4 levels. However, injection of 1μg/g/day and 2μg/g/day doses of melatonin in the evening significantly increased the plasma T_4 levels. None of the melatonin doses had any significant effect on plasma T_3 levels in both morning and evening groups except when injected with 0.5μg/g dose, which significantly increased the plasma T_3 levels in the evening group (Table 4:3).

During the regressive phase, plasma levels of T_4 and T_3 were significantly higher in the evening control group as compared to their respective morning group. Administration of melatonin during morning and evening significantly increased the plasma T_4 levels and suppressed the plasma levels of T_3 both in the morning and evening groups in a dose-dependent manner (Table 4:4).

Regression analysis of the data indicated a strong positive correlation between the doses of melatonin and the T_4 plasma levels irrespective of the time of administration and phases ($r = 0.72$ to 0.99). However, irrespective of the time of administration and phases, a strong negative correlation was found between the doses of melatonin and the plasma T_3 levels ($r = -0.95$ to -0.74) except in the evening group of the breeding phase, where the negative correlation was insignificant (Tables 4:1, 4:2, 4:3 and 4:4). T_3/T_4 ratio was decreased during all the four phases following melatonin treatment in the morning ($r = -0.95$ to -0.85) as well as in the evening ($r = -0.94$ to -0.58) groups (Tables 4:1, 4:2, 4:3 and 4:4).

DISCUSSION

There are several reports indicating inhibitory influence of melatonin on thyroid physiology of homeotherms. In mammals, increased melatonin levels following melatonin injection/implantation, blinding, or exposure to short-daylength invariably decreased, and pinealectomy or removal of superior cervical ganglia increased circulating levels of thyroid hormones (Vaughan *et al.*, 1982; Vriend *et al.*, 1982;

Vriend, 1984; Wajs and Lewiski, 1992; Ozturk *et al.*, 2000; Champney, 2001; Baltaci *et al.*, 2003, 2004). Similarly, melatonin administration decreased and pinealectomy increased thyroxine levels in birds (Sharp *et al.*, 1984; John *et al.*, 1990; Prakash *et al.*, 1998). The present study might be the first of its kind in which effects of different doses of melatonin have been studied in a fish (poikilotherm) with special reference to time of administration and all phases of the breeding cycle.

In the present study, the plasma levels of T_4 increased following evening injections of higher doses of melatonin in *C. gariepinus* during all the four phases. While melatonin treatment in the morning did not influence T_4 levels during the breeding phase, the plasma levels of T_4 was increased during the regressive phase in a dose-dependent manner. In all the experiments, strong positive correlation (correlation coefficient: 0.72 to 0.99) was evident between the doses of melatonin and the plasma levels of T_4. During the breeding phase, melatonin had no significant effect on the plasma levels of T_3 irrespective of the time of administration. However, during the quiescent, progressive and regressive phases, melatonin treatment suppressed the T_3 plasma concentrations of the fish in a dose-dependent manner in both morning and evening groups. It is important to mention that significant negative correlation was observed between the doses of melatonin and plasma levels of T_3 in all groups except in the evening groups during breeding phase. The observed increase in T_4 levels following melatonin treatment might be either due to increase in TSH secretion or sensitivity of thyroid follicles to TSH (Grau *et al.*, 1985; Rom-Bugoslavskaia and Shcherbakova,

1985) and/or due to a direct thyrotropic action of melatonin. Alternatively, the increase in T_4 concentrations in the fish blood may result due to melatonin-induced inhibition of 5'-deiodinase - the enzyme responsible for accelerating peripheral deiodination of T_4 to T_3. Melatonin treatment has been reported to decrease the activity of type-II thyroxine 5'-deiodinase enzyme in mammals (Shchervakova and Rom-Bugoslavskaia, 1988; Puig-Domingo *et al.*, 1988). Therefore, there is a strong possibility that the recorded increase in the plasma levels of T_4 and inhibition of T_3 levels in this fish following melatonin administration resulted also due to inhibition of peripheral conversion of T_4 to T_3 following inhibition of the 5'-deiodinase enzyme by melatonin. This suggestion is further supported by the dose-dependent inhibition of T_3/T_4 ratio in the fish plasma following melatonin treatment (Tables 4:1, 4:2, 4:3 and 4:4). It has been reported that in rats melatonin administration during summer suppressed T_3 plasma level with a significant increase in T_4 and TSH levels (Rom-Bugoslavskaia and Shcherbakova, 1985). Further, exogenous melatonin as subcutaneous implants has also been reported to elevate plasma T_4 levels in mink, *Mustela vison* (Mustonen *et al.*, 2000). An earlier report regarding decreased plasma levels of T_4 and increased T_3 levels following pinealectomy in another air-breathing fish *Clarias batrachus* during gonadal development and maturation phase (Nayak and Singh, 1987a) is in conformity with the present findings. Similar to melatonin, 5-methoxy-tryptamine (5-MT) has also been reported to inhibit thyroid hormone levels in an air-breathing fish, *Clarias batrachus* during pre-spawning period (Nayak and Singh, 1987b).

No significant difference was observed between the morning and evening plasma levels of T_3 and T_4 during the breeding phase. Similarly, there was no significant difference between morning and evening groups in the levels of T_4 during quiescent phase, and also that of T_3 during the progressive phase. The plasma levels of T_4 in the evening groups were significantly higher than that of the respective morning groups during progressive and regressive phases. However, the evening plasma levels of T_3 were found to be significantly higher as compared to that of morning during the quiescent and regressive phases. The evening level of T_3 in the quiescent was also significantly higher than that of the evening values of the other three phases. Further, the evening level of T_3 in the quiescent and regressive phases were also significantly higher than that of the evening values of the progressive and breeding phases. The observed increase in T_3 levels at evening during quiescent and regressive phases might be a response to the gradual increase in water temperature during the daytime, which might be helping, directly and/or indirectly, in maintaining the basal metabolic rate of the fish to ensure its survival at very low water temperature (6-8° C) during winter nights. T_3 has been found to potentiate the stimulatory effects of catecholamines on the respiratory rate of vital tissues of *C. batrachus* during winter (Lynshiang and Gupta, 2000).

Present findings indicate that, unlike its inhibitory effects on both T_3 and T_4 in mammals, melatonin seems to exert a differential control over the levels of T_3 and T_4 in this fish. Stimulatory and inhibitory effects of melatonin on T_4 and T_3 seem to depend

on seasons, dose and the time of administration. There are several reports suggesting a major role of T_4 in regulation of growth, development, sexual maturation and reproduction, while T_3 is primarily involved in metabolic regulation. Therefore, melatonin-induced inhibition of T_3 might be of adaptive significance in lowering the metabolic rate as well as in conserving energy reserves. Melatonin-induced increase in T_4 might be promoting growth and breeding related processes. Further, there is a possibility that low temperature and short photoperiod, which increase melatonin production in fish (Samejima *et al.*, 2000), are involved in the regulation of circulating levels of thyroid hormones in the fish via a neuroendocrine mechanism involving the pineal organ and melatonin.

Thyroid hormones exhibit annual variations in fishes (Bau and Parent, 2000; Pavlidis *et al.*, 2000). The circannual rhythms of thyroid hormones in fish is regulated mainly by ambient temperature and photoperiod (Cyr *et al.*, 1988, 1998). In addition to temperature and photoperiod, fish thyroid is also influenced by hormones such as gonadal steroids (Chakraborti and Bhattacharya, 1984; Bandyopadhyay *et al.*, 1991; Pavlidis *et al.*, 2000), glucocorticoids (Leaderland, 1987) and catecholamines (Eales *et al.*, 1986). The present findings seem to suggest for the first time that thyroid hormone levels in a fish can also be influenced significantly by melatonin. Melatonin has been reported to inhibit the secretion of T_4 under both *in vivo* and *in vitro* conditions in amphibian tadpoles and adults (Wright *et al.*, 1997; Wright and Alves, 2001). Further, melatonin administration inhibited thyroid activity in adult female turtle, *Lissemys*

punctata punctata (Sarkar *et al.*, 1997). Thus, the nature of the modulatory effects (stimulatory /inhibitory) of melatonin seems to vary in different groups of poikilotherms.

The present findings taken together with the earlier reports suggest that melatonin plays an important role in the regulation of circulating levels of thyroid hormones in both homeotherms and poikilotherms. Since melatonin synthesis in fishes is influenced by both photoperiod (Zachmann *et al.*, 1992; Randall *et al.*, 1995; Pavlidis *et al.*, 1999; Samejima *et al.*, 2000; Bayarri *et al.*, 2004b) and water temperature (Masuda *et al.*, 2003), the climatic factors might be influencing thyroid function in fish species by altering melatonin production. It seems that the stimulatory and inhibitory effects of melatonin on T_4 and T_3 in *Clarias gariepinus* depend on seasons, dose and the time of administration.

The findings of the present study suggest that melatonin is involved in the regulation of thyroid activity and plasma levels of thyroid hormones, and exerts a differential control over the circulating levels of T_3 and T_4 in *C. gariepinus*. These findings also suggest a probability that the circadian and circannual rhythms of melatonin might be involved in shaping the circadian and circannual rhythms of plasma T_4 and T_3 levels.

Table 4:1- Time-dependent effects of melatonin on plasma levels of thyroid hormones during quiescent phase.

Treatment	Plasma levels of thyroid hormones				
	T_4 (ng/ml)		T_3 (ng/ml)		T_3/T_4
	MORNING				
		N		N	
Control	6.05* ± 1.14	5	1.86 ± 0.25[h]	5	0.30
Melatonin (0.5µg/g/day)	7.11 ± 1.40	6	0.72 ± 0.04 [a]	6	0.10
Melatonin (1µg/g/day)	10.67 ± 0.06 [a, c]	5	0.40 ± 0.05 [b, d]	5	0.03
Melatonin (2µg/g/day)	9.35 ± 1.34	5	0.07 ± 0.01[b, e, g]	5	0.007
Correlation coefficient (r)	0.72		-0.88		-0.85
	EVENING				
Control	8.73 ± 1.11	7	7.17 ± 0.82	7	0.82
Melatonin (0.5µg/g/day)	11.72 ± 2.53	5	2.10 ± 0.31 [b]	5	0.17
Melatonin (1µg/g/day)	12.10 ± 1.09	5	1.52 ± 0.31 [b]	5	0.12
Melatonin (2µg/g/day)	16.07 ± 0.77 [b, f]	7	1.39 ± 0.13 [b]	7	0.08
Correlation coefficient (r)	0.97		-0.74		-0.75

*All values are expressed as mean ± standard error (S. E); N = 5 - 7.

[a, b] Differ significantly from their respective control group: $p < 0.01$ and 0.001, respectively.

[c, d, e] Differ significantly from their respective 0.5µg/g melatonin injected group: $p < 0.05$, 0.01 and 0.001, respectively.

[f, g] Differ significantly from their respective 1µg/g melatonin injected group: $p < 0.02$ and 0.001, respectively.

[h] Differs significantly from the T_3 evening control group of quiescent phase: $p < 0.001$.

Table 4:2- Time-dependent effects of melatonin on plasma levels of thyroid hormones during progressive phase.

Treatment	Plasma levels of thyroid hormones				
	T_4 (ng/ml)		T_3 (ng/ml)		T_3/T_4
	MORNING				
		N		N	
Control	10.17 ± 0.51* [i]	6	1.83 ± 0.05	6	0.18
Melatonin (0.5μg/g/day)	11.25 ± 0.70	5	1.18 ± 0.06 [b]	5	0.10
Melatonin (1μg/g/day)	12.25 ± 0.86	6	0.95 ± 0.05 [b, d]	6	0.07
Melatonin (2μg/g/day)	18.50 ± 1.04 [b, e, g]	6	0.85 ± 0.06 [b, c]	6	0.04
Correlation coefficient (r)	0.96		-0.85		-0.92
	EVENING				
Control	13.25 ± 0.49	6	1.95 ± 0.06	6	0.14
Melatonin (0.5μg/g/day)	13.60 ± 0.58	6	1.88 ± 0.06 [h]	6	0.13
Melatonin (1μg/g/day)	16.51 ± 0.39 [a, d]	7	1.29 ± 0.06 [b, e]	7	0.07
Melatonin (2μg/g/day)	18.34 ± 0.71 [b, e, f]	6	0.98 ± 0.07 [b, e, g]	6	0.05
Correlation coefficient (r)	0.96		-0.95		-0.94

*All values are expressed as mean ± standard error (S. E); N = 5 - 7.

[a, b] Differ significantly from their respective control group: $p < 0.01$ and 0.001, respectively.

[c, d, e] Differ significantly from their respective 0.5μg/g melatonin injected group: $p < 0.02$, 0.01 and 0.001, respectively.

[f, g, h] Differ significantly from their respective 1μg/g melatonin injected group: $p < 0.05$, 0.01 and 0.001, respectively.

[i] Differs significantly from the T_4 evening control group of progressive phase: $p < 0.001$.

Table 4:3- Time-dependent effects of melatonin on plasma levels of thyroid hormones during breeding phase.

Treatment	Plasma levels of thyroid hormones				
	T_4 (ng/ml)		T_3 (ng/ml)		T_3/T_4
	MORNING				
		N		N	
Control	19.71 ± 3.85*	5	1.72 ± 0.65	5	0.08
Melatonin (0.5μg/g/day)	24.50 ± 3.46	5	1.30 ± 0.36	5	0.05
Melatonin (1μg/g/day)	24.70 ± 2.70	5	0.81 ± 0.29	5	0.03
Melatonin (2μg/g/day)	26.87 ± 6.91	4	0.61 ± 0.14	4	0.02
Correlation coefficient (r)	0.88		-0.94		-0.91
	EVENING				
Control	17.23 ± 1.44	4	1.02 ± 0.25	4	0.05
Melatonin (0.5μg/g/day)	18.96 ± 2.18	5	2.08 ± 0.33 [b]	5	0.10
Melatonin (1μg/g/day)	40.73 ± 5.14 [a, e]	5	0.94 ± 0.19 [d]	5	0.02
Melatonin (2μg/g/day)	40.65 ± 7.98 [b, c]	5	1.08 ± 0.25 [c]	5	0.02
Correlation coefficient (r)	0.85		-0.24		-0.58

*All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[a, b] Differ significantly from their respective control group: $p < 0.05$ and 0.01, respectively.

[c, d, e] Differ significantly from their respective 0.5μg/g melatonin injected group: $p < 0.05$, 0.01 and 0.001, respectively.

Table 4:4- Time-dependent effects of melatonin on plasma levels of thyroid hormones during regressive phase.

Treatment	Plasma levels of thyroid hormones				
	T_4 (ng/ml)		T_3 (ng/ml)		T_3/T_4
	MORNING				
		N		N	
Control	4.86 ± 0.35* [h]	6	1.22 ± 0.06 [i]	6	0.25
Melatonin (0.5µg/g/day)	6.68 ± 0.26 [b]	6	0.99 ± 0.05 [a]	6	0.14
Melatonin (1µg/g/day)	8.86 ± 0.40 [c, e]	5	0.80 ± 0.04 [c, d]	5	0.09
Melatonin (2µg/g/day)	13.12 ± 0.48 [c, f, g]	6	0.39 ± 0.03 [c, f, g]	6	0.02
Correlation coefficient (r)	0.99		-0.99		-0.95
	EVENING				
Control	6.21 ± 0.40	6	1.61 ± 0.05	6	0.26
Melatonin (0.5µg/g/day)	10.36 ± 0.38 [c]	7	1.63 ± 0.03	7	0.15
Melatonin (1µg/g/day)	13.00 ± 0.37 [c, f]	7	0.66 ± 0.04 [c, f]	7	0.05
Melatonin (2µg/g/day)	14.57 ± 0.55 [c, f, g]	6	0.33 ± 0.01 [c, f, g]	6	0.02
Correlation coefficient (r)	0.92		-0.92		-0.91

*All values are expressed as mean ± standard error (S. E); N = 5 - 7.

[a, b, c] Differ significantly from their respective control group: $p < 0.02$, 0.01 and 0.001, respectively.

[d, e, f] Differ significantly from their respective 0.5µg/g melatonin injected group: $p < 0.02$, 0.01 and 0.001, respectively.

[g] Differs significantly from their respective 1µg/g melatonin injected group: $p < 0.001$.

[h, i] Differ significantly from their respective evening control group of regressive phase: $p < 0.05$ and 0.001, respectively.

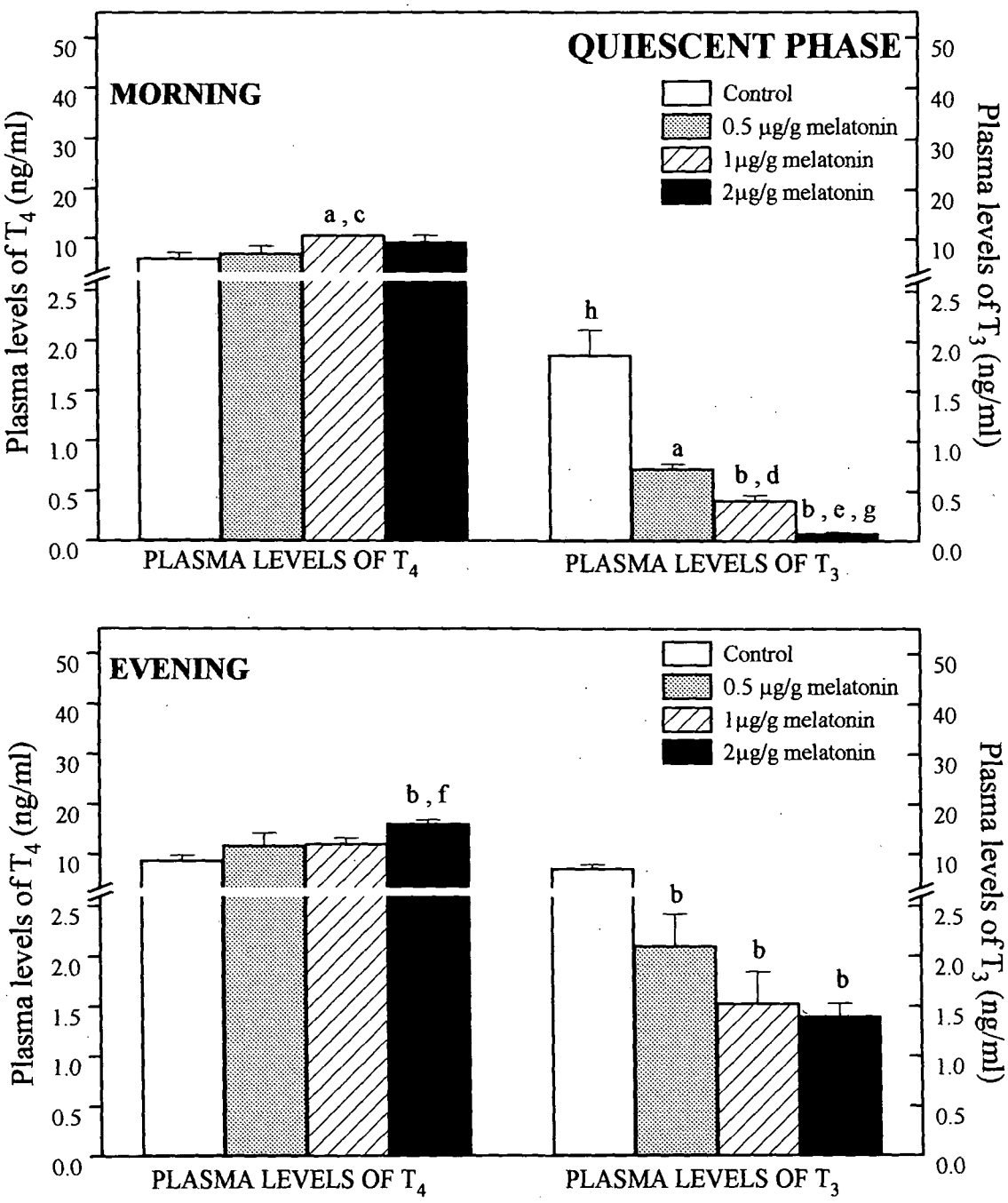


Fig. 4:1- Time-dependent effects of melatonin on plasma levels of thyroid hormones during quiescent phase.

All values are expressed as mean ± standard error (S. E); N = 5 - 7.

[a, b] Differ significantly from their respective control group: $p < 0.01$ and 0.001, respectively.

[c, d, e] Differ significantly from their respective 0.5µg/g melatonin injected group: $p < 0.05$, 0.01 and 0.001, respectively.

[f, g] Differ significantly from their respective 1µg/g melatonin injected group: $p < 0.02$ and 0.001, respectively.

[h] Differs significantly from the T_3 evening control group of quiescent phase: $p < 0.001$.

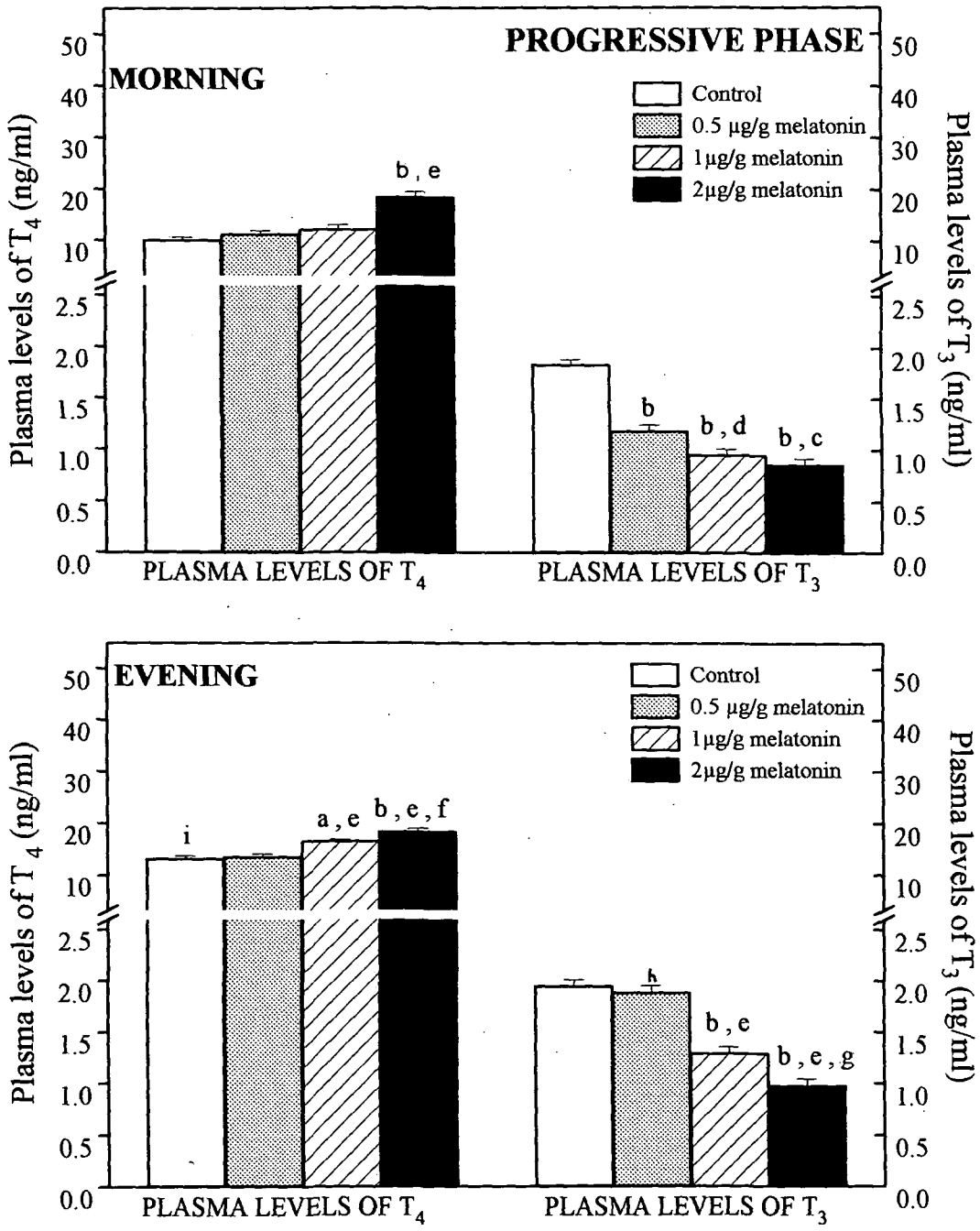


Fig. 4:2- Time-dependent effects of melatonin on plasma levels of thyroid hormones during progressive phase.

All values are expressed as mean ± standard error (S. E); N = 5 - 7.

[a, b] Differ significantly from their respective control group: $p < 0.01$ and 0.001, respectively.

[c, d, e] Differ significantly from their respective 0.5µg/g melatonin injected group: $p < 0.02$, 0.01 and 0.001, respectively.

[f, g, h] Differ significantly from their respective 1µg/g melatonin injected group: $p < 0.05$, 0.01 and 0.001, respectively.

[i] Differs significantly from the T4 evening control group of progressive phase: $p < 0.001$.

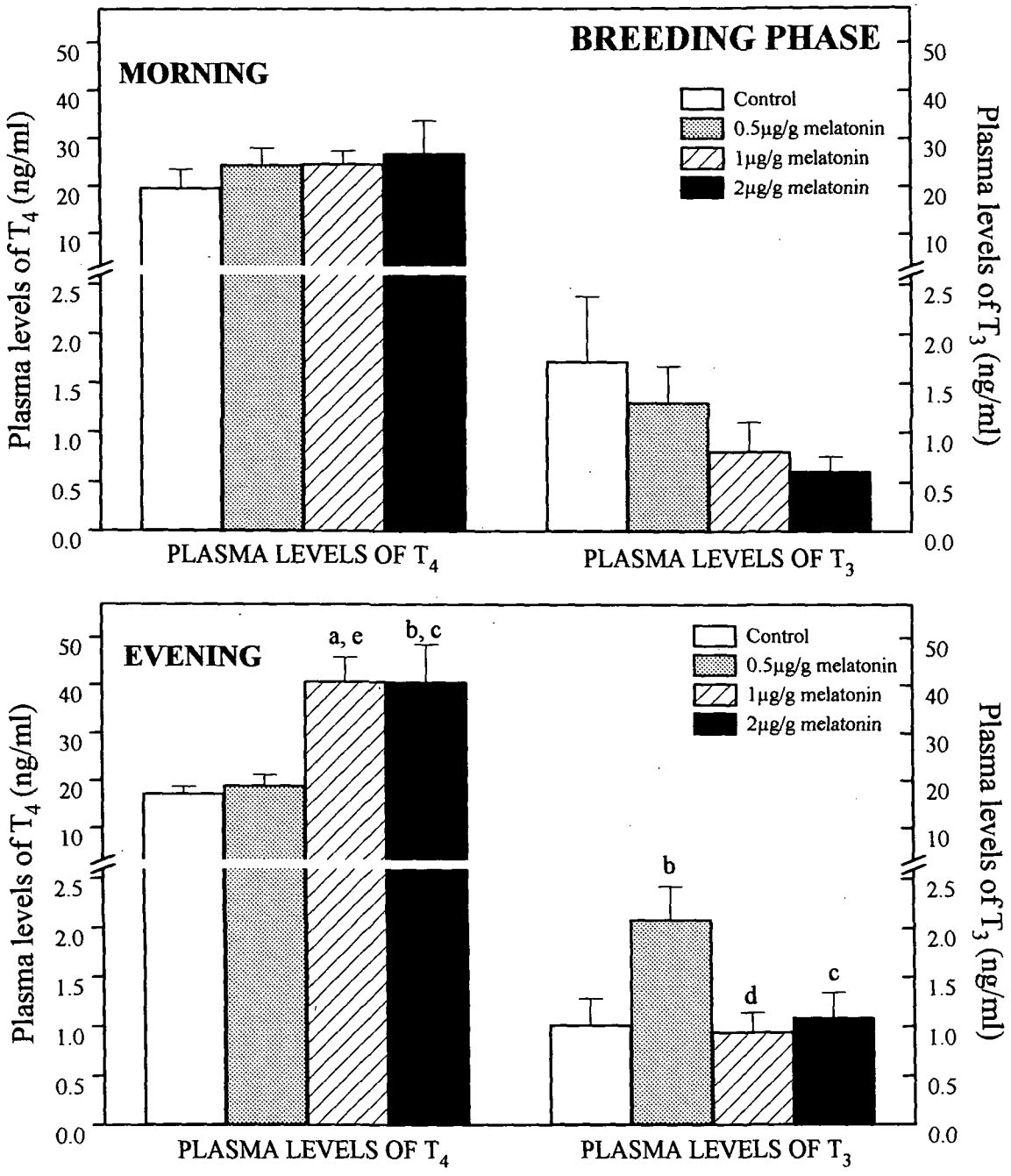


Fig. 4:3- Time-dependent effects of melatonin on plasma levels of thyroid hormones during breeding phase.

All values are expressed as mean ± standard error (S. E); N = 4 - 5.

[a,b] Differ significantly from their respective control group: $p < 0.05$ and 0.01, respectively.

[c,d, e] Differ significantly from their respective 0.5µg/g melatonin injected group: $p < 0.05$, 0.01 and 0.001, respectively.

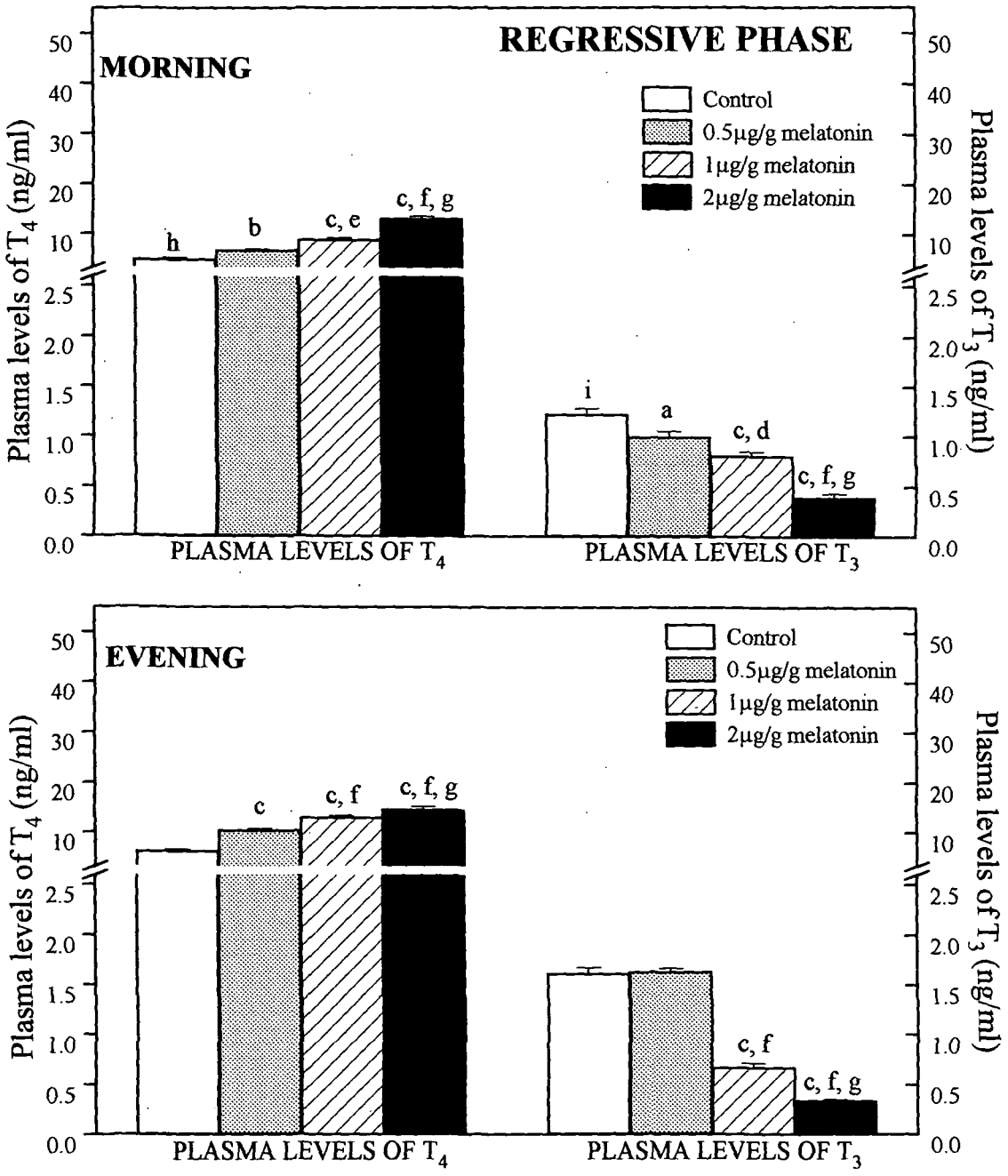


Fig. 4:4- Time-dependent effects of melatonin on plasma levels of thyroid hormones during regressive phase.

All values are expressed as mean ± standard error (S. E); N = 5 - 7.

[a, b, c] Differ significantly from their respective control group: $p < 0.02$, 0.01 and 0.001, respectively.

[d, e, f] Differ significantly from their respective 0.5µg/g melatonin injected group: $p < 0.02$, 0.01 and 0.001, respectively.

[g] Differs significantly from their respective 1µg/g melatonin injected group: $p < 0.001$.

[h, i] Differ significantly from their respective evening control group of regressive phase: $p < 0.05$ and 0.001, respectively.

CHAPTER - 5

Effects of thyroid hormones and propylthiouracil on the circadian rhythms in pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity

INTRODUCTION

The effects of environmental factors on pineal and melatonin on mammals are mediated via SCN (Zucker *et al.*, 1983). The SCN seems to use different neurotransmitters released at different times along light:dark cycle for each of the different connection types regulating different functions (Buijs and Kalsbeek, 2001; Gillette and Mitchell, 2002; Kalsbeek and Buijs, 2002). In mammals, SCN regulates the circadian rhythms of pineal activity and melatonin synthesis (Vollrath, 1981; Perreau-Lenz *et al.*, 2003, 2004; Gupta *et al.*, 2005). Further, SCN has also been reported to have dual control mechanism for thyroid activity in mammals by affecting neuroendocrine control of TSH release on the one hand and the autonomic input to the thyroid gland on the other (Kalsbeek *et al.*, 2000). Since SCN plays a critical role in regulation of circadian rhythms of activities of both pineal and thyroid, there is a possibility that SCN also determines the temporal relationship between the circadian rhythms of melatonin and thyroid hormones. Accordingly, pineal and the thyroid gland may influence activities of each other through their hormones (melatonin and thyroid hormones, respectively) directly or indirectly via the hypothalamus and/or SCN.

Melatonin has been reported to influence thyroid activity and thyroid hormones in mammals, birds, amphibian and fish. Levels of thyroid hormones are reportedly inhibited by melatonin administration and increased after pinealectomy in mammals (Vriend *et al.*, 1982; Vaughan *et al.*, 1982; Vriend, 1984; Vaughan and Pruitt, 1985; Wajs and Lewiski, 1992; Ozturk *et al.*, 2000) and birds (Sharp *et al.*, 1984; John *et al.*, 1990; Prakash *et al.*, 1998). Pinealectomy in *Clarias batrachus* during develop-ment and maturation phase increased thyroid hormones (Nayak and Singh, 1987a). Depending on the phase of the breeding cycle and seasons, melatonin has been found to differentially affect T_4 and T_3 levels in *Clarias gariepinus* (Gupta and Premabati, 2002a; Chapter - 4 of this thesis).

There are several reports on effects of hypo- and hyper-thyroidism on circadian rhythm of melatonin and AA-NAT activity in mammals. Thyroxine has been reported to inhibit nighttime plasma melatonin level and AA-NAT activity in rats and Syrian hamsters (Champney *et al.*, 1985). In cultured rat pineal, melatonin level was increased during light phase and decreased during dark phase following treatment with T_3 suggesting a direct effect of T_3 on synthesis and release of melatonin in the rat pineal (Catala *et al.*, 1988). In women, melatonin concentrations were found to be positively correlated with TSH levels in hypo-thyroidism, and negatively correlated with T_3 in hyper-thyroidism (Soszynski *et al.*, 1988). It has been reported that hypo-thyroidism in rats increased melatonin levels in the pineal, hypothalamus and serum at an early age (25 days), but in adults melatonin levels were lowered in the hypothalamus and serum

yet unaltered in the pineal (Catala *et. al.*, 1987). The nocturnal increase of pineal melatonin has been reported to be phase-advanced in the hypothyroid group (Bauer *et al.*, 1989). Injection of T_4 induced a decrease in plasma melatonin in *Rana catasbeina* (Wright and Alves, 2001). Further, it has been reported that T_4 caused a reduction in nighttime plasma melatonin level in Atlantic salmon, *Salmon salar* (Kulczykowska *et al.*, 2004). These reports clearly suggest that hypo- and hyper-thyroidism produce significant influence on AA-NAT activity and/or melatonin synthesis and release.

In fish, thyroid hormones play a major role in the regulation of the oxidative metabolism (Peter and Oomen, 1989; Gupta and Thapliyal, 1991), growth and development (Cyr *et al.*, 1998; Power *et al.*, 2001), sexual maturation (Ueda *et al.*, 1984; Cyr et al., 1998; Pavlidis et al., 2000), breeding cycle (Volkoff *et al.*, 1999), migration (Ueda *et al.*, 1984; Matty, 1985a), electrolyte and water metabolism (Peter *et al.*, 2000) etc. Similarly, melatonin has also been reported to play a role in the regulation of glucose and ions, reproduction, feeding rhythm and locomotor activity in fish species (Pavlidis *et al.*, 1999; Garcia-Allegue *et al.*, 2001; Bayarri *et al.*, 2004a). Therefore, there is a possibility that thyroid hormones influence reproduction and metabolism directly as well as indirectly by influencing AA-NAT activity and melatonin synthesis via the hypothalamo-hypophyseal complex. Since melatonin affects plasma levels of thyroid hormones in *Clarias gariepinus* (Gupta and Premabati, 2002a; Chapter - 4 of this thesis), there is a possibility that a feedback mechanism exists between thyroid hormones and melatonin. The data presented in Chapter - 2 and

Chapter - 3 also suggest an inverse relation between thyroid hormones and AA-NAT activity. But there is practically no information on the effects of hypo-thyroidism and hyper-thyroidism on the circadian rhythm of AA-NAT activity or melatonin synthesis in any species of vertebrate in general and in the fish in particular. Therefore, it was thought worthwhile to investigate effects of hypo-thyroidism and hyper-thyroidism on the circadian rhythm of AA-NAT activity in the fish *Clarias gariepinus* during winter (quiescent phase) and summer seasons (breeding phase).

MATERIALS AND METHODS

All the experiments were conducted on male *Clarias gariepinus* (body weight: 90-100g; body length: 23-27 cm) purchased from the local fish suppliers. Fishes were maintained in 100 liter aquaria and acclimatized before starting the experiment at least for 15 days in the laboratory under natural climatic conditions at Shillong (Latitude 25°30′ N, Longitude 91°52′ E; Altitude 1450 ASL; Minimum water temperature: 4° C; maximum water temperature: 24.5° C). The experiments were conducted during winter (February; water temperature: 6.0° - 22.0° C under LD 12:12) and summer (August; water temperature: 16.0° - 24.2° C under LD 12:12) seasons. Thyroid hormones (L-T_4 and L-T_3) were dissolved in few drops of 0.1 NaOH and diluted in fish saline. During both winter and summer seasons, the acclimatized fishes were divided into four groups of 32 each. Group 1 was immersed in water and acted as control, while groups 2, 3 and 4 were immersed in water containing L-T_3 (20 μg/l), L-T_4 (20 μg/l) and PTU (150 mg/l), respectively under LD 12:12 and ambient water temperature (lights "on" at 6.00 h; Lights

"off" at 18.00 h). The concentratins of L-T_4, L-T_3 and PTU were selected on the basis of earlier studies (Cyr and Eales, 1986; Omeljaniuk and Eales, 1986; Matta *et al.*, 2002). The fishes of all the groups were fed daily with minced earthworms and commercial fish food *ad libitum*, and water was changed everyday. The fishes were kept immersed in thyroid hormones (L-T_4 and L-T_3) and PTU for 10 days. Then four fishes from each group were decapitated at 8 time-points at an interval of 3 h over a 24 h period, and the pineals were rapidly removed and frozen in liquid nitrogen for the measurement of AA-NAT activity. In order to know the effects of immersion in water with the thyroid hormones and PTU on the circulating levels of thyroid hormones, blood samples were also collected from the fishes of the control and treated groups during summer for the measurement of thyroid hormones. AA-NAT activity and the plasma levels T_4 and T_3 were measured following the procedures described in details in the Chapter 1. The data were analyzed statistically with the help of Student's 't' test (Snedecor, 1961). Acro program based on least square fitting of single cosine function (Nelson, *et al.*, 1979) was used for analyzing the circadian rhythm. A $p < 0.05$ was considered as significant.

The experimental protocol for the proposed study is given below:

EXPERIMENTAL PROTOCOL

Experiments	Concentration	Time of Experiment	Photoperiod	Ambient water Temperature	Duration	Sampling Time
Effects of immersion in L-T_4, L-T_3 and PTU solution on circadian rhythms in pineal AA-NAT activity	i) L-T_4: 20 µg/ l ii) L-T_3: 20 µg/ l iii) PTU: 150mg/l	i) Winter (February) ii) Summer (August)	LD 12:12 - do -	6.0 – 22.0° C 16.0 – 24.2° C	10 days -do -	8 - time points at an interval of 3 hours over a 24 h period

RESULTS

The data are presented in Tables 5:1, 5:2, 5:3A, 5:3B, 5:4A and 5:4B; Figs. 5:1 and 5:2. Plasma T_4 and T_3 levels were significantly increased following immersion of the fishes in water containing L-T_4 and L-T_3, respectively at all the eight time points over the 24 h time scale during summer. Depending on the time point of the 24 h scale, immersion of the fishes in water containing PTU resulted in 14 to 24% and 10 to 16% decrease in the plasma levels of L-T_4 and L-T_3, respectively. Further, PTU treatment marginally increased the T_3/T_4 ratio.

Fish pineal AA-NAT activity exhibited prominent circadian rhythm in all the groups irrespective of the treatment during both winter and summer seasons. AA-NAT activity was always found to be at basal level during the light phase, increased after the

lights "off" and remained high during the dark phase irrespective of the treaments and seasons. In all the groups during both winter and summer seasons, pineal AA-NAT activity decreased after lights "on" at 6.00 h. The patterns of diurnal increase and decrease in pineal AA-NAT activity were different under different treatments and seasons. Depending on the treatment and seasons, the pineal AA-NAT activity increased slowly or rapidly reaching to a high level at early-, mid-, or late-dark phase.

The average of pineal AA-NAT activity was found to be significantly high during the dark phase than that of the light phase in the treated as well as in control groups irrespective of seasons (Table 5:3A). During both winter and summer seasons, as compared to the control group, L-T_4 treatment had no significant effect on the average AA-NAT activity during both light and dark phases. However, L-T_3 treatment significantly decreased the average AA-NAT activity in both light and dark phases. During winter season, PTU treatments significantly decreased the average AA-NAT activity in both light phase and dark phases. However, during summer seasons, PTU had no significant effect on the average pineal AA-NAT activity during the light phase, but it significantly decreased the enzyme activity during the dark phase.

The results of acro analysis of the data are presented in Table 5:3B. During both winter and summer seasons, the mesor (24 h average) of the circadian rhythm of AA-NAT activity was significantly decreased in the pineal of the fishes immersed in water containing L-T_3 and PTU, while immersion in water containing L-T_4 had no significant

effect on the mesor of the AA-NAT activity rhythm. During both summer and winter seasons, the mesor of the AA-NAT activity rhythm decreased significantly following immersion in PTU containing water (Table 5:3A).

The amplitude of the pineal AA-NAT activity circadian rhythm was decreased significantly following immersion in water containing L-T_4, L-T_3 or PTU as compared to the control group irrespective of seasons. However, immersion in water containing L-T_4 was more effective in reducing the amplitude of AA-NAT activity rhythm as compared to immersion in water with L-T_3 and PTU during winter. Unlike L-T_4, PTU treatment was more effective during the summer season in reducing the amplitude of AA-NAT activity rhythm as compared to that of the L-T_3 and L-T_4 treated groups.

Irrespective of seasons and treatments, the acrophase of pineal AA-NAT activity circadian rhythm occurred at 23.00 h except in L-T_4 group during winter and PTU treated group during summer, when the acrophase was advanced by 3 hours and occurred at 20.00 h.

DISCUSSION

Present findings clearly indicate that plasma T_4 and T_3 levels were significantly increased following immersion of the fishes in water containing the respective thyroid hormones, and were decreased following by immersion in water with PTU. There are several reports which suggest that hypo- and/or hyper-thyroidism

produce significant effects on AA-NAT activity or melatonin levels in pineals of human and other mammals (Champney *et al.*, 1985; Catala *et al.*, 1987, 1988; Soszynski *et al.*, 1988; Bauer *et al.*, 1989; Mazzoccoli *et al.*, 2004), frogs (Wright and Alves, 2001), and fishes (Kulczykowska *et al.*, 2004). However, there is practically no information on effects of thyroid hormones on the circadian rhythms of AA-NAT activity with special reference to seasons in any vertebrates in general, and in any fish species in particular. To the best of our knowledge, present study seems be the first of its kind in which the effects of both hypo- and hyper-thyroidism on the circadian rhythm of pineal AA-NAT activity have been studied in a fish during winter and summer seasons.

In the present study, levels of AA-NAT activity exhibited prominent circadian rhythms in all the groups (Tables 5:1 and 5:2, Figs. 5:1 and 5:2) suggesting that the circadian oscillator of AA-NAT activity in the fish pineal is not influenced by hyper- and hypo-thyroid conditions. Irrespective of the seasons, immersion in L-T_4 had no significant effect on average AA-NAT activity during both light and dark phases separately as well as on the 24 h average of the enzyme activity, while immersion in L-T_3 significantly decreased the average AA-NAT activity during both light and dark phases separately as well as the 24 h average of AA-NAT activity. These findings seem to suggest that while increased T_4 levels do not affect AA-NAT activity, increased T_3 concentrations produce inhibitory effect on AA-NAT activity in the fish pineal.

The enzyme deiodinase II (DII), which converts T_4 to T_3, is expressed in mammalian pineal (Osuna *et al.*, 1993a; Murakami *et al.*, 1997; Engel *et al.*, 2005) and its expression is under adrenergic control (Guerrero *et al.*, 1988a,b; Osuna *et al.*, 1993b). In rat pineal, DII activity has been reported to increase during mid night (Murakami *et al.*, 1997). Whether mid-night increase in DII activity and resultant increased conversion of T_4 to T_3 plays any role in decreasing AA-NAT activity and melatonin synthesis in rat pineal during the second half of the dark phase remains to be investigated. Hyper-thyroidism in rat did not alter DII activity in either the pineal gland or Harderian gland (Osuna *et al.*, 1993a) suggesting that hyper-thyroidism does not influence the conversion of T_4 to T_3. In the present study also, L-T_4 immersion-induced increase in plasma T_4 did not increase plasma T_3 levels (5:4A and 5:4B).

Though the circadian rhythms of AA-NAT and melatonin synthesis do not depend on the cyclic production of T_3 in the rat pineal, mid-night increase in DII activity and T_3 may play a role in the regulation of characteristics of the rhythms (Guerrero and Reiter, 1992). On the similar line, in the present study, immersion-induced increase in T_3 had no effect on the circadian rhythm of AA-NAT activity, but significantly decreased the mesor (24 h average) and amplitude of the rhythm. Unlike mammalian pinealocytes, in the fish pineal photoreceptor cells, which contain photodetector, oscillator as well as the melatonin synthesis mechinery, T_3 may play comparatively a different role in the regulation of the AA-NAT activity by its direct action on the AA-NAT activity of photoreceptor cells via thyroid hormone receptors

(Ng *et al.*, 2001; Baas *et al.*, 2002). It has been reported that T_3, but not T_4, stimulates chick myofibrillar proteolysis by stimulating proteasome activity (Nakashima *et al.*, 1998; Doi *et al.*, 2003). Further, T_3 has been reported to inactivate the deiodinase II (DII) enzyme by stimulating proteasomal proteolysis in cerebral cortex and pituitary cells (Germain, 1988; Steinsapir *et al.*, 1998, 2000). Stimulation of proteasomal proteolysis has been reported to inhibit AA-NAT activity in the pineal and/or retina of mammals (Gastel *et al.*, 1998; Stehle *et al.*, 2001; Gupta *et al.*, 2005), birds (Zatz *et al.*, 2000; Natesan *et al.*, 2002) and fishes (Kroeber *et al.*, 2000; Falcon *et al.*, 2001). Since T_3 stimulates proteasome activity, the observed decrease in AA-NAT activity seems to be a result of T_3-induced increased proteasomal proteolysis of AA-NAT protein in the fish pineal. However, this possibility needs to be experimentally established.

In rats, T_3 treatment has been reported to increase oxidative capacity and lipid peroxidase activity in liver and heart (Venditti *et al.*, 1997, 1999) as well as the susceptibility to oxidative stress, and to decrease antioxidant levels (Venditti *et al.*, 1999). Further, recent findings suggest that antioxidant defence parameters of adult rat brain are considerably influenced by thyroid states of the body, and T_3 treatment significantly increases the oxidative stress parameters in mitochondrial fraction of cerebral cortex (Das and Chainy, 2004). It has been reported that the formation and cleavage of the disulfide bond between Cys^{61} and Cys^{177} of the AA-NAT protein act as a switch and produce the active and inactive states of AA-NAT. Further, it has been found that oxidative stress affects AA-NAT activity *in vivo* through the formation and

cleavage of the disulfide bond between Cys^{61} and Cys^{177} (Tsuboi *et al.*, 2002). Therefore, it remains to be investigated whether the observed inhibition of AA-NAT activity in the fish pineal is a result of inhibitory effect of L-T_3-induced oxidative stress on AA-NAT molecule via the formation and cleavage of the disulfide bond.

Thyroid hormone receptors have been reported to be expressed in the mammalian retinal cells (Ng *et al.*, 2001; Bass *et al.*, 2002), which possess indigenous oscillator and produce melatonin in a rhythmic fashion (Herzog and Block, 1999; Tosini and Fukuhara, 2002). T_3 signaling has been found to be essential in vivo to promote complete differentiation of oligodendrocyte precursor cells (Bass *et al.*, 2002). Further, thyroid hormone receptors β2 (TRβ2) are also essential for the differentiation of short ('blue') and medium ('green') cone photoreceptors (Ng *et al.*, 2001). The deletion of thyroid hormone receptor β2 gene leads to selective loss of M-cones and concomitant increase in S-cones. It has also been reported that combinations of T_3 and *9-cis* retinoic acid cause isolated progenitor cell to differentiate as either rods or cones, depending on the relative concentration of the ligands (Kelley *et al.*, 1995). These findings suggest that T_3 plays a critical role in the differentiation of the photoreceptor cells. The photoreceptor cells of the fish pineal resemble to retinal cone cells (Gupta and Premabati, 2002b). Since S-cones and M-cones are also found in the pineal and retina of several teleosts (Meissl and Ekstrom, 1988; Kusmic *et al.*, 1993; Forsell *et al.*, 2001), T_3 may play a functional role in the regulation of photopigments and AA-NAT activity in the fish pineal as found in the present study.

Similar to the effect of T_3, PTU treatment also significantly decreased the mesor (24 h average) of the fish pineal AA-NAT activity rhythm during both the seasons (Table 5:3A). In the male Syrian hamster, AA-NAT activity was reduced by methimazole-induced hypo-thyroidism in the Harderian gland (Buzzel *et al.*, 1989). Further, hypo-thyroidism was found to enhance the nocturnal increase in deiodinase II activity in both pineal and Harderian gland in rats, while hyper-thyroidism had no effect on DII activity (Osuna *et al.*, 1993a). Findings of the present study indicate that PTU treatment increased the 24 h average T_3/T_4 ratio marginally (Tables 5:4A and 5:4B) suggesting increased conversion of T_4 to T_3 probably due to PTU-induced increased DII activity as mentioned earlier in rats (Osuna *et al.*, 1993a). Theses findings when taken together with the inhibitory effect of T_3 on AA-NAT activity seem to suggest that the observed decrease in fish AA-NAT activity by PTU-induced hypo-thyroidism might be due to increased T_3 concentrations following increased DII activity, and T_3 might be finally inhibiting AA-NAT activity by increasing proteasomal proteolysis and/or by increasing oxidative stress leading to inactivation of AA-NAT via the disulfide bond switch.

Immersion in L-T_3, L-T_4 or PTU solution decreased the amplitudes of the circadian pineal AA-NAT activity rhythms significantly irrespective of seasons. L-T_4 and L-T_3 were comparatively were more effective in inhibiting the amplitude during winter and summer, respectively. As compared to L-T_3, PTU was found to be more effective in inhibiting the amplitude of the AA-NAT activity rhythm during both the

seasons, while it was more effective than L-T_4 only during summer (Table 5:3B). It, thus, seems that both hypo- and hyper-thyroidism adversely affect the amplitude of the AA-NAT activity circadian rhythm, their degree of effectiveness seems to be influenced by the seasons.

Unlike the mesor and amplitude of the AA-NAT rhythm, the acrophase of pineal AA-NAT activity circadian rhythm occurred at 23.00 h irrespective of seasons and treatments except in L-T_4 group during winter and PTU treated group during summer, when the acrophase was advanced by 3 hours and occurred at 20.00 h. The season-dependent differential effects of the treatments on the mesor (24 h average) and amplitude but not on the acrophase of the AA-NAT rhythm seem to suggest that hypo- and hyper-thyroidism have significant effect on the melatonin synthesizing machinery, and the biological clock (oscillator) that regulates melatonin synthesis in the pineal photoreceptor cells is not influenced by the circulating levels of thyroid hormones in the fish, *Clarias gariepinus*. The optimum plasma levels of thyroid hormones seem to be essential for the normal circadian rhythm of AA-NAT activity, and both hypo- and hyper-thyroidism affect the mesor and amplitude of the AA-NAT rhythm adversely.

Table 5:1- Effects of thyroid hormones and propylthiouracil (PTU) on circadian rhythm in pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity during winter season.

Time (h)	Water tempe-rature (°C)	Arylalkylamine-N-acetyltransferase activity (nmol/pineal/hour)			
		Control	L- T_4	L- T_3	PTU
6.00	8.0	3.17 ± 0.21*	2.79 ± 0.26^{g}	2.17 ± 0.29^{f}	2.45 ± 0.27^{e}
9.00	10.0	2.73 ± 0.23^{f}	2.98 ± 0.23^{f}	1.74 ± 0.21^{g}	1.82 ± 0.21^{g}
12.00	12.2	2.56 ± 0.20^{g}	3.34 ± 0.24^{f}	1.95 ± 0.26^{g}	2.38 ± 0.24^{f}
15.00	20.5	$3.32 \pm 0.22^{a, f, h}$	3.18 ± 0.23^{f}	2.50 ± 0.29^{f}	2.09 ± 0.22^{g}
18.00	13.4	$4.54 \pm 0.24^{b, k}$	$5.10 \pm 0.31^{c, k}$	$2.91 \pm 0.28^{f, i}$	$3.36 \pm 0.17^{c, e, j}$
21.00	12.6	$5.12 \pm 0.35^{a, k}$	5.33 ± 0.37^{k}	$3.30 \pm 0.33^{g, j}$	$4.63 \pm 0.35^{b, k}$
24.00	8.2	5.81 ± 0.35^{k}	4.33 ± 0.36^{i}	$4.65 \pm 0.31^{a, k}$	4.07 ± 0.24^{k}
3.00	5.4	5.43 ± 0.27^{k}	3.47 ± 0.39^{e}	$2.74 \pm 0.23^{c, f, i}$	$2.44 \pm 0.16^{c, f}$
6.00	8.0	3.17 ± 0.21	2.79 ± 0.26	2.17 ± 0.29	2.45 ± 0.27

*All the values are expressed as mean ± standard error (S. E); N= 4.

[a, b, c] Differ from the preceding group of the respective group, $p < 0.05$, 0.02 and 0.01, respectively.

[e, f, g] Differ from the maximum value of the respective group, $p < 0.02$, 0.01 and 0.001, respectively.

[h, i, j, k] Differ from the maximum value of the respective group, $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

Table 5:2- Effects of thyroid hormones and propylthiouracil (PTU) on circadian rhythm in pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity during summer season.

Time (h)	Water tempe-rature (°C)	Arylalkylamine-N-acetyltransferase activity (nmol/pineal/hour)			
		Control	L- T_4	L- T_3	PTU
6.00	19.2	$3.27 \pm 0.34^{*\,f,j}$	2.29 ± 0.24^{g}	1.52 ± 0.20^{f}	1.93 ± 0.24^{f}
9.00	20.4	$1.64 \pm 0.12^{c,g}$	2.53 ± 0.20^{g}	1.83 ± 0.27^{f}	2.07 ± 0.21^{f}
12.00	23.2	2.02 ± 0.28^{g}	2.07 ± 0.28^{g}	1.32 ± 0.28^{f}	1.94 ± 0.25^{f}
15.00	24.5	$2.90 \pm 0.21^{f,j}$	2.60 ± 0.24^{f}	2.01 ± 0.21^{f}	2.25 ± 0.20^{f}
18.00	23.4	$3.33 \pm 0.35^{f,j}$	$3.82 \pm 0.22^{b,d,j}$	2.27 ± 0.26^{e}	$3.52 \pm 0.28^{b,j}$
21.00	22.6	$4.71 \pm 0.30^{a,k}$	$4.85 \pm 0.27^{a,k}$	$3.89 \pm 0.38^{b,j}$	4.37 ± 0.39^{j}
24.00	20.2	5.22 ± 0.32^{k}	5.04 ± 0.35^{k}	3.62 ± 0.31 j	3.31 ± 0.27^{h}
3.00	18.5	4.50 ± 0.38^{k}	$3.56 \pm 0.31^{a,d,i}$	$2.02 \pm 0.26^{c,f}$	2.35 ± 0.28
6.00	19.2	3.27 ± 0.34	2.29 ± 0.24^{b}	1.52 ± 0.20	1.93 ± 0.24

*All values are expressed as mean ± standard error (S. E); (N = 4).

[a, b, c] Differ from the preceding group of the respective group: $p < 0.05$, 0.02 and 0.01, respectively.

[d, e, f, g] Differ from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

[h, i, j, k] Differ from the maximum value of the respective group: $p < 0.05$, 0.02, 0.01 and 0.001, respectively.

Table 5:3A- Effects of thyroid hormones and propylthiouracil on pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity in light and dark phases during winter and summer seasons.

Aylalkylamine-N-acetyltransferase (nmol/pineal/hour)			
WINTER			
Treatment	Light phase (L)	Dark phase (D)	LD
Control	2.94 ± 0.13*	5.23 ± 0.19	4.09 ± 0.23
T_4	3.07 ± 0.13	4.56 ± 0.36 [b]	3.75 ± 0.17
T_3	2.09 ± 0.15 [d]	3.40 ± 0.23 [d]	2.75 ± 0.18 [d]
PTU	2.19 ±0.13 [d]	3.63 ± 0.23 [d]	2.91 ± 0.18 [d]
SUMMER			
Control	2.46 ± 0.20	4.44 ± 0.24	3.45 ± 0.23
T_4	2.37 ± 0.13	4.32 ± 0.22	3.30 ± 0.21
T_3	1.67 ± 0.14 [c]	2.95 ± 0.25 [d]	2.31 ± 0.18 [d]
PTU	2.14 ± 0.11	3.39 ± 0.24 [c]	2.76 ± 0.17 [a]

*All the values are expressed as mean ± standard error (S. E).

Light phase: N = 16; Dark phase: N = 16; Light and Dark phases: N = 32.

[a, b, c, d] Differ from the respective control value, $p< 0.05$, 0.02, 0.01, and 0.001 respectively.

Table 5:3B- Effects of thyroid hormones and propylthiouracil on the mesor (24 h average), amplitude and acrophase of pineal AA-NAT activity circadian rhythms during winter and summer seasons.

		WINTER	
Treatment	Mesor (24 h average) (nmol/pineal/hour)	Amplitude (nmol/pineal/hour)	Acrophase (h)
Control	4.09 ± 0.23*	1.62	23
L-T_4	3.75 ± 0.17	1.26	20
L-T_3	2.75 ± 0.18 [b]	1.45	23
PTU	2.91 ± 0.18 [b]	1.40	23
		SUMMER	
Treatment	Mesor (24 h average) (nmol/pineal/hour)	Amplitude (nmol/pineal/hour)	Acrophase (h)
control	3.45 ± 0.23	1.78	23
L-T_4	3.35 ± 0.21	1.48	23
L-T_3	2.31 ± 0.18 [b]	1.28	23
PTU	2.76 ± 0.17 [a]	1.22	20

*Mesor values are expressed as Mean ± standard error (S. E); N = 32.

[a, b] Differ significantly from the respective control group: $p < 0.05$ and 0.001, respectively.

Table 5:4A- Effects of L- T_4, L- T_3 and PTU on circadian rhythm in plasma levels of T_4 under LD 12:12 at ambient water temperature during summer season.

Time (hour)	Water temperature (^{o}C)	Plasma levels of T_4 (ng/ml)				
		Control	L- T_4 immersed group	L- T_3 immersed group	PTU immersed group	% decrease by PTU
6.00	19.2	14.50 ± 0.63*	25.12 ± 0.59 ^{c}	15.35 ± 0.81	12.50 ± 0.63	13.79
9.00	20.4	18.25 ± 0.67	28.62 ± 1.05 ^{c}	14.37 ± 0.48 ^{b}	14.12 ± 0.79	22.63
12.00	23.2	18.37 ± 0.77	30.62 ± 1.05 ^{c}	16.12 ± 0.83	15.32 ± 0.85 ^{a}	16.60
15.00	24.5	16.52 ± 0.81	29.12 ± 0.83 ^{c}	15.62 ± 0.71	14.85 ± 0.71	14.95
18.00	23.4	14.35 ± 0.7	28.50 ± 1.14 ^{c}	14.50 ± 0.84	13.87 ± 0.84	16.15
21.00	22.6	11.62 ± 1.16	25.12 ± 1.20 ^{c}	11.87 ± 1.12	13.54 ± 1.12	23.80
24.00	20.2	13.12 ± 1.00	27.25 ± 0.71 ^{c}	13.37 ± 0.77	12.87 ± 0.71	21.72
3.00	18.5	13.00 ± 1.23	26.12 ± 1.03 ^{c}	13.25 ± 1.39	11.32 ± 1.39	20.61
6.00	19.2	14.50 ± 0.63	25.12 ± 0.59 ^{c}	15.35 ± 0.81	12.5 ± 0.63	13.79

*All the values are expressed as Mean ± standard; (S. E); N = 4.

$^{a, b, c}$ Differ significantly from the respective control group: $p < 0.05, 0.01, 0.001$, respectively.

Table 5:4B- Effects of L- T_4, L- T_3 and PTU on circadian rhythm in plasma levels of T_3 under LD 12:12 at ambient water temperature during summer season.

Time (hour)	Water tempe-rature (^{o} C)	Plasma levels of T_3 (ng/ml)				
		Control	L- T_4 immersed group	L- T_3 immersed group	PTU immersed group	% decrease by PTU
6.00	19.2	0.85 ± 0.03*	1.04 ± 0.08	2.26 ± 0.22 [c]	0.76 ± 0.07	10.58
9.00	20.4	1.21 ± 0.11	1.13 ± 0.03	2.35 ± 0.16 [c]	1.05 ± 0.08	13.22
12.00	23.2	1.21 ± 0.15	0.99 ± 0.06	2.40 ± 0.24 [b]	1.02 ± 0.1	15.70
15.00	24.5	1.19 ± 0.06	0.99 ± 0.07	1.66 ± 0.14 [a]	1.05 ± 0.09	11.76
18.00	23.4	1.24 ± 0.03	1.12 ± 0.04	1.72 ± 0.1 [b]	1.11 ± 0.04 [a]	10.48
21.00	22.6	1.11 ± 0.05	0.98 ± 0.04	1.84 ± 0.07 [c]	0.85 ± 0.05 [b]	16.21
24.00	20.2	0.76 ± 0.03	0.81 ± 0.04	1.79 ± 0.05 [c]	0.74 ± 0.05	15.78
3.00	18.5	0.98 ± 0.11	0.99 ± 0.08	1.705 ± 0.05 [c]	0.99 ± 0.05	10.20
6.00	19.2	0.85 ± 0.03	1.04 ± 0.08	2.26 ± 0.22 [c]	0.76 ± 0.07	10.58

*All the values are expressed as Mean ± standard; (S. E); N = 4.

[a, b, c] Differ significantly from the respective control group: $p < 0.05, 0.01, 0.001$, respectively.

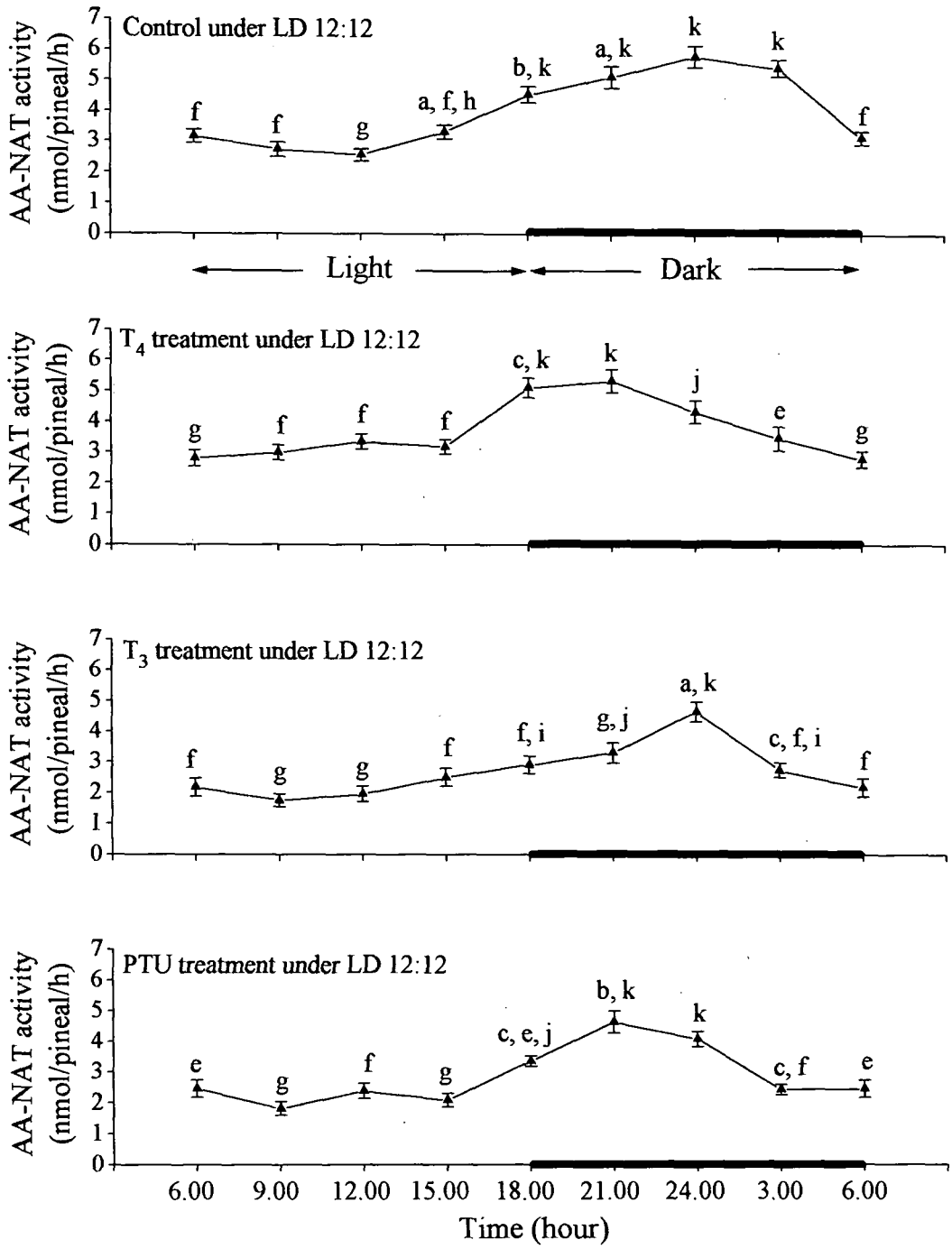


Fig. 5:1- Effects of L- T_4, L- T_3 and propylthiouracil (PTU) on circadian rhythm in pineal AA-N-acetyltransferase (AA-NAT) activity during winter season.

All values are expressed as mean ± standard error (S. E); N = 4.

[a, b, c] Differ significantly from the preceding value of the respective group: $p < 0.05$, 0.02, and 0.01, respectively.

[e, f, g] Differ significantly from the maximum value of the respective group: $p < 0.02$, 0.01, and 0.001, respectively.

[h, i, j, k] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

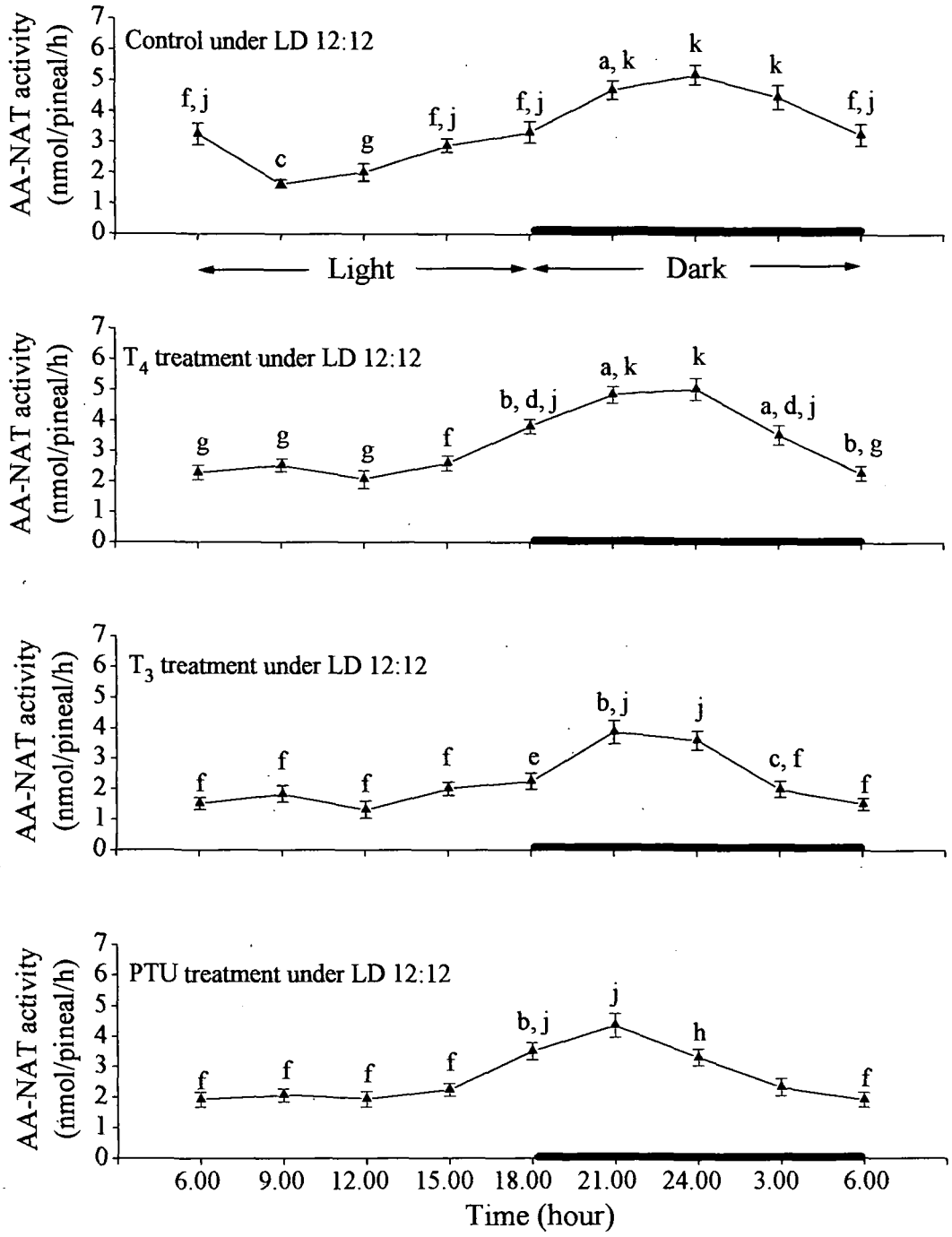


Fig. 5:2- Effects of L- T_4, L- T_3 and propylthiouracil (PTU) on circadian rhythm in pineal AA-N-acetyltransferase (AA-NAT) activity during summer season.

All values are expressed as mean ± standard error (S. E); N = 4.

[a, b, c] Differ significantly from the preceding value of the respective group: $p < 0.05$, 0.02, 0.01, respectively.

[d, e, f, g] Differ significantly from the maximum value of the respective group:$p < 0.05$, 0.02, 0.01, and 0.001, respectively.

[h, i, j, k] Differ significantly from the minimum value of the respective group: $p < 0.05$, 0.02, 0.01, and 0.001, respectively.

CHAPTER - 6

Summary and Conclusions

Melatonin and thyroid hormones play a major role in regulation of a wide range of physiological processes as well as the gonadal cycles of fishes. But so far no attempt has been made to investigate the interrelationship between pineal and thyroid in any fish species with special reference to annual gonadal cycles, seasons, photoperiod and temperature. Therefore, keeping in view the scarcity of information and importance of melatonin, thyroid hormones, temperature and photoperiod in the fish physiology, a comprehensive study was undertaken to explore the nature of interrelationship between the thyroid and the pineal in an air-breathing fish, *Clarias gariepinus*. *Clarias gariepinus* is a fish commonly found all over India and other tropical countries. At Shillong, gonadal activity of the fish remains minimum (quiescent) during the months of January and February, and increases gradually during the months of March to May (progressive phase). Breeding occurs from June to August (breeding phase) and gonads undergo regression during the months of November and December (regression phase).

For this dissertation, all experiments were conducted on adult male *Clarias gariepinus* (body weight: 90-100g; body length: 23-27 cm) purchased from the local fish suppliers. Before starting any experiment, fishes were maintained in clear plastic tubs and acclimatized at least for 15 days in the laboratory under natural climatic conditions at Shillong (Latitude 25°.30′ N, Longitude 91°.52′ E; Altitude 1450 ASL; Minimum water

temperature: 4° C; maximum water temperature: 24.5° C). During acclimatization, the fishes were fed daily with minced earthworms and commercial fish food *ad libitum*. Water was changed everyday to avoid infections.

The present Ph. D dissertation has been divided into six chapters. A brief summary of the chapters has been given below.

Chapter - 1: Materials and Methods

This chapter deals with the details of the experimental animals, experimental conditions, methods for maintaining the fishes, RIA of thyroid hormones, radio-enzyme assay of pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity, biostatistical methods and acro analysis.

Chapter - 2: Studies on the interrelationship between circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal arylalkylamine-N-acetyltransferase (AA-NAT) activity

This chapter deals with the study of circadian rhythms in the plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity in the fish maintained under natural climatic conditions during quiescent, progressive, breeding and regressive phases of the annual breeding cycle. The present study, to the best of our knowledge, seems to be the first of its kind in which the interrelationship between

the circadian rhythms of thyroid hormones and pineal AA-NAT activity have been studied in a fish maintained under natural day length and temperature during all the four phases of the breeding cycle. The summary of major findings and conclusions based on the experiments included in this chapter are mentioned below:

1. *Prominent circadian rhythms in the plasma levels of thyroid hormones* (T_4 *and* T_3) were observed during all the four phases of the breeding cycle.
2. Levels of plasma T_4 were found to be higher than those of T_3 during all the phases.
3. Plasma levels of the thyroid hormones were found to be elevated during the daytime in all the four phases. Further, the levels of thyroid hormones were significantly higher during progressive and breeding phases than that during the regressive and the quiescent phases.
4. Regression analysis of the data revealed a positive correlation between water temperature and plasma levels of thyroid hormones (T_3 and T_4) during all phases of the breeding cycle.
5. T_3/T_4 ratio was found to be comparatively higher during the regressive and the quiescent phases (pre-winter and winter months) than that during the progressive and the breeding phases.
6. Irrespective of the phase of the breeding cycle, the maximum T_3/T_4 ratio was invariably recorded during midnight/early morning.
7. A temporal shift in the T_3/T_4 ratio was observed during the dark phase in different phase of the cycle.

8. The mesor (24 h average) of T_4 and T_3 circadian rhythms increased significantly during progressive and breeding phases (summer/rainy season) as compared to regressive and quiescent phases (pre-winter and winter months).
9. The amplitude of T_4 circadian rhythm was, depending on the phase/season, six to 21-folds higher than that of the T_3 rhythm.
10. The temporal difference between the amplitudes of T_4 and T_3 rhythms was found to be minimum during the quiescent phase (6 folds), increased during the progressive phase (9 folds) and breeding phase (13 folds), and became maximum during the regressive phase (21 folds).
11. The acrophase of the circadian rhythm of thyroid hormones was always recorded during the day-time.
12. AA-NAT activity was found to be the minimum during the daytime and increased by several folds during the night.
13. The mesor (24 h average) of the AA-NAT activity rhythm was significantly higher during the regressive and the quiescent phases than that of the breeding phase.
14. The amplitude of the AA-NAT rhythm was found to be maximum during the quiescent phase, which declined during the progressive phase, reached to a minimum during the breeding phase and then increased during the regressive phase.
15. The acrophase of the AA-NAT circadian rhythm always occurred around the mid-night.
16. Unlike the amplitude, the acrophase of the thyroid hormones (T_3 and T_4) and pineal AA-NAT activity rhythms remained more or less constant.

17. In contrast to thyroid hormones, a negative correlation was found between AA-NAT activity and water temperature irrespective of phase of the breeding cycle.

18. A significant negative correlation exists between the plasma levels of thyroid hormones (T_3 and T_4) and the pineal AA-NAT activity in *Clarias gariepinus*.

On the basis of the above findings, we conclude that:

i). The secretion of T_4 is dominant over T_3 secretion in this species.

ii). The elevated levels of the thyroid hormones during the daytime as well as during progressive and breeding phases seem to be associated with increased water temperature. This assumption is supported by a positive correlation between temperature and thyroid hormones.

iii). Low water temperature favors increased peripheral conversion of T_4 to T_3 leading to higher T_3/T_4 ratio, which seems to help the fish in maintaining metabolic rate at low temperature.

iv). The recorded shift in the maximum and the minimum T_3/T_4 ratio over the circadian scale can play a role in shaping the annual breeding cycle and/or reflects shifts in energy requirements in relation to activity of the fish.

v). The high T_3/T_4 ratio recorded at night during all the phases seems to be directly correlated with the increased activity of the fish during the nighttime.

vi). The synthesis and release of the thyroid hormones is increased during the summer/rainy seasons and decreased during the pre-winter and winter months.

vii). The high levels of both T_3 and T_4 during reproductively active seasons seem to be associated with increased breeding activity and energy metabolism of the fish, *Clarias gariepinus*.

viii). The minimum and the maximum amplitude of the T_4 rhythm during quiescent (winter) and breeding (summer/rainy season) phases respectively seem to be directly related to the feeding status of the fish.

ix). The increased melatonin synthesis during the regressive and the quiescent phases (as indicated by the mesor of AA-NAT) seems to be responsible for the suppression of the testicular activity, while decrease in melatonin synthesis during the progressive phase (as indicated by the mesor of AA-NAT) allows the activation of the hypothalamo-hypophyseal-testicular axis.

x). The phase-dependent changes in the amplitude of the AA-NAT rhythm seem to be closely associated with the testicular cycle of the fish. While higher amplitudes of the rhythm seem to suppress the testicular activity, lower amplitudes of AA-NAT rhythm seem to favor testicular activation.

xi). Unlike the amplitude, the acrophase of T_3, T_4 and AA-NAT rhythms is not influenced by the phase of the breeding cycle and seasons.

xii). Low temperature stimulates and high temperature inhibits the fish pineal AA-NAT activity. In contrast to temperate zone fish species, an inverse correlation exists between the water temperature and pineal AA-NAT activity in *Clarias gariepinus*. It seems that water temperature produces differential effects on the

amplitude of melatonin/AA-NAT activity rhythms in temperate and tropical/subtropical fish species.

xiii). An inverse interrelationship exists between the circulating levels of the thyroid hormones and AA-NAT activity/melatonin synthesis.

xiv). A direct relationship between water temperature and plasma levels of thyroid hormones and an inverse relationship between water temperature and AA-NAT activity seem to be responsible for the observed inverse interrelationship between the plasma levels of thyroid hormones and AA-NAT activity.

xv). It seems that the long daylength and high ambient temperature during progressive and breeding phases (summer/rainy seasons) inhibit AA-NAT activity and thereby decrease melatonin synthesis resulting in increased breeding activity, while short daylength and low ambient temperature during the regressive and the quiescent phases (winter months) seem to stimulate AA-NAT activity/melatonin synthesis leading to testicular inactivation in the fish.

Chapter - 3: Effects of photoperiods and simulated temperatures on circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal arylalkylamine-N-acetyltransferase activity (AA-NAT) activity

This chapter deals with the effects of long photoperiod (LD 15:9), LD 12:12 and short photoperiod (LD 9:15) at a constant temperature ($25^{\circ} \pm 2^{\circ}$ C) on the circadian

rhythms in plasma levels of thyroid hormones (T_3 and T_4) and pineal AA-NAT activity during winter and summer seasons. In addition, it also deals with the effects of different simulated temperatures (i.e., 15° C, 25° C and 35° C) under LD 12:12 photoperiod on plasma levels of thyroid hormones (T_3 and T_4) and pineal AA-NAT activity during winter and summer seasons. The present study seems be the first of its kind in which the interrelationship between the circadian rhythms of thyroid hormones and pineal AA-NAT activity have been studied in a fish maintained under different photoperiods and simulated temperatures during both winter and summer seasons. The summary of major findings and conclusions of this chapter are listed below:

Effects of photoperiods on circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during winter and summer seasons:

1. Plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity exhibited prominent circadian rhythm under all photoperiodic regimes (i.e., LD 9:15, LD 12:12 and LD 15:9) during both summer and winter seasons.
2. The maximum levels of thyroid hormones were recorded during the light phase of all the photoperiodic regimes irrespective of the seasons.
3. The maximum AA-NAT activity was always found during the dark phase irrespective of photoperiod and seasons.
4. The patterns of circadian increase and decrease in the levels of thyroid hormones were different under different photoperiods and seasons.

5. During both the seasons, the average plasma levels of both T_4 and T_3 were significantly higher during the light phase than that of the dark phase under all the photoperiodic regimes except under LD 15:9 in winter when the average level of T_4 was found to be similar during light and dark phases.
6. The mesor (24 h average) of T_4 and T_3 circadian rhythms were found to be minimum in fishes under LD 9:15 and maximum in the fishes exposed to LD 15:9.
7. The mesor of T_4 circadian rhythm was found to be 14 and 16-18 folds higher than that of T_3 during winter and summer seasons, respectively.
8. Regression analysis of the data indicated a positive correlation between the length of photoperiod and mesors of T_4 and T_3 during both winter and summer seasons.
9. The amplitude of T_4 circadian rhythm was higher under LD 9:15 and LD 15:9 during winter than the respective photoperiods during summer, while under LD 12:12 the amplitude of T_4 rhythm was higher during summer than during winter.
10. The amplitude of the T_3 rhythm was higher under LD 9:15 and LD 15:9 during summer as compared to winter, while under LD 12:12 the amplitude was higher during winter.
11. Regression analysis of the data indicated a negative correlation between the amplitude of T_4 rhythm and the length of photoperiod only during winter, while

a positive correlation was found between the amplitude of T_3 rhythm and the length of photoperiod only during summer.

12. The acrophase of T_4 occurred between 8.00 and 11.00 h during winter and between 11.00 and 14.00 h during summer season.
13. The acrophase of T_3 circadian rhythm invariably occurred at 14.00 h irrespective of photoperiods and seasons except under LD 9:15 during summer, where the acrophase was advanced by 3 h and occurred at 11.00 h.
14. Irrespective of photoperiods and seasons, the diurnal rhythm of AA-NAT activity invariably exhibited a basal level of the enzyme during the light phase, which increased rapidly after the onset of dark phase, remained significantly higher during the dark phase and declined sharply after the onset of light phase.
15. The mesor (24 h average) of pineal AA-NAT activity was found to be significantly lower under LD 15:9 as compared to that under both LD 12:12 and LD 9:15 during both the seasons.
16. Regression analysis of the data indicated a strong negative correlation between the length of photoperiod and the mesor of AA-NAT activity rhythm during both winter and summer seasons.
17. The amplitude of the AA-NAT activity circadian rhythm was found to be invariably higher during winter as compared to the respective photoperiods during summer.

18. Regression analysis of the data indicated a strong negative correlation between the amplitude of pineal AA-NAT activity circadian rhythm and length of the photoperiod during both winter and summer seasons.
19. During summer, the acrophase of pineal AA-NAT activity circadian rhythm occurred at 23.00 h under all photoperiods. However, during winter the acrophase of the AA-NAT activity rhythm under LD 12:12 and LD 15:9 occurred at 02.00 h, while the acrophase was advanced by 3 hours under LD 9:15 and occurred at 23.00 h.

Effects of simulated temperatures on circadian rhythms in plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity during winter and summer seasons:

1. Plasma levels of thyroid hormones (T_4 and T_3) and pineal AA-NAT activity exhibited prominent circadian rhythms under all the simulated temperatures during both summer and winter seasons.
2. The maximum levels of thyroid hormones were recorded during the light phase of all the temperatures irrespective of the seasons.
3. Irrespective of temperatures and seasons, plasma levels of T_4 and T_3 increased after lights "on" at 6.00 h except at 15° C when the plasma level of T_3 was increased at 12.00 h during both the seasons.

4. The average plasma levels of both T_4 and T_3 were significantly higher during the light phase than that of the dark phase under all three temperatures during both the seasons except at 15° C when the average level of T_3 was found to be similar during light and dark phases.
5. The mesor (24 h average) of T_4 and T_3 circadian rhythms were found to be highest in the fishes exposed to 35° C and lowest in the fishes at 15° C during both the seasons.
6. The mesor of T_4 circadian rhythm was found to be 13-17 and 12-14 folds higher than that of T_3 during winter and summer season, respectively.
7. Regression analysis of the data indicated a strong positive correlation between water temperature and mesors of T_4 and T_3 rhythms during both winter and summer seasons.
8. The amplitude of T_4 circadian rhythm was found to be maximum at 25° C and was greater than those at 15 ° C and 35 ° C during both the seasons.
9. The amplitude of the T_3 rhythm increased with increase in water temperature.
10. Regression analysis of the data indicated a positive correlation between the amplitude of T_3 rhythm and temperature during both winter and summer seasons, while no significant correlation was found between water temperature and the amplitude of the T_4 rhythm.
11. The acrophase of T_4 circadian rhythm occurred between 11.00 and 14.00 h during both winter and summer seasons.

12. The acrophase of the T_3 circadian rhythm occurred between 14.00 and 17.00 h during both winter and summer seasons.
13. Pineal AA-NAT activity was always found to be low during the light phase.
14. AA-NAT activity increased rapidly after the onset of the dark phase irrespective of temperatures and seasons and remained high during the dark phase.
15. The mesor (24 h average) of pineal AA-NAT activity was found to be maximum at 15° C and decreased significantly at both 25° C and 35° C during both the seasons.
16. Regression analysis of the data indicated a strong negative correlation between water temperature and mesor of the AA-NAT activity rhythm during both the seasons.
17. The amplitude of the AA-NAT activity circadian rhythm was found to be higher at 15° C and 35° C during summer as compared to the respective temperatures during winter.
18. The amplitude of pineal AA-NAT activity decreased with the increase in temperature irrespective of the seasons.
19. Regression analysis of the data indicated a strong negative correlation between the amplitude of pineal AA-NAT activity circadian rhythm and water temperature during both winter and summer seasons.
20. Irrespective of the simulated temperature, the acrophase of pineal AA-NAT activity circadian rhythm occurred at 23.00 h during summer season, while during winter it was delayed by three hours and occurred at 2.00 h (Table 3.8B).

On the basis of the above mentioned findings, we conclude the following:

i) Both photoperiod and temperature produce opposite effects on the levels of thyroid hormones and AA-NAT activity and hence melatonin synthesis.

ii) A direct relationship between photoperiod as well as temperature and thyroid hormones, and an inverse relationship between photoperiod as well as temperature and AA-NAT activity jointly regulate the opposite/inverse temporal relationship between the circadian rhythms of thyroid hormones and AA-NAT activity (hence melatonin synthesis) in the fish during both winter and summer seasons.

iii) There is a direct relationship between environmental photoperiod and temperature and the plasma levels of the thyroid hormones in *Clarias gariepinus*.

iv) Photoperiods seem to produce season-dependent differential effects on the amplitudes of T_4 and T_3 circadian rhythms.

v) Water temperature seems to produce differential effects on the amplitudes of the two thyroid hormones.

vi) While the amplitude of T_4 rhythm is closely associated with the preferred water temperature, the amplitude of T_3 rhythm is directly controlled by water temperature.

vii) The acrophase of the T_4 rhythm is regulated jointly by photoperiod and water temperature, while the acrophase of the T_3 rhythm is regulated only by water temperature.

viii) Long daylength and higher ambient temperature decrease AA-NAT activity (hence melatonin synthesis) during summer months, when the fish breeds. In contrast, short

daylength and low temperature stimulate AA-NAT activity and (hence melatonin synthesis during winter months when the testis remains quiescent.

ix) The circannual changes in environmental photoperiod and temperature regulate the circannual changes in AA-NAT activity and melatonin synthesis, which in turn may be regulating the circannual rhythm of testicular activity of the fish.

x) The acrophase of AA-NAT activity rhythm in the fish pineal seems to be controlled by an indigenous clock, which is not influenced either by environmental temperature or photoperiod.

Thus, the findings on effects of different photoperiods and simulated temperatures taken together suggest that both temperatures and photoperiods have opposite effects on the plasma levels of thyroid hormones and pineal AA-NAT activity. It seems that circannual rhythm of photoperiod and temperature maintain the inverse relationship between thyroid activity and AA-NAT activity. Long daylength and high ambient temperature of summer stimulate synthesis of thyroid hormones and inhibit melatonin synthesis, both essential for active gonads and successful breeding. In contrast, low temperature and short daylength of winter decrease the synthesis of thyroid hormones and simultaneously increase AA-NAT activity and melatonin synthesis leading to regression and inactivation of gonads.

Chapter - 4: *In vivo* effects of melatonin on plasma levels of thyroxine (T_4) and triiodothyronine (T_3)

This chapter deals with *in vivo* effects of different doses of melatonin on plasma levels of T_4 and T_3 during quiescent, progressive, breeding and regressive phases of the annual breeding cycle. The major findings and conclusions based on the experiments included in this chapter are mentioned below:

1. Morning injection of melatonin during the quiescent phase had no significant effect on plasma T_4 levels, except in 1μg/g/day melatonin injected group which increased plasma T_4 levels significantly.
2. The evening group injected with 2μg/g/day dose of melatonin induced significant increase in plasma T_4 levels during the quiescent phase.
3. Administration of melatonin during the quiescent phase suppressed T_3 levels significantly in a dose-dependent manner during both morning and evening hours.
4. Administration of 2μg/g/day of melatonin during morning and 1μg/g/day and 2μg/g/day during evening significantly increased plasma T_4 levels during the progressive phase.
5. Administration of melatonin suppressed plasma T_3 level in a dose-dependent manner during morning, higher doses of melatonin (1μg/g/day and 2μg/g/day) suppressed it during evening during the progressive phase.

6. Administration of melatonin during morning hour of breeding phase had no significant effect on the plasma T_4 levels.
7. Injection of 1μg/g/day and 2μg/g/day doses of melatonin in the evening significantly increased the plasma T_4 levels during the breeding phase.
8. None of the melatonin doses had any significant effect on plasma T_3 levels during the breeding phase in both morning and evening groups except when injected with 0.5μg/g/day, which significantly increased the plasma T_3 levels in the evening group.
9. Administration of melatonin during morning and evening significantly increased the plasma T_4 levels and suppressed the plasma levels of T_3 both in the morning and evening groups in a dose-dependent manner during the regressive phase.
10. Regression analysis of the data indicated a strong positive correlation between the doses of melatonin and the plasma T_4 levels irrespective of the time of administration and phase of the breeding cycle.
11. Irrespective of the time of administration and phases, a strong negative correlation was found between the doses of melatonin and the plasma T_3 levels except in the evening group of the breeding phase, where the negative correlation was insignificant.
12. T_3/T_4 ratio was decreased during all the four phases following melatonin treatment in the morning as well as in the evening groups.

Present findings indicate that melatonin is involved in the regulation of thyroid activity and plasma levels of thyroid hormones, and exerts a differential control over the circulating levels of T_3 and T_4 in *C. gariepinus*. The climatic factors might be influencing thyroid function in fishes by altering melatonin production. It seems that the stimulatory and inhibitory effects of melatonin on T_4 and T_3 in this species seem to depend on seasons, dose and the time of administration. Melatonin-induced inhibition of T_3 might be of adaptive significance in lowering the metabolic rate as well as in conserving energy reserves. Melatonin-induced increase in T_4 seems to promote growth and breeding related processes. Further, there is a possibility that low temperature and short photoperiod, which increase melatonin production in fish, might influence circulating levels of thyroid hormones in the fish via a neuroendocrine mechanism involving the pineal organ and melatonin. These findings also suggest a probability that the circadian and circannual rhythms of melatonin might be involved in shaping the circadian and circannual rhythms of plasma T_4 and T_3 levels in *C. gariepinus*.

Chapter - 5: Effects of thyroid hormones and propylthiouracil on the circadian rhythms in pineal Arylalkylamine-N-acetyltransferase (AA-NAT) activity

This chapter deals with the effects of hyper- and hypo-thyroidism on circadian rhythms in pineal AA-NAT activity during winter and summer seasons. In order to produce hyper- and hypo-thyroidism, the fishes were immersed in water containing L-T_3,

L-T_4 and propyl thiouracil (PTU). The summary of major findings and conclusions based on the experiments included in this chapter are listed below:

1. Plasma T_4 and T_3 levels were significantly increased following immersion of the fishes in water containing L-T_4 and L-T_3, respectively at all the eight time points over the 24 h time scale.
2. Depending on the time point of the 24 h scale, immersion of the fishes in water containing PTU resulted in 14 to 24% and 10 to 16% decrease in the plasma levels of L-T_4 and L-T_3, respectively. Further, PTU treatment marginal increased the T_3/T_4 ratio.
3. Fish pineal AA-NAT activity exhibited prominent circadian rhythm in all the groups irrespective of the treatment during both winter and summer seasons.
4. The patterns of diurnal increase and decrease in pineal AA-NAT activity were different under different treatments and seasons.
5. Irrespective of seasons, L-T_4 treatment had no significant effect on the average AA-NAT activity during both light and dark phases.
6. L-T_3 treatment significantly decreased the average AA-NAT activity in both light and dark phases.
7. During winter season, PTU treatments significantly decreased the average AA-NAT activity in both light and dark phases. However, during summer seasons PTU had no significant effect on the average pineal AA-NAT activity during the

light phase, but it significantly decreased the enzyme activity during the dark phase.

8. Irrespective of seasons, the mesor (24 h average) of the circadian rhythm of AA-NAT activity was significantly decreased in the pineal of the fishes immersed in water containing L-T_3 and PTU, while immersion in water containing L-T_4 had no significant effect on the mesor of the AA-NAT activity rhythm.
9. The amplitude of the pineal AA-NAT activity circadian rhythm was decreased significantly following immersion in water containing L-T_4, L-T_3 or PTU as compared to the control group irrespective of seasons.
10. Immersion in water containing L-T_4 was more effective in reducing the amplitude of AA-NAT activity rhythm as compared to immersion in water with L-T_3 or PTU during winter.
11. PTU treatment was more effective during the summer season in reducing the amplitude of AA-NAT activity rhythm as compared to L-T_3 and L-T_4 treated groups.
12. Irrespective of seasons and treatments, the acrophase of pineal AA-NAT activity circadian rhythm occurred at 23.00 h except in L-T_4 group during winter and PTU treated group during summer, when the acrophase was advanced by 3 hours and occurred at 20.00 h.

On the basis of the present findings, we conclude the following:

i) The optimum plasma levels of thyroid hormones are essential for the normal circadian rhythm of AA-NAT activity, and both hypo- and hyper-thyroidism adversely affect the AA-NAT rhythm.

ii) While increased T_4 levels do not affect AA-NAT activity, increased T_3 concentrations produce inhibitory effect on AA-NAT activity in the fish pineal.

iii) The observed decrease in AA-NAT activity following immersion in water containing L-T_3 seems to be a result of T_3-induced increased proteasomal proteolysis of AA-NAT protein in the fish pineal. Alternatively, the observed inhibition of AA-NAT activity in the fish pineal might be a result of inhibitory effect of L-T_3-induced oxidative stress on AA-NAT molecule via the formation and cleavage of the disulfide bond between Cysteine^{61} and Cysteine^{177}.

iv) The observed decrease in fish AA-NAT activity by PTU-induced hypo-thyroidism might be due to increased T_3 concentrations following increased DII activity, and T_3 might be finally inhibiting AA-NAT activity by increasing proteasomal proteolysis and/or by increasing oxidative stress leading to inactivation of AA-NAT via the disulfide bond switch.

v) Both hypo- and hyper-thyroidism inhibit the amplitude of the AA-NAT activity circadian rhythm, and their inhibitory effect seems to be influenced by the seasons.

vi) Hypo- and hyper-thyroidism have significant effect on the melatonin synthesizing machinery. However, the biological clock (oscillator) that regulates melatonin

synthesis in the pineal photoreceptor cells is not influenced by the circulating levels of thyroid hormones in the fish, *Clarias gariepinus*.

Thus, physiological plasma levels of thyroid hormones seem to be essential for the normal circadian rhythm of AA-NAT activity in the fish pineal. Both hypo- and hyper-thyroidism adversely affect the mesor and amplitude of the AA-NAT rhythm.

CONCLUSIONS

On the basis of the major findings of the present Ph.D. dissertation, it can be concluded that the plasma levels of thyroid hormones and pineal AA-NAT activity of the fish *Clarias gariepinus* exhibit a circadian rhythm during all the phases of the breeding cycle, and under different photoperiods and simulated temperatures irrespective of the seasons. An inverse relationship exists between the circadian rhythms of thyroid hormones and pineal AA-NAT activity. Both temperatures and photoperiods have opposite effects on the plasma levels of thyroid hormones and pineal AA-NAT activity. Circannual rhythm of photoperiod and temperature seems to maintain the inverse relationship between thyroid activity and AA-NAT activity. It seems that the long daylength and high ambient temperature during progressive and breeding phases (summer/rainy seasons) stimulate synthesis and secretion of thyroid hormones and simultaneously inhibit AA-NAT activity/melatonin synthesis leading to increased breeding activity, while short daylength and low ambient temperature during the regressive and the quiescent phases (winter months) seem to inhibit synthesis and

secretion of thyroid hormones and simultaneously stimulate AA-NAT activity/ melatonin synthesis leading to testicular inactivation in the fish. Melatonin seems to be actively involved in the regulation of thyroid activity and plasma levels of thyroid hormones. However, it exerts a differential control over the circulating levels of T_4 and T_3 in *C. gariepinus*. Thus, the climatic factors may alter thyroid hormone levels in fishes by altering pineal AA-NAT activity and melatonin production. Melatonin-induced inhibition of T_3 might be of adaptive significance in lowering the metabolic rate as well as in conserving energy reserves. Melatonin-induced increase in T_4 seems to promote growth and breeding related processes. Present findings clearly indicate that the circadian rhythms of thyroid hormones and melatonin influence each other as well as get influenced by climatic factors. Since melatonin had pronounced effects on the plasma levels of thyroid hormones and pineal AA-NAT activity is controlled by highly predictive circannual changes in daylength/photoperiod, it seems that the circadian and circannual rhythms of melatonin play an important role in shaping the circadian and the circannual rhythms of plasma T_4 and T_3 levels. At the same time, the physiological plasma levels of thyroid hormones seem to be essential for the normal circadian rhythm of AA-NAT activity and melatonin synthesis. Thus, the inversely related thyroid hormones and AA-NAT activity/melatonin synthesis influence each other, and seem to jointly regulate the circadian and circannual events of the air breathing fish, *Clarias gariepinus*.

REFERENCES

Agha, A. K., and Joy, K. P. (1989). Effect of pinealectomy on the histology of the adrenacortical homologues and plasma cortisol level in the Indian catfish, *Heteropneustes fossilis* (Bloch). J. Pineal Res., **6**(4), 335-340.

Akema, T., Chiba, A., Ikeda, T., Nagami, Y., Kimura, F., and Toyoda, J. (1997). Melatonin inhibits naloxone-induced luteinizing hormone release in ovariectomized estrogen-primed rats. J. Neuroendocrinol., **9**(11), 849-857.

Akiyama, M., Kouzu, Y., Takahashi, S., Wakamatsu, H., Moriya, T., Maetani, M., Watanabe, S., Tei, H., Sakaki, Y., and Shibata, S. (1999). Inhibition of light-or glutamate-induced *mPer1* expression represses the phase-shifts into the mouse circadian locomotor and suprachaismatic firing rhythms. J. Neurosci., **19**, 1115-1121.

Alonso, R., Abreu, P., Fajardo, N., Hernandez-Diaz, F., Diaz-Cruz, A., Hernandez, G., and Sanchez-Criado, J. (1995). Ovarian hormones regulate alpha 1- and beta-adrenoceptor interactions in female rat pinealocytes. Neuroreport, **6**, 345-348.

Alonso-Gomez, A. C., Valenciano, A. I., Alonso-Bedate, M., and Delgado, M. J. (2000). Melatonin synthesis in the green frog retina in culture: I. Modulation by the light/dark cycle, forskolin and inhibitors of protein synthesis. Life Sci., **66**(8), 675-685.

Alshaikh, M. A., Salah, M. S., Kraidees, M. S., al-Saiady, M. Y., Abouheif, M. A., and Aldabeeb, S. N. (1997). Plasma concentration of thyroid hormones in lambs fed poultry offal meal in replacement of soybean meal at two energy levels. Dtsch. Tierarztl. Wochenschr., **104**(6), 213-215.

Appelbaum, L., Toyama, R., Dawid, I. B., Klein, D. C., Baler, R., and Gothilf, Y. (2004). Zebrafish serotonin-N-acetyltransferase-2 gene regulation: pineal-restrictive downstream module contains a functional E-box and three photoreceptor conserved elements. Mol. Endocrinol., **18**(5), 1210-1221.

Armstrong, S. M., Cassone, V. M., Chesworth, M. J., Redman, J. R., and Short, R. V. (1986). Synchronization of mammalian circadian rhythms by melatonin. J. Neural Transm. Suppl., **21**, 375-394.

Augee, M. L., Raison, J. K., and Hulbert, A. J. (1979). Seasonal changes in membrane lipid transitions and thyroid function in the hedgehog. Am. J. Physiol., **236**(6), 589-593.

Aydogan, S., Yerer, M. B., and Yapislar, H. (2004). *In vitro* effects of melatonin on the filtrability of erythrocytes in SNP-induced oxidative stress. Clin. Hemorhoel Microcric., **30**(3-4), 317-322.

Baas, D., Legrand, C., Samarut, J., and Flamant, F. (2002). Persistence of oligodendrocyte precursor cells and altered myelination in optic nerve associated to retina degeneration in mice devoid of all thyroid hormone receptors. Proc. Natl. Acad. Sci., U S A, **99**(5), 2907-2911.

Baler, R., and Klein, D. C. (1995). Circadian expression of transcription factor Fra-2 in the rat pineal gland. J. Biol. Chem., **270**(45), 27319-27325.

Baltaci, A. K., Mogulkoc, R., Kul, A., Bediz, C. S., and Ugur, A. (2003). Pinealectomy and zinc deficiency have opposite effects on thyroid hormones in rats. Endocr. Res., 29(4), 473-481.

Baltaci, A. K., Mogulkoc, R., Kul, A., Bediz, C. S., and Ugur, A. (2004). Opposite effects of zinc and melatonin on thyroid hormones in rats. Toxicol., **195**(1), 69-75.

Bandyopadhyay, S., Banerjee, P. P., and Bhattacharya, S. (1991). 17β-estradiol releases thyoxine from the thyroid follicles of a teleost, *Chana gachua* (Ham). Gen. Comp. Endocrinol., **81**(2), 227-233.

Barassin, S., Kalsbeek, A., Saboureau, M., Bothorel, B., Vivien-Roels, B., Malan, A., Buijs, R. M., and Pevet, P. (2000). Potentiation effect of vasopressin on melatonin secretion as determined by trans-pineal microdialysis in the Rat. J. Neuroendocrinol., **12**(1), 61-68.

Barnes, J. W., Tischkau, S. A., Barnes, J. A., Mitchell, J. W., Burgoon, P. W., Hickok, J. R., and Gillette, M. U. (2003). Requirement of mammalian *Timeless* for circadian rhythmicity. Science, **302**, 439-442.

Barre, H., and Rouanet, J. L. (1983). Calorigenic effect of glucagon and catecholamines in king penguin chicks. Amer. Physiol. Society, **244**, 758-763.

Barrell, G. K., and Montgomery, G. W. (1989). Absence of circadian patterns of secretion of melatonin or cortisol in Weddell seals under continuous natural daylight. J. Endocrinol., **122**, 445-449.

Barrett, R. K., and Takahashi, J. S. (1995). Temperature compensation and temperature entrainment of the chick pineal cell circadian clock. J. Neurosci., **15**(8), 5681-5692.

Barrett, R. K., and Takahashi, J. S. (1997). Lability of circadian pacemaker amplitude in chick pineal cells: a temperature-dependent process. J. Biol. Rhythms, **12**(4), 309-318.

Bartness, T. J., Powers, J. B., Hastings, M. H., Bittman, E L., and Goldman, B. D. (1993). The time infusion paradigm for melatonin delivery: what has it taught us about

the melatonin signal, its reception, and the photoperiodic control of seasonal responses. J. Pineal Res., **15**, 161-190.

Bau, F., and Parent, J. P. (2000). Seasonal variation of thyroid hormone levels in wild fish. C. R. Acad. Sci. III, **323**, 365-372.

Bauer, M. S., Poland, R. E., Whybrowm P. C., and Frazer, A. (1989). Pituitary-adrenal and thyroid effects on melatonin content of the rat pineal gland. Psychoneuroendocrinology, **14**(3), 165-175.

Bayarri, M. J., Munoz-Cueto, J. A., Lopez-Olmeda, J. F., Vera, L. M., Rol de Lama, M. A., Madrid, J. A., and Sanchez-Vazquez, F. J. (2004a). Daily locomotor activity and melatonin rhythms in Senegal sole (*Solea senegalensis*). Physiol. Behav., **81**(4), 577-583.

Bayarri, M. J., Rodriguez, L., Zanuy, S., Madrid, J. A., Sanchez-Vazquez, F. J., Kagawa, H., Okuzawa, K., and Carrillo, M. (2004b). Effect of photoperiod manipulation on the daily rhythms of melatonin and reproductive hormones in caged European sea bass (*Dicentrachus labrax*). Gen. Comp. Endocrinol., **136**(1), 72-81.

Bednarek, J., Wysocki, H., and Sowinski, J. (2004). Oxidation products and antioxidant makers in plasma of patients with Graves' disease and toxic multinodular goiter: effect of methimazole treatment. Free Radic. Res., **38**(6), 659-664.

Begay, V., Falcon, J., Cahill, G. M., Klein, D. C., and Coon, S. L. (1998). Transcripts encoding two melatonin synthesis enzymes in the teleosts pineal organ: circadian regulation in pike and zebrafish, but not in trout. Endocrinology, **139**, 905-912.

Ben Saad, M. M., and Maurel, D. L. (2004). Reciprocal interaction between seasonal testis and thyroid activity in Zembra island wild rabbits (*Oryctolagus cuniculus*): effects of castration, thyroidectomy, temperature, and photoperiod. Biol. Reprod., **70**(4), 1001-1009.

Bernard, M., Iuvone, P. M., Cassone, V. M., Roseboom, P. H., Coon, S. L., and Klein, D. C. (1997). Avain melatonin synthesis: photic and circadian regulation of serotonin N-acetyltransferase mRNA in the chicken pineal gland and retina. J. Neurochem., **68**(1), 213-224.

Bertolucci, C., Sovrano, V. A., Magnone, M. C., and Foa, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. Am. J. Physiol. Regul. Integr. Comp. Physiol., **279**(6), 2121-2131.

Bertolucci, C., Wagner, G., Foa, A., Gwinner, E., and Brandstatter, R (2003). Photoperiod affects amplitude but not duration of *in vitro* melatonin production in the ruin lizard (*Podarcis sicula*). J. Biol. Rhythms, **18**(1), 63-70.

Bhattacharya, S. (1987). Influence of thyroid on piscine reproduction. In "Proc. 1st Congress, Asia and Oceanic Soc. Comp. Endocrinol." (E. Ohnishi, Y. Nagahama and H. Ishizaki, eds.), pp. 343-344. Nagoya University Corporation, Nagoya, Japan.

Binkley, S. (1981). Pineal biochemistry: Comparative aspects and circadian rhythms. In "The pineal Gland Vol. VI.", (R. J. Reiter, ed.), pp. 115-172, Boca Raton, F. L. C R C Press.

Binkley, S. (1993). Structures and molecules involved in generation and regulation of biological rhythms in vertebrates and invertebrates. Experientia, **49**(8), 648-653.

Binkley, S., and Geller, E. B. (1975). Pineal enzymes in chickens: development of daily rhythmicity. Gen. Comp. Endocrinol., **27**(4), 424-429.

Binkley, S., and Moser, K. (1984). Proir light alters the circadian clock in the chick gland. J. Exp. Zool., **232**(2), 551-556.

Binkley, S., Reilly, K. B., and Hryshchyshyn, M. (1980). N-acetyltransferase in the chick retina I: Circadian rhythms controlled by environmental lighting are similar to those in the pineal gland. J. Comp. Physiol. B, **139**(2), 103-109.

Binkley, S., Muller, G., and Hernandez, T. (1981). Circadian rhythm in pineal N-acetyltransferase activity: phase shifting by light pulses I. J. Neurochem., **37**, 798-800.

Binkley, S., Mosher, K., and Reilly, K. B. (1983). Circadian rhythms in house sparrows: lighting *ad lib*. Physiol. Behav., **31**(6), 829-837.

Binkley, S., Mosher, K., and White, B. H. (1985). Circadian rhythm in pineal N-acetyltransferase activity: phase shifting by dark pulses (III). J. Neurochem., **45**(3), 875-878.

Binkley, S., Mosher, K., and White, B. H. (1989). Photoperiod modifies daily maps of light and dark sensitivity for N-acrtyltransferase activity in pineal glands of 3-week old *Gallus domesticus*. J. Comp. Physiol. B, **159**(1), 37-42.

Binkley, S., Macbride, S., Klein, D. C., and Ralph, C. (1973). Pineal enzymes: regulation of avian melatonin synthesis. Science, **181**, 273-275.

Blask, D. E., and Nodelman, J. L. (1980). An interaction between the pineal gland and olfactory deprivation in potentiating the effects of melatonin on gonads, accessory sex organs, and prolactin in male rats. J. Neurosci. Res., **5**(2), 129-136.

Bobek, S., Niezgoda, J., Pietras, M., Zacinska, M., and Ewy, Z. (1980). The effect of acute cold and warm ambient temperatures on the thyroid hormone concentration in blood plasma, blood supply, and oxygen consumption in Japanese quail. Gen. Comp. Endocrinol., **40**(2), 201-210.

Boiko, N. E., Vorob'eva, O. A., Grigor'ian, R. A., and Kornienko, G. G. (2004). Dynamics of thyroid hormones during the early stages in the development of the sturgeon *Acipenser guldenstadti*. Zh. Evol. Biokhim. Fiziol., **40**(2), 142-146.

Bolliet, V., Ali, M. A., Anctil, M., and Zachmann, A. (1993). Melatonin secretion *in vitro* from the pineal complex of the lamprey *Petromyzon marinus*. Gen. Comp. Endocrinol., **89**(1), 101-106.

Bolliet, V., Begay, V., Taragnat, C., Ravault, J. P., Collin, J. P., and Falcon, J. (1997). Photoreceptor cells of the pike pineal organ as cellular circadian oscillators. Eur. J. Neurosci., **9**(4), 643-653.

Bona-Gallo, A., Licht, P., MacKenzie, D. S., and Lofts, B. (1980). Annual cycles in levels of pituitary and plasma gonadotropin, gonadal steriods, and thyroid activity in the Chinese cobra (*Naja naja*). Gen. Comp. Endocrinol., **42**(4), 477-493.

Bondarenko, L. A. (1991). Effects of excess and deficiency of thyroid hormones in the body upon blood melatonin in pubertal male rats. Biull. Eksp. Biol. Med., **111**(6), 590-591.

Borg, B., and Ekstrom, P. (1981). Gonadal effects of melatonin in the three-spined stickleback, *Gasterosteus aculeatus* L., during different seasons and photoperiods. Reprod. Nutr. Dev., **21**(6), 919-927.

Brandstatter, R. (2003). Encoding of day and time of year by the avain circadian system. J. Neuroendocrinol., **15**(4), 398-404.

Bridges, C. R. (1993). Adaptation of vertebrates to the inter-tidal environment. In "The Vertebrate Gas Transport Cascade- Adaptation and Environment and Mode of Live" (J. E. P. W. Bicudo, ed.), pp.12-22. Boca Raton, F.L. C R C Press.

Bronstein, D. M., Haak, K. A., Torres, G., and Lytle, L. D. (1990). Light-induced changes in pineal gland N-acetyltransferase activity: developmental aspects. Neuroendocrinology, **51**(2), 139-146.

Brzezinska-Slebodzinska, E. (2003). Influence of hypothyroidism on lipid peroxidation, erythrocyte resistance and antioxidant plasma properties in rabbits. Acta. Vet. Hung., **51**(3), 343-351.

Buijs, R. M., and Kalsbeek, A. (2001). Hyppothalamic integration of central and peripheral clocks. Mat. Rev. Neurosci., **2**, 521-526.

Buzzell, G. R., Chen, Z., Vaughan, M. K., and Reiter, R. J. (1989). Effects of inhibition of thyroid function and of cold on melatonin synthesis and porphyrin content in the Harderian glands of male Syrian hamsters, *Mesocricetus auratus*. Comp. Biochem. Physiol. A, **94**(3), 427-429.

Cahill, G. M. (2002). Clock mechanisms in zebrafish. Cell Tissue Res., **309**, 27-34.

Cardinali, D. P., Ritta, M. N., Fuentes, A. M., Gimeno, M. F., and Gimeno, A. L. (1980). Prostaglandin E release by rat medial basal hypothalamus *in vitro*. Inhibition by melatonin at submicromolar concentrations. Eur. J. Pharmacol., **67**(1), 151-153.

Cardinali, D. P., Vacas, M. I., Keller Sarmiento, M. I., Etchegoyen, G. S., Pereyra, E. N., and Chuluyan, H. E. (1987). Neuroendocrine integrative mechanisms in mammalian pineal gland: effects of steroid and adenohypophysial hormones on melatonin synthesis *in vitro*. J. Steroid Biochem., **27**(1-3), 565-571.

Carter, D. S., and Goldman, B. D. (1983). Antigonadal effects of timed melatonin infusion in pinealectomised male djungarian hamsters (*Phodopus sungorus sungorus*); duration is the critical parameter. Endocrinology, **113**, 1261-1267.

Cassone, V. M., Lane, R. F., and Menaker, M. (1983). Daily rhythms of serotonin metabolism in the medial hypothalamus of the chicken: effects of pinealectomy and exogenous melatonin. Brain Res., **289**(1-2),129-134.

Catala, M. D., Quay, W. B., Vibat, C. R. T., and Timiras, P. S. (1987). Effect of hypothyroidism and rehabilitation on day and night melatonin levels in pineal, hypothalamus and serum of male rats. Neuroendocr. Lett., **9**, 378-388.

Catala, M. D., Quay, W. B., and Timiras, P. S. (1988). Effects of thyroid hormone on light/dark melatonin synthesis and release by young and maturing rat pineal glands *in vitro*. Int. J. Devl. Neurosci., **6**, 285-288.

Cerda-Reverter, J. M., Zanuy, S., Carrillo, M., and Kah, O. (1996). Development of enzyme immunoassays for 3,5,3'-triiodo-L-thyronine and L-thyroxine: time-course studies on the effect of food deprivation on plasma thyroid hormones in two marine teleosts, sea bass (*Dicentrarchus labrax* L.) and sea bream (*Sparus aurata* L.). Gen. Comp. Endocrinol., **103**(3), 290-300.

Cermakian, N., Whitmore, D., Foulkes, N. S., and Sassoni-Corsi, P. (2000). Asynchronous oscillations of two zebrafish CLOCK partners reveal differential clock control and function. Proc. Natl. Acad. Sci. USA, **97**, 4339-4344.

Chabot, C. C., and Menaker, M. (1992). Circadian feeding and locomotor rhythms in pigeons and house sparrows. J. Biol. Rhythms, **7**(4), 287-299.

Chakraborti, P., and Bhattacharya, S. (1984). Plasma thyroxine levels in freshwater perch: influence of season, gonadotropins, and gonadal hormones. Gen. Comp. Endocrinol., **53**(2), 179-186.

Chakravorty, K., Sharma, K. K., and Bhatt, D. (1985). Control of seasonal reproduction in tropical weaver bird. In "The Endocrine System and the Environment" (B. K. Follett, S. Ishii, and A. Chandola, eds.), pp. 23-32. Japan Sci. Soc., Press Tokyo/Springer-Verlag, Berlin.

Challet, E., Poirel, V. J., Malan, A., and Pevet, P. (2003). Light exposure during daytime modulates expression of *Per1* and *Per2* clock genes in the suprachiasmatic nuclei of mice. J. Neurosci. Res., **72**(5), 629-637.

Champier, J., Jouvet, A., Rey, C., Guyotat, J., and Fevre-Montange, M. (2003). Differential somatostatin receptor subtype expression in human normal pineal gland and pineal parenchymal tumors. Cell Mol. Neurobiol., **23**(1), 101-113.

Champney, T. H. (2001). Reductions in hamster serum thyroxine levels by melatonin are not altered by changes in serum testosterone. Gen. Comp. Endocrinol., **123**(2), 121-126.

Champney, T. H., Webb, S. M., Richardson, B. A., and Reiter, R. J. (1985). Hormonal modulation of cyclic melatonin production in the pineal gland of rats and Syrian hamsters: effects of thyroidectomy or thyroxine implant. Chronobiol. Intl., **2**(3), 177-183.

Charron, G., Souloumiac, J., Fournier, M. C., and Canivenc, R. (1991). Pineal rhythm of N-acetyltransferase activity and melatonin in the male badger, *Meles meles* L, under natural daylight: relationship with the photoperiod. J. Pineal Res., **11**(2), 80-85.

Chen W. J., Lin, K. H., Lai, Y. J., Yang, S. H., and Pang, J. H. (2004). Protective effect of propylthiouracil independent of its hypothyroid effect on atherogenesis in cholesterol-fed rabbits: PTEN induction and inhibition of vascular smooth muscle cell proliferation and migration. Circulation, **110**(10), 1313-1319.

Claus, R., and Weiler, U. (1985). Influence of light and photoperiodicity on pig prolificacy. J. Reprod. Fertil. Suppl., **33**, 185-197.

Cockrem, J. F. (1991). Plasma melatonin in the Adelie penguin (*Pygoscelis adeliae*) under continuous daylight in Antartica. J. Pineal Res., **10**, 2-8.

Cogburn, L. A., and Harrison, P. C. (1980). Adrenal, thyroid, and rectal temperature responses of pinealectomized cockerels to different ambient temperatures. Poultry Science, **59**, 1132-1141.

Collin, A., Buyse, J., van As, P., Darras, V. M., Malheiros, R. D., Moraes, V. M., Reyns, G. E., Taouis, M., and Decuypere, E. (2003). Cold-induced enhancement of avian uncoupling protein expression, heat production, and triiodothyronine concentrations in broiler chicks. Gen. Comp. Endocrinol., **130**(1), 70-77.

Coon, S. L., Begay, V., Falcon, J., Klein, D. C. (1998). Expression of melatonin synthesis genes is controlled by a circadian clock in the pike pineal organ but not in the trout. Biol. Cell, **90**(5), 399-405.

Coon, S. L., Begay, V., Deurloo, D., Falcon, J., and Klein D. C. (1999). Two arylalkylamine-N-acetyltransferase genes mediate melatonin synthesis in fish. J. Biol. Chem., **274**(13), 9076-9082.

Cossins, A. R., and Bowler, K. (1987). Temperature Biology of Animals. Chapman and Hall, New York.

Cyr, D. G., and Eales, J. G. (1986). The effects of sodium ipodate (ORAgrafin) on thyroid function in rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol., **63**(1), 86-92.

Cyr, D. G., Bromage, N. R., Duston, J., and Eales, J. G. (1988). Seasonal patterns in serum levels of thyroid hormones and sex steroids in relation to photoperiod-induced changes in spawning time in rainbow trout, *Salmo gardneri*. Gen. Comp. Endocrinol., **69**, 217-225.

Cyr, D. G., Idler, D. R., Audet, C., McLeese, J. M., and Eales, J. G. (1998). Effects of long-term temperature acclimation on thyroid hormone deiodinase function, plasma thyroid hormone levels, growth, and reproductive status of male Atlantic cod, *Gadus morhua*. Gen. Comp. Endocrinol., **109**, 24-36.

Das, K., and Chainy, G. B. (2004). Thyroid hormone influences antioxidanr defense system in adult rat brain. Neurochem. Res., **29**(9), 1755-1766.

Dawson, A., and Thapliyal, J. P. (2001). The thyroid and photoperiodism. In "Avian endocrinology" (A. Dawson, and C. M. Chaturvedi, eds.), pp. 141-151. Narosa Publishing House, New Delhi, India.

Dawson, A., and Van't, H. T. (2002). Ontogeny of the daily profile of plasma melatonin in European starlings raised under long or short photoperiods. J. Biol. Rhythms, **17**(3), 259-265.

Dawson, A., King, V. M., Bentley, G. E., and Ball, G. F. (2001). Photoperiodic control of seasonality in birds. J. Biol. Rhythms, **16**(4), 365-380.

Deguchi T. (1979). A circadian oscillator in cultured cells of chicken pineal gland. Nature, **282**(5734), 94-96.

Deguchi, T. (1981). *Rhodopsin-like photosensitivity of isolated chicken pineal gland.* Nature, **290**(5808), 706-707.

Deguchi, T., and Axelrod, J. (1972). Sensitive assay for serotonin N-acetyltransferase activity in rat pineal. Anal. Biochem., **50**(1), 174-179.

Delgado, M. J., and Vivien-Roels, B. (1989). Effects of environmental temperature and photoperiod on the melatonin levels in the pineal, lateral eye, and plasma of the Frog, *Rana perezi*: Importance of ocular melatonin. Gen. Comp. Endocrinol., **75**, 46-53.

Demeneix, B. A., and Henderson, N. E. (1978). Serum T_4 and T_3 in active and torpid ground squirrels, *Spermophilus richardsoni*. Gen. Comp. Endocrinol., **35**(1), 77-85.

d'Istria, M., Momteleone, P., Serino, I., and Chieffi, G. (1994). Seasonal varations in the daily rhythm of melatonin and NAT activity in the Harderian gland, retina, pineal gland and serum of the green frog, *Rana esculenta*. Gen. Comp. Endocrinol., **96**, 6-11.

Doi, O., Koyama, E., Nakamura, T., and Tanabe, Y. (1983). Photoperodic regulation of serotonin N-acetyltransferase activity in the pineal gland of the chicken. Comp. Biochem. Physiol. A, **74**(2), 195-198.

Doi, J., Ohtsubo, A., Ohtsuka, A., and Hayashi, K. (2003). Triidothyronine but not thyroxine accelerates myofibrillar proteolysis via ATP production in cultured muscle cells. Biosci. Biotechnol. Biochem., **67**(11), 2451-2454.

Eales, J.G. (1979). Thyroid functions in cyclostomes and fishes. In "Hormones and Evolution" (E. J. W. Barrington, ed.), Vol. 1, pp. 341-436. Academic Press, New York.

Eales, J. G., Ranson, M., Shostak, S., and Primeau, D. (1986). Effects of catecholamines on plasma thyroid hormone levels in arctic charr, *Salvelinus alpinus*. Gen. Comp. Endocrinol., **63**, 393-399.

Eales, J. G., Chang, J. P., van der Kraak, G., Omeljaniuk, R. J., and Uin, L. (1982). Effects of temperature on plasma thyroxine and iodide kinetics in rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol., **47**(3), 295-307.

Eddy, J. M. (1969). Metamorphosis and the pineal complex in the brook lamprey, *Lampetra planeri*. J. Endocrinol., **44**(3), 451-452.

Engel, L., Vollrath, L., and Spessert, R. (2004a). Arylalkylamine N-acetyltransferase gene expression in retina and pineal gland of rats under various photoperiods. Biochem. Biophys. Res. Commun., **318**(4), 983-986.

Engel, L., Mathes, A., Schwerdtle, I., Heinrich, B., Pogorzelski, B., Holthues, H., Vollrath, L., and Spessert, R. (2004b). Rat pineal arylalkylamine-N-acetyltrans-ferase: cyclic AMP inducibility of its gene depends on prior entrained photoperiod. Mol. Brain Res., **123**(1-2), 45-55.

Engel, L., Gupta, B. B. P., Lorenzkowski, V., Heinrich, B., Schwerdtle, I., Gerhold, S., Holthues, H., Vollrath, L., and Spessert, R. (2005). Fos related antigen 2 (Fra-2) memorizes photoperiod in the rat pineal gland. Neuroscience, **132** (2), 511-518.

Esquifino, A., Agrasal, C., Velazquez, E., Villanua, M. A., and Cardinali, D. P. (1997). Effect of melatonin on serum cholesterol and phospholipid levels, and on prolactin, thyroid-stimulating hormone and thyroid hormone levels, in hyperprolactinemic rats. Life Sci., **61**(11), 1051-1058.

Falcon, J., Marmillon, J. B., Claustrat, B., and Collin, J. P. (1989). Regulation of melatonin secretion in a photoreceptive pineal organ: an *in vitro* study in the pike. J. Neurosci., **9**(6), 1943-1950.

Falcon, J., Galarneau, K. M., Weller,, J. L., Ron, B., Chen, G., Coon, S. L., and Klein, D. C. (2001). Regulation of arylalkylamine N-acetyltransferase-2 (AANAT2, EC 2.3.1.87) in the fish pineal organ: evidence for a role of proteasomal proteolysis. Endocrinology, **142**(5), 1804-1813.

Falcon, J., Gothilf, Y., Coon, S. L., Boeuf, G., and Klein, D. C. (2003). Genetic, temporal and developmental differences between melatonin rhythm generating systems in the teleost fish pineal organ and retina. J. Neuroendocrinol., **15**(4), 378-382.

Ferreira, A. C., de Carvalho, L., Rosenthal, D., and de Carvalho, D. P. (2003). Thyroid Ca^{2+}/NADPH-dependent H_2O_2 generation is partially inhibited by propylthiouracil and methimazole. Euro. J. Biochem., **270**(11), 2363-2368.

Firth, B. T., and Kennaway, D. J. (1989). Thermoperoid and photoperiod interact to affect the phase of the plasma melatonin rhythm in the lizard, *Tiliqua rugosa*. Neurosci. Lett., **106** (1-2), 125-130.

Firth, B. T, Kennaway, D. J., and Belan, I. (1991). Thermoperiodic influences on plasma melatonin rhythms in the lizard *Tiliqua rugosa*; Effect of thermophase duration. Neurosci. Lett., **121**(1-2), 139-142.

Fleury, F., and Naulleau, G. (1987). Relations between hibernation and the resumption of endocrine, testicular and thyroid activities, in *Vipera aspis* L. (Reptilia, Viperidae). Gen. Comp. Endocrinol., **68**(2), 271-277.

Forsell, J., Ekstrom, P., Flamarique, I. N., and Holmqvist, B. (2001). Expression of pineal ultraviolet-and green-like opsins in the pineal organ and retina of teleosts. J. Expt. Biol., **204**, 2517-2525.

Foulkes, N. S., Borjegin, J., Snyder, S. H., and Sassone-Corsi, P. (1996). Transcriptional control of circadian hormone synthesis via the CREM feedback loop. Proc. Acad. Sci. U. S. A, **93**, 14140-14145.

Fowler, P. A. (1988). Seasonal endocrine cycles in the European hedgehog, *Erinaceus europaeus*. J. Reprod. Fertil., **84**(1), 259-272.

Gamse, J. T., Shen, Y. C., Thisse, C., Thisse, B., Raymond, P. A., Halpern, M. E., and Liang, J. O. (2002). Otx5 regulates genes that show circadian expression in the zebrafish pineal complex. Nat. Genet., **30**(1), 117-121.

Gancedo, B., Alonso-Gomez, A. L., de Pedro, N., Delgado, M. J., and Alonso-Bedate, M. (1996). Daily changes in thyroid activity in the frog *Rana perezi*: variation with season. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol., **114**(1), 79-87.

Garcia-Allegue, R., Madrid, J. A., and Sanchez-Vazquez, F. J. (2001). Melatonin rhythms in European sea bass plasma and eye: influence of seasonal photoperiod and water temperature. J. Pineal Res., **31**(1), 68-75.

Garcia, A., Landete-Castillejos, T., Zarazaga, L., Garde, J., and Gallego, L. (2003). Seasonal changes in melatonin concentrations in female Iberian red deer (*Cervus elaphus hispanicus*). J. Pineal Res., **34**(3), 161-166.

Garg, S. K. (1989). Effect of pinealectomy, eye enucleation, and melatonin treatment on ovarian activity and vitillogenin levels in the catfish exposed to short photoperiod or long photoperiod. J. Pineal. Res., 7(2), 91-104.

Garg, S. K., and Sundararaj, B. I. (1986). Role of pineal in the regulation of some aspects of circadian rhythmicity in the catfish, *Heteropneustes fossilis* (Bloch). Chronobiologia, **13** (1), 1-11.

Gastel, J. A., Roseboom, P. H., Rinaldi, P. A., Weller, J. L., and Klein, D. C. (1998). Melatonin production: proteasomal proteolysis in serotonin N-acetyltransferase regulation. Science, **279**(5355), 1358-1360.

Gekakis, N., Staknis, D., Nguyen, H. B., Davis, F. C., Wilsbacher, L. D., King, D. P., Takahashi, J. S., and Weitz, C. J. (1998). Role of the CLOCK protein in the mammalian circadian mechanism. Science, **280,** 1564–1569.

Germain, D. L. (1988). The effects and interactions of substrates, inhibitors, and the cellular thiol-disulfide balance on the regulation of type II iodothyronine 5'-deiodenase. Endocrinology, **122**(5), 1860-1868.

Gern, W. A., and Norris, D. O. (1979). Plasma melatonin in the neotenic tiger salamander (*Ambystoma tigrinum*): effects of photoperiod and pinealectomy. Gen. Comp. Endocrinol., **38**(4), 393-398.

Gern, W. A., and Greenhouse, S. S. (1988). Examination of *in vitro* melatonin secretion from superfused trout (*Salmo gairdneri*) pineal organs maintained under diel illumination or continuous darkness. Gen. Comp. Endocrinol., **71**(1), 163-174.

Gillette, M. U., and Mitchell, J. W. (2002). Signalling in the suprachaismatic nucleus: selectively responsive and integrative. Cell Tissues Res., **309**, 99-107.

Goldman, B. D., and Darrow, J. M. (1983). The pineal gland and mammalian photoperiodism. Prog. Neuroendocrinol., **37**(5), 386-396.

Gomez, J. M., Boujard, T., Boeuf, G., Solari, A., and Le Bail, P. Y. (1997). Individual diurnal plasma profiles of thyroid hormones in rainbow trout (*Oncorhynchus mykiss*) in relation to cortisol, growth hormone, and growth rate. Gen. Comp. Endocrinol., **107**(1), 74-83.

Gothilf, Y., Coon, S. L., Toyama, R., Chitnis, A., Namboodiri, M. A., and Klein, D. C. (1999). Zebrafish serotonin N-acetyltransferase-2: marker for development of pineal photoreceptors and circadian clock function. Endocrinology, **140**(10), 4895-4903.

Gothilf, Y., Toyama, R., Coon, S. L., Du, J. S., Dawid, I. B., and Klein, D. C. (2002). Pineal-specific expression of green fluorescent protein under the control of the serotonin-N-acetyltransferase gene regulatory regions in transgenic zebrafish. Dev. Dyn., **225**(3), 241-249.

Gower, B. A., Nagy, T. R., and Stetson, M. H. (1996). Influence of photoperiod, time, and sex on hormone concentrations in collared lemmings (*Dicrostonyx groenlandicus*). Gen. Comp. Endocrinol., **101**(1), 53-62.

Graham, L. H., Swanson, W. F., Wildt, D. E., and Brown, J. L. (2004). Influence of oral melatonin on natural and gonadotropin-induced ovarian function in the domestic cat. Theriogenology, **61**(6), 1061-1076.

Grau, E. G., Brown, C. L., and Stetson, M. H. (1985). Photoperoidic regulation of thyroid responsiveness to TSH in *Fundulus heteroclitus*. J. Exp. Zool., **234**, 199-205.

Grechez-Cassiau, A., Panda, S., Lachoche, S., Teboul, M., Azmi, S., Laudet, V., Hogensch, J. B., Taneja, R., and Delaunay, F. (2004). The transcriptional repressor STRA13 regulates a subset of peripheral circadian outputs. J. Biol. Chem., **279**, 1141-1150.

Guerrero, J. M., and Reiter, R. J. (1992). Iodothyronine 5'-deiodenating activity in the pineal gland. Int. J Biochem., **24**(10), 1513-1523.

Guerrero, J. M., Santana, C., and Reiter, R. J. (1988a). Effect of isoproterenol and dibutyl cyclic AMP on thyroxine type II 5'-deiodenase and N-acetyltransferase activities in rat pineal organ cultures. Neurosci. Lett., **89**(2), 229-233.

Guerrero, J. M., Puig-Domingo, M., Santana, C., Menendez-Pelaez, A., Gonzalez-Brito, A., and Reiter, R. J. (1988b). Differential responses of rat pineal thyroxine type II 5'-deiodenase and N-acetyltransferase activities to either light exposure, isoproterenol, phenylephrine, or propanolol. Cell Mol. Neurobiol., **8**(4), 447-458.

Guerrero, H. Y., Caceres, G., Paiva, C. L., and Marceno, G. (1990a). Hypothalamic and telencephalic catecholamines content in the brain of teleost fish, *Pygocentrus notolus*, during the annual reproductive cycle. Gen. Comp. Endocrinol., **80**, 257-263.

Guerrero, J. M., Santana, C., and Reiter, R. J. (1990b). Type II thyroxine 5'-deiodinase activity in the rat brown adipose tissue, pineal gland, harderian gland, and cerebral cortex: effect of acute cold exposure and lack of relationship to pineal melatonin synthesis. J. Pineal Res., **9**(2), 159-166.

Guillaumond, F., Sage, D., Deperz, P., Bosler, O., Becquet, D., and Francois-Bellan, A. M. (2000). Circadain binding activity of AP-1, a regulator of the arylalkylmine N-acetyltransferase gens in the rat pineal gland, depends on circadian Fra-2, c-jun, and Jun-D expression and is regulated by the clock's zietgebers. J. Neurochem., **75**, 1398-1407.

Gupta, B. B. P., and Thapliyal, J. P. (1991). Endocrine regulation of the oxidative metabolism in poikilothermic vertebrates. Zool. Sci., **8**, 625-634.

Gupta, B. B. P., and Premabati, Y. (2002a). Differential effects of melatonin on plasma levels of thyroxine and triiodothyronine levels in the air-breathing fish, *Clarias gariepinus* during breeding and quiescent periods. Gen. Comp. Endocrinol., **129**, 146-151.

Gupta, B. B. P., and Premabati, Y. (2002b). Fish Pineal: Structure, Function and Regulation. In "Treatise on Pineal Gland" (C. Haldar, M. Singaravel, and S. K. Maitra, eds.), pp. 77-102. Science Publishers, Inc., Enfield (NH), U.S.A.

Gupta, B. B. P., Spessert, R., and Vollrath, L. (2005). Molecular components and mechanism of adrenergic signal transduction in mammalian pineal gland: Regulation of melatonin synthesis. India J. Exp. Biol., **43**, 115-149.

Gupta, B. B. P., Spessert, R., Rimoldi, S., and Vollrath, L. (2001). Sulfhydryl G proteins and phospholipase A2-associated G proteins are involved in adrenergic signal transduction in the rat pineal gland. Gen. Comp. Endocrinol., **122**, 320-328.

Gupta, Y. K., Gupta, M., and Kohli, K. (2003). Neuroprotective role of melatonin in oxidative stress vulnerable brain. Indian J. Physiol. Pharmacol., **47**(4), 373-386.

Gutjarh, G. H., van Rensburg, L. J., Malpaux, B., Richter, T. A., and Bennett, N. C. (2004). The endogenous rhythm of plasma melatonin and its regulation by light in the high veld mole-rat (*Cryptomys hottentotus pretoriae*): a microphthalmic, seasonally breeding rodent. J. Pineal Res., **37**(3), 185-192.

Gwinner, E. (1989). Melatonin in the circadian system of birds: model of internal resonance. In "Circadian clocks and ecology", (T. Hiroshige, K. Honma, eds). pp. 127-145. Hokkaido Univ. Press, Sappora, Japan.

Gwinner, E., Hau, M., and Heigl, S. (1997). Melatonin: generation and modulation of avian circadian rhythms. Brain Res. Bull., **44**(4), 439-444.

Hamm, H. E., Takahashi, J. S., and Menaker, M. (1983). Light induced decrease of serotonin N-acetyltransferase activity and melatonin in the chicken pineal gland and retina. Brain Res., **266** (2), 287-294.

Hanew, K., Shiino, M., and Rennels, E. G. (1980). Effect of indoles, AVT, oxytocin, and AVP on prolactin secretion in rat pituitary clonal (2B8) cells. Proc. Soc. Exp. Biol. Med., **164**(3), 257-261.

Harvey, S., and Klandorf, H. (1983). Reduced adrenocortical function and increased thyroid function in fasted and refed chickens. J. Endocrinol., **98** (1), 129-135.

Harvey, S., Davidson, T. F., Klandorf, H., and Philips, J. G. (1980). Diurnal changes in the plasma concentrations of thyroxine and triiodothyronine and their binding to plasma protein in the domestic chick (*Anas platyrhynchos*). Gen. Comp. Endocrinol., **42**, 500-504.

Hastings, M. H., and Follett, B. K. (2001). Toward a molecular biological calendar? J. Biol. Rhythms, **16**(4), 424-430.

Hau, M., Romero, L. M., Brawn, J. D., and Van't Hof, T. J. (2002). Effect of polar day on plasma profiles of melatonin, testosterone, and estradiol in high-Arctic *Lapland Longspurs*. Gen. Comp. Endocrinol., **126**(1), 101-112.

Hayashi, K., and Okatani, Y. (1999). Mechanisms underlying the effects of estrogen on nocturnal melatonin synthesis in the peripubertal female rats: relation to norepinephrine and adenylate cyclase. J. Pineal Res., **26**, 178-183.

Hazel, J. R. (1993). Thermal biology. In "The physiology of fishes". (D. H. Evans, ed.), pp. 427-467. Boca Raton. F. L. C R C Press.

Heldmaier, G., Klaus, S., Wesinger, H., Friedrich, U., and Wenzel, M. (1989). In "Living in the Cold II" (A. Malanand and B. Canguilhem, eds.), pp. 347-358. John Libbey Eurotext Ltd.

Herbute, S., Pintat, R., and Bayle, J. D. (1981). Nycthermeral variations of thyroxine levels in the quail: effect of ambient temperature. C. R. Scances Soc. Biol. Fil., **175**(4), 485-489.

Hernandez-Diaz, F. J., Sanchez, J. J., Abreu, P., Lopez-Coviella, I., Tabares, L., Prieto, L., and Alonso, R. (2001). Estrogen modulates alpha (1)/beta-adrenoceptor-induced signaling and melatonin production in female rat pinealocytes. Neuroendocrinology, **73**, 111-122.

Herzog, E. D., and Block, G. D. (1999). Keeping an eye on the retinal clocks. Chronobiol. Int., **16**(3), 229-247.

Hill, S. M., Spriggs, L. L., Lawson, N. O., and Harlan, R. E. (1996). Effects of melatonin on estrogen receptor expression in the forebrain of outbred (Lak.LVG) golden hamsters. Brain Res., **742**(1-2), 107-114.

Hirota, T., and Fukada, Y. (2004). Resetting mechanism of central and peripheral circadian clocks in mmammals. Zooligical Sci., **21**, 359-368.

Hofman, M. A. (2004). The brain's calendar: neural mechanisms of seasonal timing. Biol. Rev. Camb. Philos. Soc., **79**(1), 61-77.

Hoffmann, K., Illnerova, H., and Vanecek, J. (1981). Effect of photoperiod and of one minute light at night-time on the pineal rhythm on N-acetyltransferase activity in the Djungarian hamster *Phodopus sungorus*. Biol. Reprod., **24**(3), 551-556.

Hogenesch, J. B., Chan, W. K., Jackiw, V. H., Brown, R. C, Gu, Y. Z., Pray-Grant, M., Perdew, G. H., and Bradfield, C. A. (1997). Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. J. Biol. Chem., **272**(13), 8581-8593.

Holthues, H., and Vollrath, L. (2004). The phototransduction cascade in the isolated chick pineal gland revisited. Brain Res., **999**(2), 175-180.

Hoover, P. A., Vaughan, M. K., Little, J. C., and Reiter, R. J. (1992). N-methyl-D-aspartate does not prevent effects of melatonin on the reproductive and thyroid axes of male Syrian hamsters. J. Endocrinol., **133**(1), 51-58.

Hosaka, T., Mimuro, T., Hamada, N., Itoh, M. T., and Ishizuka, B. (2002). Stimulatory effects of LH on release of melatonin and activities of its synthesizing enzymes NAT and HIOMT in organ-cultured pineal glands of female rats. Horm. Metab. Res., **34**(8), 441-445.

Hulbert, A. J. (1985). A comparative study of thyroid function in reptiles and mammals. In "The endocrine system and the environment" (B. K. Follett, S. Ishii and A. Chandola, eds.), pp.105-115. Japan Sci. Soc. Press, Tokyo/Springer-verlag, Berlin.

Hulbert, A. J., and Williams, C. A. (1988). Thyroid function in a lizard, a tortoise and a crocodile, compared with mammals. Comp. Biochem. Physiol. A, **90**(1), 41-48.

Hulbert, A. J., Hinds, D. S., and MacMillen, R. E. (1985). Minimal metabolism, summit metabolism and plasma thyroxine in rodents from different environments. Comp. Biochem. Physiol. A, **81**(3), 687-693.

Humlova, M., and Illnerova, H. (1992). Resetting of the rat circadian clock after a shift in the light/dark cycle depends on the photoperiod. Neurosci. Res., **13**(2), 147-153.

Iigo, M., Kezuka, H., Aida, K., and Hanyu, I. (1991). Circadian rhythms of melatonin secretion from superfused goldfish (*Carassius auratus*) pineal glands *in vitro*. Gen. Comp. Endocrinol., **83**(1), 152-158.

Iigo, M., Hara, M., Ohtani-Kancko, R., Hirata, K., Tabata, M., and Aida, K. (1997). Photic and circadian regulations of melatonin rhythms in fishes. Biol. Signals, **6**, 225-232.

Iigo, M., Fujimoto, Y., Gunji-Suzuki, M., Yokosuka, M., Hara, M., Ohtani-Kaneko, R., Tabata, M., Aida, K., and Hirata, K. (2004). Circadian rhythms of melatonin release from the photoreceptor pineal organ of a teleosts, ayu (*Plecoglossus altivelis*) in flow – through culture. J. Neuroendocrinol., **16**(1), 45-51.

Ikeda, M., and Nomura, M. (1997). cDNA cloning and tissue-specific expression of a novel basic helix-loop-helix/PAS protein (BMAL1) and identification of alternatively spliced variants with alternative translation initiation site usage. Biochem. Biophys. Res. Commun., **233**(1), 258-264.

Illnerova, H., and Vanecek, J. (1979). Response of rat pineal serotonin N-acetyltransferase to one min light pulse at different night times. Brain Res., **167**(2), 431-434.

Illnerova, H., and Sumova, A. (1997). Photic entrainment of the mammalian rhythm in melatonin production. J. Biol. Rhythms, **12**(6), 547-555.

Illnerova, H., and Vanecek, J. (1985). Regulation of the circadian rhythm in pineal melatonin production. Physiol. Bohemoslov. Suppl., **34**, 57-61.

Illnerova, H., Vanecek, J., and Hoffmann, K. (1983). Regulation of the pineal melatonin concentration in the rat (*Rattus norvegicus*) and in the Djungarian hamster (*Phodopus sungorus*). Comp. Biochem. Physiol. A, **74**(1), 155-159.

Illnerova, H., Hoffmann, K., and Vanecek J. (1984). Adjustment of pineal melatonin and N-acetyltransferase rhythms to change from long to short photoperiod in the Djungarian hamster *Phodopus sungorus*. Neuroendocrinology, **38**(3), 226-231.

Illnerova, H., Vanecek, J., Krecek, J., Wetterberg, L., and Saaf, J. (1979). Effect of one minute exposure to light at night on rat pineal serotonin N-acetyltransferase and melatonin. J. Neurochem., **32**(2), 673-675.

Ishikawa, T., Hirayama, J., Kobayashi, Y., and Todo, T. (2002). Zebrafish CRY represses transcription mediated by CLOCK-BMAL heterodimer without inhibiting its binding to DNA. Genes Cells, **7**, 1073–1086.

Jacob, N., Vuillez, P., and Pevet, P. (1997). Photoperiod does not act on the suprachiasmatic nucleus photosensitive phase through the endogenous melatonin, in the Syrian hamster. Neurosci. Lett., **229**(2), 117-120.

John-Alder, H. B., and Bennett, A. F. (1987). Thermal adaptations in lizard muscle function. J. Comp. Physiol. B, **157**(2), 241-252.

John, T. M., Vishwanathan, M., George, J. C., and Scanes, C. G. (1990). Influence of chronic melatonin implantation on circulating levels of catecholamines, growth hormone, thyroid hormones, glucose, and free fatty acids in the pigeon. Gen. Comp. Endocrinol., **79**, 226-232.

Johnston, J. D. (2004). Photoperiodic regulation of prolactin secretion: changes in intra-pituitary signalling and lactotroph heterogeneity. J. Endocrinol., **180**(3), 351-356.

Joshi, B. N., and Udaykumar, K. (2000). Melatonin counteracts the stimulatory effects of blinding or exposure to red light on reproduction in the skipper frog *Rana cyanophlyctis*. Gen. Comp. Endocrinol., **118**(1), 90-95.

Joy, K. P., and Senthilkumaran, B. (1998). Annual and diurnal variations in, and effects of altered photoperiod and temperature, ovariectomy, and estradiol-17 beta replacement on catechol-O-methyltransferase level in brain regions of the catfish, *Heteropneustes fossilis*. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol., **119**(1), 37-44.

Jung, H., Choe, Y., Kim, H., Park, N., Son, G. H., Khang, I., and Kim, K. (2003). Involvement of CLOCK:BMAL 1 heterodimer in serum-responsive *mPer1* induction. Neuroreport, **14**, 15-19.

Kalsbeek, A., and Buijs, R. M. (2002). Output pathways of the mammalian suprachiasmatic nucleus: coding circadian time by transmitter selection and specific targeting. Cell Tissue Res., **309**, 109-118.

Kalsbeek, A., Fliers, E., Franke, A. N., Wortel, J., and Buijs, R. M. (2000). Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. Endocrinology, **141**(10), 3832-3841.

Kamis, A. B., and Robinson, G. A.,(1978). Serum T_3 and T_4 concentrations of Japanese quail treated with thyrotropin-releasing hormone. Gen. Comp. Endocrinol., **36**(4), 636-638.

Kanematsu, S., and Mikami, S. I. (1970). Effects of hypothalamic lesions on protein-bound 131-iodine and thyroidal ^{131}-I uptake in the chicken. Gen. Comp. Endocrinol., **14**(1), 25-34.

Karasek, M., and Stepien, H. (1980). Ultrastructure of the rat pineal gland after administration of triiodothyronine and thyroidectomy. Acta. Med. Pol., **21**(4), 357-358.

Karbownik, M., and Lewinski, A. (2003a). The role of oxidative stress in physiological and pathological processes in the thyroid gland: possible involvement in pineal-thyroid interactions. Neuro. Endocrinol. Lett., **24**(5), 293-303.

Karbownik, M., and Lewinski, A. (2003b). Melatonin reduces Fenton reaction-induced lipid peroxidation in porcine thyroid tissue. J. Cell Biochem., **90**(4), 806-811.

Karolczak, M., Burbach, G. J., Sties, G., Korf, H. W., and Stehle, J. H. (2004). Clock gene mRNA and protein rhythms in the pineal gland of mice. Eur. J. Neurosci., **19**(12), 3382-3388.

Karsch, F. J., Woodfill, C. C. I., Maplaux, B., Robinson, J. E., and Wayne, N. L. (1991). Melatonin and mammalian photoperiodism: synchronization of annual reproductive cycles. In "Suprachiasmatic nuleus: The mind's clock" (D. C. Klein, R. Y. Moore and S. M. Reppert, eds.), pp. 217-232. Oxford University Press.

Kato, H., Fu, Z., Kotera, N., Sugahara, K., and Kuba, T. (1999). Regulation of the expression of serotonin N-acrtyltransferase gene in Japanese quail (*Coturnia japonica*): I. Rhythmic pattern and effect of light. J. Pineal Res., **27**(1), 24-33.

Kavaliers, M. (1981). Circadian variations in the effects of cyclic nucleotides on the thermoregulatory behaviour of a teleost fish. Neuropharmacology, **20**(3), 293-296.

Kawamoto, T., Noshiro, M., Sato, F., Maemura, K., Takeda, N., Nagai, R., Iwata, T., Fujimoto, K., Furukawa, M., Miyazaki, K., Honma, S., Honma, K., and Kato, Y. (2004). A novel autofeedback loop of *Dec1* transcription involved in circadian rhythm regulation. Biochem. Biophys. Res. Commun., **313**, 117-124.

Kelley, M. W., Turner, J, K., and Reh, T. A. (1995). Ligands of steroid/thyroid receptors induce cone photoreceptors in vertebrate retina. Developmental, **121**, 3777-3785.

Kellner, M., Yassouridis, A., Manz, B., Steiger, A., Holsboer, F., and Wiedemann, K. (1997). Corticotropin-releasing hormone inhibits melatonin secretion in healthy volunteers- a potential link to low-melatonin syndrome in depression? Neuroendocrinology, **65**, 284-290.

Kezuka, H., Aida, K., and Honey, I. (1989). Melatonin secretion from goldfish pineal gland in organ culture. Gen. Comp. Endocrinol., **75**, 217-221.

Kezuka, H., Furukawa, K., Aida, K., and Hanyu, I. (1988). Daily cycles in plasma melatonin levels under long or short photoperiod in the common carp, *Cyprinus carpio*. Gen. Comp. Endocrinol., **72**(2), 296-302.

King, D. P., and Takahashi, J. S. (2000). Molecular genetics of circadian rhythms in mammals. Annu. Rev. Neurosci., **23**, 713-742.

King, D. B., King, C. R., and Eshleman, J. R. (1977). Serum triiodothyronine levels in the embryonic and post-hatching chicken, with particular reference to feeding-induced changes. Gen. Comp. Endocrinol., **31** (2), 216-223.

Klandorf, H., Sharp., P. J., and Duncan, I. J. (1978). Variations in levels of plasma thyroxine and triiodothyronine in juvenile female chickens during 24- and 16-hr lighting cycles. Gen. Comp. Endocrinol., **36** (2), 238-243.

Klandorf, H., Sharp., P. J., and Macleod, M. G. (1981). The relationship between heat production and concentrations of plasma thyroid hormones in the domestic hen. Gen. Comp. Endocrinol., **45**, 513-520.

Klein, D. C., and Weller, J. (1970). Indole metabolism in the pineal gland. A circadian rhythm in N-acetyltransferase activity. Science, **169**, 1093-1095.

Klein, D. C., and Voisin, P. (1999). Melatonin synthesis pathway: circadian regulation of the genes encoding the eye enzymes in the chicken pineal gland and retina. Reprod. Nutr. Dev., **39**(3), 325-334.

Klein, D. C., Smoot, R., Weller, J. L., Higa, S., Markey, S. P., Creed, G. J., and Jacobowitz, D. M. (1983). Lesions of the paraventricular nucleus area of the hypothalamus disrupt the suprachiasmatic leads to spinal cord circuit in the melatonin rhythm generating system. Brain Res. Bull., **10**(5), 647-652.

Klein, D. C., Coon, S. L., Roseboom, P. H., Weller, J. L., Bernard, M., Gastel, J. A., Zatz, M., Iuvone, P. M., Rodeiguez, I. R., Begay, V., Falcon, G. M., Cahill, V. M., Cassone, V. M., and Baler, R. (1997). The melatonin rhythm-generating enzyme: molecular regulation of serotonin-N-acetyltransferase in their at pineal gland, Rec. Prog. Horm. Res., **52**, 307-358.

Klemcke, H. G., Bartke, A., and Goldman, B.D. (1981). Plasma prolactin concentrations and testicular human chorionic gonadotropin binding sites during short photoperiod-induced testicular regression and recrudescence in the golden hamster. Biol. Reprod., **25**(3), 536-548.

Kohel, K. A., MacKenzie, D. S., Rostal, D. C., Grumbles, J. S., and Lance, V. A. (2001). Seasonality in plasma thyroxine in the desert tortoise, *Gopherus agassizii*. Gen. Comp. Endocrinol., **121**(2), 214-222.

Korf, H. W., Schomerus, C., and Stehle, J. H. (1998). The pineal organ, its hormone melatonin, and the photoneuroendocrine system. Adv. Anat. Embryol. Cell. Biol., **146**, 1-100.

Kose, K., Utas, S., Yazici, C., Akdas, A., and Kelestimur, F. (2001). Effect of propylthiouracil on adenosine deaminase activity and thyroid function in patients with psoriasis. Br. J. Dermatol., **144**(6), 1121-1126.

Kroeber, S., Meissl, H., Maronde, E., and Korf, H. W. (2000). Analyses of signal transduction cascades reveal an essential role of calcium ions for regulation of melatonin biosynthesis in the light-sensitive pineal organ of the rainbow trout (*Oncorhynchus mykiss*). J. Neurochem., **74**(6), 2478-2489.

Kuhn, E. R., and Nouwen, E. J. (1978). Serum levels of triiodothyronine and thyroxine in the domestic fowl following mild cold exposure and injection of synthetic thyrotropin-releasing hormone. Gen. Comp. Endocrinol., **34**, 336-342.

Kuhn, E. R., Delmotte, N. M., and Darras, V. M. (1983). Persistence of a circadian rhythmicity for thyroid hormones in plasma and thyroid of hibernating male *Rana ridibunda*. Gen. Comp. Endocrinol., **50**(3), 383-394.

Kuhn, E. R., Gevaerts, H., Vandorpe, G., and Jacobs, G. F. (1987). Plasma concentrations of testosterone and thyroxine in males of the giant swamp frog *Dicroglossus occipitalis* at the equator. Gen. Comp. Endocrinol., **68**(3), 492-493.

Kuhn, E. R., Darras, V. M., Gysemans, C., Decuypere, E., Berghman, L. R., and Buyse, J. (1996). The use of intermittent lighting in broiler raising. 2. Effects on the somatotrophic and thyroid axes and on plasma testosterone levels. Poult. Sci., **75**(5), 595-600.

Kukner, A. S., Kukner, A., Naziroglu, M., Colakoglu, N., Celebi, S., Yilmaz, T., and Aydemir, O. (2004). Protective effects of the intraperitoneal vitamin C, aprotinin and melatonin administration on retinal edema during experimental uveitis in the guinea pig. Cell Biochem. Funct., **22**(5), 299-305.

Kulczykowska, E., Sokolowska, E., Takvam, B., Stefansson, S., and Ebbesson, L. (2004). Influence of exogenous thyroxine on plasma melatonin in juvenile Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. B Biochem. Mol. Biol., **137**(1), 43-47.

Kusmic, C., Barsanti, L., Passarelli, V., and Gaultieri, P. (1993). Photoreceptor morphology and visual pigment content in the pineal organ and in the retina of the juvenile and adult trout *Salmo irideus*. Micron., **24**, 279-286.

Laidley, C. W., and Leatherland, J. F. (1988). Circadian studies of plasma cortisol, thyroid hormone, protein, glucose and ion concentration, liver glycogen concentration and liver and spleen weight in rainbow trout, *Salmo gairdneri* Richardson. Comp. Biochem. Physiol. A, **89**(3), 495-502.

Lakatua, D. J., Nicolau, G. Y., Bogdan, C., Petrescu, E., Sackett-Lundeen, L. L., Irvine, P. W., and Haus, E. (1984). Circadian endocrine time structure in humans above 80 years of age. J. Gerontol., **39**(6), 648-654.

Lal, P. (1987). Effect of pinealectomy on sexual and body weight cycles of migratory redheaded bunting, *Emberiza bruniceps*. Indian J. Exp. Biol., **25**, 439-441.

Larkin, J. E., Freeman, D. A., and Zucker, I. (2001). Low ambient temperature accelerates short-day responses in Siberian hamsters by altering responsiveness to melatonin. J. Biol. Rhythms, **16**(1), 76-86.

Larsen, R. P., Davies, T. F., Schlumberger, M. J., and Hay, I. D. (2003). Thyroid physiology and diagnostic evaluation of patients with thyroid disorders. In "Williams textbook of endocrinology" (R. P. Larsen, H. M. Kronenberg, S. Melmed, and K. S. Polonsky, eds.), pp. 331-373. Elsevier Science, U. S. A. Saunders.

Lea, R. W., Sharp, P. J., Klandorf, H., Harvey, S., Dunn, I. C., and Vowles, D. M. (1986). Seasonal changes in concentrations of plasma hormones in the male ring dove (*Streptopelia risoria*). J. Endocrinol., **108**(3), 385-391.

Leatherland, J. F. (1987). Thyroid response to ovine thyrotropin challenge in cortisol- and dexamethasone-treated rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., **86**(2), 383-387.

Lee, P. P., Allen, A. E., and Pang, S. F. (1990). Cold stress during scotophase elicited differential responses in quail pineal, retinal, and serum melatonin levels. Acta. Endocrinol. (Copenh)., **122**(4), 535-539.

Lee, J. H., Hung, C. F., Ho, C. C., Chang, S. H., Lai, Y. S., and Chung, J. G. (1997). Light-induced changes in frog pineal gland N-acetyltransferase activity. Neurochem. Int., **31**(4), 533-540.

Leiner, K. A., and Mackenzie, D. S. (2001). The effects of photoperiod on growth rate and circulating thyroid hormone levels in the red drum, *Sciaenops ocellatus*: evidence for a free-running circadian rhythm of T_4 secretion. Comp. Biochem. Physiol. Mol. Integr. Physiol., **130**, 141-149.

Leiner, K. A., and Mackenzie, D. S. (2003). Central regulation of thyroidal status in a teleost fish: nutrient stimulation of T_4 secretion and negative feedback of T_3. J. Exp. Zool. Part A Comp. Exp. Biol., **298**(1), 32-43.

Leiner, K. A., Han, G. S., and Mackenzie, D. S. (2000). The effect of photoperiod and feeding on the diurnal rhythm of circulating thyroid hormones in the red drum, *Sciaenops ocellatus*. Gen. Comp. Endocrinol., **120**, 88-98.

Leloup, J., and Fontaine, M. (1960). Iodine metabolism in lower vertebrates. Ann. N Y Acad. Sci., **86**, 316-353.

Leloup, J., and De-Luze, A. (1985). Environmental effects of temperature and salinity of thyroid function in teleost fishes. In "The Endocrine System and the Environment" (B. K. Follett, S. Ishii, and A. Chandola, eds.), pp. 23-32. Japan Sci. Soc. Press Tokyo/Springer-Verlag, Berlin.

Lema, S. C., and Nevitt, G. A. (2004). Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. J. Exp. Biol., **207**(19), 3317-3327.

Lemay, A., Brouillette, A., Denizeau, F., and Lavoie, M. (1979). Melatonin-and serotonin-stimulated release of vasopressin from rat neurohypophysis *in vitro*. Mol. Cell Endocrinol., **14**(2), 157-166.

Lena, P. J., and Subramanian, P. (2004). Effects of melatonin on the levels of antioxidants and lipid peroxidation products in rats treated with ammonium acetate. Pharmazie, **59**(8), 636-639.

Lewinski, A. (1986). Evidence for pineal gland inhibition of thyroid growth: Contribution to the hypothesis of a negative feedback between the thyroid and the pineal. Adv. Pineal Res., **1**, 167-176.

Lewinski, A., Wajs, E., and Karbownik, M. (1992). Effects of pineal-derived indolic compounds and of certain neuropeptides on the growth processes in the thyroid gland. Thyroidology, **4**(1), 11-15.

Liang, F. Q., Green, L., Wang, C., Alssadi, R., and Godley, B. F. (2004). Melatonin protects human retinal pigment epithelial (RPE) cells against oxidative stress. Exp. Eye Res., **78**(6), 1069-1075.

Licht, P., Breitenbach, G. L., and Congdon, J. D. (1985a). Seasonal cycles in testicular activity, gonadotropin, and thyroxine in the painted turtle, *Chrysemys picta*, under natural conditions. Gen. Comp. Endocrinol., **59**(1), 130-139.

Licht, P., Wood, J. F., and Wood, F. E. (1985b). Annual and diurnal cycles in plasma testosterone and thyroxine in the male green sea turtle *Chelonia mydas*. Gen. Comp. Endocrinol., **57**(3), 335-344.

Licht, P., Denver, R. J., and Pavgi, S. (1989). Temperature dependence of *in vitro* pituitary, testis, and thyroid secretion in a turtle, *Pseudemys scripta*. Gen. Comp. Endocrinol., **76**(2), 274-285.

Lincoln, G. A., Andersson, H., and Clarke, I. J. (2003a). Prolactin cycles in sheep under constant photoperiod: evidence that photorefractoriness develops within the pituitary gland independently of the prolactin output signal. Biol. Reprod., **69**(4), 1416-1423.

Lincoln, G. A., Andersson, H., and Hazlerigg, D. (2003b). Clock genes and the long-term regulation of prolactin secretion: evidence for a photoperiod/circannual timer in the pars tuberalis. J. Neuroendocrinol., **15**(4), 390-397.

Lincoln, G. A., Andersson, H., and Loudon, A. (2003c). Clock genes in calendar cells as the basis of annual timekeeping in mammals- a unifying hypothesis. J. Endocrinol., **179**(1), 1-13.

Luboshitzky, R., Lavi, S., Thuma, I., and Lavie, P. (1996a). Testosterone treatment alters melatonin concentrations in male patients with gonadotropin-releasing hormone deficiency. J. Clin. Endocrinol. Metab., **81**(2), 770-774.

Luboshitzky, R., Lavi, S., Thuma, I., and Lavie, P. (1996b). Nocturnal melatonin and luteinizing hormone rhythms in women with hyperprolactinemic amenorrhea. J. Pineal Res., **20**(2), 72-78.

Lutterschmidth, D. I., Lutterschmidth, W. I., Ford, N.B., and Hutchison, V. H. (2002). Behavioural thermoregulation and the role of melatonin in a nocturnal snake. Horm. Behav., **41**(1), 41-50.

Lynshiang, D. S., and Gupta, B. B. P. (2000). Role of thyroidal and testicular hormones in regulation of tissue respiration in the male air-breathing fish, *Clarias batrachus*. Indian J. Exp. Biol., **38**, 705-712.

Maeda, K., Mori, Y., Sawasaki, T., and Kano, Y. (1984). Diurnal changes in peripheral melatonin concentration in goats and effects of light or dark interruption. Nippon Juigaku Zasshi., **46**(6), 837-842.

Mahapatra, M. S., Mahata, S. K., and Maiti, B. R. (1988). Circadian rhythms and influence of light on serotonin, nor-epinephrine and epinephrine content in the pineal, paraphyseal complex of soft shelled turtles (*Lissemys punctata punctata*). Gen. Comp. Endocrinol., **71**(1), 183-188.

Mahapatra, M. S., Mahata, S. K., and Maiti, B. R. (1989). Effects of ambient temperature on serotonin, norepinephrine and epinephrine contents in the pineal, paraphyseal complex of soft shelled turtles (*Lissemys punctata punctata*). Gen. Comp. Endocrinol., **74**(2), 215-220.

Maitra, S. K., Huesgen, A., and Vollrath, L. (1986). The effects of short pulses of light at night on numbers of pineal "synaptic" ribbons and serotonin N-acetyltransferase activity in male Sprague-Dawley rats. Cell Tissue Res., **246**(1), 133-136.

Malpaux, B., Thiery, J. C., and Chemineau, P. (1999). Melatonin and the seasonal control of reproduction. Reprod. Nutr. Dev., **39**(3), 355-366.

Markowska, M., Bialicka, B., Ciechanowska, M., Koter, Z., Laskowska, H., Karkucinska-Wieckowska, A., and Skwarlo-Sonta, K. (2000). Effect of immunization on nocturnal NAT activity in chicken pineal gland. Neuro. Endocrinol. Lett., **21**(50), 367-373.

Maronde, E., Pfeffer, M., Olcese, J., Molina, C. A., Schlotter, F., Dehghani, F., Korf, H. W., and Stehle, J. H. (1999). Transcription factors in neuroendocrine regulation: rhythmic changes in pCREB and ICER levels frame melatonin synthesis. J. Neurosci., **19**, 3326-3336.

Martin, S. M., and Touitou, Y. (2000). DHEA-sulphate causes a phase-dependent increase in melatonin secretion: a study of perifused rat pineal glands. Steroids, **65**, 491-496.

Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Aida, K., and Tabata, M. (2003). Variations in plasma melatonin levels of the rainbow trout (*Oncorhynchus mykiss*) under various light and temperature conditions. Zoo. Sci., **20**(8), 1011-1016.

Matta, S. L., Vilela, D. A., Godinho, H. P., and franca, L. R. (2002). The goitrogen 6-n-propylthiouracil (PTU) given during testis development increases Sertoli and germ cell numbers per cyst in fish: the tilapia (*Oreochromis niloticus*) model. Endocrinology, **143**(3), 970-978.

Matty, A. J. (1985a). The thyroid gland. In "Fish Endocrinology", (A. J. Matty, ed.), pp. 54-83. Croom Helm, London.

Matty, A. J. (1985b). The corpuscles of stannous, urophysis and pineal. In "Fish Endocrinology", (A. J. Matty, ed.), pp. 174-197. Croom Helm, London.

Mazzoccoli, G., Giuliani, A., Carughi, S., De Cata, A., Puzzolante, F., La Viola, M., Urbano, N., Perfetto, F., and Tarquini, R. (2004). The hypothalamic-pituitary-thyroid axis and melatonin in humans: possible interactions in the control of body temperature. Neuro. Endocrinol. Lett., **25**(5), 368-372.

McArthur, A. J., Gillette, M. U., and Prosser, R. A. (1991). Melatonin directly resets the rat suprachiasmatic circadian clock *in vitro*. Brain Res., **565**, 158-161.

McCormick, S. D., Moriyama, S., and Bjornsson, B. T. (2000). Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. Am. J. Physiol-Reg. Integr. Comp. Physiol., **278**, 1352-1361.

Meissl, H. (1986). Photoneurophysiology of pinealocytes. In "Pineal and Retinal Relationships", (P. J. O'Brien and D. C. Klein, eds.), pp. 33-46. Academic Press, Orlando.

Meissl, H., and Ekstrom, P. (1988). Photoreceptor responses to light in the isolated pineal organ of the trout, *Salmo gairdneri*. Neurosci., **24**, 1071-1076.

Melamed, P., Eliahu, N., Levavi-Sivan, B., Ofir, M., Farchi-Pisanty, O., Rentier-Delrue, F., Smal, J., Yaron, Z., and Naor, Z. (1995). Hypothalamic and thyroidal regulation of growth hormone in tilapia. Gen. Comp. Endocrinol., **97**(1), 13-30.

Menendez-Pelaez, A., Buzzell, G. R., Nonaka, K. O., and Reiter, R. J. (1990). *In vivo* administration of isoproterenol or forskolin during the light phase induces increases in the melatonin content of the Syrian hamster pineal gland without a rise in N-acetyltransferase activity. Neurosci. Lett., **110**(3), 314-318.

Messager, S., Hazlerigg, D. G., Mercer, J. G., and Morgan, P. J. (2000). Photoperiod differentially regulates the expression of Per1 and ICER in the pars tuberalis and the suprachiasmatic nucleus of the Siberian hamster. Eur. J. Neurosci., **12**(8), 2865-2870.

Mitsui, S., Yamaguchi, S., Matsuo, T., Ishida, Y., and Okamura, H. (2001). Antagonistic role of E4BP4 and PAR proteins in the circadian oscillatory mechanism. Genes Dev., **15**, 995-1006.

Mizusawa, K., Iigo, M., Masuda, T., and Aida, K. (2000). Photic regulation of arylalkylamine-N-acetyltransferase 1 mRNA in trout retina. Neuroreport, **11**(16), 3473-3477.

Morita, Y., Samejima, M., and Tamotsu, S. (1989). Response patterns and neuronal networks of photosensory pineal organs. Arch. Histol. Cytol., **52**, 469-475.

Morton, D. J., and Forbes, H. J. (1988). Pineal gland N-acetyltranferase and hydroxyindole-O-methytransferase activity in the rainbow trout (*Salmo gairdneri*): seasonal variation link to photoperiod. Neurosci. Lett., **94**(3), 333-337.

Moyer, R. W. Firth, B. T., and Kennaway, D. J. (1997). Effect of variable temperatures, darkness and light on the secretion of melatonin by pineal explants in the Gecko, *Christinus marmoratus*. Brain Res., **747**(2), 230-235.

Murakami, M., Hosoi, Y., Negeshi, T., Kamiya, Y., Ogiwara, T., Mizuma, H., Yamada, M., Iriuchijima, T., and Mori, M. (1997). Expression and nocturnal increase of type II iodothyronine deiodenase mRNA in rat pineal gland. Neurosci. Lett., **227**(1), 65-67.

Murakami, M., Kawano, T., Nakahara, K., Nasu, T., and Shiota, K. (2001). Effect of melatonin on circadian rhythm, locomotor activity and body temperature in the intact house sparrow, Japanese quail and owl. Brain Res., **889**(1-2), 220-224.

Mustonen, A. M., Nieminen, P., Hyvarinen, H., and Asikainen, J. (2000). Exogenous melatonin elevates the plasma leptin and thyroxine concentrations of the mink (*Mustela vison*). Z. Naturforsch. C, **55**(9-10), 806-813.

Mylonas, C. C., Scott, A. P., and Zohar, Y. (1997). Plasma gonadotropin II, sex steroids, and thyroid hormones in wild striped bass (*Morone saxatilis*) during spermiation and final oocyte maturation. Gen. Comp. Endocrinol., **108**(2), 223-236.

Nagy, T. R., Gower, B. A., and Stetson, M. H. (1993). Threshold photoperiods for the induction of short day traits in collared lemmings (*Dicrostonyx groenlandicus*). J. Exp. Zool., **267**(1), 57-66.

Nagy, T. R., Gower, B. A., and Stetson, M. H. (1994). Response of collared lemmings to melatonin: I. Implants and photoperiod. J. Pineal Res., **17**(4), 177-184.

Nakashima, K., Ohtsuka, A., and Hayashi, K. (1998). Effects of thyroid hormones on myofibrillar proteolysis and activities of calpain, proteasome, and cathepsin in primary cultured chick muscle cells. J. Nutr. Sci. Vitaminol., **44**(6), 799-807.

Narula, L., and Saxena, R. N. (1981). Variation in hypothalamic LHRH, pituitary and plasma LH in relation to testis cycle of the male Indian weaver bird, *Ploceus philippinus*. Indian J. Exp. Biol., **19**, 995-997.

Natesan, A., Geetha, L., and Zatz, M. (2002). Rhythm and soul in avian pineal. Cell Tiss. Res., **309**, 35-45.

Naulleau, G., Fleury, F., and Boissin, J. (1987). Annual cycles in plasma testosterone and thyroxine in the male aspic viper *Vipera aspis* L., (Reptilia, Viperidae), in relation to the sexual cycle and hibernation. Gen. Comp. Endocrinol., **65**(2), 254-263.

Nayak, P. K., and Singh, T. P. (1987a). Effect of melatonin and 5-methoxytryptamine on sex steroids and thyroid hormones during the prespawning phase of the annual reproductive cycle in the freshwater teleosts, *Clarias batrachus*. J. Pineal Res., **4**(4), 377-386.

Nayak, P. K., and Singh, T. P. (1987b). Effect of pinealectomy on thyroid hormone (T_4 and T_3) levels in plasma during annual reproductive cycle in the freshwater catfish, *Clarias batrachus*. J. Pineal Res., **4**(4), 387-394.

Nayak, P. K., and Singh, T. P. (1991). Seasonal and diurnal changes of thyroid hormone (T_4 and T_3) in tropical female catfish, *Clarias batrachus* (Linn.), Zoologi. Schejahrbuchev., **95**(1), 51-60.

Nelson, W., Tong, Y. L., Lee, J. K., and Halberg, F. (1979). Methods for cosinor-rhythmometry. Chronobiologia, **6**, 305-323.

Nelson, B. D., Mutvei, A., and Joste, V. (1984). Regulation of biosynthesis of the rat liver inner mitochondrial membrane by thyroid hormone. Arch. Biochem. Biophys., **228**, 41-48.

Ng, T. B. (1987). Effects of pineal indoles on corticosterone and aldosterone production by isolated rat adrenal cells. Biochem. Int., **14**(4), 635-641.

Ng, L., Hurley, J. B., Dierks, B., Srinivas, M., Salto, C., Vennstrom, B., Reh, T. A., and Forrest, D. (2001). A thyroid hormone receptor that is required for the development of green cone photoreceptors. Nat. Genet., **27**(1), 94-98.

Nicholls, T. J., Goldsmith, A. R., and Dawson, A. (1988). Photorefractoriness in birds and comparison with mammals. Physiol. Rev., **68**, 133-176.

Nuesslein-Hildesheim, B., O'Brien, J. A., Ebling, F. J., Maywood, E. S., and Hastings, M. H. (2000). The circadian cycle of mPER clock gene products in the suprachiasmatic nucleus of the siberian hamster encodes both daily and seasonal time. Eur. J. Neurosci., **12**(8), 2856-2864.

Ohshima, K., Hirai, S., and Hiramatsu, K. (1999). Seasonal variations in serotonin immunoreactivity and ultra structure in the pineal organ of the Japanese grass lizard, with special reference to environmemtal temperature. Tissue Cell, **31**(4), 441-450.

Ohta, Y., Kongo, M., and Kishikawa, T. (2003). Therapeutic effect of melatonin on cholestatic liver injury in rats with bile duct ligation. Adv. Exp. Med. Biol., **527**, 559-565.

Oishi, T., and Konishi, T. (1978). Effects of photoperiod and temperature on testicular and thyroid activity of the Japanese quail. Gen. Comp. Endocrinol., **36**(2), 250-254.

Oishi, K., Sakamoto, K., Okada, T., Nagase, T., and Ishida, N. (1998). Antiphase circadian expression between BMAL1 and period homologue mRNA in the suprachias-

matic nucleus and peripheral tissues of rats. Biochem. Biophys. Res. Commun., **253**(2), 199-203.

Okatani, Y., Watanabe, K., Morioka, N., Hayashi, K., and Sagara, Y. (1997). Nocturnal changes in the pineal melatonin synthesis during puberty: relation to estrogen and progesterone levels in female rats. J. Pineal. Res., **22**, 33-41.

Okatani, Y., Morioka, N., and Hayashi, K. (1999). Changes in nocturnal pineal melatonin synthesis during the perimenopausal period: relation to estrogen levels in rat. J. Pineal. Res., **27**, 65-72.

Okatani, Y., Morioka, N., and Wakatsuki, A. (2000). Changes in nocturnal pineal melatonin secretion in perimenopausal women: correlation with endogenous estrogen concentrations. J. Pineal. Res., **28**, 111-118.

Okimoto, D. K., and Stetson, M. H. (1999). Properties of the melatonin-generating system of the sailfin molly, *Poecilia velifera*. Gen. Comp. Endocrinol., **114**(2), 293-303.

Omeljaniuk, R. J., and Eales, J. G. (1986). The effect of 3,5,3'-triiodo-thyronine on gill Na^+/K^+-ATPase of rainbow trout *Salmo gairdneri*, in fresh water. Comp. Biochem. Physiol. A. **84**(3), 427-429.

Onur, R., Semercioz, A., Orhan, I., and Yekeler, H. (2004). The effects of melatonin and the anti oxidant defence system on apoptosis regulator proteins (Bax and Bcl-2) in experimentally induced varicocele. Urol. Res., **32**(3), 204-208.

Osei, P., Robbins, K. R., and Shirley, H. V. (1989). Effects of exogenous melatonin on growth and energy metabolism of chickens. Nutrition Research, **9**, 69-81.

Osol, J. G., Foss, D. C., and Carew, L. B. (1980). Pinealectomy and light environ-ment effects on testicular and comb development in the 46-day-old broiler cockerel. Poult. Sci., **59**(4), 918-920.

O'Steen, S., and Janzen, F. J. (1999). Embryonic temperature affects metabolic compensation and thyroid hormones in hatchling snapping turtles. Physiol. Biochem. Zool., **72**(5), 520-533.

Oster, H., Werner, C., Magnone, M. C., Mayser, H., Feil, R., Seeliger, M. W., and Hofmann, F., and Albrecht, U. (2003). cGMP-dependent protein kinase II modulates *mPer1* and *mPer2* gene induction and influences phase shifts of the circadian clock. Curr. Biol., **13**, 725-733.

Osuna, C., Orta, J. M., Rubio, A., Molinero, P., and Guerrero, J. M. (1993a). Thyroxine type II 5'-deiodenase activity in pineal and Harderian glands is enhanced by

hypothyroidism but is independent of serum thyroxine concentrations during hyper-thyroidism. Int. J. Biochem., **25**(7), 1041-1046.

Osuna, C., Rubio, A., and Guerrero, J. M. (1993b). Potentiating effect of phenylephrine on isoproterenol activation of thyroxine type II deiodenase in the pineal gland of adult rats. Experientia, **49**(4), 329-331.

Ottenweller, J. E., and Hedge, G. A. (1982a). Diurnal variations of plasma thyrotropin, thyroxine and triiodinethyronine in female rats are phase shifted after inversion of the photoperiod. Endocrinology, **111**, 509-514.

Ottenweller, J. E., and Hedge, G. A. (1982b). Thyrotropin-like immunoreactivity in the pituitary and three brain regions of the female rat: diurnal variations and the effect of thyroidectomy. Endocrinology, **111**, 515-521.

Özturk, G., Coskun, S., Erbas, D., and Hasanoglu, E. (2000). The effect of melatonin on liver superoxide dismutase activity, serum nitrate and thyroid hormone levels. Jpn. J. Physiol., **50**, 149-153.

Pavlidis, M., Greenwood, L., Paalavo, M., Molsa, H., and Laitinen, J. T. (1999). The effect of photoperiod on diel rhythms in serum melatonin, cortisol, glucose, and electrolytes in the common dentex, *Dentex dentex*. Gen. Comp. Endocrinol., **113**, 240-250.

Pavlidis, M., Greenwood, L., Mourot, B., Kokkari, C., Le Menn, F., Divanach, P., and Scott, A. P. (2000). Seasonal variations and maturity stages in relation to differences in serum levels of gonadal steroids, vitellogenin, and thyroid hormones in the common dentex (*Dentex dentex*). Gen. Comp. Endocrinol., **118**, 14-25.

Perreau-Lenz, S., Kalsbeek, A., Garidou, M. L., Wortel, J., Van Der Vliet, J., Van Heijningen, C., Simmonneaux, V., Pevet, P., and Buijs, R. M. (2003). Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. Eur. J. Neurosci., **17**, 221-228.

Perreau-Lenz, S., Pevet, P., Buijs, R. M., and Kalsbeek, A. (2004). The biological clock: the bodyguard of temporal homeostasis. Chronobiol. Int., **21**(1), 1-25.

Perrier, H., Perrier, C., Peres, G., and Gras, J. (1979). Immediate effects of thermal shocks on the plasma level of various componemts in the rainbow trout: compound indicating stress and protein fraction. Rev. Can. Biol., **38**(1), 37-41.

Peter, M. C. S., and Oommen, O. V. (1989). Oxidative metabolism in a teleost, *Anabas testudineus* Bloch: effect of thyroid hormones on hepatic enzyme activities. Gen. Comp. Endocrinol., **73**(1), 96-107.

Peter, M. C. S., Lock, R. A., and Bonga, S. E. W. (2000). Evidence for an osmoregulatory role of thyroid hormones in the fresh water Masambique tilapia, *Oreochromis mossambicus*. Gen. Comp. Endocrinol., **120**, 157-167.

Pevet, P. (2000). Melatonin and biological rhythms. Biol. Signals Recept., **9**(3-4), 203-212.

Pevet, P., Bothorel, B., Slotten, H., and Saboureau, M. (2002). The chronobiotic properties of melatonin. Cell Tissue Res., **309**(1), 183-191.

Plisetskaya, E., Woo N. Y., and Murat, J. C. (1983). Thyroid hormones in cyclo-stomes and fish and their role in regulation of intermediary metabolism. Comp. Biochem. Physiol. A, **74**(2), 179-187.

Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high Artic summer. Physiol. Behav., **67**, 327-337.

Pohl, H. (2000). Circadian control of migratory restlessness and the effects of exogenous melatonin in the brambling, *Fringilla montifringilla*. Chronobiol. Int., **17**(4), 471-488.

Power, D. M., Llewellyn, L., Faustino, M., Nowell, M. A., Bjornsson, B. T., Einarsdottir, I. E., Canario, A. V., and Sweeney, G. E. (2001). Thyroid hormones in growth and development of fish. Comp. Biochem. Physiol., **130** (C), 447-459.

Prakash, P., Laloraya, M., and Kumar, P. (1998). Influence of a melatonin implant on the free radical load in avain thyroid and its relation with thyroid hormonogenesis. Biochem. Mol. Biol. Int., **46**, 1249-1258.

Preitner, N., Damiola, F., Lopez-Molina, L., Zakany, J., Duboule, D., Albrecht, U., and Schibler, U. (2002). The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell, **110**, 251-260.

Privat, K., Ravault, J. P., Chesneau, D., and Fevre-Montange, M. (1999). Day/night variation of tryptophan hydroxylase and serotonin N-acetyltransferase mRNA levels in the ovine pineal gland and retina. J. Pineal Res., **26** (4), 193-203.

Puig- Domingo, M., Guerrero, J. M., Reiter, R. J., Tannenbaum, M. J., Hurlbut, E. C., Gonzalez- Brito, A., and Santana, C. (1988). Thyroxine 5'- deiodination in brown adipose tissue and pineal gland: implications for thermogenic regulation and role of melatonin. Endocrinology, **123**, 677-680.

Randall, C. F., Bromage, N. R., Thrope, J. E., Miles, M. S., and Muir, J. S. (1995). Melatonin rhythms in Atlantic salmon (*Salmo salar*) maintained under natural and out-of-phase photoperiods. Gen. Comp. Endocrinol., **98**, 73-86.

Redins, C. A., Novaes, J. C., and Torres, K.B. (1999). The effects of testosterone on the mice pinealocytes: a quantitative study. Tissue Cell, **31**(2), 233-239.

Redman, J., Armstrong, S., and Ng, K. T. (1983). Free-running activity rhythmsin the rat: entrainment by melatonin. Science, **219**, 1089-1091.

Regard, E., Taurog, A., and Nakashima, T. (1978). Plasma thyroxine and triiodothyronine levels in spontaneously metamorphosing *Rana catesbeina* tadpoles and in adult anuran amphibia. Endocrinology, **102**(3), 674-684.

Reierth, E., and Stokkan, K. A. (1998). Activity rhythm in high Artic Syalbard ptarmigan (*Lagopus mutus hyperbereus*). Can. J. Zool., **76**, 2031-2039.

Reiter, R. J. (1980). The pineal and its hormones in the control of reproduction in mammals. Endocrine Rev., **1**, 109-131.

Reiter, R. J. (1993a). The melatonin rhythm: both a clock and a calendar. Experientia, **49**, 654-664.

Reiter, R. J. (1993b). Interactions of the pineal hormone melatonin with oxygen-centered free radicals: a brief review. Braz. J. Med. Biol. Res., **26** (11), 1141-1155.

Reiter, R. J., and Maestroni, G. J. (1999). Melatonin in relation to the antioxidative defense and immune systems: possible implications for cell and organ transplantation. J. Mol. Med., **77**(1), 36-39.

Reiter, R. J., Richardson, B. A., and King, T. S. (1983). The pineal gland and its indole product: Their importance in the control of reporduction in mammals. In "The pineal gland" (R. Relkin, ed.), pp. 151-200. Elsevier Science Publishing Co. Inc.

Reiter, R. J., Melchiorri, D., Sewerynek, E., Poeggeler, B., Barlow-Walden, L., Chuang, J., Ortiz, G. G., and Acuna-Castroviejo, D. (1995). A review of the evidence supporting melatonin's role as an antioxidant. J. Pineal Res., **18**(1), 1-11.

Reiter, R. J., Tan, D. X., Manchester, L. C., Lopez-Burillo, S., Sainz, R. M., and Mayo, J. C. (2003). Melatonin: detoxification of oxygen and nitrogen-bases toxic reactants. Adv. Exp. Med. Biol., **527**, 539-548.

Reiter, R. J., Tan, D. X., Sainz, R. M., Mayo, J. C., Leon, J., Manchester, L. C., Vijaya-laxmi, Kilic, E., and Kilic, U. (2004). Pharmachological utility of melatonin in reducing oxidative cellular and molecular damage. Pol. J. Pharmacol., **56**(2), 159-170.

Rekasi, Z., and Czompoly, T. (2002). Accumulation of rat pineal serotonin N-acetyltransferase mRNA induced by pituitary adenylate cyclase activating polypeptide and vasoactive intestinal peptide *in vitro*. J. Mol. Endocrinol., **28**(1), 19-31.

Relkin, R. (1983). Pineal-hormonal interactions. In "The pineal gland" (R. Relkin, ed.), pp. 225-246. Elsevier Science Publishing Co. Inc.

Renden, J. A., Lien, R. J., Oates, S. S., and Bilgili, S. F. (1994). Plasma concentra-tions of corticosterone and thyroid hormones in broilers provided various lighting schedules. Poult. Sci., **73**(1), 186-193.

Rensing, L., and Ruoff, P. (2002). Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. Chronobiol. Int., **19**(5), 807-864.

Reppert, S. M., and Weaver, D. R. (2002). Coordination of circadian timing in mammals. Nature, **418**, 935-941.

Reuss, S., Mattern, E., Spessert, R., Riemann, R., Weber, A., and Vollrath, L. (1993). Lack of effect of oxytocin on the numbers of "synaptic" ribbons, cyclic guanosine monophosphate and serotonin N-acetyltransferase activity in organ-cultured pineals of three strains of rats. Cell Tissue Res., **274**(2), 337-342.

Ribelayga, C., Garidou, M. L., Malan, A., Gauer, F., Calgari, C., Pevet, P., and Simonneaux, V. (1999). Photoperiodic control of the rat pineal arylalkylamine-N-acetyltransferase and hydroxyindole-O-methyltransferase gene expression and its effect on melatonin synthesis. J. Biol. Rhythms, **14**(2), 105-115.

Rintamaki, H., Reijonen, H., and Hissa, R. (1985). The effect of pinealectomy on plasma levels of thyroid hormones in pigeons reared under different photoperiods. Comp. Biochem. Physiol. A, **82**(1), 97-100.

Robertson, L. M., and Takahashi, J. S. (1988). Circadian clock in cell culture: II. *In vitro* photic entrainment of melatonin oscillation from dissociated chick pineal cells. J. Neurosci., **8**(1), 22-30.

Roche, J. F., Foster, D. L., Karsch, F. J., and Dziuk, P. J. (1970). Effect of castration and infusion of melatonin on levels of luteinizing hormone in sera and pituitaries of ewes. Endocrinology, **87**(6), 1205-1210.

Rom-Bugoslavskaia, E. S., and Shcherbakova, V S. (1985).Comparative experimental study of the effect of melatonin and 5-methoxytryptamine on the thyroid gland of rats. Farmakol. Toksikol., **48**(5), 84-89.

Rookh, H. V., Azukizawa, M., DiStefano, J. J., Ogihara, T., and Hershman, J. M. (1979). Pituitary-thyroid hormone periodicities in serially sampled plasma of unanesthetized rats. Endocrinology, **104**(4), 851-856.

Rose, M. F., and Rose, S. R. (1998). Melatonin accelerates metamorphosis in *Xenopus laevis*. J. Pineal Res., **24**(2), 90-95.

Ross, M. G., Leake, R. D., Stegner, H., Ervin, G., and Fisher, D. A. (1985). Oxytocin release induced by melatonin in the ewe. Dev. Pharmacol. Ther., **8**(4), 254-259.

Rudeen, P. K., Creighton, J., Bylund, D. B., Petterborg, L. J., and Paredez, S. (1990). Ontogeny of light-induced decrease of N-acetyltransferase activity in explanted chick pineal glands. J. Pineal Res., **8(2)**, 153-158.

Ruzsas, L., and Mess, B. (1987). The role of the pineal body in the adaptive reactions of the thyroid gland and possible involvement of the habenular complex. Adv. Pineal Res., **2**, 155-169.

Saito, D., Shi, Q., Ando, H., and Urano, A. (2004). Attenuation of diurnal rhythms in plasms levels of melatonin and cortisol, and hypothalamic contents of vasotocin and isotocin mRNAs in pre-spawning chum salmon. Gen. Comp. Endocrinol., **137**(1), 62-68.

Sakamoto, S., Nakamura, K., Inoue, K., and Sakai, T. (2000). Melatonin stimulates thyroid-stimulating hormone accumulation in the thyrotropes of the rat pars tuberalis. Histochem. Cell Biol., **114**(3), 213-218.

Samejima, M., Tamotsu, S., Uchida, K., Moriguchi, Y., and Morita, Y. (1997). Melatonin excretion rhythms in the cultured pineal organ of the lamprey, *Lampetra japonica*. Biol. Signals, **6**(4-6), 241-246.

Samejima, M., Shavali, S., Tamotsu, S., Uchida, K., Morita, Y., and Fukada, A. (2000). Light and temperature-dependence of the melatonin secretion rhythm in the pineal organ of the lamprey, *Lampetra japonica*. Jpn. J. Physiol., **50**(4), 437-442.

Sangoram, A. M., Saez, L., Antoch, M. P., Gekakis, N., Staknis, D., Whiteley, A., Fruechte, E. M., Vitaterna, M. H., Shimomura, K., King, D. P., Young, M. W., Weitz, C. J., and Takahashi, J. S. (1998). Mammalian circadian autoregulatory loop: a timeless ortholog and mPer1 interact and negatively regulate CLOCK-BMAL1-induced transcription. Neuron, **21**(5), 1101-1113.

Sarkar, S., Sarkar, N. K., Bhattacharyya, S., and Das, P. (1997). Melatonin action on thyroid activity in the soft-shelled turtle, *Lissemys punctata punctata*. Folia Biol., **45**, 109-112.

Saxena, R. N., Malhotra, L., Kant, R., and Baweja, P. K. (1979). Effect of pinealectomy and seasonal changes on pineal antigonadotropic activity of male Indian weaver bird, *Ploceus philippinus*. Indian J. Exp. Biol., **17**, 732-735.

Sayan, H., Haktan Ozacmak, V., Ozen, O. A., Coskun, O., Oktay Arslan, S., Cem Sezen, S., and Gulhan Aktas, R. (2004). Beneficial effects of melatonin on reperfusion injury in rat sciatic nerve. J. Pineal Res., **37**(3), 143-148.

Sayer, M. D. J., and Davenport, J. (1991). Amphibians fish. why do they leave water? Rev. Fish Biol. Fisheries, **1**, 159-181.

Sharp, P. J., and Klandorf, H. (1985). Environmental and physiological factors controlling thyroid function in Galliformes. In "The Endocrine System and the Environment" (B. K. Follett, S. Ishii, and A. Chandola, eds.), pp. 175-188. Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.

Sharp, P. J., Klandorf, H., and Lea, R. W. (1984). Influence of lighting cycles on daily rhythms in concentrations of plasma triiodothyronine and thyroxine in intact and pinealectomized immature broiler hens (*Gallus domesticus*). J. Endocrinol., **103**, 337-345.

Shavali, S. S., and Haldar, C. (1998). Effects of continuous light, continuous darkness and pinealectomy on pineal-thyroid-gonadal axis of the female Indian palm squirrel, *Funambulus pennanti*. J. Neural Transm., **105**(4-5), 407-413.

Shchervakova, V. S., and Rom-Bugoslavskaia, E. S. (1988). Features of the response of the rat thyroid gland to melatonin in pinealectomized rats. Probl. Endokrinol., **34**(5), 75-78.

Shearman, L. P., and Weaver, D. R. (1999). Photic induction of *Period* gene expression is reduced in *Clock* mutant mice. Neuroreport, **10**, 613-618.

Siguenza, A. F., Recio, J. M., and Agapito, M. T. (1988). Radioimmunoassay for melatonin and its application to fowl pineal research. Rev. Esp. Fisiol., **44**(2), 221-226.

Silverin, B., Viebke, P. A, Westin, J., and Scanes, C. G. (1989). Seasonal changes in body weight, fat depots, and plasma levels of thyroxine and growth hormone in free-living great tits (*Parus major*) and willow tits (*P. montanus*). Gen. Comp. Endocrinol., **73**(3), 404-416.

Simonneaux, V., Poirel, V. J., Garidou, M. L., Nguyen, D., Diaz-Rodriguez, E., and Pevet, P. (2004). Daily rhythm and regulation of clock gene expression in the rat pineal gland. Mol. Brain Res., **120**(2), 164-172.

Singh, D., and Turner, C. S. (1972). Effect of melatonin upon thyroid hormone secretion rate in female hamsters and male rats. Acta endocr. (Hh)., **69**, 35-40.

Singh, U. P., Krishna, A., and Bhatnagar, K. P. (2002). Seasonal changes in thyroid activity in the female sheath-tailed bat, *Taphozous longimanus* (Chiroptera: Emballonuridae). Acta Biol. Hung., **53**(3), 267-278.

Skene, D. J., Vivien-Roels, B., and Pevet, P. (1989). Pineal 5-methoxytryptophol rhythms in the box turtle: effect of photoperiod and environmental temperature. Neurosci. Lett., **98**(1), 69-73.

Skene, D. J., Pevet, P., Vivien-Roels, B., Masson-Pevet, M., and Arendt, J. (1987). Effects of different photoperiod on concentrations of 5-methoxytryptophol and melatonin in the pineal gland of the Syrian hamster. J. Endocrinol., **114**, 301-309.

Smith, K. A., Schoen, M. W., and Czeisler, C. A. (2004). Adaptation of human pineal melatonin suppression by recent photic history. J. Clin. Endocrinol. Metab., **89**(7), 3610-3614.

Snedecor, G. W. (1961). Statistical methods, Pacific Private Ltd., Bombay, India.

Snyder, P. J., and Utiger, R. D. (1972). Inhibition of thyrotropin response to thyrotropin-releasing hormone by small quantities of thyroid hormones. J. Clin. Invest., **51**(8), 2077-2084.

Song, C. K., Bartness, T. J., Petersen, S. L., and Bittman, E. L. (1999). SCN cells expressing mt1 receptor mRNA coexpress AVP mRNA in Syrian and Siberian hamsters. Adv. Exp. Med. Biol., **460**, 229-232.

Soszynski, P., Zgliczynski, S., and Pucilowska, J. (1988). The circadian rhythm of melatonin in hypothyroidism and hyperthyroidism. Acta Endocrinol. (Copenh)., **119**(2), 240-244.

Soybir, G., Topuzlu, C., Odabas, O., Dolay, K., Bilir, A., and Koksoy, F. (2003). The effcts of melatonin on angiogenesis and wound healing. Surg. Today, **33**(12), 896-901.

Spessert, R., Rapp, M., Jastrow, H., Karabul, N., Blum, F., and Vollrath, L. (2000). A differential role of CREB phosphorylation in cAMP-inducible gene expression in the rat pineal. Brain Res., **864**(2), 270-280.

Spieler, R. E. (1990). Its role in clinical medicine, general biology and agriculture. In "Chronobiology". Part B, pp. 905-920.

Stehle, J. H., von Gall, C., and Korf, H. W. (2003). Melatonin: A clock-output, a clock-input. J. Neuroendocrinol., **15**, 383-389.

Stehle, J. H., von Gall, C., Schomerus, C., and Korf, H. W. (2001). Of rodents and ungulates and melatonin: creating a uniform code for darkness by different signaling mechanisms. J. Biol. Rhythms, **16**(4), 312-325.

Stehle, J. H., foulkes, N. S., Molina, C. A., Simonneaux, V., Pevet, P., and Sassone-corsi, P. (1993). Adrenergic signals direct rhythmic expression of transcriptional repressor CREM in the pineal gland. Nature, **365**, 314-320.

Steinlechner, S., Buchberger, A., and Heldmaier, G. (1987). Circadian rhythms of pineal N-acethyltransferase activity in the Djungarian hamster, *Phodupus sungorus*, in response to seasonal changes of natural photoperiod. J. Comp. Physiol. A, **160**(5), 593-597.

Steinsapir, J., Harney, J., and Larsen, P. R. (1998). Type 2 iodothyronine deiodenase in rat pituitary tumor cells is inactivated in proteasomes. J. Clin. Invest., **102**(11), 1895-1899.

Steinsapir, J., Bianco, A. C., Buettner, C., Harney, J., and Larsen, P. R. (2000). Substrate-induced down-regulation of human type 2 deiodenase (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. Endocrinology, **141**(3), 1127-1135.

Stetson, M. H., and Watson-Whitmyre, M. (1986). Effects of exogenous and endogenous melatonin on gonadal function in hamsters. J. Neural Transm. Suppl., **21**, 55-80.

Stetson, M. H., Watson-Whitmyre, M., and Matt, K. S. (1977). Termination of photorefractoriness in golden hamsters-photoperiodic requirements. J. Exp. Zool., **202**(1), 81-88.

Stieglitz, A., Steinlechner, S., Ruf, T., and Heldmaier, G. (1991). Cold prevents the light induced inactivation of pineal N-acetyltransferase in the Djungarian hamster, *Phodopus sungorus*. J. Comp. Physiol. A, **168**(5), 599-603.

Stokkan, K. A., Nonaka, K. O., Lerchl, A., Vaughan, M. K., and Reiter, R. J. (1991). Low temperature stimulates pineal activity in Syrian hamsters. J. Pineal Res., **10**(1), 43-48.

Sudhakumari, C. C., Haldar, C., and Senthilkumaran, B. (2001). Seasonal changes in adrenal and gonadal activity in the quail, *Perdicula asiatica*: involvement of the pineal gland. Comp. Biochem. Physiol. B Biochem. Mol. Biol., **128**(4), 793-804.

Sumova, A., Jac, M., Sladek, M., Sauman, I., and Illnerova, H. (2003). Clock gene daily profiles and their phase relationship in the rat suprachiasmatic nucleus are affected by photoperiod. J. Biol. Rhythms, **18**(2), 134-144.

Tabata, M., and Meissl, H. (1993). Thermal responses of acromatic ganglion cells in the photosensory pineal organ of rainbow trout, *Onchorhynchus mykiss*. Comp. Biochem. Physiol. A, **105**, 453-457.

Tabata, M., Minh-Nyo, M., and Oguri, M. (1988). Involvement of retinal and extraretinal photoreceptors in the mediation of nocturnal locomotor activity rhythms in the catfish, *Silurus asotus*. Exp. Biol., **47**(4), 219-225.

Takahashi, J. S., and Menaker, M. (1982). Role of suprachiasmatic nuclei in the circadian system of the house sparrow, *Passer domesticus*. J. Neurosci. Lett., **2**, 815-828.

Takahashi, J. S., Hamm, H., and Menaker, M. (1980). Circadian rhythms of melatonin release from individual superfused chicken pineal glands *in vitro*. Proc. Nat. Acad. Sci. USA, **77**, 2319-2322.

Takahashi, J. S., Murakami, N., Nikaido, S. S., Pratt, B. L., and Robertson, L. M. (1989). The avian pineal, a vertebrate model system of the circadian oscillator: cellular regulation of circadian rhythms by light, second messengers, and macromolecular synthesis. In "Recent Progress in Hormone Research", (J. Clark, ed.). Vol. 45, pp. 279-348. Academic Press.

Tannenbaum, M. G., Haigh, G. R., Vaughan, M. K., and Reiter, R. J. (1990). Effects of acute cold exposure at night on pineal N-acetyltransferase activity and melatonin content in white-footed mice, *Peromyscus leucopus*. Comp. Biochem. Physiol. A, **95**(3), 363-366.

Tannenbaum, M. G., Reiter, R. J., Vaughan, M. K., Troiani, M. E., and Gonzalez-Brito, A. (1988). Effects of short-term cold exposure on pineal biosynthetic function in rats. Cryobiology, **25**(3), 227-232.

Thapliyal, J. P. (1980). Thyroid in reptiles and birds. In "Hormones, Adaptation and Evolution" (S. Ishii, M. Wada and T. Hirang, eds.), pp. 241-250. Japan Sci. Soc. Press, Tokyo/ Springer-Verlag, Berlin.

Thapliyal, J. P. (1981). Endocrinology of avain reproduction. Proc. 68th session, Indian Sci. Cong. Assoc., Section: Zoology, Entomology and Fisheries, pp. 1-30.

Thapliyal, J. P., and Gupta, B. B. P. (1989). Reproductive cycles of birds. In "Reproductive cycles of Indian Vertebrates." (S. K. Saidapur, ed.), pp. 273-310. Alied Publishers, New Delhi.

Thomas, K. B., Brown, A. D., and Iuvone, P. M. (1998). Elevation of melatonin in chicken retina by 5-hydroxytryptophan: differential light/dark responses. Neuroreport, **9**(18), 4041-4044.

Tilden, A. R., and Hutchinson, V. H. (1993). Influence of photoperiod and temperature on serum melatonin in the diamondback water snake, *Nerodia rhombifera*. Gen. Comp. Endocrinol., **92**(3), 347-354.

Tischkau, S. A., Mitchell, J. W., Tyan, S. H., Buchanan, G. F., and Gillette, M. U. (2003a). $Ca2^{+}$/cAMP response element-binding protein (CREB)-dependent activation of *Per1* is required for light-induced signaling in the suprachiasmatic nucleus circadian clock. J. Biol. Chem., **278**, 718-723.

Tischkau, S. A., Weber, E. T., Abbott, S. M., Mitchell, J. W., and Gillette, M. U. (2003b). Circadian clock-controlled regulation of cAMP-protein kinase G in the nocturnal domain. J. Neurosci., **23**, 7543-7550.

Tŏpal, T., Oter, S., Corkmasz, A., Sadir, S., Metinyurt, G., Korkmazhan, E. T., Serdar, M. A., Bilgic, H., and Reiter, R. J. (2004). Exogenously administered and endogenously produced melatonin reduce hyperbaric oxygen-induced oxidative stress in rat lung. Life Sci., **75**(4), 461-467.

Torres, G., Haak, K. A., and Lytle, L. D. (1989). Catecholaminergic mechanisms mediate hypothermia-induced elevations in pineal gland N-acetyltransferase in neonatal rats. J. Pineal Res., **6**(1), 43-53.

Tosini, G., and Fukuhara, C. (2002). The mammalian retina as a clock. Cell Tissue Res., **309**(1), 119-126.

Tosini, G., and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in the green iguana. J. Comp. Physiol. A, **179**(1), 135-142.

Tournier, B. B., Menet, J. S., Dardente, H., Poirel, V. J., Malan, A., Masson-Pevet, M., Pevet, P., and Vuillez, P. (2003). Photoperiod differentially regulates clock genes' expression in the suprachiasmatic nucleus of Syrian hamster. Neuroscience, **118**(2), 317-322.

Tripathi, G., and Verma, P. (2003). Differential effects of thyroxine on metabolic enzymes and other macromolecules in a freshwater teleost. J. Exp. Zool. A Comp. Exp. Biol., **296**(2), 117-124.

Troiani, M. E., Reiter, R. J., Vaughan, M. K., Gonzalez-Brito, A., and Herbert, D. C. (1988). The depression in rat pineal melatonin production after saline injection at night may be elicited by corticosterone. Brain Res., **450**, 18-24.

Tsuboi. S., Kotani, Y., Ogawa, K., Hatanaka, T., Yatsushiro, S., Otsuka, M., and Moriyama, Y. (2002). An intramolecular disulfide bridge as a catalytic switch for serotonin N-acetyltransferase. J. Biol. Chem., **277**(46), 44229-44235.

Ueda, H., Hiroi, O., Hara, A., Yamauchi, K., and Nagahama, Y. (1984). Changes in serum concentrations of steroid hormones, thyroxine, and vitellogenin during spawning migration the chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol., **53**(2), 203-211.

Ueda, H. R., Chen, W., Adachi, A., Wakamatsu, H., Hayashi, S., Takasugi, T., Nagano, M., Nakahama, K., Suzuki, Y., Sugano, S., Iino, M., Shigeyoshi, Y., and Hashimoto, S. (2002). A transcription factor response element for gene expression during circadian night. Nature, **418**, 534-539.

Undeger, U., Giray, B., Zorlu, A. F., Oge, K., and Bacaran, N. (2004). Protective effects of melatonin on the ionizing radiation induced DNA damage in the rat brain. Exp. Toxicol. Pathol., **55**(5), 379-384.

Underwood, H. (1989). The pineal and melatonin: regulators of circadian function in lower vertebrates. Experientia, **45**(10), 914-922.

Underwood, H., and Siopes, T. (1985). Melatonin rhythms in quail: regulation of photoperiod and circadian parmeters. J. Pineal Res., **2**(2), 133-144.

Underwood, H., and Calaban, M. (1987). Pineal melatonin rhythms in the lizard *Anolis carolinensis*: I. Response to light and temperature cycles. J. Biol. rhythms, **2** (3), 179-193.

Underwood, H., Steel, C. T., and Zivkovic, B. (2001). Circadian organization and the role of the pineal in birds. Micorsc. Res. Tech., **53** (1), 48-62.

Underwood, H., Binkley, S., Siopes, T., and Mosher, K. (1984). Melatonin rhythms in the eyes, pineal bodies, and blood of Japanese quail (*Coturnix coturnix japonica*). Gen. Comp. Endocrinol., **56**(1), 70-81.

Vanecek, J., and Illnerova, H. (1979). Changes of a rhythm in rat pineal serotonin N-acetyltransferase following a one-minute light pulse at night. Prog.Brain Res., **52**, 245-248.

Vanecek, J., and Illnerova, H. (1982a). Effect of photoperiod on the growth of reproductive organs and on pineal N-acetyltransferase rhythm in male rats treated neonatally with testosterone propionate. Biol. Reprod., **27**(3), 517-522.

Vanecek, J., and Illnerova, H. (1982b). Effect of light at night on the pineal rhythm in N-acetyltransferase activity in the Syrian hamster *Mesocricetus auratus*. Experientia, **38**(4), 513-514.

Varghese, S., Shameena, B., and Oommen, O. V. (2001). Thyroid hormones regulate lipid peroxidation and antioxidant enzyme activities in *Anabas testudineus* (Bloch). Comp. Biochem. Physiol. B Biochem. Mol. Biol., **128**(1), 167-171.

Vaughan, G. M., and Pruitt, B. A. (1985). Pineal-induced depression of free thyroxine in Syrian hamsters. J. Pineal Res., **2**, 325-330.

Vaughan, G. M., Vaughan, M. K., Seraile, L. G., and Reiter, R. J. (1982). Thyroid hormones in male hamsters with activiated pineals or melatonin treatment. In "Proceedings of the Symposium on Pineal and its Hormones", (R. J. Reiter, ed.), pp. 187-196. Liss, New York.

Vaughan, M. K., Menendez-Pelaez, A., Buzzell, G. R., Vaughan, G. M., Little, J. C., and Reiter, R. J. (1994). Circadian rhythms in reproductive and thyroid hormones in gonadally regressed male hamsters exposed to natural autumn photoperiod and temperature conditions. Neuroendocrinology, **60**(1), 96-104.

Venditti, P., Balestrieri, M., Di Meo, S., and De Leo, T. (1997). Effect of thyroid state on lipid proxidation, antioxidant defences, and susceptibility to oxidative stress in rat tissues. J. Endocrinol., **155**(1), 151-157.

Venditti, P., Daniele, M. C., Masullo, P., and Di Meo, S. (1999). Antioxidant-sensitive triiodothyronine effects on characteristics of rat liver mitochondrial population. Cell Physiol. Biochem., **9**(1), 38-52.

Verma, K., Ldawa, T., Verma, A. K., and Alroel. (1996). Seasonal variations in testicular weight, fat body and gonad-somatic index. Journal of Hill Research, **9**(1), 1-4.

Vivien-Roels, B. (1981). Pineal control of reproduction in non-mammalian vertebrates. Dev. Endocrinol., **14**, 315-334.

Vivien-Roels, B., and Arendt, J. (1979). Circadian and circannual fluctuations of pineal metatonin content in *Testudo hermanni* G. (Reptilia-Chelonia) under natural conditions of photoperiod and temperature. Ann. Endocrinol., **40**(1), 93-94.

Vivien-Roels, B., and Arendt, J. (1981). Relative role of environmental factors, photoperiod and temperature in the control of serotonin and melatonin circadian variation in the pineal organ and plasma of tortoise *Testudo hermani* G meline. In "Advancement in Bioscience", (N. Birau and W. Schtoot, eds.), vol 29, pp. 401-406.

Vivien-Roels, B., and Arendt, J. (1983). How does the indoleamine production of the pineal gland respond to variations of the environment in a non-mammalian vertebrate, *Testudo hermanni* Gmelin? Psychoneuroendocrinology, **8**, 327-332.

Vivien-Roels, B., Pevet, P., and Claustrat, B. (1988). Pineal and circulating melatonin rhythms in the box turtle, *Terrapene carolina triunguis*: effect of photoperiod, light pulse, and environmental temperature. Gen. Comp. Endocrinol., **69**(2), 163-73.

Vivien-Roels, B., Pitrosky, B., Zitouni, M., Malan, A., Canguilhem, B., Bonn, D., and Pevet, P. (1997). Environmental control of the seasonal variations in the daily pattern of melatonin synthesis in the European hamster, *Cricetus cricetus*. Gen. Comp. Endocrinol., **106**(1), 85-94.

Vodicnik, M. J., and de Vlaming, V. L. (1978). The effect of pinealectomy on pituitary prolactin levels in *Carassius auratus* exposed to various photoperiod-temperature regimes. Endocr. Res. Commun., **5**, 199-210.

Volkoff, H., Wourms, J. P., Amsebury, E., and Snelson, F. F. (1999). Structure of the thyroid gland, serum thyroid hormones, and the reproductive cycle of the Atlantic Stingray, *Dasyatis Sabina*. J. Exp. Zool., **284**, 505-516.

Vollrath, L. (1981). The pineal complex in lampreys and fishes. In: The Pineal Organ, (L. Vollrath, ed.), pp. 321-367. Springer-Verlag, Berlin/ Heidelberg.

Vollrath, L., Seidel, A., Huesgen, A., Manz, B., Pollow, K., and Leiderer, P. (1989). One millisecond of light suffices to suppress nighttime pineal melatonin synthesis in rats. Neurosci. Lett., **98**(3), 297-298.

von Gall, C., Stehle, J. H., and Weaver, D. R. (2002). Mammalian melatonin receptors: molecular biology and signal transduction. Cell Tissue Res., **309**(1), 151-162.

Voordouw, B. C., Euser, R., Verdonk, R. E., Alberda, B. T., de Jong, F. H., Drogendijk, A. C., Fauser, B. C., and Cohen, M. (1992). Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. J. Clin. Endocrinol. Metab., **74**(1), 108-117.

Vriend, J. (1983a). Evidence for pineal gland modulation of the neuroendocrine-thyroid axis. Neuroendocrinology, **36**, 68-78.

Vriend, J. (1983b). Pineal-thyroid interaction. Pineal Res. Rev., **1**, 183-206.

Vriend, J. (1984). Influence of the pineal gland and circadian rhythms in circulating levels of thyroid hormones of male hamsters. J. Pineal Res., **1**, 15-22.

Vriend, J., and Reiter, R. J. (1977). Free thyroxine index in normal, melatonin-treated and blind hamsters. Horm. Metab. Res., **9**(3), 231-234.

Vriend, J., and Wilber, J. F. (1983). Influence of the pineal gland on hypothalamic content of TRH in the Syrian hamster. Horm. Res., **17**, 108-113.

Vriend, J., Richardson, B. A., Vaughan, M. K., Johnson, L. Y., and Reiter, R. J. (1982). Effects of melatonin on thyroid physiology of female hamsters. Neuroendocrinology, **35**, 79-85.

Vuillez, P., Jacob, N., Teclemariam-Mesbah, R., and Pevet, P. (1996). In Syrian and European hamsters, the duration of sensitive phase to light of the suprachiasmatic nuclei depends on the photoperiod. Neurosci. Lett., **208**(1), 37-40.

Wainwright, S. D. (1980). Diurnal cycles in serotonin acetyltransferase activity and cyclic GMP content of cultured chick pineal glands. Nature, **285**(5765), 478-480

Wainwright, S. D., and Wainwright, L. K. (1980). Regulation of the cycle in chick pineal serotonin N-acetyltransferase activity *in vitro* by light. J. Neurochem., **35**(2), 451-457.

Wajs, E., and Lewiski, A. (1992). Inhibitory influence of late afternoon melatonin injections and the counter-inhibitory action of melatonin-containing pellets on thyroid growth process in male Wistar rats: comparison with effects of other indole substances. J. Pineal Res., **13**, 158-166.

Weinberg, U., Weitzman, E.D., Fukushima, D. K., Cancel, G. F., and Rosenfeld, R. S. (1980). Melatonin does not suppress the pituitary luteinizing hormone response to luteinizing hormone-releasing hormone in men. J. Clin. Endocrinol. Metab., **51**(1), 161-162.

Wever, R. (1980). Circadian rhythms of finches under bright light: Is self sustainment a precondition for circadian rhythmicity? J. Comp. Physiol., **139**, 49-58.

White, B. H., Mosher, K., and Binkley, S. (1984). Daily profiles of N-acetyltransferase measured at a single time in rat pineal glands, retinas, and Harderian glands. J. Pineal Res., **1**(2), 129-137.

Whitmore, D., Foulkes, N. S., and Sassoni-Corsi, P. (2000). Light acts directly on organs and cells in culture to set the vertebrate circadian clock. Nature, **404**, 87-91.

Whitmore, D., Foulkes, N. S., Strahle, U., and Sassoni-Corsi, P. (1998). Zebrafish clock rhythmic expression reveals independent peripheral circadian oscillators. Nat. Neurosci., **1**, 701-707.

Wilson, F. W., and Donham. (1988). Daylength and control of seasonal reproduction in male birds. In "Processing of environmental information in vertebrates", (M. H. Stetson, ed.). pp. 101-111, Springer Verlag, New York, Inc.

Wilson, R., McKillop, J. H., MacLean, M., Walker, J. J., Fraser, W. D., Gray, C., Dryburgh, F., and Thomson, J. A. (1992). Thyroid function tests are rarely abnormal in patients with severe hyperemesis gravidarum. Clin. Endocrinol., **37**(4), 331-334.

Wolfe, M. S., and Zatz, M. (1994). Synthesis of heat shock proteins in cultured chick pineal cells. Brain Res., **31**, 662 (1-2), 273-277.

Wright, M. L. (2002). Melatonin, diel rhythms, and metamorphosis in anuran amphibians. Gen. Comp. Endocrinol., **126**(3), 251-254.

Wright, M. L., and Alves, C. D. (2001). The decrease in plasma melatonin at metamorphic climax in *Rana catesbeiana* (bullfrog) tadpoles is induced by thyroxine. Comp. Biochem. Physiol. A. Mol. Integr. Physiol., **129**(2-3), 653-663.

Wright, M. L., Frim, E. K., Bonak, V. A., and Baril, C. (1986). Metamorphic rate in *Rana pipiens* larvae treated with thyroxine or prolactin at different times in the light/dark cycle. Gen. Comp. Endocrinol., **63**(1), 51-61.

Wright, M. L., Blanchard, L. S., Pikula, A., and Labieniec, K. E. (1995). Circadian rhythms of thyroid secretion, morphometry, and cell division in prometamorphic and climax Rana tadpoles. Gen. Comp. Endocrinol., **99**(1), 75-84.

Wright, M. L., Pikula, A., Babski, A. M., Labienice, K. E., and Wolan, R. B. (1997). Effect of melatonin on the response of the thyroid to thyrotropin stimulation *in vitro*. Gen. Comp. Endocrinol., **108**, 298-305.

Wright, M. L., Duffy, J. L., Guertin, C. J., Alves, C. D., Szatkowski, M. C., and Visconti, R. F. (2003). Developmental and diel changes in plasma thyroxine and plasma and ocular melatonin in the larval and juvenile bullfrog, *Rana catesbeiana*. Gen. Comp. Endocrinol., **130**(2), 120-128.

Yalcin, S., Solak, K., and Akyurt, T. (2001). Certain reproductive characteristics of the catfish (*Clarias gariepinus*, Burchell, 1822) living in the river Asi, Turkey. Turk. J. Zool., **25**, 453-460.

Yamada, K. (1990). Effects of melatonin on adrenal function in male rats. Res. Commun. Chem. Pathol. Pharmacol., **69**(2), 241-244.

Yamaguchi, S., Mitsui, S., Yan, L., Yagita, K., Miyake, S., and Okamura, H. (2000). Role of DBP in the circadian oscillatory mechanism. Mol. Cell Biol., **20**, 4773-4781.

Yokota, T., and Oishi, T. (1992). Seasonal change in the locomotor activity rhythm of the medaka, *Oryzias latipes*. Int. J. Biometeorol., **36**(1), 39-44.

Yoshimura, T., Yasuo, S., Watanabe, M., Iigo, M., Yamamura, T., Hirunagi, K., and Ebihara, S. (2003). Light induced hormone conversion of T_4 and T_3 regulates photoperiodic response of gonads in birds. Nature, **426** (6963), 178-181.

Yuwiler, A., and Brammer, G. L. (1981). Neonatal hormone treatment and maturation of the pineal noradrenergic system: hydrocortisone and thyroxine. J. Neurochem., **37**, 985-992.

Zachmann, A., Falcon, J., Knijff, S. C. M., Bolliet, V., and Ali, M. A. (1992). Effects of photoperiod and temperature on rhythmic melatonin secretion from the pineal organ of the white sucker (*Catostomus commersoni*) *in vitro*. Gen. Comp. Endocrinol., **86**, 26-33.

Zatz, M., Mullen, D. A., and Moskal, J. R. (1988). Photoendocrine transduction in cultured chick pineal cells: effects of light, dark, and potassium on the melatonin rhythm. Brain Res., **438**(1-2), 199-215.

Zatz, M., Lange, G. D., and Rollag, M. D. (1994). What does changing the temperature do to the melatonin rhythm in cultured chick pineal cells? Am. J. Physiol., **266**, 50-58.

Zatz, M., Gastel, J. A., Heath, J. R., and Klein, D. C. (2000). Chick pineal melatonin synthesis: light and cyclic AMP control abundance of serotonin N-acetyltransferase protein. J. Neurochem., **74**(6), 2315-2321.

Zeman, M., and Illnernova, H. (1988). Rapid adjustment of the pineal N-acetyltransferase rhythm to change from long to short photoperiod in the Japanese quail (*Coturnix coturnix japonica*). J. Pineal Res., **5**(6), 565-571.

Zeman, M., and Illnerova, H. (1990). Ontogeny of N-acetyltransferase activity rhythm in pineal gland of chick embryo. Comp. Biochem. Physiol. A, **97**(2), 175-178.

Zucker, I., Boshes, M., and Dark, J. (1983). Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. Am. J. Physiol., **244**(4), 472-480.

APPENDIX

Name: **YUMKHAIBAM PREMABATI**

Title of dissertation: **Study of thyroid-pineal interrelationship in the fish, *Clarias gariepinus*.**

Date of payment of admission fees: 11-11-1999

Approval of research proposal:

i). B. P. G. S: 07-04-2000

ii). School board: 18-04-2000

iii). Registration no. & date of registration: 441 18-04-2000

BIO-DATA

Name: YUMKHAIBAM PREMABATI

Father's name: YUMKHAIBAM KAMAL SINGH

Mother's name: YUMKHAIBAM JANORIE DEVI

Addresses-

Present: C/o Prof. B. B. P. Gupta
Department of Zoology
North-Eastern Hill University
Shillong- 793022 (Meghalaya)

Permanent: Khurai Lamlong Bazaar
Imphal-East- 795 010 (Manipur)

E-Mail: premabati@yahoo.com

Date of birth: 19-03-76

Academic qualifications:

Sl. No.	Exam. passed	Div.	Year	Board/Univ	Subjects
1	B. Sc (Hons.)	First	1996	Manipur University, Manipur	Botany, Chemistry, Zoology (Hons.).
2	M. Sc	First	1999	North-Eastern Hill University, Shillong, Meghalaya	Zoology *Specialization:* Endocrinology and reproductive physiology

Other qualification/achievement:

- Won first prize in the "Project Contest" 1992, Junior Science Refresher, Bright Careers Institute, New Delhi
- Cetificate of SWIFT INDIA computer course from NIIT, 2000
- SRF (CSIR)- 2003 - 2005

Research experience: 5 years

Publications:

- Gupta, B. B. P., and Premabati, Y. (2002). Differential effects of melatonin on plasma levels of thyroxine and triiodothyronine levels in the air-breathing fish, *Clarias gariepinus* during breeding and quiescent periods. Gen. Comp. Endocrinol., **129**, 146-151.
- Gupta, B. B. P., and Premabati, Y. (2002). Fish Pineal: Structure, Function and Regulation. In "Treatise on Pineal Gland" (C. Haldar, M. Singaravel, and S. K. Maitra, eds.), pp. 77-102. Science Publishers, Inc., Enfield (NH), U.S.A.

List of papers presented in Seminar/School/Symposium:

- "Study of annual variations in the rate of tissue respiration in a hibernating species, *Limnonectes limnocharis* and a non-hibernating species, *Euphylctis cyanophlyctis.*" In the "Regional Seminar on Recent Trends in Zoology" held on 14th September 2000, Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya.
- "Melatonin rhythm in Fish." In the "First School in Chronobiology" held from 17th - 27th October 2002, Department of Zoology, University of Lucknow, Lucknow, India.
- "Effects of thyroid hormones on the arylalkylamine-N-acetyltransferase (AA-NAT) activity circadian rhythms in the fish, *Clarias gariepinus* during winter

and summer seasons." In the "XXIII National Symposium on Reproductive Biology and Comparative Endocrinology" held from 07th–09th February 2005, Department of Zoology, Visva-Bharati, Santiniketan, India.

Seminar/School/Training Course/Symposium attended:

- Participated and presented a paper in the "Regional Seminar on Recent Trends in Zoology" held on 14th September 2000, Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya.
- Participated and presented a paper in the "First School in Chronobiology" held from 17th - 27th October 2002, Department of Zoology, University of Lucknow, Lucknow, India.
- Attended the training course on "Basic Techniques in Animal Cell and Tissue Culture," held from 15th - 20th September 2003, Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya.
- Attended the "13th National Symposium on Environment," held from 5th - 7th June, 2004, North-Eastern Hill University, Shillong, Meghalaya.
- Participated and presented a paper in the "XXIII National Symposium on Reproductive Biology and Comparative Endocrinology" held from 07th–09th February 2005, Department of Zoology, Visva-Bharati, Santiniketan, India.