บทบาทของต่อมไพเนียลต่อการเจริญเติบโตของรังไข่ของปลานิล (Sarotherodon niloticus)

Role of the pineal in ovarian development in Sarotherodon niloticus

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Master of Science Thesis in Biological Science

Prince of Songkla University

2530

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บัณฑิตวิทยาลัย มหาวิทยาลัยสงขลานครินทร์ อนุมัติให้นับวิทยานิพนธ์ฉบับนี้ เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิทยาศาสตร์ชีวภาพ

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PROJECT IMPLEMENTATION

Place of study - Department of Biology,
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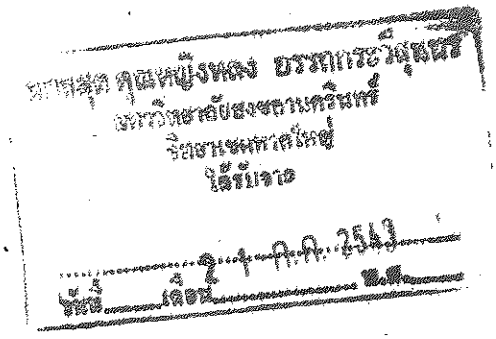
Period of study - June, 1985 - December, 1986.



ACKNOWLEDGEMENT

I wish to express my sincere appreciation and profound gratitude to Dr. Alan F. Geater, advisor and chairman of the Advisory Committee, not only for his valuable advice and encouragement throughout the period of study, but also for his kind and warm guidance, and his help in correcting the author's English from the first draft to the final draft.

Sincere thanks are extended to the members of my advisory committee, Associate Professor Dr. Reungchai Tansakul and Assistant Professor Nonglak Supakorndech, for their comments, suggestions and critical reading of the manuscript.

I am very much indebted to Assistant Professor Nithi Rithipornpan for the use of laboratory space and other facilities and for his valuable suggestions.

Thanks are due to all technical staff of the Department of Biology for their help during the study.

Grateful acknowledgement is due to the Graduate School, Prince of Songkla University and to Harvard Alumni Graduate Research Grants' 85 for grants which allowed this study to be made.

Finally, gratitude is especially extended to my parents for their encouragement, understanding and inspiration throughout my period of studies.

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บทคัดย่อ

ได้ทำการศึกษาถึงความเป็นไปได้ในการเปลี่ยนแปลงระบบของไพเนียล เพื่อควบคุมการพัฒนาการของระบบสืบพันธุ์ของปลานิล (Sarotherodon niloticus) ปลานิลที่ใช้นำมาจากบ่อปลาที่มีลักษณะกึ่งธรรมชาติ ที่ ทุ่งลุง จ. สงขลา ระหว่างเดือนพฤศจิกายน พ.ศ. 2528 ถึง เดือนกรกฎาคม พ.ศ. 2529 จะแสดงถึงรอบการสืบพันธุ์ที่เกิดขึ้นไม่พร้อมกันและไม่เป็นฤดูกาล อัตราส่วนของน้ำหนักของอวัยวะสืบพันธุ์ ต่อน้ำหนักตัวในรูปของลอการิทึม มีความเหมาะสมที่จะใช้เป็นพารามิเตอร์ที่แสดงถึงการเจริญเติบโตของรังไข่ การเริ่มการเจริญเติบโตของรังไข่ จะพบในปลาที่มีน้ำหนักตัวตั้งแต่ 7 กรัมขึ้นไป และปลาที่มีรังไข่ที่เจริญเติบโตเต็มที่จะมีน้ำหนักประมาณ 32 กรัมขึ้นไป การตัดต่อมไพเนียลในปลานิลเพศเมียที่ยังไม่เจริญเติบโตเต็มที่ (น้ำหนักตัวโดยเฉลี่ยประมาณ 11 กรัม) ในช่วงเดือนตุลาคม ถึงเดือนพฤศจิกายน 2529 จะไม่สามารถยับยั้งการเริ่มต้นการเจริญของรังไข่ (โดยการวัดค่า log GSI) ทั้งในสภาวะที่มีแสง 18 ชั่วโมงและสภาวะที่มืดตลอด การใช้ต่อมไพเนียลของวัวที่บดจนละเอียดและฉีดเข้าช่องท้องของปลานิลเพศเมียที่ยังไม่เจริญเติบโตเต็มที่ (น้ำหนักตัวโดยเฉลี่ยประมาณ 13.40 ± 0.65 กรัม) โดยฉีดวันเว้นวันเป็นเวลา 3 สัปดาห์ โดยใช้ขนาด 7.5 มิลลิกรัม และ 30 มิลลิกรัม ต่อ ปลา 1 ตัว ภายใต้สภาวะช่วงแสงธรรมชาติ ที่เลี้ยงในกระชังและสภาวะที่มีแสงตลอด 24 ชั่วโมง ที่เลี้ยงในถังเลี้ยงปลา ช่วงเดือนตุลาคม ถึง เดือนพฤศจิกายน พบว่าจะไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญ ของการแจกแจงในรูปลอการิทึมของน้ำหนักของรังไข่ ต่อน้ำหนักตัวของปลาภายใต้สภาวะของช่วงแสงทั้งสอง อย่างไรก็ตามจากการตรวจสอบเนื้อเยื่อ และการตรวจสอบเซลล์ของรังไข่ของปลากลุ่มที่อยู่ภายใต้สภาวะที่มีแสงตลอด พบว่ามีการเพิ่มขนาดของไข่ที่อยู่ในระยะ tertiary yolk และ maturation ร่วมกับการเพิ่มของปริมาตรของไข่แดงและขนาดของ yolk platelets ในปลาที่ได้รับเนื้อเยื่อของต่อมไพเนียล 30 มิลลิกรัม ต่อการฉีดแต่ละครั้ง ในปลากลุ่มที่ได้รับการฉีดทั้ง Ringer's solution อย่างเดียว หรือ Ringer's solution ร่วมกับต่อมไพเนียลของวัว

ที่บดละเอียด พบว่าจำนวนไข่ในรังไข่จะน้อยกว่าในกลุ่มควบคุม นั่นคือต่อมไพเนียลอาจไม่เกี่ยวข้องกับการเจริญเติบโตของรังไข่ในระยะเริ่มต้น ขณะที่สารที่ได้จากต่อมไพเนียลของวัวอาจเป็นเหตุให้เกิดการสร้าง yolk ของไข่ภายในรังไข่ได้

สีตัวของปลานิลเพศเมีย (S. niloticus) ที่อยู่ภายใต้สภาวะที่มีแสงตลอดและมืดตลอด ร่วมกับการฉีด fish Ringer's solution อย่างเดียวหรือการฉีด fish Ringer's solution ร่วมกับต่อมไพเนียลของวัวที่บดละเอียด (30 มิลลิกรัมต่อครั้ง) แสดงให้เห็นถึงความเข้มของสีผิวของปลาในสภาวะมืดตลอด เมื่อเปรียบ เทียบกับในสภาวะมีแสงตลอด และแสดงถึงความแตกต่างอย่างมีนัยสำคัญของความเข้มของสีผิวของปลาหลังจากฉีดเนื้อเยื่อต่อมไพเนียลที่บดละเอียด ภายใต้สภาวะที่มีแสงตลอด การตอบสนองของสีผิวต่อสภาวะที่มีแสงตามธรรมชาติ และสภาวะที่เพิ่มสารจากต่อมไพเนียลในปลานิล จะแตกต่างจากการตอบสนองของปลาและสัตว์ครึ่งบกครึ่งน้ำชนิดอื่น ๆ ที่ตอบสนองต่อช่วงแสงและต่อมไพเนียล หรือเมลาโทนิน ที่บันทึกในเอกสารอ้างอิง ซึ่งได้เสนอว่าสารจากต่อมไพเนียล (อาจจะเป็นเมลาโทนิน) เป็นตัวหลักในการควบคุมการเปลี่ยนแปลงของสีผิว โดยจะหลั่งออกมาในช่วงที่มืด และถูกยับยั้งในสภาวะที่มีแสง ซึ่งสารที่หลั่งออกมาจากต่อมไพเนียลจะทำให้เกิดการกระจายของเม็ดสีภายในเมลาโนฟอร์

ดังนั้นจึงมีความเป็นไปได้น้อยมากในการควบคุมการสืบพันธุ์ของปลานิล โดยใช้การเปลี่ยนแปลงการทำงานของต่อมไพเนียล

ABSTRACT

The possibility of controlling reproductive development of Sarotherodon niloticus by modifying the pineal system has been investigated. S. niloticus collected from a semi-natural pond at Tung Lung between November, 1985 and July, 1986 showed nonseasonal asynchronous breeding cycles. Logarithmic ratio of gonad weight to body weight was shown to be a suitable parameter of ovarian maturation. Initial ovarian maturation occurred in fish with a body weight greater than about 7 g and full maturation achieved by fish of body weight about 32 g. Pinealectomy of immature (mean body weight 11.Ø3 ± Ø.69 g) female fish in October to November failed to inhibit initial ovarian development (as measured by change in log GSI) either in 18 h :6 h light:dark cycle or in constant darkness. Administration of macerated bovine pineal (MBP) by intraperitoneal injection of immature fish (mean body weight 13.42 ± Ø.65 g) on alternate days for 3 weeks at doses of 7.5 mg and 3Ø mg pineal per injection under natural light in cages and constant light in tanks in October to November caused no significant changes in gonad weight vs. body weight logarithmic distribution under either photoperiodic condition. However, histological observation and cytometric determinations of ovaries of the constant light groups revealed enlarged tertiary-yolk and maturation oocytes associated with increased yolk content and enlarged yolk

platelets in fish receiving 30 mg pineal tissue per injection. All injected fish, whether receiving fish Ringer's solution alone or Ringer's solution + MBP showed reduced numbers of oocytes in ovaries. Thus, the pineal is therefore not required for initial ovarian maturation while bovine pineal derived substances may induce increased vitellogenesis.

Skin colour of female S. niloticus held under conditions of constant light or constant darkness and with daily injection of fish Ringer's solution alone or fish Ringer's solution + MBP (30 mg pineal/injection) showed a darkening of skin in constant dark compared with constant light and significant darkening of skin following MBP administration of fish kept under constant light. The skin colour responses to environmental light condition and to pineal administration differ from the responses of several other fish and amphibian species to photoperiod and pineal or melatonin administration reported in the literature. It is suggested that the pineal colour-controlling principle (presumed to be melatonin), is secreted in the dark phase, inhibited in light, and causes dispersion of pigment in the dermal melanophores.

It is concluded that the prospects for limiting reproductive activity of female S. niloticus by modifying pineal activity are poor.

CHAPTER 1

GENERAL INTRODUCTION

Cichlid fish

Cichlid fish are valuable pond fish and almost all genera are principally herbivores. Some prefer higher plants, filamentous algae, or plankton, while others consume various types of aquatic flora. Several species are voracious feeders and are used to control weeds (Rifai, 1980).

Cichlid fish, especially those of the genera Tilapia and Sarotherodon, are an important food fish in many tropical and subtropical countries. Many species of Tilapia and Sarotherodon have been cultured in developing countries where mammalian protein is insufficient (Guerrero, 1982). Cichlid fish are used not only as human food fish, but also as animal food and, because of their largely herbivorous habit, to control weeds and thus, indirectly, to control malaria and bilhazia (Balarin and Hatton, 1979). They are particularly suitable for culture because of their high tolerance to adverse environmental conditions, their relatively fast growth and ease with which they can be bred (Guerrero, 1982).

The main drawback to the worldwide culture of cichlids

is manifest when they are allowed to grow without control in ponds of limited size. The fish increase in number and, under such conditions, individual fish reach maturity at a much smaller size than they would under natural conditions, usually as a direct result of inadequate food supplies. Consequently, at harvest, most fish produced in ponds are too small to be acceptable as human food. The problem is made worse by poor pond conditions and the occurrence of social hierarchy among the fish (Chen, 1965). This can become a critical problem.

In general, cichlid fish have high breeding rates. Their fecundity ranges from a few hundred eggs to several thousand per spawning (Lowe-McConnell, 1982). The efficiency of reproduction in Sarotherodon niloticus has paradoxical consequences: on one hand, this aptitude which allows easy and rapid propagation in various tropical and subtropical environments partially explains the economic interest in this species for fish culture; on the other hand this reproductive efficiency can be a source of problems because uncontrolled multiplication within a limited environment in a situation of food competition is liable to produce dwarf fish populations of little value (Jalabert and Zohar, 1982).

Another aspect of the reproductive efficiency of Tilapia or Sarotherodon is precocious sexual maturation which can occur as early as 3 months in some species and depends on, probably in addition to genetic factors, environmental factors like temperature (Huet, 1979) and food

availability and probably many intrinsic factors such as levels of hormonal activity (Sundararaj, 1981) etc.

As soon as sexual maturity is attained, and provided temperature is suitable, most cichlid females are able to undergo successive breeding cycles, producing new broods at 4 to 6 week intervals. Especially in equatorial lakes and ponds, breeding occurs throughout the year, without well-defined seasons (Lowe-McConnell, 1982). This usually results in a continuous production of fry throughout a population, with the exception those in certain environments subject to substantial seasonal variations. However, the relative asynchrony between the sexual cycles of individual females can be a problem when mass production of homogeneous fry is required for intensive fish farming (Jalabert and Zohar, 1982).

Thus, for pratical reasons dictated by fish farming conditions, it would often be advantageous either to inhibit or delay sexual maturation, or in some cases to favour synchronous spawning and breeding for mass production of fry (Huet, 1979).

There are various methods for controlling the reproduction and recruitment of Tilapia and Sarotherodon. These include predator association, monosex culture, differentiation of sexes, irradiation as a technique for the reproductive sterilization of tilapia, chemical castration and sex reversal, such as the use of methallibure for gonadal repression and methyltestosterone for sex reversal,

stocking at high densities and cage culture (Balarin and Hatton, 1979; Huet, 1979; Rifai, 1980; Sundararaj, 1981; Guerrero, 1982; Hepher and Pruginin, 1982). Each method, however, has certain disadvantages.

Finally, the great plasticity of tilapia growth in natural waters suggests that concentrating reseach on environmental and behavioral factors affecting growth and the switch to reproduction is likely to be more helpful for tilapia culture than the search for faster-growing genetic strains (Lowe-McConnell, 1982).

The pineal complex and its roles

The remarkably wide variation of the pineal complex in vertebrates has been the subject of reseach since the turn of century (Meiniel, 1981).

The diversity in structure and function characteristic of the chordate pineal complex reflects a surprisingly high degree of evolutionary and adaptive capacity inherent to this ancient component of the brain. Despite the striking variations in morphology and function (Axelrod, 1974), the pineal complex of all vertebrates exhibits the following common feature:

1) It develops as an evagination of the roof of the diencephalon.

2) Its fine structure and function can be influenced by natural and artificial photoperiods.

3) Some of its cellular components are capable of indoleamine metabolism (Reiter, 1974; Hartwig and Oksche, 1981).

There is considerable evidence at present, in vertebrate classes other than fish, attesting to the fact that the pineal complex may be involved in the regulation of reproductive processes, especially gonad maturation. In photoperiodic mammals the pineal organ has an antigonadal role since removal of the pineal typically causes growth and maturation of the gonads of animals maintained under nonstimulatory photoperiodic conditions (Underwood, 1981). This suggests that the primitive function of the pineal complex may have been in regulation of the reproductive function.

In animals, especially mammals, where the photoperiod appears to be an important trigger of seasonal reproductive rhythms, the pineal complex is considered as a link between annual photoperiodic changes and seasonal reproduction (Vivien-Roels, 1981; Underwood, 1981). In non-mammalian vertebrates, as far as the influence of the pineal complex appears to be progonadal, antigonadal or without effects on gonads (Ralph, 1978). However, most investigations are somewhat limited and often have not taken into consideration the environmental or seasonal cues to which animals were exposed (Vivien-Roels, 1981).

Two different groups of compounds, considered to be responsible for its endocrine capabilities, are elaborated

by the pineal gland: (i) indoleamines (e.g. serotonin, melatonin, 5-methoxytryptophol, 5-methoxytryptamine),and (ii) peptidergic compounds (Reiter, 1974; Pévet, 1981).

From the time of Lerner's discovery of melatonin (Lerner et al., 1958) to the present, a great proportion of pineal research has been concentrated on the role of melatonin in pineal function. Wurtman and Axelrod (1965) suggested that melatonin is formed specifically by the pineal organ, is released into the circulation and concentrated in specific target organ, and depresses ovarian functions in rodents (Wurtman and Axelrod, 1965 lit. cit. Fenwick, 1970 a). Furthermore, the rate of melatonin synthesis by the pineal is inhibited by continuous light. Wurtman et al. (1963) have reported a delay in the vaginal opening and a reduction of ovarian weight following melatonin treatment in female rats, and in male rats serum melatonin demonstrated diurnal changes in all age groups studied (Pang, Tang and Tang, 1984). The pineal gonad of golden-mantled ground squirrel evidently is involved in the expression of the annual hibernatory cycle (Ralph, Harlow, and Phillips, 1983). Bittman (1984) has suggested that the pineal controls the reproductive response of ewes to both stimulatory (short) and inhibitory (long) daylength and Almeida (1984) showed reproductive photorefractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion after exposure to alternating 16-week cycles of long and short days (16 hrs light: 8 hrs dark

and 8 hrs light: 16 hrs dark). Pinealectomy of birds (Breneman, 1950 lit. cit. Fenwick, 1970; Underwood, 1981; Vivien-Roels, 1981) and lizards (Stebbins, 1960 lit. cit. Fenwick, 1970 b; Cuellar, 1978; Underwood, 1981; Vivien-Roels, 1981; Maier, and Singer, 1982) has been found to provoke some degree of gonadal maturation and in this respect agrees with findings in mammals.

Among the vertebrate classes the pineal complex varies widely in its gross location, form and size, and in its histological composition (Ueck, 1981). The pineal organ in fish is located on the dorsal roof of the brain, between the telencephalon and the optic tectum. It is composed of three kinds of cells: photoreceptor cells (pinealocytes), ganglion cells (neurons) and ependymal-like supportive or interstitial cells (Fenwick, 1970 c; Hafeez, 1971; Tamura and Hanya, 1980; Sundararaj, 1981; Meiniel, 1981). Though the pineal organ in fish has long been a subject of research and review, knowledge of its physiological role in fish is still far from clear. It is generally believed that the fish pineal has a photosensory or photoreceptive function though some authors have also attributed to it a secretory function (Sundararaj, 1981).

The available evidence indicates that in teleost fish the pineal organ may play an important role in the regulation of several physiological processes, possibly including reproduction (Jalabert and Zohar, 1982).

Many studies have been undertaken to examine the role

of the pineal or melatonin in gonadal function in fish. Under certain circumstances the pineal seems to have antigonadal properties in teleosts (Sundararaj, 1981) and pineal melatonin can induce marked changes in neuroendocrine and gonadal activities in a number of fish (Fenwick, 1970 b). Recent studies have demonstrated that pineal melatonin has progonadal properties also and that it can inhibit the antigonadal activities of the pineal. This ambivalent action of melatonin has been shown to depend on dose, mode and time of administration and the photoperiod to which the experimental animals are exposed. Fenwick (1969) has also shown that in the goldfish gonadal response to experimental light-dark regimes varied during the year; there was a seasonal variation in responsiveness to photoperiod and the pineal organ of the goldfish was found to be specifically related to photoperiod regimes and this relationship was associated with responsiveness of the gonad to light (Fenwick, 1970 a). However, all the observed antigonadal activities of the pineal extracts cannot be explained by its melatonin content. Fenwick (1970 b) demonstrated that injections of melatonin inhibited the normal increase of gonadal size usually induced by an increasing photoperiod. More recently, Urasaki (1976) suggested that in Oryzias latipes, pineal effect may be stimulatory under continous illumination or long photoperiods and inhibitory under constant darkness or short photoperiods. In another species, Notemigonus crysoleucus , De Vlaming (1975 lit. cit. Vivien-

Roels, 1981) has shown that the pineal may be inhibitory or stimulatory with respect to gonadal function, depending on the combination of water temperature and photoperiods.

The effect of pinealectomy on reproduction in teleost fish varies considerably with the species, season and condition of photoperiod. In the Japanese killifish, O. latipes, melatonin inhibited gonadal development of both intact and pinealectomized fish exposed to a 16 h-photoperiod, while the same treatment had no effect in fish maintained on an 8h-photoperiod (Urazaki, 1972). In the same species, Urazaki (1976) showed that fish pinealectomized during winter and the maintained under short photoperiods had larger ovaries than controls, while the ovaries of animals maintained under long photoperiods were smaller than controls. De Vlaming (1975 lit. cit. Vivien-Roels, 1981) observed that pinealectomy caused either gonadal regression or retarded maturation if the fish were maintained on a short photoperiod-warm temperature regime. Day et al. (1983) suggested that pinealectomized fish did not remain sexually active when exposed to short days and in Fundulus heteroclitus neither the eyes nor the pineal organ were essential for the initiation or maintenance of seasonal reproduction. Vodicnik et al (1978 lit. cit. Peter, 1980) reported that pinealectomy of recrudescing female goldfish held on a long photoperiod and warm temperature for 22 days caused a decrease in plasma gonadotrophin hormone levels at 4 hours but not at 10 hours after the onset of the

photophase. These results are partly consistent with the significantly lower GSI (gonadosomatic index: gonad weight expressed as percent of body weight) in the pinealectomized fish (Peter, 1980). Therefore, the pineal organ can be antigonadal or progonadal during different seasons depending on daylength conditions.

An overall review of the functional aspects of the pineal in fish suggests that the pineal modulates physiological activities to facilitate breeding during the proper season and to prevent breeding during the unfavourable seasons of the year (De Vlaming and Vodicnik, 1978; Vodicnik et al, 1979; Vivien-Roels, 1981). Precision in breeding periodicity has been described as an adaptation to synchronize the emergence of fry with the availability of proper food, which itself is subject to cycles of seasonal abundance (Sundararaj, 1981).

The importance of reproductive control of S. niloticus by the pineal complex

The tilapia, S. niloticus has become increasingly important in fish culture in Thailand in recent years because it is appreciated by customers and can produce high yields on relatively low inputs. Nevertheless, the culture of S. niloticus is beset with the management problems referred to above, viz. they breed in production ponds while still young and small, thereby leading to a greatly

increased and supra-optimal population size. The resulting competition for food lowers the growth rate and causes frequent stunting. Only by controlling reproduction in these production ponds can yields of fish of marketable size per unit pond area be obtained. With present techniques for reproduction control still largely unsatisfactory , it is considered worthwhile to explore the possibilities for new techniques of control. With abundant evidence for a key role for the pineal in reproductive control in other vertebrate groups and at least some evidence for pineal involvement in the control of reproduction in certain teleosts, it is possible that a method for reproductive control (inhibition) in S. nilotiçus based on a pineal mediated system could provide a feasible means of population control in production ponds of this species.

The aim of the study reported here was to investigate the dependency of ovarian development on pineal function in female S. nilotiçus. The immediate objectives were 1) to determine the size of fish at which initial ovarian development occurred 2) to find an easily measurable parameter of ovarian development and 3) to determine if simple alterations in pineal status (pinealectomy and/or injection of macerated bovine pineal tissue) could produce detectable changes in ovarian development.

CHAPTER 2

A STUDY OF S. niloticus FROM A CULTIVATION POND (AT TUNG LUNG); CONSIDERATION OF BODY WEIGHT, GONAD WEIGHT AND DEVELOPMENTAL STAGES OF OOGENESIS

Previous studies (Fenwick, 1969 and 1970 a; Urasaki, 1972; Joss, 1973; De Vlaming and Vodicnik, 1979) have indicated that the season at which pinealectomy or melatonin injection are performed has a profound influence on the effects of these procedures. Thus, recent investigations of de Vlaming and Vodicnik (1978) suggested that the pineal may be stimulatory or inhibitory to reproductive activity in female C. auratus depending on the photoperiod-temperature regimes to which that fish are exposed during a certain season. Therefore, the pineal complex could potentially be involved in mediating the effects of photoperiod on reproduction in teleosts.

Most of these studies have been done on temperate fish under temperate conditions. It is normal under these conditions for fish to show seasonal breeding. Reproductive cycles have been shown to be regulated or synchronized by seasonally changing daylength (de Vlaming and Vodicnik, 1978). However, the present investigation was carried out on a tropical fish under tropical conditions where seasons were not clearly marked, and photoperiod and temperature

variations throughout the year are not of great magnitude. Most tropical fish breed throughout the year, though the number of breeding fish in any population might be slightly higher during the wet season (Lowe-McConnell, 1958 lit. cit. Jalabert and Zohar, 1982).

It was considered necessary, therefore, to examine the reproductive status of a population of S. niloticus under semi-natural conditions to determine if any seasonal breeding cycle occurred, and so that the most suitable time or size of fish could be selected for studies of the effects of pineal manipulation.

This was done by investigating the relationships among body weight, gonad weight and developmental condition of the ovaries as judged by histological observation and examining the distribution of these parameters in the population at different times of the year.

The time at which the pineal might exert an influence was believed to be during the period of gonadal development either during initial maturation or development within the adult breeding cycle.

PART A BODY WEIGHT/GONAD WEIGHT RELATIONSHIP

PROCEDURES

A population of S. niloticus in a cultivation pond located at Tung Lung, Songkhla province (06° 50' 45" N, 100°

28' 25" E) was examined from November, 1985 to July, 1986. This was an artificial pond, earth-banked, and of roughly rectangular surface area, approximately 60 m x 100 m, and of a water depth approximately 2 m. The pond had been stocked with fingerlings 3-4 years previously, and fish of marketable size (>15 cm body length) removed in small batches for sale continuously and all large fish harvested once or twice a year. A battery hen unit was operated above the pond and covered approximately 5 % of the pond area. Waste from the hen unit (spilled food, faeces etc.) fell through to the pond.

Samples of fish were collected 2 times a month for 5 months and one time per month for another 4 months. In each sample, 20 to 40 female S. niloticus of various sizes were collected each time by using throwing nets of 20 - 25 mm mesh size. After collection, the fish were immediately transported in aerated water to the laboratory, a journey of about 30 minutes. The length of each fish was measured, and the body weighed. Fish were then killed by transection of the spinal cord dorsal to the posterior borders of the operoula. Ovaries were removed, weighed, their length and width measured and then fixed in Bouin's fluid. The gonadosomatic index was culculated (GSI = gonad weight expressed as percentage of body weight).

Fixed ovaries were dehydrated in ethyl alcohol for about 3 hours and overnight in cellosove, cleared in benzene, embedded in paraplast (M.Pt. 56 C Sherwood Medical,

St. Louis, USA) and sectioned at 7 μm. Sections were stained in Harris's hematoxylin, counter-stained in Bowie's eosin and mounted in D.P.X.

RESULTS AND DISCUSSION

i. Throughout the collection period, fish showed a wide variation of gonad weight for a similar body weight. The relationships between body weight and gonad weight at each sampling time are shown in Fig. 1-13. The changes in value of mean GSI of this population throughout the collection period are shown in Table 1. One-way analysis of variance failed to show any significant differences between collection groups (Table 2), since the within groups variation was large. Relative standard error values within groups ranged from 10 % to 20 %. The median test was also used to examine the distribution of GSI values at each collection time but again no significant differences were apparent (Table 3).

ii. Since no differences appeared throughout the collection period, all data were combined, and the overall distribution of this population is shown in Fig. 14. The wide variation in gonad weight for any given body weight was reduced by doing a logarithmic transformation of the data (both body weight and gonad weight) as shown in Fig. 15. Although the general tendency for gonad weight to increase with log body weight is apparent, considerably more

understanding of this relationship of gonad weight and body weight is possible by employing the concept of the 'envelope curve'. In Fig. 15 the envelope curve, and the "minimum curve" have been drawn. The envelope curve can be considered as representing the probable maximum gonad weight at any given body weight. The curve reaches a plateau at about 100 g body weight (log B.Wt. = 5.00) of fish. The minimum curve shows clearly that the minimum weight of gonad increased with body weight at least in fish greater than about 32 g (log B.Wt. = 4.50). In small fish (<~ 7 g, log B.Wt. = 3.85) the range between maximum and minimum gonad weight was narrow but in fish heavier than 7 g it became gradually wider, up to a body weight of about 60 g (log B.Wt. = 4.78).

Throughout this range (7 g - 100 g), the GSI value of the envelope curve increases rapidly (from about 0.35 % to 10 %) and probably coincides with the period at which initial ovarian maturation occurs (at least in some fish of the population). In fish larger than about 60 g and up to about 180 g, maximum GSI (i.e. the envelope curve) is relatively constant but above this level falls with increasing body weight to about 2 % at 320 g.

Table 1 Value of mean changes in gonadosomatic index of female S. niloticus (n = 418) in 9 months.

	Time (yr.mon.date)	number of fish	GSI (mean ± S.E. g/100g)
1.	85.11.26	35	1.19 ± 0.23
2.	85.12.06	24	1.42 ± 0.29
3.	85.12.20	32	1.51 ± 0.27
4.	86.01.06	40	1.06 ± 0.18
5.	86.01.23	34	0.83 ± 0.17
6.	86.02.06	43	1.30 ± 0.20
7.	86.02.27	21	1.57 ± 0.25
8.	86.03.17	36	1.26 ± 0.19
9.	86.03.31	38	1.00 ± 0.17
10.	86.04.15	20	1.15 ± 0.16
11.	86.05.01	34	1.17 ± 0.16
12.	86.06.03	30	1.26 ± 0.14
13.	86.07.28	31	1.53 ± 0.30

Table 2 The analysis of variance of GSI of female S. niloticus of each times in 9 months.

SOV	d.f.	SS	MS
Total	417	600.2500	
between groups	12	17.8000	1.4800
within groups	405	582.4500	1.4400

$F = 1.8278^{ns}$

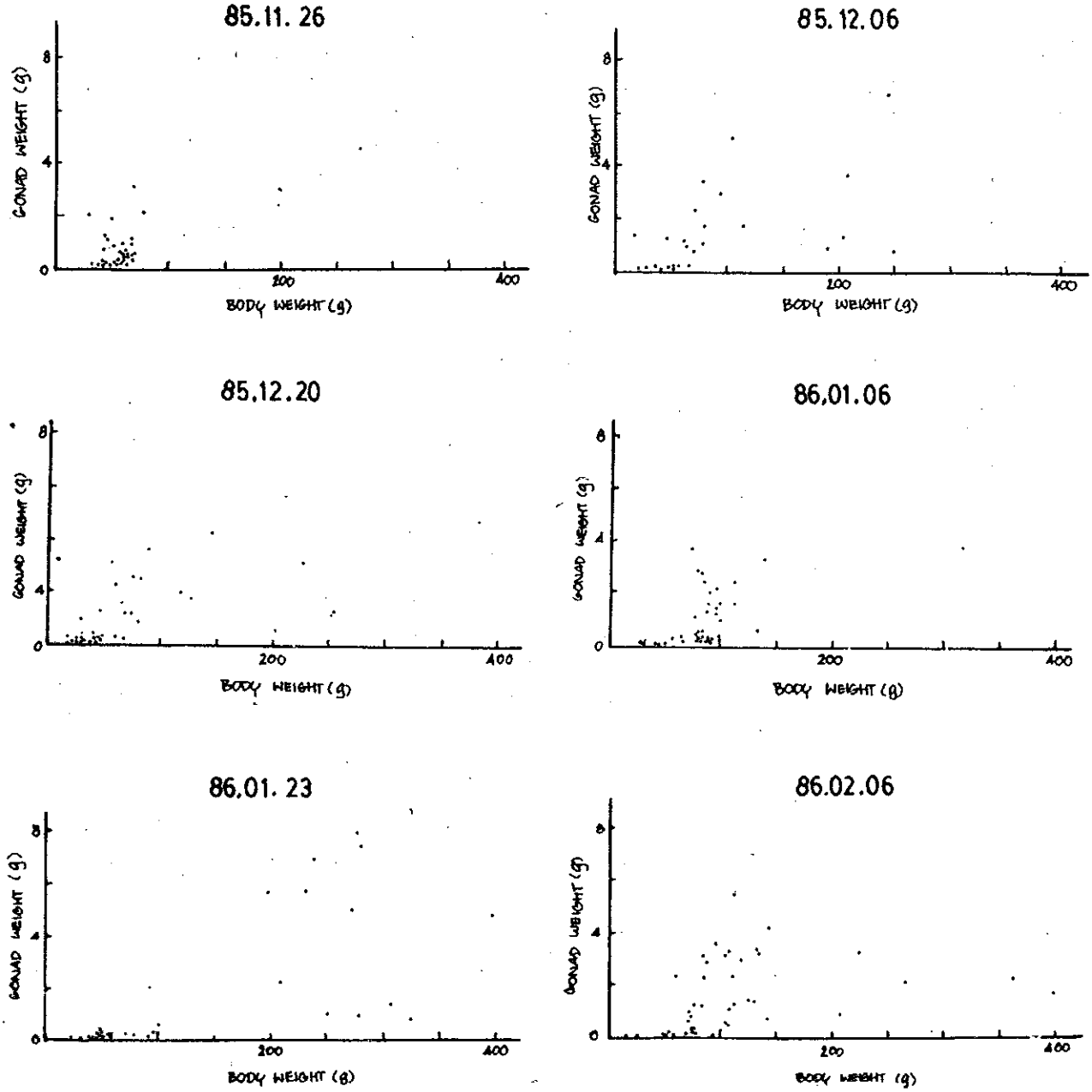
ns = not significantly different (P>0.5)

Table 3 Median test for body weight/gonad weight relationship in GSI during 9 months.

	Time (yr.mon.date)	GSI > grand median	GSI < grand median	Total
1.	85.11.26	16	19	35
2.	85.12.06	12	12	24
3.	85.12.20	17	15	32
4.	86.01.06	17	23	40
5.	86.01.23	10	24	34
6.	86.02.06	19	24	43
7.	86.02.27	13	8	21
8.	86.03.17	18	18	36
9.	86.03.31	16	22	38
10.	86.04.15	13	7	20
11.	86.05.01	20	14	34
12.	86.06.03	19	11	30
13.	86.07.28	15	16	31
	Total	205	213	418

$P > 0.05$ (Chi-square test)

Fig. 1-13 The relationship between body weight and gonad weight at each sampling time in 9 months



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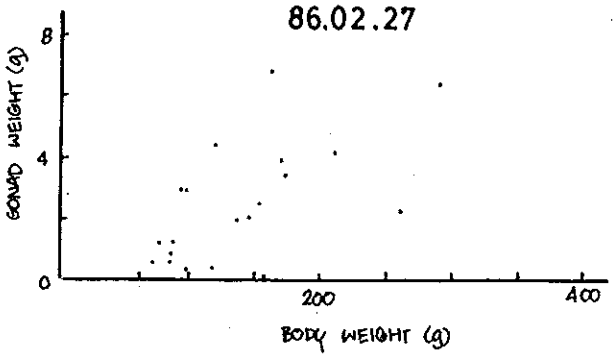

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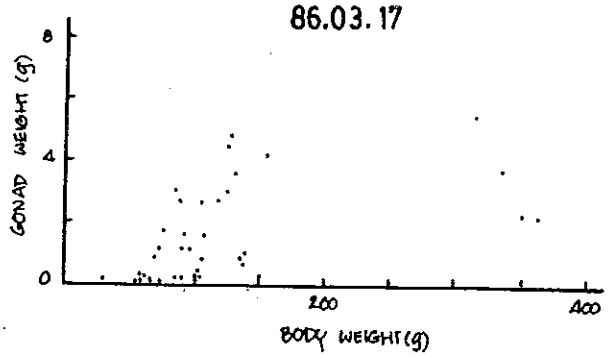

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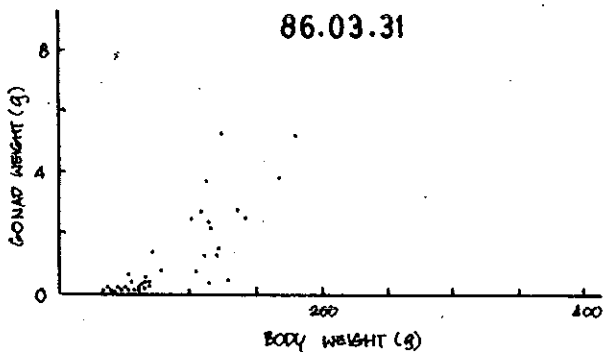

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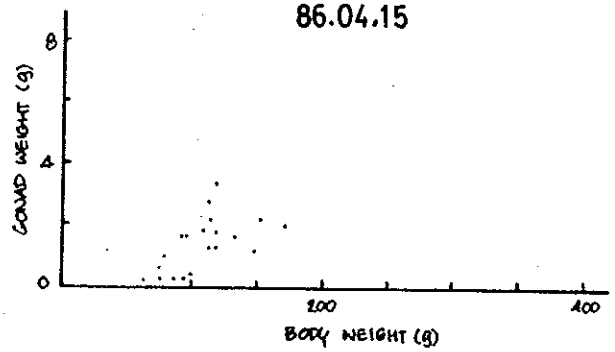

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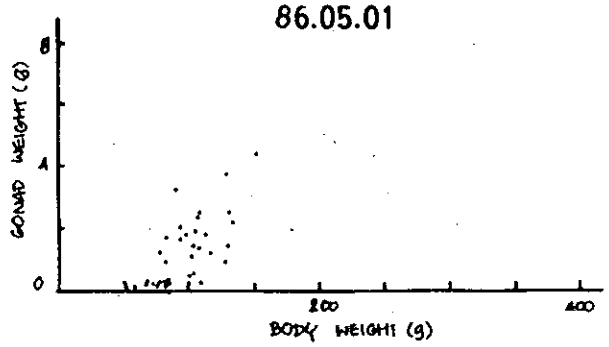

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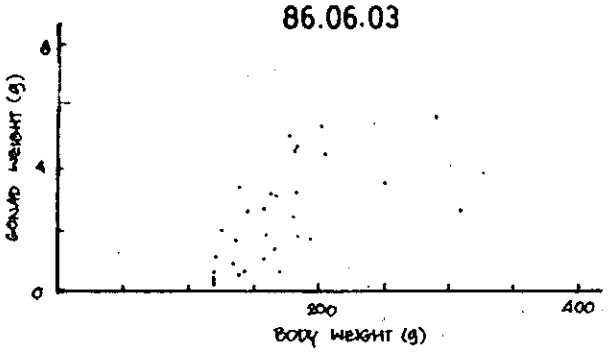

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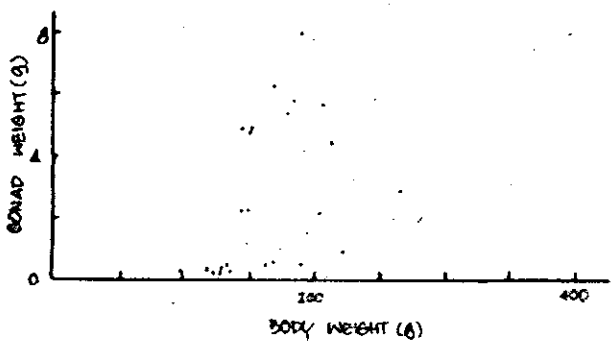


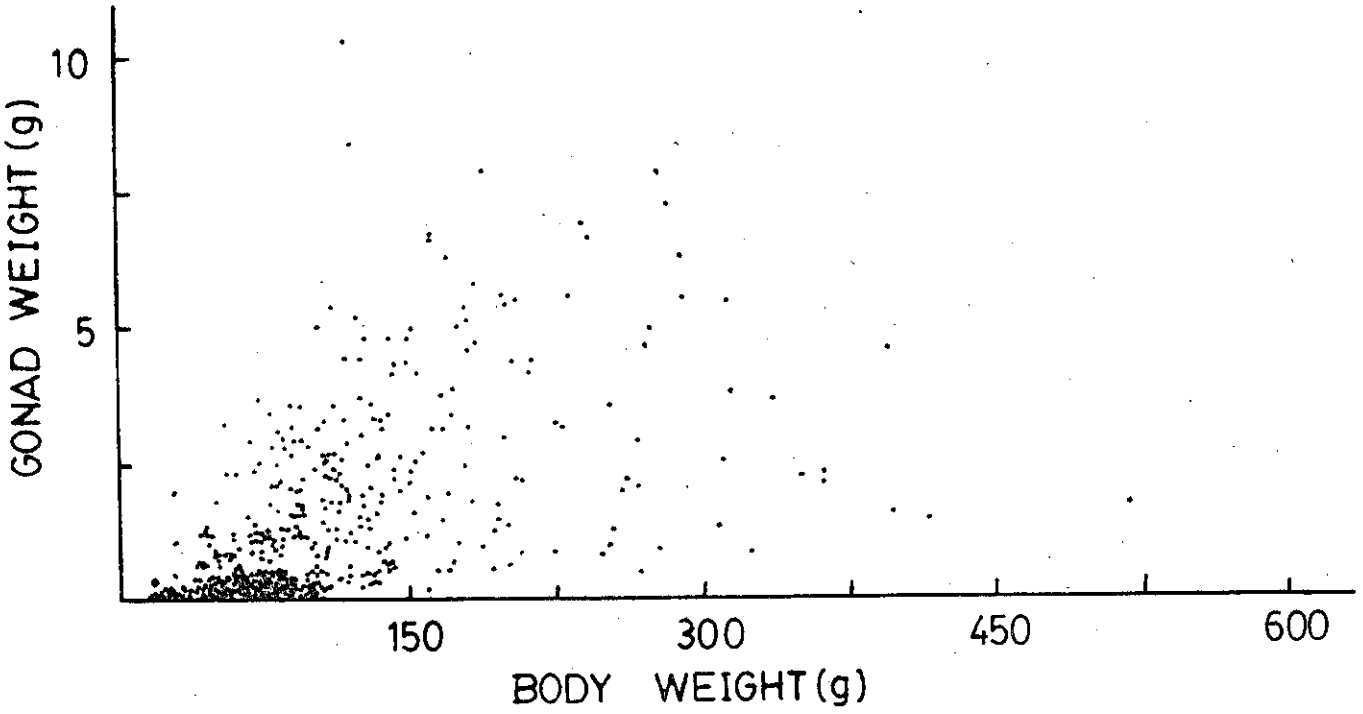


Fig. 14 The relationship between body weight and gonad weight throughout 9 months

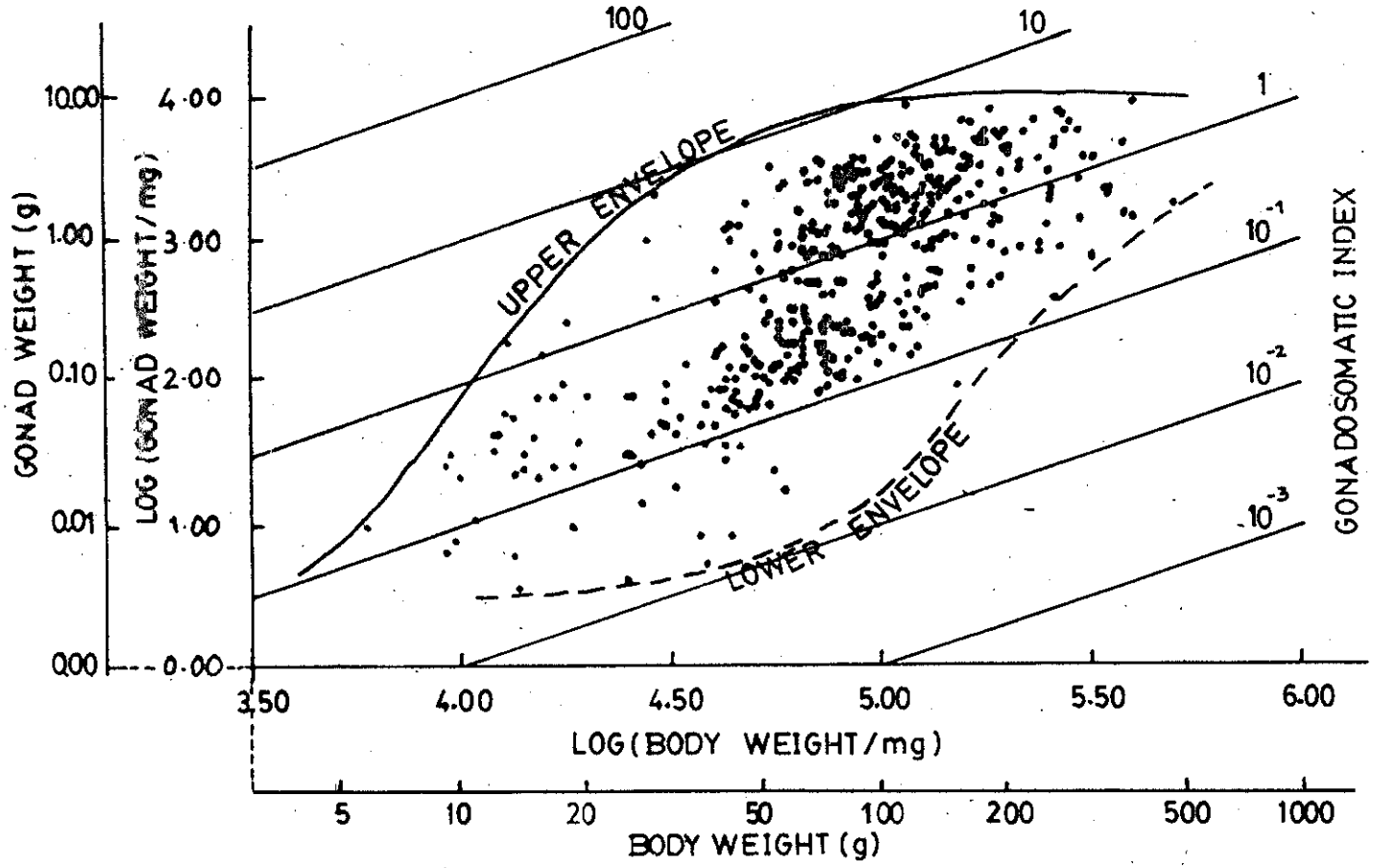


Fig. 15 The logarithmic transformation of body weight and gonad weight throughout 9 months

PART B RELATIONSHIP OF OVARIAN DEVELOPMENTAL STAGE TO BODY WEIGHT/GONAD WEIGHT DISTRIBUTION

PROCEDURES

A subsample of 8 to 12 ovaries from the fish sample at each collection time was made for histological examination. Subsampling was done by first plotting the distribution of gonad weight against body weight on logarithmic axes and then selecting ovaries from the high, mid and low range of log gonad weight in each of 4 to 5 log body weight intervals of Ø.1.

The various cytological stages of oogenesis were determined in the histological study by following the methods and principles outlined by Nagahama (1983) to ascertain the state of maturation of the ovaries.

Ovaries generally contain several different stages of oocyte development but the particular stages present, and their relative numbers vary. Ovaries of S. nilotieus were classified according to the oocyte developmental stages present.

RESULTS AND DISCUSSION

In this experiment, the developmental stage of S. nilotieus is divided into 8 stages (Nagahama, 1983) :-

1. Oogonium stage :- range in diameter from 5 to 1Ø μm.

and consist of a large round nucleus, a single prominent nucleolus and pale indistinst cytoplasm. They are grouped in cell 'nests'.

2. Perinucleolus stage

a) Early perinucleolus stage :- The nucleus increases in size, and multiple nucleoli become located around the periphery of the large pale nucleus and basophilic cytoplasm. Oocyte diameters for this stage ranged from 35 to 52 μm.

b) Late perinucleolus stage :- can be distinguished from previous stage by the enlargement of oocyte with yolk nucleus in slightly basophilic cytoplasm. An increased oocyte diameter ranges from 75 to 210 μm.

3. Yolk-vesicle stage :- The first structure to appear within the oocyte cytoplasm during the secondary growth of oocytes and first appear in the outer and midcortical zones of the oocyte. At maturity of this stage they move to the periphery of the oocyte, where they are known as cortical alveoli. In this stage, oocyte diameter ranges from 180 to 300 μm.

4. Oil-drop stage :- Oil droplets first appear in the perinucleolus area and then migrate to the periphery in later stages. An increasing in number fat drops occur in cytoplasm around nucleus. The range of diameter of oocyte is 250 to 370 μm.

5. Primary-yolk globule stage :- can be identified by the deposition of primary yolk. Yolk globules are formed in

this stage by the fusion of small, coated vesicle. In this stage, the oocyte has a slightly eosinophilic cytoplasm, and the diameter of oocytes ranges from 280 to 450 μm.

6. Secondary-yolk globule stage :- oocyte diameter ranges from 434 to 824 μm. Oocytes show secondary yolk granules (size about 3.7-7.5 μm.) within the eosinophilic cytoplasm. Nucleus is eosinophilic with nucleoli.

7. Tertiary yolk globule stage :- is characterized by a rapid increased in size of oocytes ranging form 890 to 1260 μm. The yolk globules from a single mass of yolk. Increasing numbers of eosinophilic yolk globules indicating tertiary yolk deposition appear in the cytoplasm.

8. Maturation stage :- Oocyte diameter ranges from 1300 to 1628 μm. The nucleus was frequently eccentric in its location within the cell, and the nulear membrane and nycleoli show signs of disintegration. The oocytes. with strongly eosinophilic cytoplasm are filled with tertiary yolk globules (size about 12.5 μm) which coalesce to form a solid mass.

Composition of ovaries examined is shown in Table A1 (Appendix).

More than one developmental stage was present in most ovaries. On the basis of developmental stages of gonads represented in the ovary, ovaries could be distinguished as belonging to one of six "combonations".

Combination 1. Oogonia only (Fig. 16).

2. Oogonia - preprimary yolk globule

oocytes only (Fig. 17).

3. Oogonia - pre-maturation oocytes only (Fig. 18).
4. Oogonia - maturation oocytes (Fig. 19).
5. Post-oogonium stages - pre-maturation oocytes only (Fig. 20).
6. Post-oogonium stages - maturation oocytes only (Fig. 21).

The distribution of fish with ovaries in each of these combinations is shown in Fig. 22. The distributions are clearly defined. The ranges of gonad weight of ovaries in these different combinations are shown in Table 4.

A test of significant differences among 4 combinations (combinations 3 and 5, and combinations 4 and 6 combined) by comparing the slopes of regression lines is shown in Table 5. These regression lines are shown in Fig. 23. Comparison of slopes and elevations of these regression lines by analysis of ovariance showed no significant differences between slopes but highly significant differences in elevation ($P<0.0005$). Multiple comparison procedures to determine between which elevations the differences occur, revealed the elevation of each line is significantly different from all others $P<0.001$ (Table 6).

Table 4 The range of gonad weight of ovaries with different combinations.

Combination	range of gonad weight min. - max. (g)
1. Oogonia only	0.004 - 0.017
2. Oogonia - pre-primary yolk globule oocytes only	0.005 - 1.121
3. Oogonia - pre-maturation oocytes only	0.096 - 5.346
4. Ooginia - maturation oocytes	2.281 - 6.632
5. Post-oogonium stages - pre-maturation oocytes only	0.194 - 3.500
6. Post-oogonium stages - maturation oocytes only	1.986 - 8.947

Table 5 Calculations for testing for significant difference between slopes and elevations of regression lines (log body weight vs. log gonad weight) for each combination .

		x^2	xy	y^2	Res. SS	Res. DF	F_1-v	F_2-v
Regression combination	of 1	0.0745	0.1173	0.1987	0.0060	1	2.31 ns	80.10 **
	2	2.8555	2.4967	9.0020	5.9694	45		
	3 (3+5)	2.7893	1.2693	6.9278	6.3582	56		
	4 (4+6)	0.7577	0.4447	0.4878	0.2276	13		
"Pooled" regression					12.5524	115		
"Common" regression		5.6770	4.3280	16.6083	13.3087	118		
Total regression		7.0264	12.0205	60.9776	48.4134	121		

F_1-v = F-value of test for differences between slopes
F_2-v = F-value of test for differences between elevations
ns = not significantly different at 0.05 confidence interval
** = significantly different at 0.0005 confidence interval

Table 6 Multiple comparisons among elevations of those continuous (combination 3+5 = 3, combination 4+6 = 4) by The Newman-Keuls multiple range test.

Comp. (B vs. A)	Difference $(\bar{X}_B-\bar{X}_A)$	Difference $(\bar{Y}_B-\bar{Y}_A)$	SE	q	$q_{\alpha,n5,p}$	Conclusion
4 vs. 1	0.4530	2.6583	0.1911	12.1033	5.4760	$\mu 4 = \mu 1$
4 vs. 2	0.2331	1.4778	0.0778	16.7106	5.2110	$\mu 4 = \mu 2$
4 vs. 3	0.0601	0.5458	0.0691	7.2356	4.7710	$\mu 4 = \mu 3$
3 vs. 1	0.3929	2.1125	0.1510	12.0063	5.2110	$\mu 3 = \mu 1$
3 vs. 2	0.1730	0.9320	0.0502	15.9383	4.7710	$\mu 3 = \mu 1$
2 vs. 1	0.2199	1.1075	0.1459	6.4417	4.7710	$\mu 2 = \mu 1$

α = The significance level
p = The total number of means being tested
overall conclusion : $\mu 1 = \mu 2 = \mu 3 = \mu 4$
each elevation was significant different ($P<0.001$)

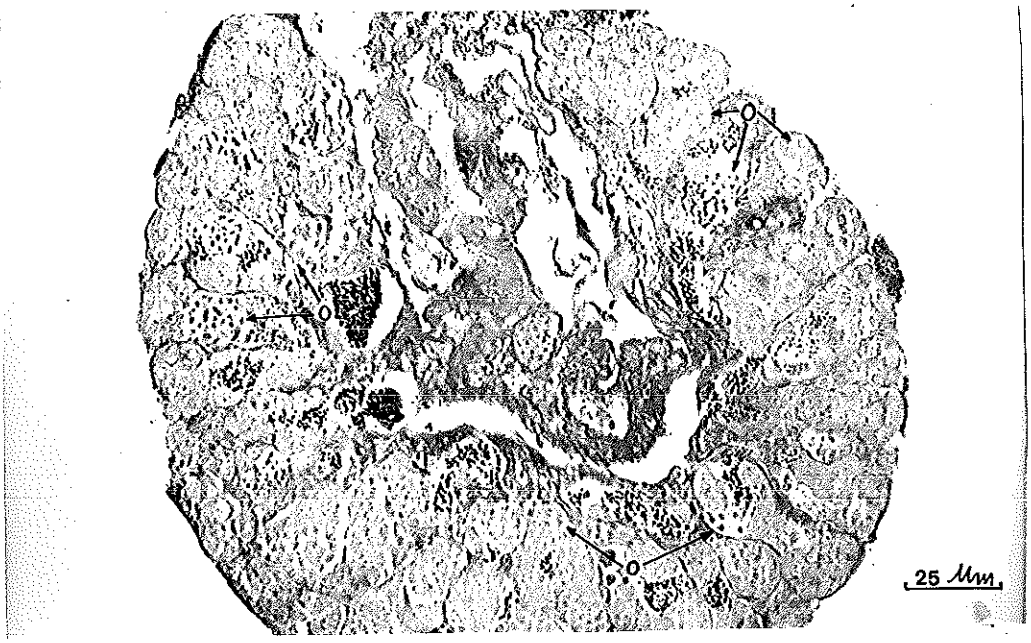


Fig. 16 Combination 1: "oogonia only"; O = Oogonia stage.

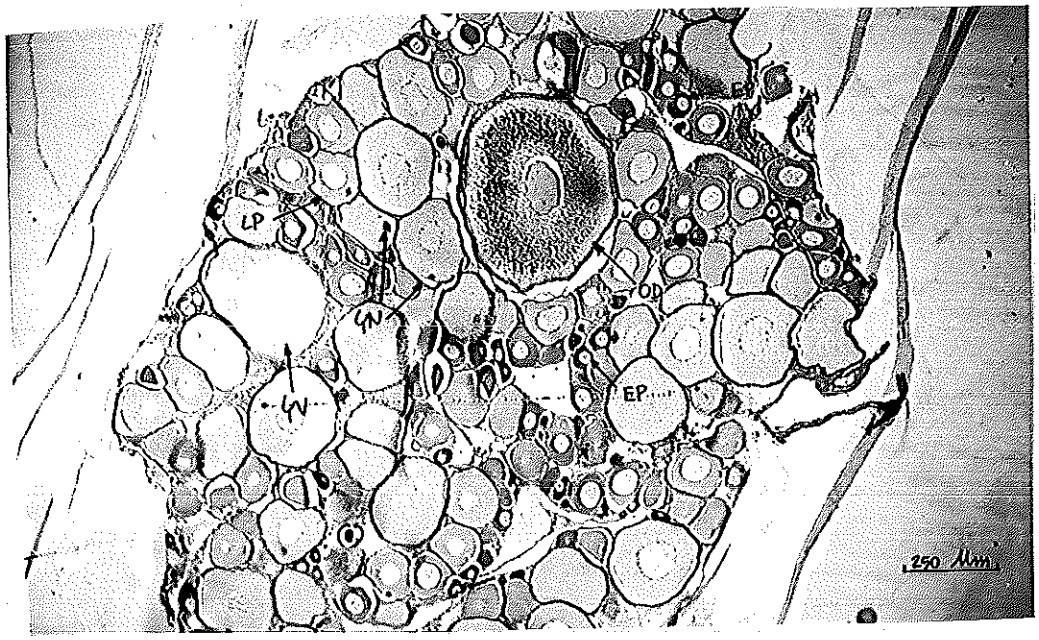


Fig. 17 Combination 2: "oogonia to pre-primary yolk globule oocytes only"; EP = Early-perinucleolus stage, LP = Late-perinucleolus stage, YN = Yolk nucleus, YV = Yolk-vesicle stage, OD = Oil-drop stage.

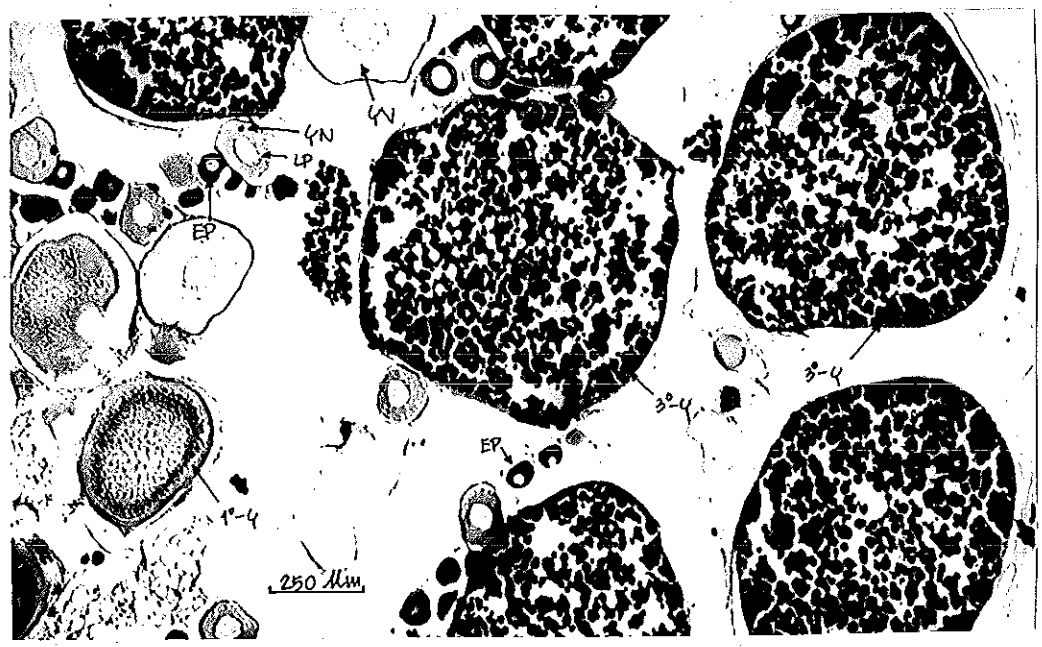


Fig. 18 Combination 3: "oogonia to pre-maturation oocytes only"; EP = Early-perinucleolus stage, LP = Late-perinucleolus stage, YN = Yolk nucleus, 1°-Y = Primary-yolk stage, 3°-Y = Tertiary-yolk stage.

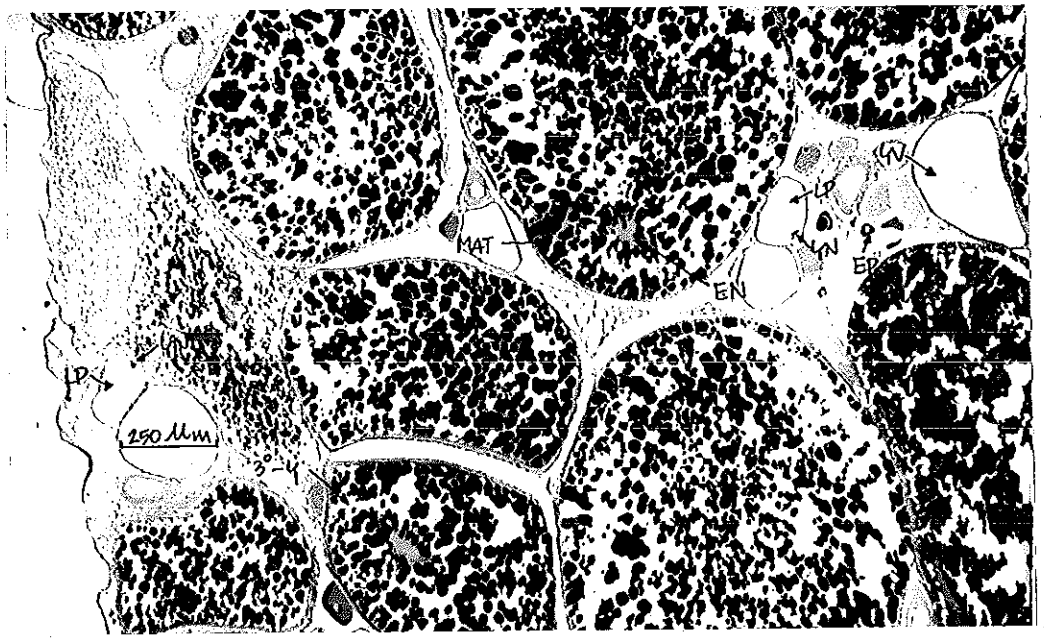


Fig. 19 Combination 4: "oogonia-maturation oocytes"; EP = Early-perinucleolus stage, LP = Late-perinucleolus stage, YN = Yolk nucleus, YV = Yolk-vesicle stage, 3°-Y = Tertiary-yolk stage, MAT = Maturation stage, EN = Eccentric nucleus.

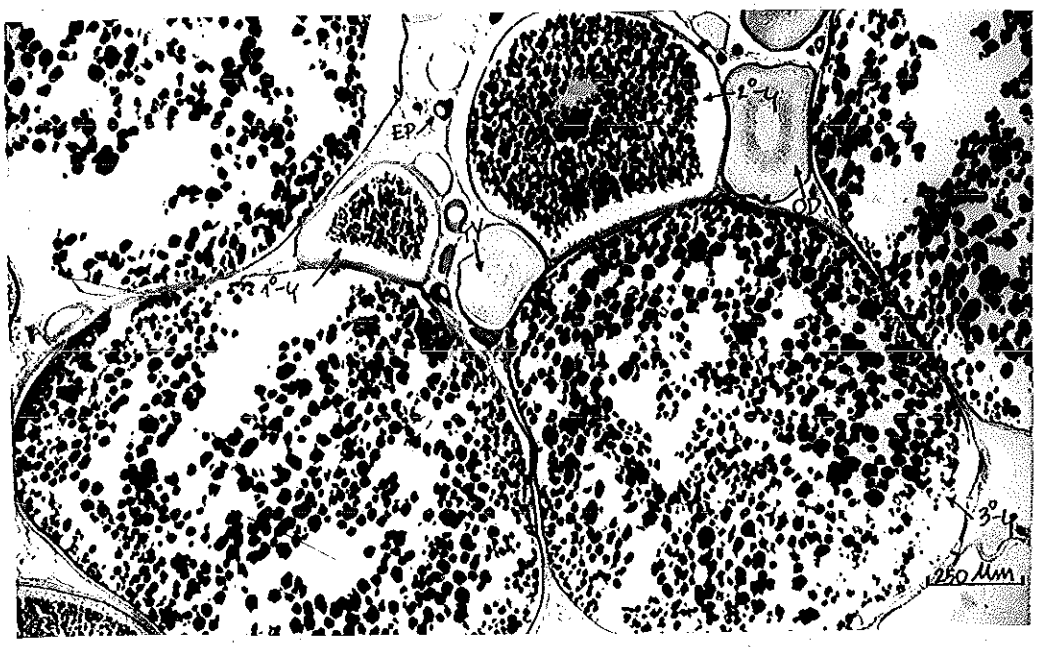


Fig. 20 Combination 5: "post-oogonium stages to pre-maturation oocytes only"; EP = Early-perinucleolus stage, YV = Yolk-vesicle stage, OD = Oil-drop stage, 1°-Y = Primary-yolk stage, 2°-Y = Secondary-yolk stage, 3°-Y = Tertiary-yolk stage.

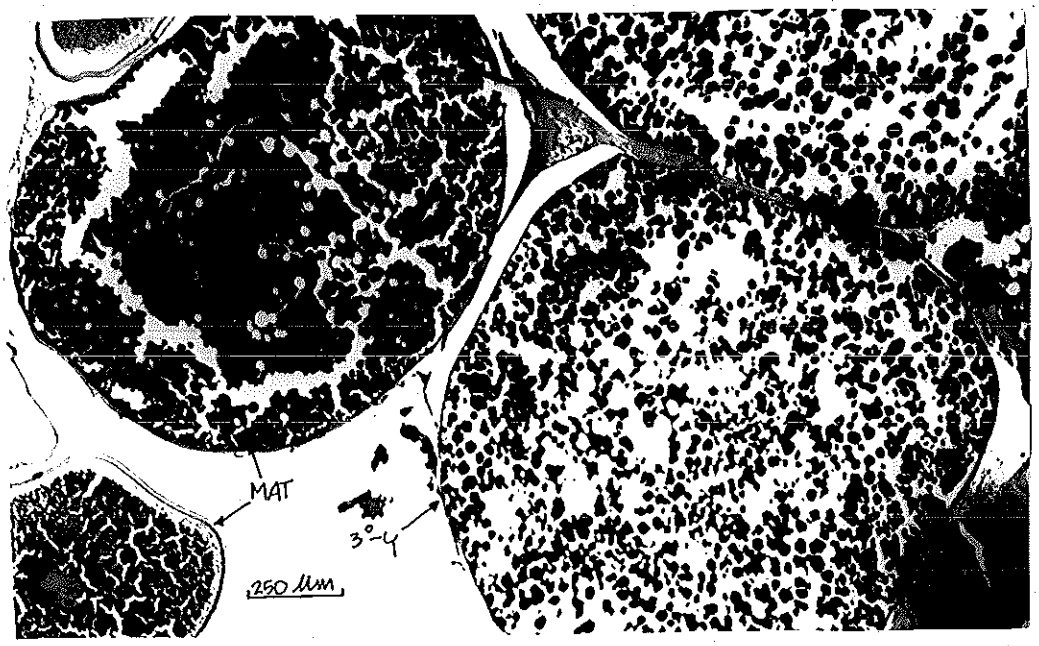


Fig. 21 Combination 6: "post-oogonium stages-maturation oocytes only"; 3°-Y = Tertiary-yolk stage, MAT = Maturation stage.

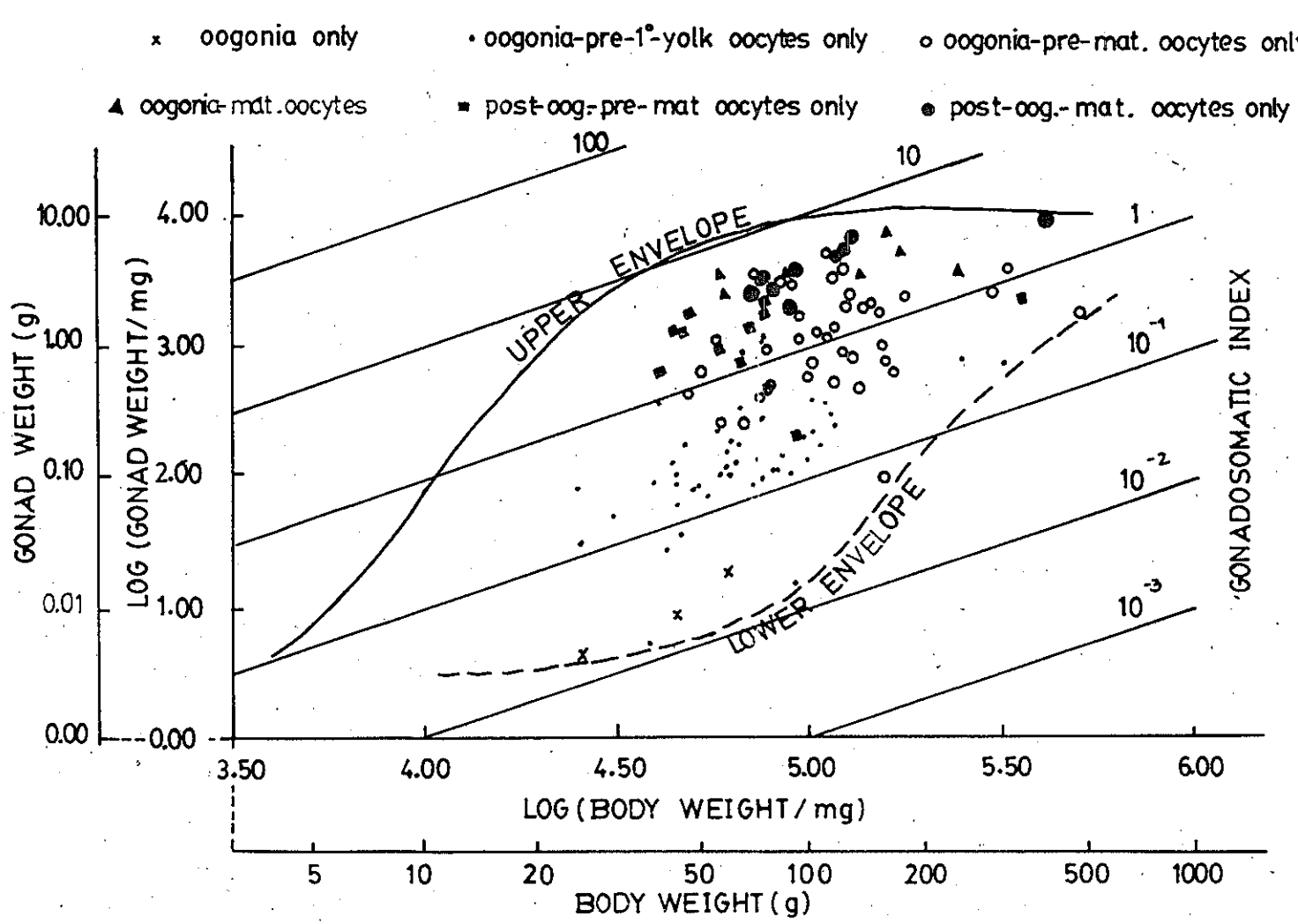


Fig. 22 The distribution of fish with ovaries in each of six combinations

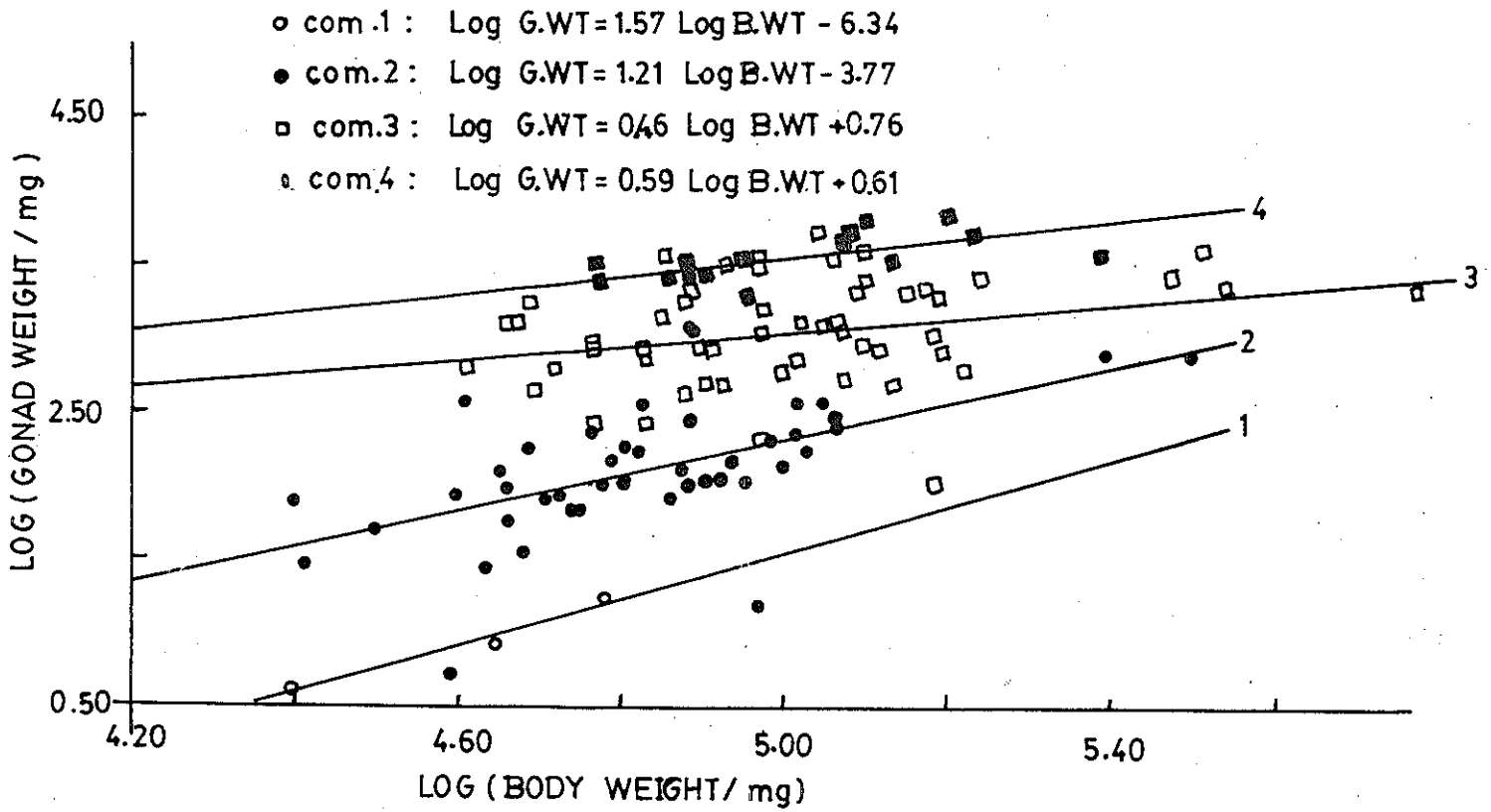


Fig 23 The regression lines of 4 combinations (combination 3 combined with 5 and combination 4 combined with 6)

EVALUATION

No evidence of a seasonal breeding season in this population during November, 1985 to July, 1986 was apparent, although the wide range of ovarian development in fish with a body weight greater than about 20 g suggests a cyclicity of reproductive development but with no synchrony among the members of the population. The use of fish greater than 20 g for an investigation of pineal effects would be unsuitable because of the heterogenity of samples. For this reason for examination of the pineal effects, it was decided to use fish of smaller size so that the period of initial development of ovaries was covered, i.e. the period of highest gradient in the envelope curve (log gonad weight/log body weight).

The similar width of the range of log gonad weight in the log gonad weight body weight distribution of fish in different ovarian development states makes the use of this distribution itself an ideal indicator of ovarian development. In this aspect it differs from the use of mean GSI, which hides the true relationships between body weight and gonad weight within the samples and is not so sound statistically. In using GSI, the estimated population variance of GSI of fish with mature gonads differs markedly from that of fish with immature or intermediate ovaries.

CHAPTER 3

PRELIMINARY EFFECT OF PINEAL ADMINISTRATION : THE COLOUR CHANGE OF S. niloticus

The effect on colour change of S. niloticus of bovine pineal injection was investigated here. This preliminary experiment was performed to determine if colour change was indeed induced by macerated bovine pineal injection. If it were, then uptake of the colour change inducing principle, presumed to be melatonin, would be confirmed.

PROCEDURES

Ten female fish from Tung Lung (12-15 cm body length and 35 -80 g body weight) were used. Five fish kept under constant light and five under constant darkness for 7 days before the beginning of this experiment. Of each group of five, three were injected intraperitoneally with a mixed solution of macerated bovine pineal and fish Ringer's solution (Hoar and Randall, 1970), and the rest with Ringer alone.

The mixed solution was prepared by homogenizing bovine pineal (bull's pineal only) with a manual glass homogenizer and adding fish Ringer's solution (30 mg pineal/injection). Homogenization was continued until the mixed solution

appeared homogeneous. Each fish was injected with approximately 0.25 cm^3 of solution at approximately 12.00 h.

The shade of colour was observed and given a "darkness score", ranging from 0 to +4, on the basis of intensity of shade colour of transverse lines on the body of fish. This observation was made at 2 and 6 hours after injection and again immediately before each injection. Each group was exposed to either constant light or constant darkness. Injection was repeated daily for 10 days.

After 10 days of culture , the fish were killed, and the body weight and gonad weight recorded.

RESULTS

After 7 days in constant light and/or constant darkness, the shade of colour of fish in constant darkness was darker than that of fish in constant light. When fish were handled prior to injection, they turned darker but the colour returned to that prior to handling after replacement in the tanks.

The shades of colour of control and treatment fish in constant light and/or constant darkness at 2, 6 and 24 hours throughout 10 days are shown in Tables 7-9.

Frequency distributions of the darkness scores are shown in figures 24-35. There were no significant differences in frequency of scores between 14.00 h, 18.00 h and 12.00 h in fish under condition of either constant

light or constant dark. However, the pattern of darkness scores of fish in constant light showed a paler colouration than that of fish in constant darkness at all three times. The difference was highly significant at 14.ØØ and 12.ØØ hr ($P<Ø.ØØ1$ and $P<Ø.Ø25$, X^2) but did not quite reach significance at 16.ØØ hr ($Ø.Ø5<P<Ø.1$, X^2).

Fish of the constant light group receiving pineal injection were significantly darker than controls ($P<Ø.Ø1$) at all times after pineal injection, and the range of darkness scores was wider (Ø to +2 in control; +1 to +4 in treatment).

Fish of the constant darkness group receiving pineal injection, showed a slight tendency to increased darkness but the frequency of darkness scores was significant only at 6 hrs after pineal injection ($P<Ø.Ø5$). The reason for this is presumably because fish of this group were already dark (Fig. 24 and 34).

The results of a test of significance of the different frequency distributions of the darkness scores is shown in Table 1Ø.

Table 7 Darkness scores of control and treated fish in constant light and/or constant darkness at 2 hours after daily injection throughout 10 days.

DAY	CONST. LT+R	CONST. LT+R	CONST. LT+P	CONST. LT+P	CONST. LT+P	CONST. DK+R	CONST. DK+R	CONST. DK+P	CONST. DK+P	CONST. DK+P
1	+1	+1	+3	+3	+3	+2	+2	+3	+3	+3
2	+2	+1	+4	+4	+3	+2	+2	+3	+3	+3
3	+2	+2	+3	+3	+2	+1	+2	+2	+2	+2
4	+1	+2	+3	+3	+2	+2	+2	+2	+2	+2
5	+1	+1	+2	+2	+2	+2	+2	+2	+2	+2
6	+1	+1	+2	+2	+2	+2	+2	+3	+2	+2
7	+1	+1	+1	+2	+2	+2	+2	+3	+2	+2
8	+1	+1	+1	+2	+2	+2	+2	+2	+2	+2
9	+1	+1	+3	+3	+2	+2	+2	+2	+2	+2
10	+1	+2	+2	+2	+1	+2	+2	+2	+2	+2

0 --> +4 = darkness score
CONST. LT+R = constant light + Ringer's solution
CONST. LT+P = constant light + macerated bovine pineal
CONST. DK+R = constant darkness + Ringer's solution
CONST. DK+P = constant darkness + macerated bovine pineal

Table 8 Darkness scores of control and treated fish in constant light and/or constant darkness at 6 hours after daily injection throughout 10 days.

DAY	CONST. LT+R	CONST. LT+R	CONST. LT+P	CONST. LT+P	CONST. LT+P	CONST. DK+R	CONST. DK+R	CONST. DK+P	CONST. DK+P	CONST. DK+P
1	+1	+1	+3	+2	+2	+1	+1	+2	+2	+2
2	+1	0	+4	+3	+3	+1	+2	+2	+2	+3
3	+2	+1	+3	+3	+2	+1	+2	+2	+2	+2
4	+1	+2	+3	+2	+2	+1	+2	+2	+2	+2
5	+1	+1	+1	+2	+2	+1	+2	+2	+2	+2
6	+1	+1	+1	+2	+2	+2	+2	+2	+2	+2
7	+1	+1	+1	+2	+2	+2	+2	+2	+2	+2
8	+1	+1	+1	+2	+1	+2	+2	+2	+2	+2
9	+2	+2	+3	+2	+2	+2	+2	+2	+2	+2
10	+1	+2	+2	+2	+1	+2	+2	+2	+2	+2

0 --> +4 = darkness score
CONST. LT+R = constant light + Ringer's solution
CONST. LT+P = constant light + macerated bovine pineal
CONST. DK+R = constant darkness + Ringer's solution
CONST. DK+P = constant darkness + macerated bovine pineal

Table 9 Darkness scores of control and treated fish in constant light and/or constant darkness at 24 hours after daily injection throughout 10 days.

DAY	CONST. LT+R	CONST. LT+R	CONST. LT+P	CONST. LT+P	CONST. LT+P	CONST. DK+R	CONST. DK+R	CONST. DK+P	CONST. DK+P	CONST. DK+P
1	+1	+1	+4	+2	+3	+2	+2	+3	+2	+2
2	+2	+1	+3	+2	+2	+1	+2	+2	+2	+2
3	+1	+2	+3	+2	+2	+1	+1	+2	+2	+2
4	+1	+2	+2	+2	+2	+1	+2	+2	+2	+2
5	+1	+1	+1	+2	+2	+2	+2	+2	+2	+2
6	+1	+1	+1	+2	+1	+2	+2	+2	+2	+2
7	+1	+1	+1	+1	+1	+2	+2	+2	+2	+2
8	+1	+1	+2	+2	+2	+2	+2	+2	+3	+2
9	+1	+1	+2	+3	+1	+1	+2	+2	+1	+2
10	+1	+2	+2	+2	+1	+2	+2	+2	+2	+2

0 --> +4 = darkness score
CONST. LT+R = constant light + Ringer's solution
CONST. LT+P = constant light + macerated bovine pineal
CONST. DK+R = constant darkness + Ringer's solution
CONST. DK+P = constant darkness + macerated bovine pineal

Table 10 The Chi-square test of differences of frequency distribution of the darkness scores of fish.

Hours after injection	uninjected const. LT compared with uninjected control in const. DK	Pineal injection in const. LT compared with uninjected control	Pineal injection in const. DK compared with uninjected control
2 hrs	P<0.001	P<0.001	P>0.10
6 hrs	P>0.05	P<0.005	P<0.05
24 hrs	P<0.025	P<0.01	P>0.10

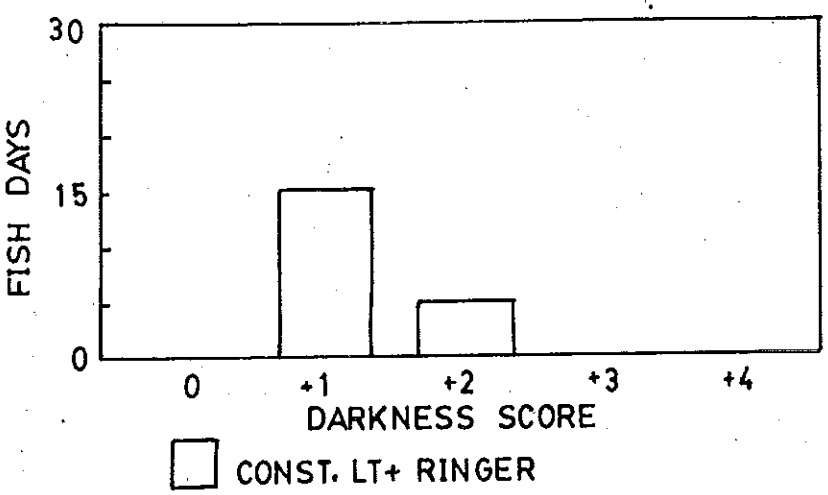


Fig. 24 The frequency distribution of the darkness scores of control group in constant light 2 h after Ringer's solution injection.

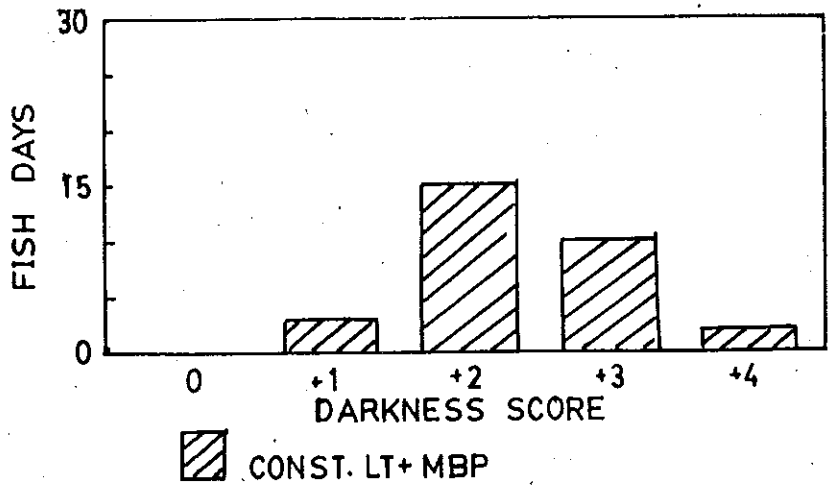


Fig. 25 The frequency distribution of the darkness scores of treatment group in constant light 2 h after MBP injection.

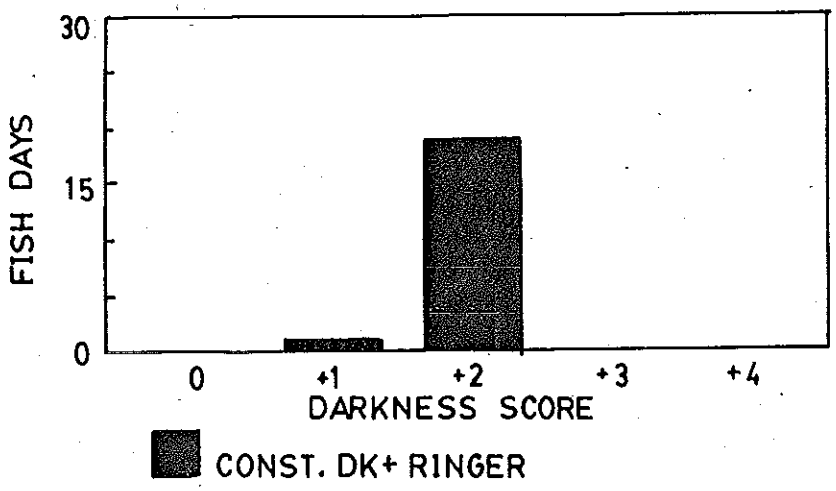


Fig. 26 The frequency distribution of the darkness scores of control group in constant darkness 2 h after Ringer's solution injection.

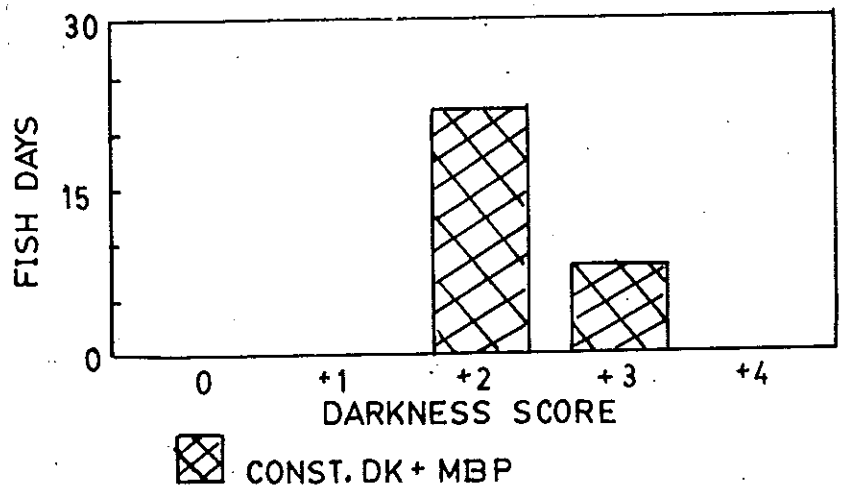


Fig. 27 The frequency distribution of the darkness scores of treatment group in constant darkness 2 h after MBP injection.

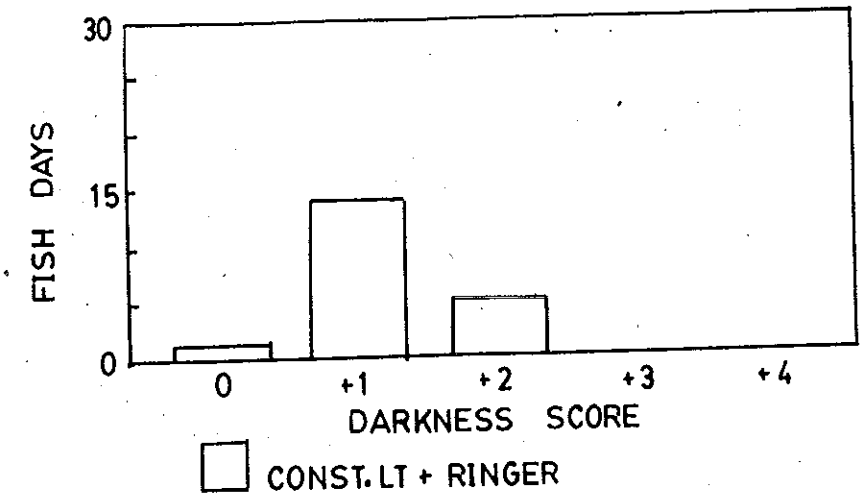


Fig. 28 The frequency distribution of the darkness scores of control group in constant light 6 h after Ringer's solution injection.

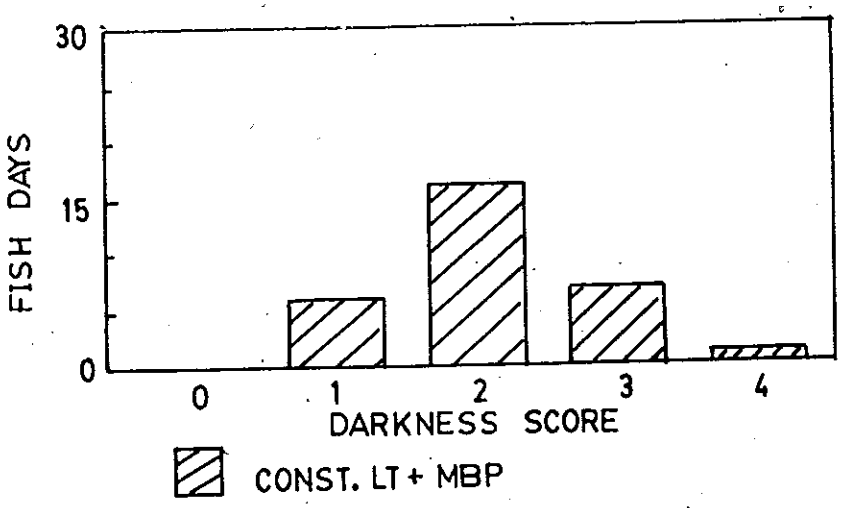


Fig. 29 The frequency distribution of the darkness scores of treatment group in constant light 6 h after MBP injection

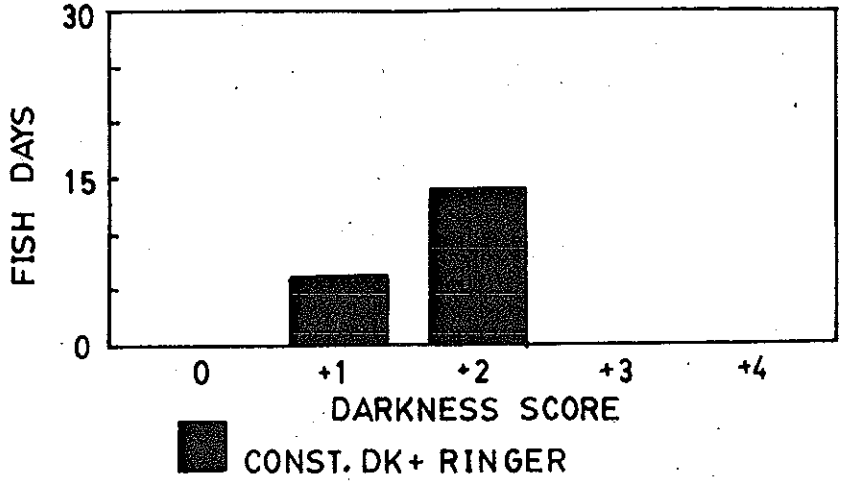


Fig. 30 The frequency distribution of the darkness scores of control group in constant darkness 6 h after Ringer's solution injection.

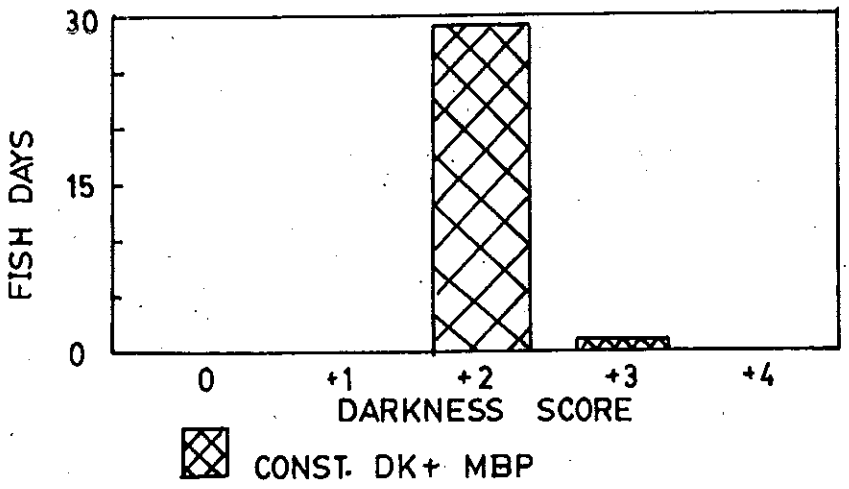


Fig. 31 The frequency distribution of the darkness scores of treatment group in constant darkness 6 h after MBP injection.

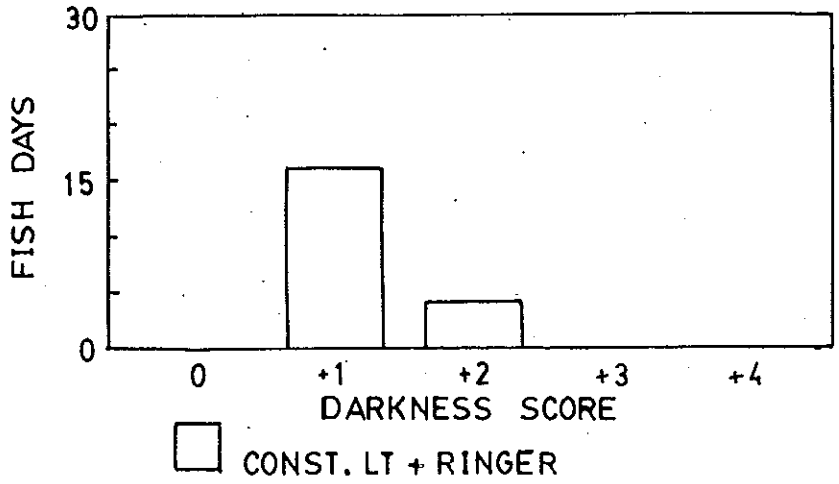


Fig. 32 The frequency distribution of the darkness scores of control group in constant light 24 h after Ringer's solution injection.

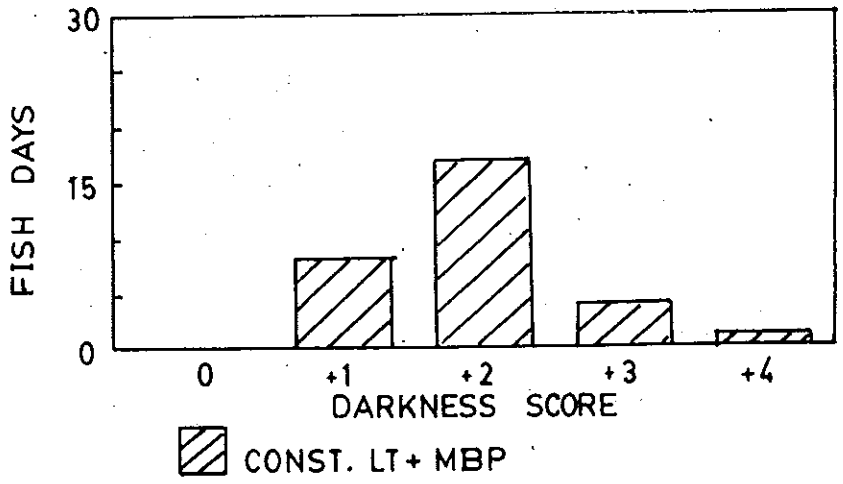


Fig. 33 The frequency distribution of the darkness scores of treatment group in constant light 24 h after MBP injection.

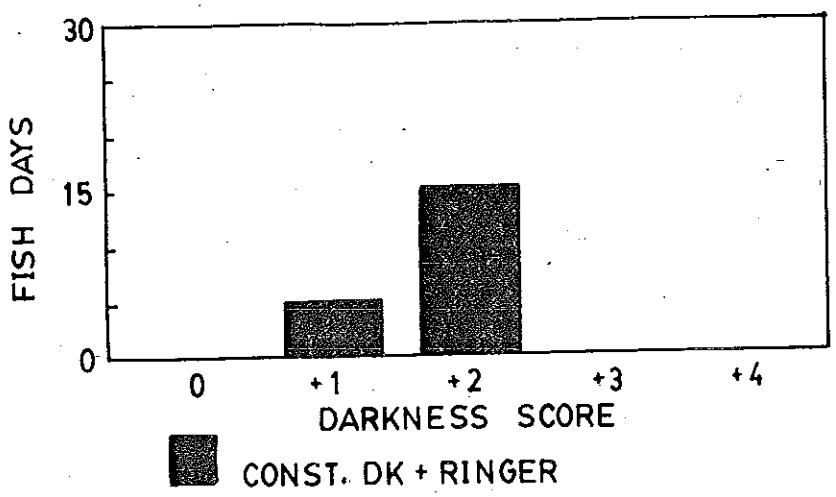


Fig. 34 The frequency distribution of the darkness scores of control group in constant darkness 24 h after Ringer's solution injection.

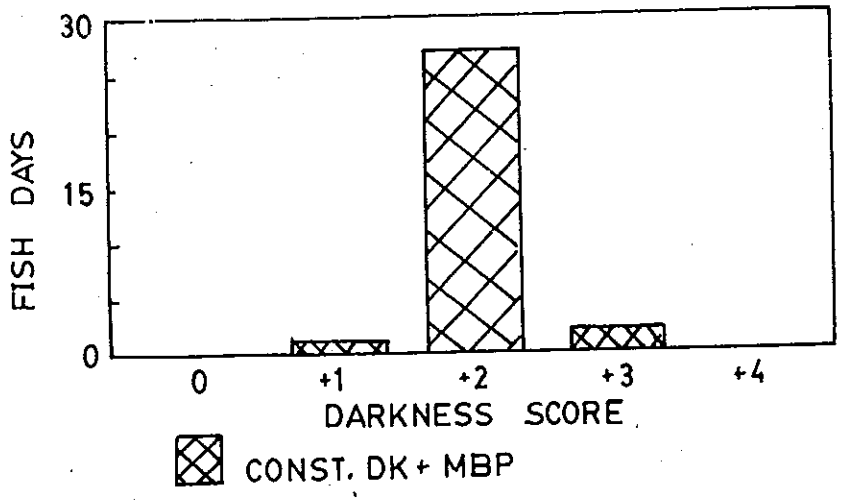


Fig. 35 The frequency distribution of the darkness scores of treatment group in constant darkness 24 h after MBP injection.

EVALUATION

At the beginning of the experiment, before injection, fish in constant darkness were darker than in those constant light. However, in general, most fish and amphibians studied on LD cycle tend to be dark coloured during the day and pale by night and, in constant conditions, the colour changes persist with periods of approximately 24 hr, i.e., Xenopus tadpoles (Bagnara and Hadley, 1970) and lampreys (Young, 1935; Joss, 1973) etc.

In S. niloticus a darkening of the skin followed pineal injection, which was readily apparent in fish which were initially pale as a result of being kept in constant light. This contrasts with results reported for Xenopus laevis tadpoles and Lampetra ammocoetes that bovine pineal extract (presumably containing melatonin) elicited a blanching of skin of these animals (Young, 1935; Lerner et al, 1958; Bagnara and Hadley, 1970; Joss, 1973).

These observations suggest that the pineal substances controlling pigmentation have an opposite effect in S.niloticus from that in these other animals (tadpoles, lamprey etc.).

However, this opposite effect on dermal melanophore combined with the opposite natural colour change in light and/or darkness suggests that the pattern of secretion of the pineal melanophore controlling substances (melatonin) is similar in S. niloticus to that in lampreys and tadpoles,

i.e. secreted during the dark phase and inhibited during the light phase.

CHAPTER 4

EFFECT OF PHOTOPERIOD AND PINEALECTOMY IN OVARIAN DEVELOPMENT OF S. niloticus

The reproductive cycles of many temperate zone fish are regulated or sychronized by seasonally changing daylength (de Vlaming, 1974 lit. cit. de Vlaming and Vodicnik , 1978). At present it is not known whether the effects of photoperiod on teleostean reproduction are mediated via retinal pathways and/or by extraretinal receptors. In teleost fish, the photoreceptive function of the pineal has been emphasized on the basis of neurophysiological studies (Dodt, 1963; Morita 1966 lit. cit. Urasaki, 1972) and electron microscopical observations (Herwing, 1963 lit. cit de Vlaming and Vodicnik, 1978). Therefore, the pineal organ could potentially be involved in mediating the effect of photoperiod on reproduction in teleosts. Furthermore, the pineal organ itself may also function as an endocrine gland (Hafeez, 1971).

Many investigators have indicated that pineal-gonadal relationship in teleosts may be manifest in many ways including a progonadal, an antigonadal or no effect of the pineal, depending upon the phase of the reproductive cycle, or on the thermal and photoperiodic conditions (Fenwick, 1970 and Hontela, 1978).

Selection of S. nilotícus in a particular phase of its reproduction would seem to present a problem since cycles are not related to season and are asynchronous within a population. The initial ovarian maturation, however, seems to occur between about 7g and 20 g body weight.

In investigating the control of ovarian maturation in S. nilotícus, the following possible roles of the pineal have been considered :-

- The ovary itself is either responsive or nonresponsive to pineal stimulation. If it is responsive, the ovary could either respond positively (i.e. develop) or negatively (i.e. remain undevelop). If this is the case, at the time of ovarian maturation the ovary must either be stimulated positively or an inhibitory response must be removed. Alternatively, the ovary could be nonresponsive to the pineal secretions.

- As for the pineal itself, during the period of ovarian maturation, it could be either secretory or non-secretory.

- Even on the assumption of a role of the pineal in regulating ovarian maturation the dependency (obligatory or not) of the ovary on the pineal activity is uncertain.

On the basis of these considerations, three hypothetical mechanisms have been considered which might operate during initial ovarian maturation.

A. Initial ovarian maturation is stimulated by the secretory activity of the pineal.

B. Ovarian maturation is allowed by the removal the effect of a pineal substance which is at other times inhibitory.

C. Pineal secretion is not involved and ovarian maturation occurs as a result of other factors.

On the basis of hypothesis A, removal of the pineal throughout this period would be expected to delay or inhibit ovarian maturation. Pinealectomy of young, preovarian-maturation fish would then be expected to produce fish with less mature ovaries of those of control groups. If this does not occur, hypothesis A can be declared false. Hypothesis A receives some support from studies on medaka, Oryzias latipes; in which pinealectomy during the period of February to May decreased GSI (Urasaki, 1972 c). In the following experiment, the effect on ovarian maturation of pinealectomy performed on small pre-ovarian initial development S. niloticus was investigated under both long photoperiod regimes (L:D = 18h:6h) and constant darkness.

PROCEDURES

FISH :-

Fish (11.Ø3 ± Ø.69 g body weight) were obtained from a semi-natural pond at Tung Lung, Songkhla province. They were kept in dechlorinated water for 7 days in a stock aquarium to allow recovery from the effects of transport. As far as possible, females were selected and kept in 20-

litre tanks under a photoperiod of either 18 hours of light alternating with 6 hours of darkness or of constant darkness and the range of daytime temperature was 26-28 C. All fish were fed daily with Hen's food (16 % protein; Sentago, Bangkok, Thailand).

OPERATIVE PROCEDURES :-

GENERAL

Fish were anaesthetized in quinaldine (Ø.Ø3 ml of Quinaldine + Ø.Ø3 ml acetone + distilled water up to 1 dm^3 (Hoar and Randall, 197Ø)) until incapable of a righting reaction. The dorsal aspect of the head was illuminated with a bright microscope lamp that could be removed to prevent undue heating of the fish. Some fish were left as untreated controls.

PINEALECTOMY AND SHAM-PINEALECTOMY

Anaesthetized fish were wrapped in a wet cloth and the position of the pineal complex roughly determined. A skull puncture was then made with a syringe needle number 17, which was then withdrawn. A glass micropipette was inserted in the aperture and the pineal gland and surrounding fat tissue then detached by gentle suction applied through this glass micropipette. Tissues from the suction pipette was examined on a microscope slide by staining with methylene

blue to confirm removal of pineal and its stalk under light microscope. After pineal ablation the wound was covered with silicone grease to reduce leakage of water into the skull cavity. Sham-operated controls were treated in a similar manner except that the pineal complex was not sucked out.

After the operation was completed the fish were held under either light:dark cycle (18h:6h) or constant darkness for 2 weeks. After this period all fish were sacrificed. Body weight and gonad weight were recorded.

RESULTS

The distribution of log body weight with log gonad weight of each experimental group of both photoperiod regimes is shown in Fig. 36 and 37. The range of log GSI value and mean log GSI of ovaries in different groups of each photoperiod condition are shown in Table 11, 12. These results indicate that gonadal development of fish occured in both experimental groups and control groups. Thus, the pinealectomy was not inhibitory to development of fish in terms of either somatic growth or gonadal development.

The distribution of ovarian development categories as indicated by log GSI values among experimental groups showed no significant difference from controls in either light:dark cycle (18h:6h, $P>0.5$, Table 13) or in constant darkness ($P>0.05$, Table 14). One-way analysis of variance of log GSI

failed to show any significant difference between control and treatment groups in either condition (P>Ø.25, Table 15, 16).

Table 11 The range of log GSI value and mean log GSI of ovaries in initial control, intact control, sham-pinealectomized and pinealectomized groups in light:dark (18h:6h) condition.

Experimental groups	range of log GSI value (min. - max.)	mean log GSI ± S.E.
1. Initial control	0.311 - 1.323	- 1.033 ± 0.116
2. Intact control	0.765 - 1.928	- 0.604 ± 0.092
3. Sham-pinealectomized	1.079 - 2.096	- 0.439 ± 0.065
4. Pinealectomized	0.569 - 2.098	- 0.443 ± 0.099

Table 12 The range of log GSI value and mean log GSI of ovaries in initial control, intact control, sham-pinealectomized and pinealectomized groups in constant darkness condition.

Experimental groups	range of log GSI value (min. - max.)	mean log GSI ± S.E.
1. Initial control	1.133 - 2.356	- 0.429 ± 0.302
2. Intact control	1.445 - 2.784	0.023 ± 0.160
3. Sham-pinealectomized	1.103 - 2.599	- 0.295 ± 0.145
4. Pinealectomized	1.236 - 2.680	- 0.038 ± 0.126

Table 13 Distribution of ovarian development categories to log GSI value in fish of each experimental groups in light:dark (18h: 6 h) condition.

ovarian development categories (log GSI value)	Number of animals (%)			Total
	Intact control	Sham-pinealectomized	Pinealectomized	
1. -1.5 to < -1.0	3 (20)	0 (0)	1	4
2. -1.0 to < -0.5	6 (40)	4 (30.77)	4	14
3. -0.5 to < 0	6 (40)	8 (61.54)	8	22
4. 0 to < 0.5	0 (0)	1 (7.69)	1	2
Total	15	13	14	42

P > 0.05 : Chi-square Test

Table 14 Distribution of ovarian development categories to log GSI value in fish of each experimental groups in constant darkness condition.

ovarian development categories (log GSI value)	Number of animals (%)			Total
	Intact control	Sham-pinealectomized	Pinealectomized	
1. -1.5 to < -1.0	4 (28.57)	6 (50)	2 (14.29)	12
2. -1.0 to < -0.5	4 (28.57)	3 (25)	6 (42.86)	13
3. -0.5 to < 0	0 (0)	2 (16.67)	4 (28.57)	6
4. 0 to < 0.5	6 (42.86)	1 (8.33)	2 (14.29)	9
Total	14	12	14	40

P > 0.05 : Chi-square Test

Table 15 The analysis of variance of log GSI in fish of each experimental group in light:dark (18h:6h) condition.

SOV	df	SS	MS
Total	41	4.4389	
between groups	2	0.2565	0.1283
within groups	39	4.1824	0.1072

F = 1.1964^{ns}
ns = not significantly different (P > 0.25)

Table 16 The analysis of variance of log GSI in fish of each experimental group in constant darkness condition.

SOV	df	SS	MS
Total	39	12.6864	
between groups	2	0.6826	0.3413
within groups	37	12.0038	0.3244

F = 1.0521^{ns}
ns = not significantly different (P > 0.25)

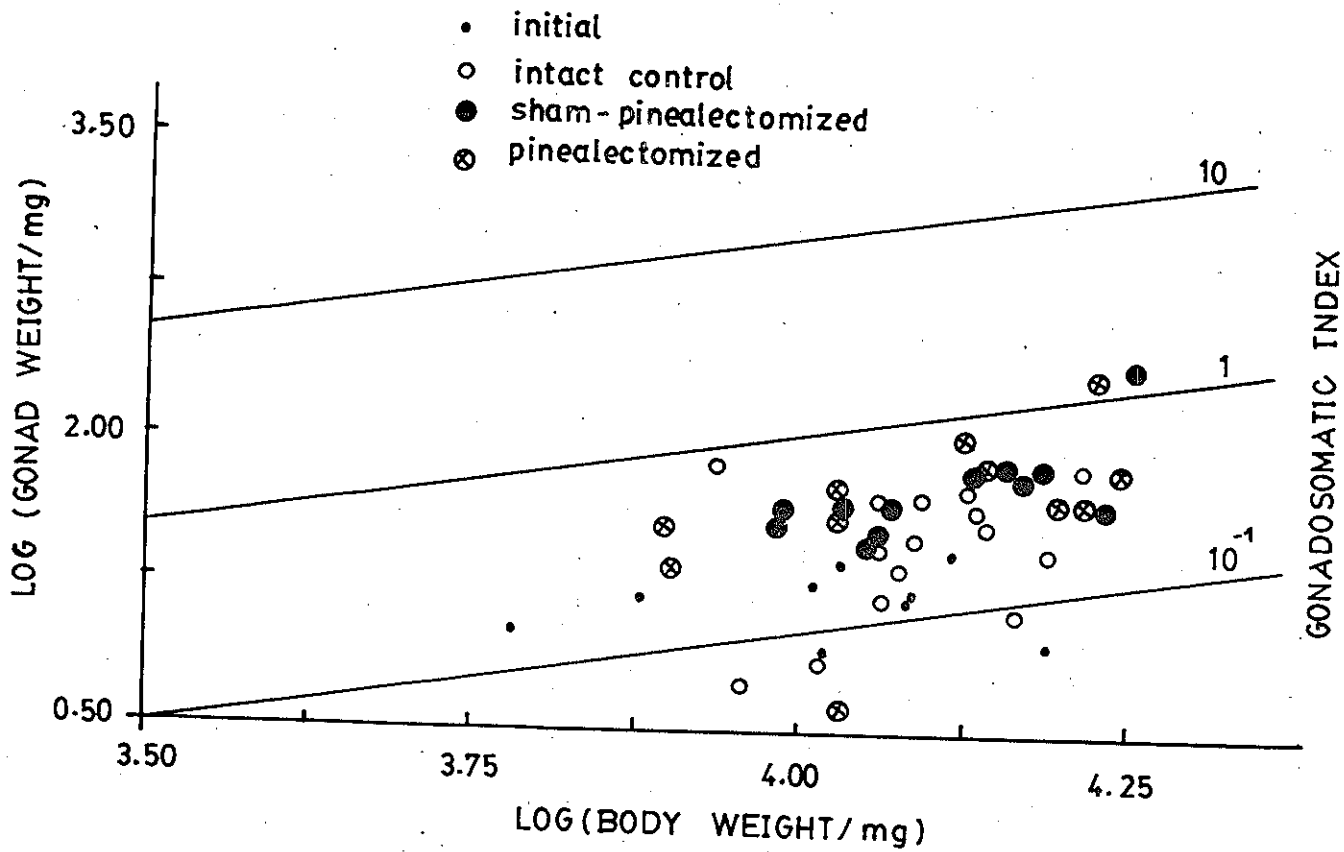


Fig. 36 The distribution of log body weight with log gonad weight of each experimental group (intact control, sham-pinealectomized and pinealectomized groups) in light: dark cycle (18 h : 6 h)

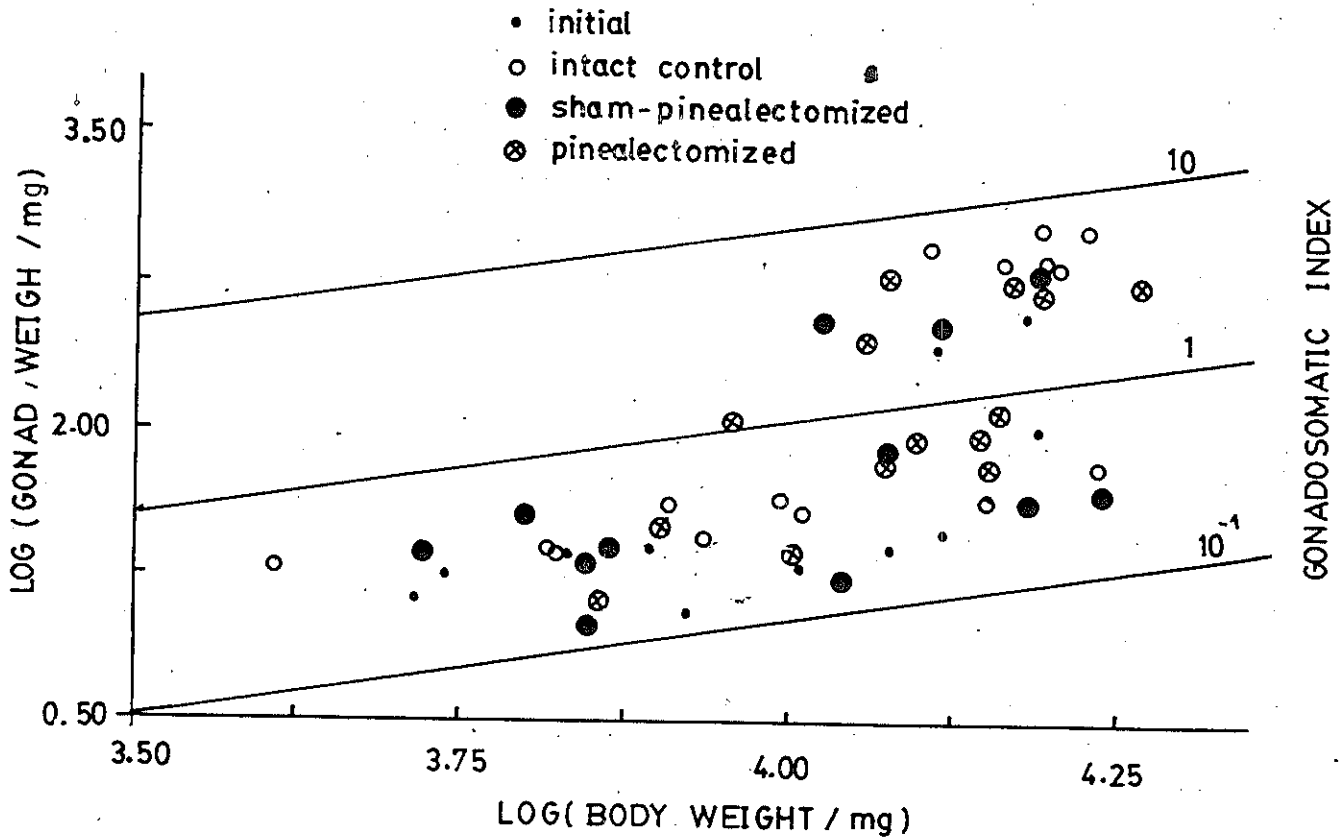


Fig. 37 The distribution of log body weight with log gonad weight of each experimental group (intact control, sham-pinealectomized and pinealectomized groups) in constant darkness

EVALUATION

Pinealectomy combined with exposure either to 18h:6h (light:dark) cycle or to constant darkness had no statistically significant effect on ovarian activity (log GSI) throughout the period of normal initial ovarian maturation in S. niloticus. These data agree with those of de Vlaming and Vodicnik (1978) who suggested that pinealectomy had no effect on ovarian development in goldfish from July through December. Other investigators (Schonherr, 1955; Rasquin, 1958; Pang, 1967 lit. cit. de Vlaming et al, 1974) have suggested that pinealectomy has little influence on gonadal activity in teleost fish, but most of these investigators failed to mention photoperiod conditions or the time of year when the experiments were conducted.

Interpretation of the present results should be guarded since the operation technique for pinealectomy could not ensure complete pineal removal. Furthermore, Pang (1967, lit cit Fenwick, 1970 a) found a difference in the degree that pinealectomy affected growth rate and gonad size when pinealectomy was performed during the initial phase of growth and sexual maturation as opposed to fish that were pinealectomized during the height of growth and reproduction. This indicates that the functions of the pineal in fish may vary at different times of the reproductive cycle, and/or in animals of different ages.

Thus, the need to carefully select the phase of reproductive cycle that the animals are in at the beginning of the experiment when testing the effect of pinealectomy on the reproductive system of fish is emphasized.

The results of pinealectomy in this chapter indicate that hypothesis A is false since ovarian maturation occurred in pinealectomized, sham-pinealectomized and control groups.

CHAPTER 5

RE-EXAMINATION OF PINEAL ROLE IN S. niloticus OVARIAN DEVELOPMENT

Of the three hypothesis presented in Chapter 4, 2 hypotheses remain to be considered :-

Hypothesis B. Ovarian maturation is prevented by the removal the effect of a pineal substance which is, at other times, inhibitory.

Hypothesis C. Pineal secretions are not involved and ovarian maturation occurs as a result of other factors.

In the case of B, the removal of an inhibitory effect could be effected either centrally or peripherally i.e. the pineal could cease to secrete the inhibitory substance (hypothesis B1) or ovarian receptors could disappear at a certain time and thereby allow ovarian maturation to proceed (Hypothesis B2).

The administration of appropriate macerated pineal during the period of ovarian maturation would be expected to have different effects in the two cases. The case of B1, ovarian development would be inhibited. In the case of B2, no effect would be expected. Ideally, the administration of fish macerated pineal would be performed. However, fish pineals are small and difficult to dissect. The bovine pineal was used in this experiment since it is several times

larger, can be dissected relatively easily, and has been shown to have an effect both on colour change (Bagnara and Hadley, 1970; Joss, 1973; in Chapter 3 of this thesis) and on gonadal development under certain conditions (Krockert, 1963. lit. cit. de Vlaming et al, 1974).

In the experiment which follows, macerated bovine pineals were administered to S. niloticus under constant light and under natural photoperiod conditions. Were a positive response (i.e. inhibition of ovarian maturation in fish receiving macerated pineal) to occur, this would lend support to hypothesis B1. However, if a positive responses were not to occur then no information would be provided as to the truth or falsity of hypothesis B1, or of hypotheses B2 and C.

This experiment was directly related to the initial ideas surrounding the concept of this investigation i.e. that the reproduction of S. niloticus might be controllable in rearing ponds, by manipulation of pineal status, possibly by feeding pineal tissue which would exert an antigonadal effect. Antigonadal activity of pineal or melatonin has been shown already in other teleosts. Melatonin injection of goldfish, C. auratus, performed and held under long photoperiod (18:6 hrs light-dark cycle) during the spring was shown to inhibit the increase in gonad size (Fenwick, 1969). Secondary sexual characteristic of guppies, Lebistes reticulatus, were delayed when fed dessicated bull pineals (Krockert, 1963 lit. cit. de Vlaming et. al., 1974), and

melatonin administration inhibited oocyte development and altered pituitary gonadotroph cytology in medaka, O. latipes, exposed to a LD 14:10, 26 C regime during March (Urasaki, 1977).

PROCEDURES

Fish (13.42 ± 0.65 g body weight) from stock culture similar to fish in Chapter 4 were used. As far as possible, 240 females were selected and divided equally into two groups : one was illuminated continuously in tanks (contained 80 litres of water and daytime temperature about 28 C), the other was transfered to "natural" condition in floating cages (width 1 m x length 1 m x height 2 m) in a 40 m x 40 m pond at the Department of Aquatic Sciences, Faculty of Natural Resources, Prince of Songkla University.

Each group was divided into 8 subgroups and these allocated to one of four treatments : uninjected control, control injected with Ringer solution alone, injected with low concentration of macerated bovine pineal (7.5 mg/injection) and injected with high concentration of macerated bovine pineal (30 mg/injection). Each fish was injected every other day with a volume of 0.1 cm^3.

All fish were fed daily (5 % of initial body weight) with Hen's food (16 % protein; Sentago, Bangkok, Thailand) and held under these conditions for a minimum of 7 days before any injection was begun. The fish from each of the

tanks and cages were then subjected to the appropriate treatment. The treatments lasted 21 days.

Following completion of the injections, all fish were killed. Body weight and gonad weight were recorded and ovaries fixed in Bouin's fixative for histological examination. The various cytological stages of oogenesis were determined in the same way as described in Chapter 2. The volume fraction and mean volume of tertiary-yolk and maturatuion oocytes of each ovary of uninjected control, injected control and high dose pineal extract fish were then estimated.

The estimation of volume fraction was done by sampling three sections from slides of each ovary. Each section was divided by micrometer into five transverse lines on the section plane, and the total length of each line was recorded. The distances along the line which overlapped tertiary-yolk or maturation oocytes were measured. The overlap value of tertiary-yolk and maturation oocytes divided by total length was taken as the volume fraction of tertiary-yolk or maturation oocytes in the ovary. Three sections of each ovary were sampled so that a mean value of volume fraction of tertiary-yolk or maturation oocytes of each sample could be estimated.

The method used to calculate the value of mean volume of tertiary-yolk and maturation oocytes of each sample assumed that oocytes were approximately spherical in shape. Three oocytes (tertiary-yolk or maturation stage) were

chosen from each sample and the section in which the largest diameter of each oocyte occurred was selected to measured one diameter of the oocyte and the process repeated to measure in the largest diameter at right angle to the first. A mean radius "r" was calculated and the volume estimated by $4/3\pi r^3$.

RESULTS :-

The distributions of log body weight with log gonad weight of each experimental group of both conditions are shown in Fig. 38 and 39. The range of ovarian weight in different groups of each photoperiod condition are shown in Table 17, 18.

The distribution of ovarian development categories as indicated by log GSI among experimental groups showed no significant differences in constant light in tanks ($P>0.25$, Table 19) but highly significant differences in natural condition in cages ($P<0.001$, Table 20). One-way analysis of variance of log GSI failed to show any significant differences between experimental groups in constant light in tanks ($P>0.25$, Table 21) but highly significant differences between uninjected control and all others in natural condition in cages ($P<0.0005$, Table 22). The Newman-Keuls multiple range test applied to reveal the differences of mean log GSI of each experimental group in natural condition in cages showed that the mean log GSI of uninjected control

was significantly lower than all injected groups [PL vs. C and CR vs. C ($P<0.001$); PH vs. C ($P<0.005$), Table 23].

Histological examination of developmental stages of those ovaries of fish kept under constant light in tanks (uninjected control, injected control and high dose macerated pineal injection) revealed the oocyte composition presented in Table A2 (Appendix). More than one developmental stage was present in most ovaries. The combination of developmental stages of the ovaries were not obviously different among uninjected control, injected control and high dose macerated pineal injection. However, microscopic observation showed a tendency to increased size of tertiary-yolk oocytes and maturation oocytes in the pineal-injected group compared with that of uninjected and injected controls (Fig. 40 a, b, c).

The mean volume fractions and mean volumes of tertiary-yolk or maturation oocytes of uninjected control, injected control and high dose injection in constant light in tanks are shown in Table 24. One-way analysis of variance and the Newman-Keuls multiple range test showed significant increases ($P<0.05$) in mean volume of oocytes in uninjected control, injected contol and high dose injection, respectively (Table 25, 26). The same test showed that gonad weights were significantly greater ($P<0.025$) in high dose injection group than in either uninjected or injected control groups while the two control groups were not significantly different (Table 27, 28).

Table 17 The range of log GSI value (%) and mean log GSI of ovaries in initial control, uninjected control, injected control, low dose and high dose macerated bovine pineal injection under constant light in tanks.

Experimemtal groups	range of log GSI value (min. - max.)	mean log GSI ± S.E.
1. Initial control	0.642 - 1.957	- 0.469 ± 0.289
2. Uninjected control	0.342 - 2.749	0.268 ± 0.080
3. Injected control	0.701 - 2.765	0.282 ± 0.102
4. Low dose injection	1.054 - 2.868	0.288 ± 0.090
5. High dose injection	1.395 - 2.854	0.446 ± 0.088

Table 18 The range of log GSI value (%) and mean log GSI of ovaries in initial control, uninjected control, injected control, low dose and high dose macerated bovine pineal injection under natural photoperiod in cages.

Experimemtal groups	range of log GSI value (min. - max.)	mean log GSI ± S.E.
1. Initial control	0.840 - 2.128	- 0.754 ± 0.077
2. Uninjected control	0.496 - 1.930	- 0.874 ± 0.081
3. Injected control	0.791 - 2.778	- 0.292 ± 0.113
4. Low dose injection	1.023 - 2.789	- 0.232 ± 0.127
5. High dose injection	0.987 - 2.480	- 0.357 ± 0.106

Table 19 Distribution of ovarian development categories to log GSI value in fish under constant light in tanks.

ovarian development categories (log GSI value)	Number of animals (% of treatment group)				Total
	uninjected control	injected control	low dose injection	high dose injection	
1. -1.5 to < -1.0	0 (0)	1 (4.35)	0 (0)	0	1
2. -1.0 to < -0.5	1 (4.76)	1 (4.35)	1 (4)	1 (4.35)	4
3. -0.5 to < 0	2 (9.53)	1 (4.35)	6 (24)	3 (13.04)	12
4. 0 to < 0.5	12 (57.14)	10 (43.48)	8 (32)	5 (21.74)	35
5. 0.5 to < 1.0	6 (28.57)	10 (43.48)	10 (40)	14 (60.87)	40
Total	21 (100)	23 (100)	25 (100)	23 (100)	92

P > 0.25 : Chi-square Test

Table 20 Distribution of ovarian development categories to log GSI value in fish under natural photoperiod in cages.

ovarian development categories (log GSI value)	Number of animals (% of treatment group)				Total
	uninjected control	injected control	low dose injection	high dose injection	
1. -1.5 to < -1.0	10 (45.45)	2 (9.09)	0 (0)	1 (5.26)	13
2. -1.0 to < -0.5	9 (40.91)	7 (31.82)	10 (45.45)	6 (31.58)	32
3. -0.5 to < 0	3 (13.64)	5 (22.73)	3 (13.64)	8 (42.11)	19
4. 0 to < 0.5	0 (0)	6 (27.27)	6 (27.27)	4 (21.05)	16
5. 0.5 to < 1.0	0 (0)	2 (9.09)	3 (13.64)	0 (0)	5
Total	22 (100)	22 (100)	22 (100)	19 (100)	85

P > 0.001 : Chi-square Test

Table 21 The analysis of variance of log GSI in uninjected control, injected control, low dose injection and high dose injection under constant light in tanks.

SOV	df	SS	MS
Total	91	17.1573	
between groups	3	0.4810	0.1603
within groups	88	16.6763	0.1895

F = 0.8459^{ns}
ns = not significantly different (P > 0.25)

Table 22 The analysis of variance of log GSI in uninjected control, injected control, low dose injection and high dose injection under natural photoperiod in cages.

SOV	df	SS	MS
Total	84	25.8783	
between groups	3	5.7019	1.9006
within groups	81	20.1764	0.2491

F = 7.6299^{**}
**. = significantly different (P < 0.0005)

Table 23 The Newman-Keuls multiple range test applied to the data of mean log GSI of each experimental group under natural photoperiod in cages.

Comparison (B vs. A)	difference $(\bar{X}_B - \bar{X}_A)$	S.E.	q	$q_{\alpha,81,p}$	Conclusion
PL vs. C	0.6412	0.1064	6.0263	5.6530	$P < 0.001$
PL vs. PH	0.1242	0.1105	1.1240	3.3990	$P > 0.05$
PL vs. CR	0.0590	0.1064	0.5545	2.8290	$P > 0.05$
CR vs. C	0.5822	0.1064	5.4718	5.3650	$P < 0.001$
CR vs. PH	0.0652	0.1105	0.5901	2.8290	$P > 0.05$
PH vs. C	0.5170	0.1105	4.6787	4.1220	$P < 0.005$

α = The significance level
p = The total number of means being tested
overall conclusion : $\mu_C \neq \mu_{CR} = \mu_{PL} = \mu_{PH}$
C = uninjected control PL = low dose injection
CR = injected control PH = high dose injection

Table 24 Value of mean changes in volume fraction, volume of cell (tertiary- yolk and/or maturation oocytes), weight estimated volume of ovary, mean number of cell per unit volume of ovary and total number of cell of uninjected control, injected control and high dose injection groups under light in tanks.

	Experimental groups		
	uninjected control	injected control	high dose injection
$\overline{VF} \pm$ S.E.	0.695 ± 0.040	0.724 ± 0.058	0.694 ± 0.041
$\overline{V} \pm$ S.E. (mm3)	0.308 ± 0.040	0.661 ± 0.090	0.995 ± 0.130
$\hat{V}_o \pm$ S.E. (mm3)	547.139 ± 89.660	631.842 ± 104.516	925.888 ± 71.258
$N_A/\hat{V}_o \pm$ S.E. (=VF/V) (cell/mm3)	2.856 ± 0.451	1.392 ± 0.285	0.836 ± 0.096
$\overline{N} \pm$ S.E. $((\overline{VF}/\overline{V})*\hat{V}_o)$ (cell/unit vol.)	1430.731 ± 246.212	880.080 ± 174.874	717.450 ± 79.047

$\overline{VF}$ = mean volume fraction

$\overline{V}$ = mean volume of cell

$\hat{V}_o$ = mean weight estimated volume of ovary

$N_A/\hat{V}_o$ = mean number of cell per unit volume of ovary

$\overline{N}$ = total number of cell

cell = tertiary-yolk oocytes and/or maturation oocytes

Table 25 The analysis of variance of volume of oocytes (tertiary-yolk and/or maturation stage) of samples in each control, intact control and high dose macerated bovine pineal injection groups under constant light in tanks.

SOV	df	SS	MS
Total	40	8.8891	
between groups	2	3.4708	1.7354
within groups	38	5.4183	0.1426

$F = 12.1697^{**}$

** = significantly different ($P < 0.05$)

Table 26 The Newman-Keuls multiple range test applied to the data of mean volume of oocytes of each experimental group under constant light in tanks.

Comparison (B vs. A)	difference $(\bar{X}_B - \bar{X}_A)$	S.E.	q	$q_{\alpha,35,p}$	Conclusion
PH vs. C	0.6952	0.1200	5.7933	5.6980	$\mu_{PH} \neq \mu_C$ $P < 0.001$
PH vs. CR	0.3335	0.1020	3.2696	2.8880	$\mu_{PH} \neq \mu_{CR}$ $P < 0.05$
CR vs. C	0.3617	0.1070	3.3803	2.8880	$\mu_{CR} \neq \mu_C$ $P > 0.05$

α = The significance level
p = The total number of means being tested
overall conclusion : $\mu_C \neq \mu_{CR} \neq \mu_{PH}$

Table 27 The analysis of variance of gonad weight in mg (weight estimated volume of ovaries in each uninjected control, injected control and high dose macerated bovine pineal injection groups under constant light in tanks.

SOV	df	SS	MS
Total	40	5155871.1	
between groups	2	1155871	577935.5
within groups	38	4000000.1	105263.16

$F = 5.4904^{**}$

** = significantly different ($P < 0.01$)

Table 28 The Newman-Keuls multiple range test applied to the data of mean gonad weights in mg (weight estimated volume of ovaries) of each experimental group under constant light in tanks.

Comparison (B vs. A)	difference $(\bar{X}_B - \bar{X}_A)$	S.E.	q	$q_{\alpha,35,p}$	Conclusion
PH vs. C	378.7567	85.6625	4.4252	3.9190	$\mu_{PH} \neq \mu_C$ $P < 0.025$
PH vs. CR	294.0458	87.6096	3.3573	3.3370	$\mu_{PH} \neq \mu_{CR}$ $P < 0.025$
CR vs. C	84.7109	91.8398	0.9227	2.8880	$\mu_{CR} \neq \mu_C$ $P > 0.05$

α = The significance level
p = The total number of means being tested
overall conclusion : $\mu_C \neq \mu_{CR} \neq \mu_{PH}$

Table 29 The analysis of variance of estimated total numbers of oocytes (tertiary-yolk or maturation stage) in each uninjeted control, injected control and high dose macerated bovine pineal injection groups under constant light in tanks.

SOV	df	SS	MS
Total	40	19119421	
between groups	2	4126312	2063156
within groups	38	14993109	394555.5

$F = 5.2291^{**}$

** = significantly different ($P < 0.025$)

Table 30 The Newman-Keuls multiple range test applied to the data of mean total numbers of oocytes of each experimental group under constant light in tanks.

Comparison (B vs. A)	difference $(\bar{X}_B - \bar{X}_A)$	S.E.	q	$q_{\alpha,35,p}$	Conclusion
C vs. PH	713.2800	165.8465	4.3009	3.9190	$\mu_C \neq \mu_{PH}$ $P < 0.025$
C vs. CR	630.6500	177.8060	3.5469	3.3370	$\mu_C \neq \mu_{CR}$ $P < 0.025$
CR vs. PH	82.6300	169.6163	0.4872	2.8880	$\mu_{CR} = \mu_{PH}$ $P > 0.05$

α = The significance level
p = The total number of means being tested
overall conclusion : $\mu_C \neq \mu_{CR} = \mu_{PH}$

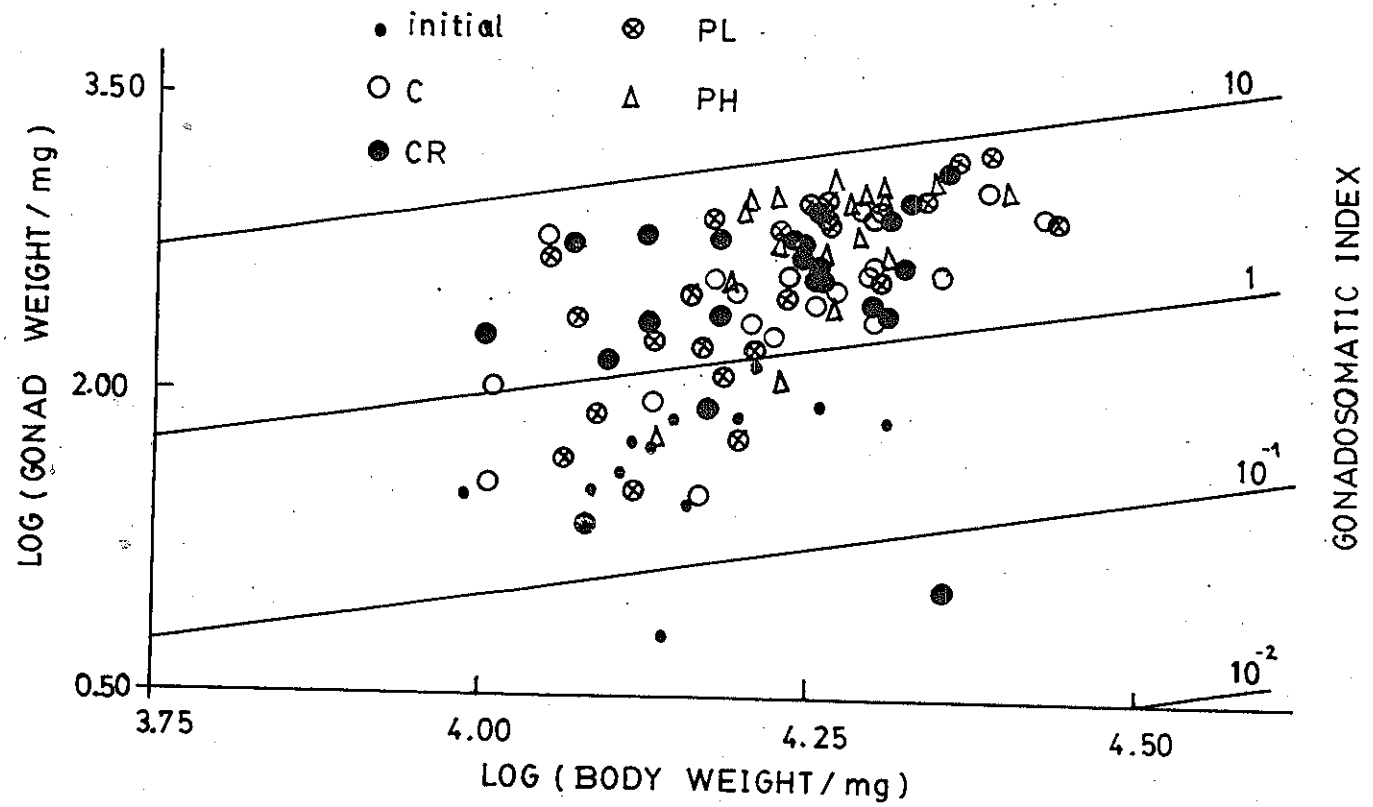


Fig. 38 The distribution of log body weight with log gonad weight of each experimental group (uninjected control[C], injected control [CR], low dose MBP injection [PL], high dose MBP injection [PH] in constant light

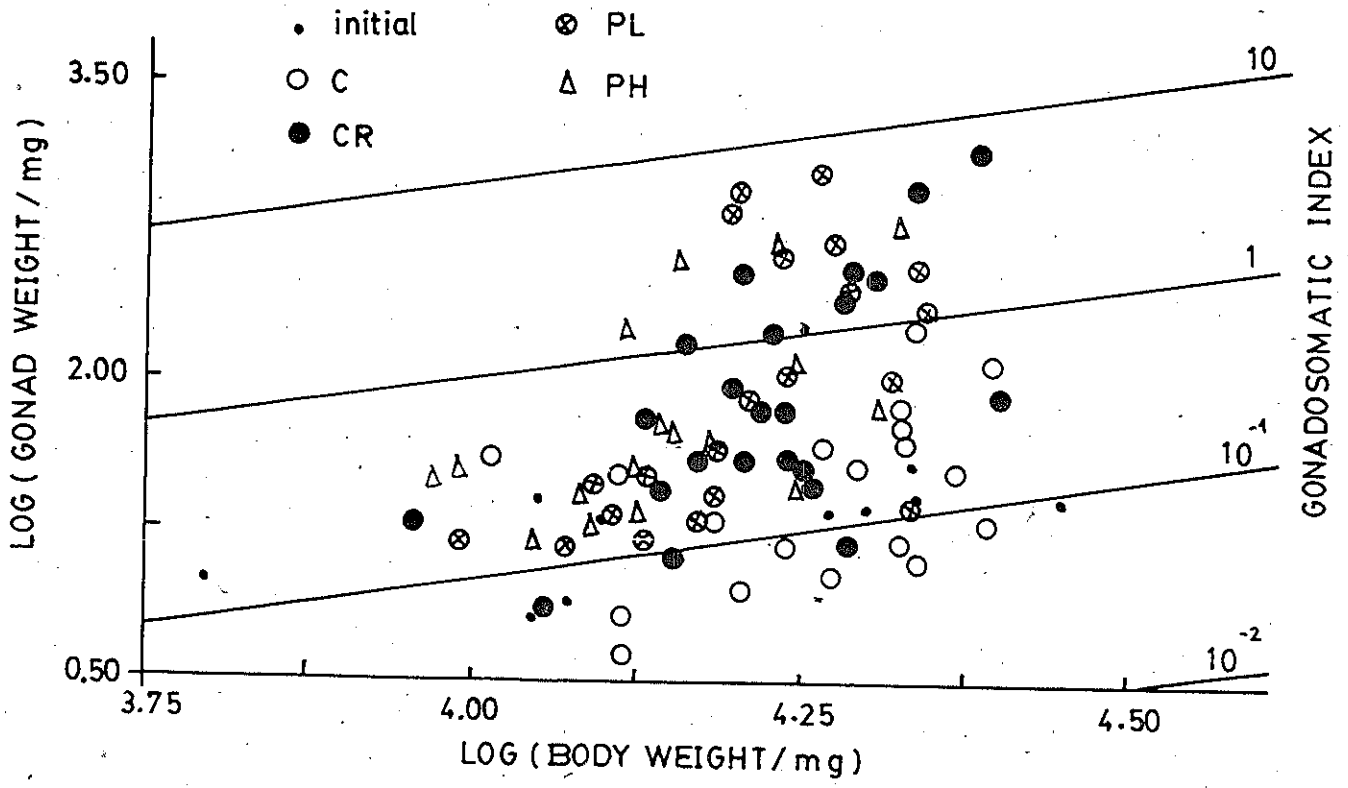


Fig. 39 The distribution of log body weight with log gonad weight of each experimental group (uninjected control [C], injected control [CR], low dose MBP injection [PL], high dose MBP injection [PH] in natural photoperiod in October to November.

Fig. 40 The comparison of the size of tertiary-yolk oocytes and maturation oocytes in the pineal-injected group compared with that of uninjected and injected control groups.

a. uninjected control; EP = Early-perinucleolus stage, LP = Late-perinucleolus stage, YN = Yolk nucleus, 3 -Y = Tertiary-yolk stage,

b. injected control; LP = Late-perinucleolus stage, YN = Yolk nucleus, OD = Oil-drop stage, 3 -Y = Tertiary-yolk stage, MAT = Maturation stage, EN = Eccentric nucleus.

c. MBP-injected group; OD = Oil-drop stage, MAT = Maturation stage

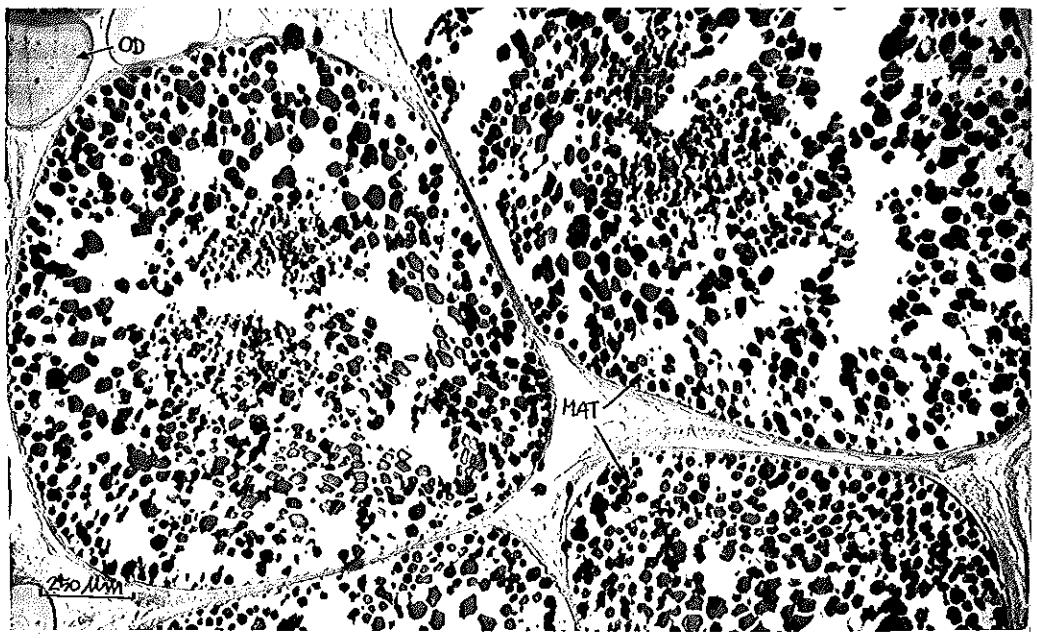
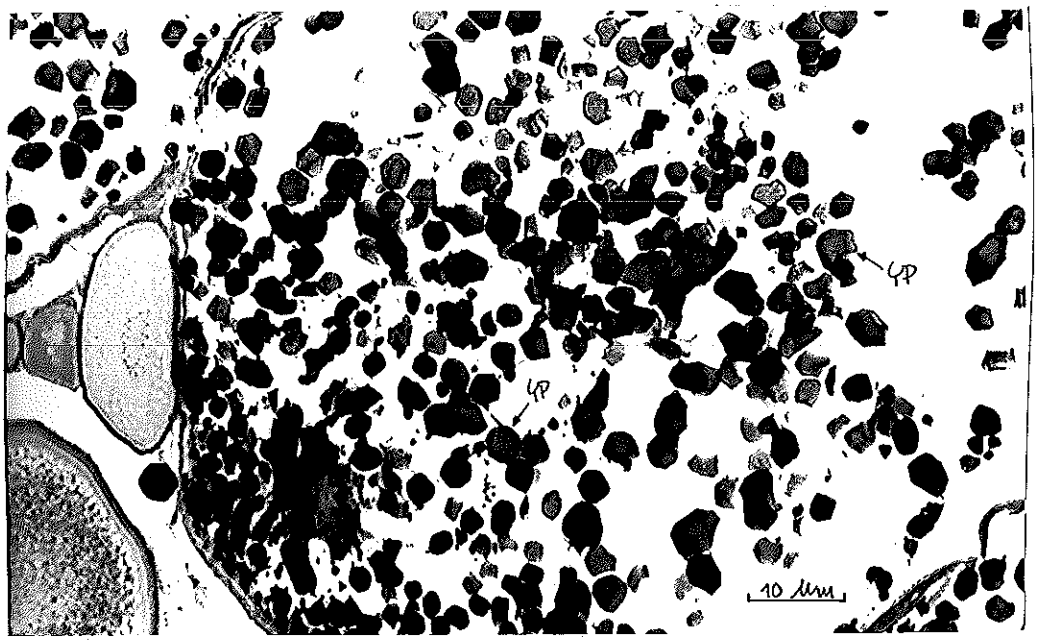


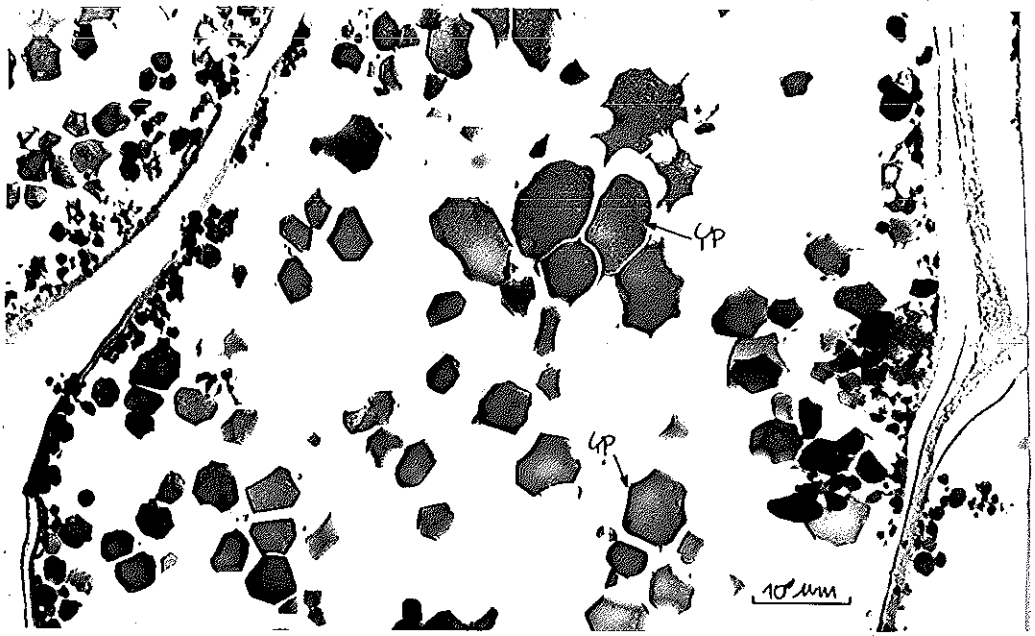
Fig. 41 The comparison of the size of yolk platelets in the pineal-injected group compared with that of uninjected and injected control groups.

a. uninjected control; YP = Yolk platelets.



b. injected control; YP = Yolk platelets.

c. MBP-injected group; YP = Yolk platelets.



EVALUATION

After 21 days of pineal administration, no reduction in ovarian maturation in the macerated bovine pineal injection group occurred in S. niloticus; and so this result cannot contribute towards an evaluation of hypotheses B1, B2 or C.

However, histological examination of ovaries of fish kept under constant light in tanks revealed some differences among uninjected control, injected control and high dose macerated pineal injection groups. In particular, the mean volume of tertiary-yolk or maturation oocytes was significantly greater in the high dose macerated bovine pineal injection group (PH) than in either injected control (CR) or uninjected control (C) groups, but also the mean volume of tertiary-yolk or maturation oocytes of injected controls was greater than that of uninjected controls. By contrast, the mean volume fraction of all groups (C, CR, PH) was nearly equal.

For any ovary, the volume fraction (VF), of oocytes of tertiary-yolk or maturaion stage can be considered as given by the following equation :-

$$VF = N \times \bar{V}/V_{\emptyset} ,$$

where $\bar{V}$ is the mean cell volume,

N ; the toatl number of cells in the ovary,

and $V_{\emptyset}$; the volume of the ovary.

Thus the value VF/V gives the number of oocytes of tertiary-yolk or maturation stage per unit ovarian volume, and $(VF/\bar{V}) \times V_{\emptyset}$, the estimated total number of oocytes of tertiary-yolk or maturation stage in the ovary.

In the present experiment, ovarian volume was estimated from ovarian fresh-weight assuming a constant density of 1 gcm^{-3}. This means of estimation was considered preferable to calculations of volume made on the basis of length and greatest width of the ovaries, since ovarian shape was very variable among all groups.

Calculations based on the values of VF and V in uninjected controls, injected controls and high dose macerated bovine pineal injection groups revealed that the mean number of tertiary-yolk or maturation oocytes per unit ovarian volume was progressively less in uninjected control, injected control and high dose macerated bovine pineal injection groups, respectively. Estimated total number of tertiary-yolk or maturation oocytes per ovary, showed a large decrease ($P<0.025$) in injected controls compared with uninjected controls, but no significant drop in the high dose macerated bovine pineal injection group compared with the injected control group (Table 29, 30). Thus the injection of Ringer's solution was associated with bigger but fewer tertiary-yolk or maturation oocytes while the injection of macerated bovine pineal resulted in enlarged oocytes but also bigger ovaries ($P<0.025$, Table 24).

The enlarged oocytes of injected control and high dose

injection groups were found to have larger yolk platelets than smaller tertiary-yolk or maturation oocytes characteristic of the uninjected control group, while there was no noticeable difference in nuclear volume. The enlargement of oocytes appears to have resulted from more or more rapid yolk deposition (Fig. 41 a, b, c).

Although, under the conditions of this experiment, macerated bovine pineal injection was not inhibitory to ovarian maturation, there is evidence of its exerting a stimulatory effects on viltellogenesis. This finding contrasts with the results of experiments in some other fish, such as female catfish, Heteropneustes fossilis, in which melatonin treatment during prespawning period inhibited vitellogenesis and induced follicular atresia, and treatment during spawning season caused ovarian regression. In both seasons, melatonin caused a reduction in the number of hypophyseal gonadotrophs (Sundararaj and Keshavanath, 1976 lit. cit. Reiter, 1980). In another Asian catfish, Mystus tengara, melatonin injection 2 hr after the light phase onset (LD 12h:12h) during the spring rapid-ovarian-recrudescence phase arrested vitellogenesis and increased the frequency of atretic oocytes (Saxena and Anand, 1977 lit. cit. Reiter, 1980).

CHAPTER 6

GENERAL DISCUSSION

A. ASSESSMENT OF OBJECTIVE ACHIEVEMENT AND POSSIBILITIES FOR APPLICATION

A fundamental reason for this study was to investigate the possibility of exploiting modified pineal function to restrict the reproduction of S. nilotieus in rearing ponds.

Functional studies of the pineal in many fish have revealed that the pineal modulates physiological activities to facilitate breeding during the proper season and to prevent breeding during the unfavourable season of the year. Precision in breeding periodicity is an adaptation to synchronize the emergence of fry with the availability of proper food, which itself is subject to cycles of seasonal abundance (Sundararaj, 1981). Pineal melatonin has been suggested to possess antigonadal properties in a number of teleost fish (Minneman and Wurtman, 1976 lit. cit. Sundararaj, 1981) as in other chordate classes. It can induce marked changes in neuroendocrine and gonadal activities in many fish (Fenwick, 1970 b; Urasaki, 1972, 1977; de Vlaming, Sage and Charlton, 1974). Recent studies, however, have demonstrated that melatonin can also have progonadal properties and that it inhibits the antigonadal

activities of the pineal (Reiter et al., 1975 lit. cit. Sundararaj, 1981). This ambivalent action of pineal cannot be explained by its melatonin content. Therefore, it has been suggested that the pineal may contain several other antigonadotropic substances (Benson et al., 1976; Ebel, 1976 lit. cit. Sundararaj, 1981) in the form of some as yet unidentified proteins and polypeptide hormones.

Early ideas of this study included the possibilities; i) if the activity of the native pineal was predominantly progonadal (stimulating ovarian maturation), then interfering with normal pineal function could inhibit ovarian maturation; and ii) if native pineal was antigonadal (and removal of this inhibitory effect associated with ovarian maturation), then increasing or stimulating prolonged pineal activity might inhibit ovarian maturation.

Many potential ways of interfering with normal pineal function could be considered, including pinealectomy, alteration of light regime or, perhaps, the use of specific metabolic blocking agents, though not all are equally attactive as potential field techniques.

In any case, this investigation has now shown that the native pineal of S. niloticus is not required for stimulation of ovarian maturation during this period.

Of the several ways in which pineal activities could be stimulated or increased (alteration of light regimes, injection of pineal derived metabolic substances including melatonin, injection or feeding of crushed pineal tissue),

administration of pineal from fish in a nonfunctional gonadal stage would probably provide the best initial procedure. However, in this investigation, fish pineal administration was replaced by bovine pineal administration for the reason described in Chapter 5.

The results of this series of experiments have shown that bovine pineal did not have an antigonadal effect (at least under the conditions of this experiment). This, of course, does not rule out the possibility that the native pineal has some pineal inhibitory action as suggested by Krockert, 1963 (lit. cit. de Vlaming et al., 1974) and others.

While ovarian development was not inhibited by bovine pineals, there is evidence of increased vitellogenesis associated with bovine pineal administration. The implication of this finding will be discussed more fully in a later section. However, it is noted here that other investigators have reported that melatonin inhibited vitellogenesis in the catfish, H. fossilis (Sundararaj and Keshavanath, 1976 lit. cit. Reiter, 1980), and in M. tengara (Saxena and Anand, 1977 lit. cit. Reiter, 1980).

Whether this discrepancy reflects species differences or the fact that the whole pineal contains a multiplicity of active substances, cannot be decided without further experiment. Furthermore, the specificity of this response to pineal tissue administration (rather than to administration of other tissues) has not been determined.

B. "PROGONADAL" AND "ANTIGONADAL" ROLE

Most investigators have used alterations in GSI value as an indicator of the role of pineal on gonadal development, and established the concepts of "progonadal" or "antigonadal" activity based on these changes (Fenwick, 1970 a, b, c; Urasaki, 1972 a, b, c, d, 1976; de Vlaming, Sage and Charlton, 1974). However, since GSI is a proportion of gonad weight to body weight, the use of GSI alone does not eliminate the possible effects of any treatment on somatic growth. Evidence from the investigation of de Vlaming (1980) indicated that pinealectomy consistently decreased the rate of linear growth in fishes exposed to a short but not to a long photoperiod and melatonin treatment accelerated growth and weight gain in goldfish maintained on a short, but not on a long photoperiod. Thus, changed GSI values may not always be indicative of altered gonadal activity (de Vlaming and Voddicnik, 1978). The ease of determination of GSI probably explains its widespread use but it is suggested that such measurements would be more useful, if considered as the gonad weight/body weight distribution or if combined with measurement of other parameters such as histological determination of degree of ovarian maturation or cytometric measurements of germ cell populations (Fenwick, 1970 c; Urasaki, 1977 lit. cit. Reiter, 1980; Iwamatsu, 1978 lit. cit. Reiter, 1980; de Vlaming and Vodicnik, 1978; Vodicnik et al., 1979) or

determinations of, for instance, gonadotrophic and/or gonadal hormones etc. (Urasaki, 1974; de Vlaming et al., 1977 lit. cit. de Vlaming and Vodicnik, 1978; de Vlaming and Vodicnik, 1978; Vodicnik et al., 1978 lit. cit. Reiter, 1980).

In general, a progonadal activity has been ascribed to the pineal (or to melatonin) under conditions where pinealectomy has resulted in a lowered GSI or pineal (or melatonin) administration has induced an elevated GSI. Changes of GSI with the opposite direction have led to the pineal (or melatonin) being described as "antigonadal". In the discussion which follows, the terms "progonadal" and "antigonadal" refer to the activities inferred from changes in GSI, unless otherwise stated.

Most studies have suggested that the pineal gland exerts both progonadal and antigonadal effects on fish, depending on other factors, such as time of year, photoperiod and temperature. Progonadal involvement of the pineal was shown in medaka, O. latipes, (decrease of GSI after pinealectomy) during the period February to May in Yokohama, Japan (Urasaki, 1972 c) and in N. crysoleucas held on a LD 15.5 h:8.5 h, 25 C regime during the preparatory (January), prespawning (March to April) and early spawning (May) periods in Wisconsin USA (pinealectomy blocked the stimulatory effect of this regime) (de Vlaming, 1975 lit. cit. Reiter, 1980). Note that these reports have in common long or increasing-light photoperiod regimes. However, the

pineal has been apparently "antigonadal" under other conditions. Urasaki (1972 a, b, 1973, 1976) showed that the pineal organ and eyes were involved in suppressing gonadal activity (increase of GSI and shortening of the period of oviposition after pinealectomy) in medaka maintained on a short photoperiod or in continuous darkness. An antigonadal involvement was also reported by Fenwick (1970 a) in goldfish, C. auratus, exposed to an LD 8h:16h, 13 C regime between January to May (prespawning) (pinealectomy caused enlarged gonads). In each report of "antigonadal" pineal activity, fish have been held under conditions of short-light or decreasing-light photoperiod. By contrast, the pineal, which at other times was progonadal, seemed to be uninvolved in ovarian development in goldfish exposed to short photoperiod conditions at 20 C during June to August, October to December and April to May (no effect on GSI after pinealectomy) (de Vlaming and Vodicnik, 1978).

Data presented by de Vlaming and Vodicnik (1977 lit. cit. Reiter, 1980) imply that, in N. crysoleucas, the pineal organ exert its effects on reproduction, at least in part, via the hypothalamus and pituitary. An experiment conducted during the fall with fish exposed to a LD 15.5h:8.5h or a LD 9h:15h photoperiod at 15 C or 25 C revealed that pinealectomy altered pituitary gonadotrophin cells under all four regimes. Pinealectomy in this experiment, also repressed ovarian activity in fish maintained on the long photoperiod regimes. de Vlaming and Vodicnik (1977 lit.

cit. Reiter, 1980) reported that epiphysectomy resulted in changes of pituitary gonadotrophin content, especially in photoperiod groups of N. crysoleucas maintained at 15 C during February. On the other hand, pinealectomy resulted in an elevated hypothalamic gonadotrophin releasing activity in fish exposed to a LD 9h:15h, 25 C regime. Evidently the pineal organ of N. crysoleucas is a component in or can modify the pathway(s) by which photoperiod affects reproductive mechanisms.

Vodicnik et al. (1978 lit. cit. Reiter, 1980) claimed that pituitary gonadotrophin levels of pinealectomized goldfish were greater than in controls when samples were taken at 10 h, but not at 4 h, after the onset of the light phase of a LD 15.5h:8.5h, 22 C regime (March experiment); plasma ganadotrophin levels, however, were lower in pinealectomized fish than in controls only at the earlier sampling time. Perhaps the pineal organ influences gonadal development through, at least in part, alteration of the daily cycles of serum gonadotrophin levels.

Furthermore, Hontela and Peter (1980 a lit. cit. Peter, 1983) found that pinealectomy and blinding both caused the daily cycle in serum GTH levels to disappear in fish held on long photoperiod and warm temperature; a significant decrease in GSI also occurred in some experimental groups although the fish were killed at only 7-9 days after exposure to the environmental condition, a relatively short time period for effect on GSI. Pinealectomy but not

blinding, of fish held on a short photoperiod and warm temperature caused a daily cycle in serum GTH levels; but no effect on GSI was found. These results suggested that the progonadal effect of the pineal and eyes under long photoperiod conditions entails the promotion of a daily cycle in serum GTH levels; however, the antigonadal effects of the pineal under short photoperiod entails suppression of daily cycles in serum GTH levels (Peter, 1983). Again, it appears that mediation of pineal-gonad effects may involve alteration of the cycles of GTH secretion. A possible mechanism might involve an effect of melatonin levels in hypothalamus-hypophyseal vessels controlling either synthesis or release of a gonadotrophic releasing hormone or synthesis or release of gonadotrophic hormone directly, although a direct effect of pineal substances in the gonads themselves cannot be ruled out.

However, in a more detailed study, Hafeez and Zerihun (1974 lit. cit. de Vlaming and Vodicnik, 1978) noted that a branch of the pineal nerve tract may project into the hypothalamus. Photoperiod information might therefore be channeled directly via a nervous pathway into centers which influence gonadotrophin secretion.

A synthesis of the above reports suggests that photoperiod, temperature, pineal action and gonadal maturation are linked in a complex relationship. A hypothetical system relating gonadal maturation to photoperiod with pineal involvement, which is consistent

with these findings, and based on a secretory pineal (melatonin) is shown in Fig. 42.

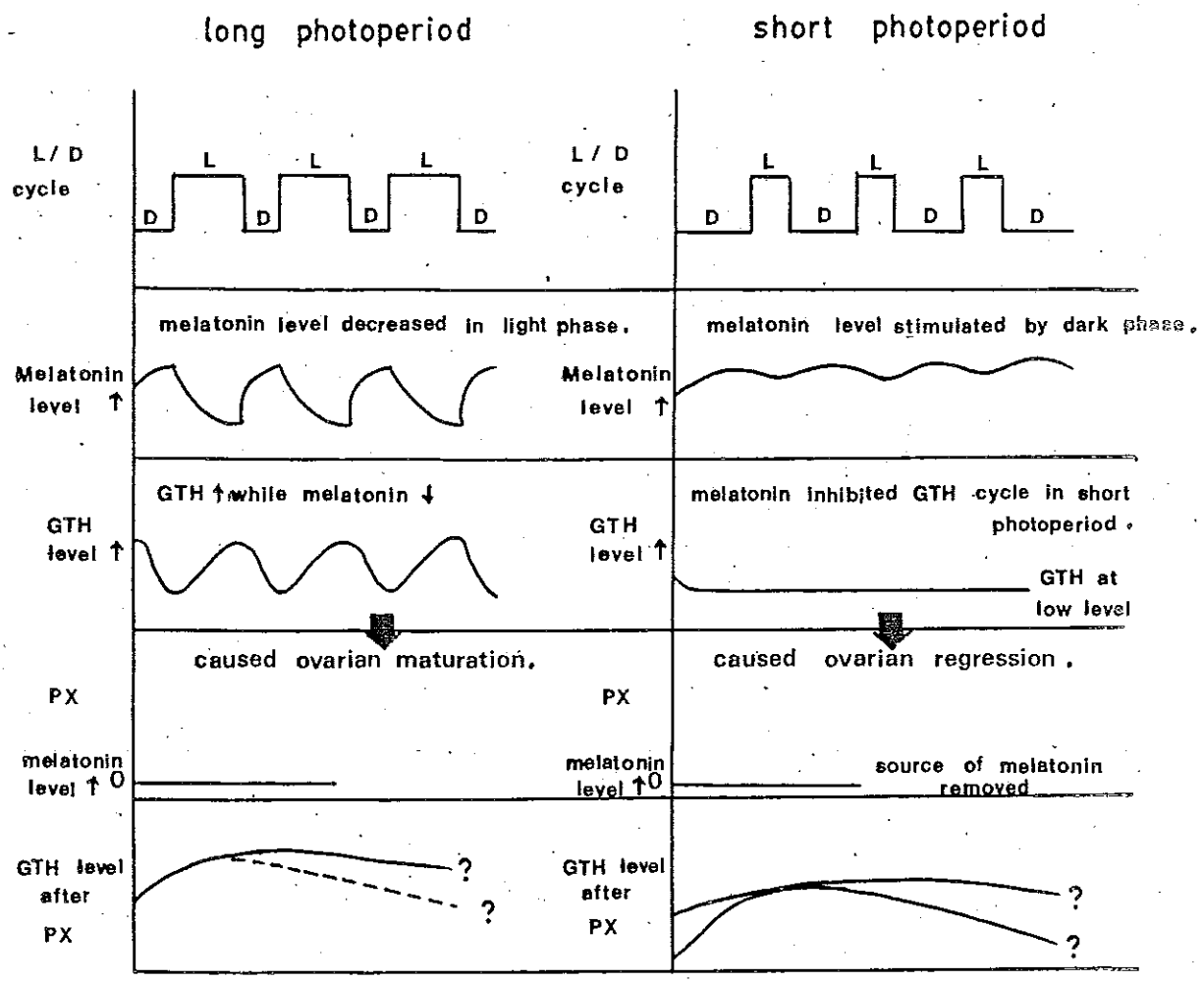


Fig. 42 A hypothetical system relating gonadal maturation to photoperiod with pineal involvement.(?=possibility changes of GTH level after pinealectomy[PX])

C. VITELLOGENESIS

In this study, macerated bovine pineal injection resulted in enlargement of oocytes, which contained larger yolk platelet than uninjected control and injected control groups.

A hypothesis for the mechanism of vitellogenesis in the goldfish was proposed by Bailey (1957 lit. cit. Ng and Idler 1983). Vitellogenin is synthesized in the liver, under stimulation of estrogen produced from the ovary, secreted into the circulation, and transported to the ovary where it is taken up into the oocytes.

The contribution of autosynthetic processes (endogenous vitellogenesis) to the yolk mass in the teleost ovary, relative to exogenous yolk acquired by in corporation of vitellogenin, has not been estimated. Exogenous vitellogenesis can be considered to consist of two phases. The first phase involves the induction of hepatic vitellogenin production under stimulation of ovarian estrogen. During the second phase, vitellogenin is taken up from the blood stream and incorporated into ovarian yolk proteins. In all stages of vitellogenesis, starting with the mobilization of lipid, the synthesis in the liver of a female-specific glycolipophospho-protein-vitellogenin and its eventual deposition in oocytes are known to be gonadotrophin dependent (Wallace, 1978; Nath and Sundararaj, 1981 lit. cit. Sundararaj, 1981). Idler and Ng (1979 lit.

cit. Bun Ng and Idler, 1983) have isolated two gonadotrophic hormones. The one with low carbohydrate content induced vitellogenesis, while the other which was rich in carbohydrate, induced maturation and ovulation. A possible means whereby the pineal could influence vitellogenis could involve a pineal-mediated regulation or modification of the secretion of GTH (possibly the low-carbohydrate GTH).

de Vlaming et al. (1977 lit. cit. de Vlaming and Vodicnik, 1978) reported that a specific lipoprotein (probably a yolk precursor) was much more frequent in the plasma of sham operated than pinealectomized goldfish exposed to a short photoperiod during early April. Since this lipoprotein was oestrogen-dependent (de Vlaming et al., 1977 lit. cit. de Vlaming and Vodicnik, 1978), these data implied that pinealectomy had an effect on plasma sex steroid levels, possibly by altering gonadotrophin secretion.

The present results, in which bovine pineal administration caused increased yolk deposition in tertiary-yolk and maturation oocytes, contrasts with the implication of increased vitellogenesis following pinealectomy of goldfish shown by de Vlaming et al. (1977 lit. cit. de Vlaming and Vodicnik, 1978).

D. RETROSPECT AND PROSPECT

The facts that adult S. niloticus have repeated

nonsynchronous reproductive cycles, and that body weight at which the onset of first ovarian maturation occurs seems to be somewhat variable, create considerable maturational heterogeneity in fish ovaries in any sample taken for experimental purposes even when the sample is homogeneous with respect to body weight or length. For experimental work where the stage of ovarian maturation is being determined, it may be advantageous to make additional checks on the ovarian condition. Taking ovarian biopsies has been considered as a possible solution to this problem.

For a tropical breeder where photoperiod throughout the year changes only from 12.5h:11.5h to 11.5h:12.5h (L:D) as in the region where the present experiments were performed, the change in photoperiod is unlikely to be a natural stimulus inducing reproductive activity. S. niloticus, moreover, breeds throughout the year. Thus, it may be questioned whether the pineal has a role in controlling reproductive activity in this fish at all, and if not, what function does it play ?

Indeed it has been shown that the pineal of S. niloticus was not required for ovarian maturation (during the actual time of ovarian maturation) and that the administration of bovine pineal was not inhibitory, (though it had some stimulatory effect on vitellogenesis). Thus, as far as reproduction is concerned, the role of the pineal may be minimal in this species.

However, to clarify further the possible reproductive

control mechanisms in which the pineal may be involved, 2 approaches might be used i) monitoring pineal activity, e.g. by ultrastructural appearance and/or histochemical and biochemical analysis for serotonin, or HIOMT (Hydroxyindol-O-methyltransferase) of fish pineals in relation to different stages of the ovarian cycle when kept under different photoperiodic regimes, and ii) determination of the response of gonadotrophin levels (high carbohydrate and low carbohydrate forms) to pinealectomy and/or melatonin or (fish) pineal administration at different phases of ovarian development.

There is much more evidence for the pineal's role in stimulating colour change in S. niloticus. Female S. niloticus darken under constant darkness, blanch under constant light and show a darkening reaction to MBP injection which is not shown in fish injected with Ringer's solution alone. This implies the increased release of melatonin from pineal under darkness and a pigment dispersing effect on dermal melanophores (the opposite of the effect reported in amphibia and lampreys). A simple experiment to confirm its peripheral action would be to observe the melanophore response to melatonin applied directly to excised skin.

While much remains to be learned before the natural function of the pineal in S. niloticus is elucidated, the prospects for limiting reproductive development in rearing ponds of this fish using a pineal mediated mechanisms, as

envisaged at the onset of this work, seem to be poor. In reality, the very reasons why there is a need to limit reproduction in rearing ponds of S. niloticus (nonseasonal, nonsychronous and repeated breeding cycles) reflect the lack of natural environmentally-induced control factors and thereby provide little opportunity for exploiting a natural regulatory system in the management of its reproduction.

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APPENDIX

Table A1 Composition of ovaries examined of *S. niloticus* from semi-natural pond at Tung Lung (- = none, 0 = a little, + --> ++++)

Time (yr.mon.date)	Log interval	Body weight (g)	Gonad weight (g)	Oogonium stage	Early-perinucleolus stage	Late-perinucleolus stage	Yolk-vesicle stage	Oil-drop stage	1-yolk stage	2-yolk stage	3-yolk stage	Maturation stage
85.11.26	4.6-4.7	39.5	0.0052	++	++++	+++	0	-	-	-	-	-
		40.3	0.0072	++	+++	++++	+	-	-	-	-	-
		41.5	0.6012	-	++	+	+++	++	+	++	-	-
		49.2	1.7377	-	0	+	++	+	+	+	+++	-
	4.7-4.8	51.2	0.0760	++	++++	+++	+	-	-	-	-	-
		59.5	0.8525	-	+	++	+++	++	+	++	+	-
	4.8-4.9	68.6	0.2482	+	++	+++	+	+	+	0	0	-
		67.1	0.0307	0	0	+	+	+++	+	++	+	-
		77.2	2.0197	-	-	0	+	+++	++++	+++	++	-
85.12.06	4.4-4.5	25.4	0.0766	++	+++	++++	-	-	-	-	-	-
	4.6-4.7	46.0	0.0997	++	++	+++	++	+	-	-	-	-
		45.5	1.1859	-	0	+	+++	+	++	+	+	-
	4.7-4.8	53.6	0.0803	+	+++	++++	+	+	-	-	-	-
		58.9	1.0768	+	0	++	+	+	0	+++	0	-
	4.8-4.9	64.1	0.1767	+	++	+++	++	0	-	-	-	-
		68.6	0.7282	-	0	+	++	+	++	+++	+	-
		76.3	3.3616	-	-	0	+	+	++	+++	+	0
	5.3-5.4	245.6	0.7821	+	-	+++	++++	0	-	-	-	-
85.12.28	4.6-4.7	46.1	0.0818	+	++++	+++	0	-	-	-	-	-
		41.1	0.3500	++	+++	++++	++	0	-	-	-	-
		47.0	1.2164	-	0	+	++	+++	++	+	+	-
	4.7-4.8	59.3	0.2232	+	++	+++	0	+	-	-	-	-
		59.1	3.2303	-	0	0	+	++	+	++	+++	+
	4.8-4.9	67.8	0.1708	0	+++	++++	0	-	-	-	-	-
		79.6	0.8418	+	+	++	+++	+	+	++	-	-
		73.7	2.4691	-	-	0	+	+	++	+	+++	+
86.01.06	4.6-4.7	43.7	0.0266	++	++	+++	-	-	-	-	-	-

Table A1 (cont.)

Time (yr.mon.date)	Log interval	Body weight (g)	Gonad weight (g)	Oogonium stage	Early-perinucleolus stage	Late-perinucleolus stage	Yolk-vesicle stage	Oil-drop stage	1-yolk stage	2-yolk stage	3-yolk stage	Maturation stage
86.01.06	4.8-4.9	64.5	0.0974	++	++	++++	+++	-	-	-	-	-
		76.3	0.3929	+	+	++	+	+	+	-	-	-
		72.6	3.5781	+	0	0	0	+	+	++	+++	++
	4.9-5.0	85.0	0.1083	++	+++	++++	+	-	-	-	-	-
		81.6	0.4698	++	+	++++	++	+++	++	+	-	-
		80.9	2.7311	-	0	0	+	+++	++	+	+	+
86.01.23	4.4-4.5	25.3	0.0041	++++	-	-	-	-	-	-	-	-
		31.8	0.0481	++	+++	++++	-	-	-	-	-	-
	4.6-4.7	48.2	0.0341	++	+++	++++	0	-	-	-	-	-
		45.7	0.1231	++	+++	++++	+	-	-	-	-	-
		49.6	0.4150	0	+	++	+	+++	+	+	-	-
	4.9-5.0	91.5	0.1031	++	+++	++++	-	-	-	-	-	-
		108.4	0.5703	++	++	+++	+	++	+	+	-	-
		91.5	1.9864	-	+	0	+	++	+	++	+++	0
	5.5-5.6	322.0	0.7288	++	+++	++	+	++	-	-	-	-
		510.8	1.7825	+++	++	++	+++	++++	-	-	-	-
		410.6	8.9472	-	+	0	+	+	++	++	+++	++
86.02.06	4.4-4.5	26.3	0.0311	+++	++	++++	-	-	-	-	-	-
	4.7-4.8	56.7	0.0671	++	++	++++	-	-	-	-	-	-
		60.4	2.2807	0	-	0	0	0	+	+++	++	0
	4.8-4.9	76.4	0.0938	++	+++	++++	-	-	-	-	-	-
		77.3	0.2746	+	+++	++	++	++	-	-	-	-
		77.2	1.1213	+	++	++++	+++	+	-	-	-	-
	4.9-5.0	93.4	0.0145	++	++++	+++	0	-	-	-	-	-
		108.4	0.1329	+	+++	++++	++	0	-	-	-	-
		94.1	3.4995	-	0	+	+++	++	+++	++	+	-
	5.0-5.1	104.9	0.3647	++	+++	++++	+	-	-	-	-	-
		111.8	1.1468	+	0	+	++	++	+++	++	+	-

Table A1 (cont.)

Time (yr.mon.date)	Log interval	Body weight (g)	Gonad weight (g)	Oogonium stage	Early-perinucleolus stage	Late-perinucleolus stage	Yolk-vesicle stage	Oil-drop stage	1-yolk stage	2-yolk stage	3-yolk stage	Maturation stage
86.02.06	5.0-5.1	111.7	5.3457	0	0	+	++	0	+	+++	+	-
	5.5-5.6	358.4	2.1613	-	0	+	++	+++	++	+	-	-
86.02.27	4.8-4.9	61.8	0.1128	+++	++	++++	+	-	-	-	-	-
		77.7	1.0979	+	0	+	++	++	+	+++	0	-
	4.9-5.0	98.2	0.1913	++	+++	++++	+	-	-	-	-	-
		84.3	0.4839	++	++	++++	+++	0	+	0	-	-
		93.3	2.8912	+	+	++	+++	+	+	++++	0	-
	5.0-5.1	117.2	0.2885	++	+++	++++	++	+	-	-	-	-
		119.0	4.3987	+	+	0	++	+	+	++	+++	0
	5.1-5.2	154.9	0.0964	+	0	0	++	+	+	+++	+	-
		143.5	1.9133	+	0	0	+	+	++	+++	+	-
	5.2-5.3	168.9	6.6323	0	0	0	+	0	+	++	+++	++
86.03.17	4.7-4.8	55.3	0.0675	+++	++++	++	+++	-	-	-	-	-
		59.4	0.2431	++	+++	++++	++	+	0	-	-	-
	4.8-4.9	73.4	0.0803	++	+++	++++	+	-	-	-	-	-
		68.3	0.7991	+	0	++	+	0	+++	-	-	-
		76.6	1.6519	-	0	0	+	+++	++++	++	0	-
	4.9-5.0	87.3	0.1398	+++	++	++++	0	-	-	-	-	-
		95.4	1.0911	+	+	+++	++++	++	+	-	-	-
		85.6	2.9875	0	0	+	++	+	+	+++	+	-
	5.0-5.1	104.1	0.2123	++	+++	++++	+	0	-	-	-	-
		105.1	0.7402	+	+++	++	+	++++	++	+++	+++	-
	5.5-5.6	331.2	3.6355	+	++	+++	++	+	++	+	-	-
86.03.31	4.6-4.7	45.1	0.0084	++++	-	-	-	-	-	-	-	-
		46.4	0.0578	++	+++	++	-	-	-	-	-	-
		48.7	0.1783	+	++	++++	+++	-	-	-	-	-

Table A1 (cont.)

Time (yr.mon.date)	Log interval	Body weight (g)	Gonad weight (g)	Oogonium stage	Early-perinucleolus stage	Late-perinucleolus stage	Yolk-vesicle stage	Oil-drop stage	1-yolk stage	2-yolk stage	3-yolk stage	Maturation stage
86.03.31	4.7-4.8	60.6	0.0170	++++	-	-	-	-	-	-	-	-
		60.4	0.1003	++	+++	++	-	-	-	-	-	-
		52.6	0.5928	+	+	++	+	+	+	+	+	-
	4.8-4.9	65.3	0.1237	++	+++	++	+	-	-	-	-	-
		67.7	0.3359	0	++	+++	+	+	-	-	-	-
		71.3	0.3852	-	0	0	++	+	+	+++	+	-
	5.0-5.1	113.8	0.3500	+	+++	++	+	+	-	-	-	-
		118.5	1.1697	0	0	+	+	+++	++	+	+	-
		121.6	5.0970	-	0	+	+	++	+	++	+++	+
86.04.15	4.8-4.9	62.5	0.1505	+++	++++	++	0	-	-	-	-	-
		79.8	0.8757	+	+	++	+	++	+++	+	-	-
	4.9-5.0	93.3	0.1939	-	0	+	+	++	+++	0	-	-
		95.1	1.5798	0	0	+	0	+	+++	+	-	-
	5.0-5.1	118.4	1.2128	0	0	++	+	++	+++	0	-	-
		116.6	3.2521	0	0	0	0	+	++	+++	0	-
	5.1-5.2	149.7	2.8921	0	0	+	+	++	++	+++	0	-
86.05.01	4.8-4.9	74.7	0.1293	+++	++	++++	-	-	-	-	-	-
		77.1	1.1724	0	0	0	+	+	0	+++	-	-
	4.9-5.0	81.6	0.1040	+++	++	++++	-	-	-	-	-	-
		82.3	0.8256	++	+	+++	+	++	+	-	-	-
		89.6	3.1944	+	0	0	+	+	++	+++	+	0
	5.0-5.1	109.0	0.1656	++	+++	++++	0	-	-	-	-	-
		106.9	1.2341	+	++	+++	+	0	+	++	0	-
		126.1	3.6881	0	0	0	+	+++	++	++++	+	-
	5.1-5.2	126.8	0.8508	++	+	++	+	+++	0	-	-	-
		128.9	2.3643	0	+	++	+	+	++	+++	+	-
		129.2	6.4818	-	0	0	0	+	++	+++	++++	0

Table A1 (cont.)

Time (yr.mon.date)	Log interval	Body weight (g)	Gonad weight (g)	Oogonium stage	Early-perinucleolus stage	Late-perinucleolus stage	Yolk-vesicle stage	Oil-drop stage	1-yolk stage	2-yolk stage	3-yolk stage	Maturation stage
86.06.03	5.0-5.1	118.5	0.2354	+++	++	++++	0	-	-	-	-	-
		119.0	0.5242	++	+	++	+++	++	+	+	-	-
		120.5	1.0380	+	0	++	0	+	+	+++	-	-
		124.4	1.8966	+	0	++	0	+	++	+++	0	-
	5.1-5.2	137.4	0.4582	++	+	+++	++	+++	+	-	-	-
		131.9	0.8100	++	++	+++	+	++	++	+	-	-
		156.2	1.0215	+	+	++	+	++	+++	0	-	-
		158.0	1.0097	+	0	+	0	++	++	+++	+	-
		137.6	3.2951	+	0	+	0	+	++	+++	++++	0
	5.2-5.3	169.2	0.5763	++	++	++++	+	+++	0	-	-	-
		198.7	0.7879	+	+	++	+++	+	++	0	-	-
		178.3	2.3984	0	0	++	+	++	+	+++	0	-
		175.1	4.9685	0	0	+	+	++	+++	++++	++	0
	5.3-5.4	249.2	3.4412	0	0	0	0	+	+	+++	++	0
	5.4-5.5	305.1	2.4586	+	++	+++	+	+	+	++	-	-

Table A2 Composition of ovaries examined of S. niloticus kept under constant light in tanks (uninjected control, injected control, high dose injection).

Experimental group	Fish No.	Body weight (g)	Gonad weight (g)	Oogonium stage	Early-perinucleolus stage	Late-perinucleolus stage	Yolk-vesicle stage	Oil-drop stage	1-yolk stage	2-yolk stage	3-yolk stage	Maturation stage
uninjected control	1	16.6	0.2877	+	++	+++	0	+	−	0	++	−
	2	16.1	0.02390	+	++	+++	+	0	−	0	++	−
	3	17.2	0.4298	+	++	+++	0	0	0	+	+++	0
	4	19.5	0.9535	0	0	+	+	+	−	0	++	0
	5	11.2	0.6286	0	0	+	0	+	0	+	++	+
	6	20.0	0.2591	++	++	+++	+	0	0	+	+	−
	7	20.0	0.4531	0	+	++	0	+	+	+++	−	−
	8	10.1	0.0352	++	++	+++	++	0	0	+	−	−
	9	13.5	0.0922	++	+++	++	0	−	+	−	−	−
	10	15.1	0.3966	+	+	++	0	+	0	0	++	−
	11	26.7	0.8748	+	0	++	+	+++	0	−	++	−
	12	19.8	0.4202	+	+	+	+++	0	0	++	−	−
	13	18.9	0.3322	+	+	++	0	0	+	+	++	−
	14	22.5	0.4193	+	++	+	0	+	0	+	++	0
	15	14.5	0.0319	++	+++	++	−	−	−	−	−	−
	16	24.5	1.1776	+	+	++	0	++	−	0	++	−
	17	20.0	0.8534	+	+	++	+	+	+	+++	−	−
	18	20.5	0.8983	+	+	+	+	0	++	+	+	−
	19	17.9	0.2878	+	++	+++	+	−	−	−	+	−
	20	15.8	0.3518	+	+	++	0	++	+	−	−	−
	21	10.2	0.1088	+	0	++	+	0	0	+	−	−
injected control	1	18.2	0.8716	+	+	+	+	+	++	+++	++	+
	2	14.9	0.0874	+	++	+++	0	0	+	++	+	−
	3	22.5	0.0113	+++	++	++++	−	−	−	−	−	−
	4	26.9	0.9828	+	+	+	0	+	0	+	+	++
	5	17.6	0.4628	++	++	+++	++	+	0	+	−	−
	6	15.2	0.6326	+	+	++	+	+++	0	+	+	−
	7	20.7	0.8268	0	+	+	0	+	0	+	++	−
	8	11.8	0.5651	+	++	+	0	+	+	0	++	0
	9	12.6	0.1528	+	+	++	0	0	+	+++	0	−
	10	13.4	0.6366	++	0	+	0	+	0	++	−	−
	11	12.1	0.0222	+++	++	++++	0	−	−	−	−	0
	12	21.4	0.9288	+	0	+	0	0	+	++	+	−
	13	16.9	0.6718	+	+	++	0	+++	0	++	−	−
	14	17.6	0.5959	++	++	+	+	++	+	+++	0	−
	15	21.2	0.4572	++	++	+++	+	0	++	−	−	−

Table A2 (cont.)

Experimental group	Fish No.	Body weight (g)	Gonad weight (g)	Oogonium stage	Early-perinucleolus stage	Late-perinucleolus stage	Yolk-vesicle stage	Oil-drop stage	1-yolk stage	2-yolk stage	3-yolk stage	Maturation stage
injected control	16	20.0	0.2800	+	+++	++	0	0	+	++	0	-
	17	18.4	0.4137	+	+	+++	+	0	0	+	++	-
	18	15.2	0.2670	++	+	+	+++	+	++	+	-	-
	19	12.0	0.1152	+	++	+++	+	+	+	++	-	-
	20	22.8	1.3260	+	0	+	0	+	++	+	+++	-
	21	18.2	0.3785	++	++	+++	+	++	0	+	-	-
	22	18.0	0.1911	++	++	+	+	+	0	+	-	-
	23	13.4	0.2522	+	+	++	0	+	+++	++	-	-
high dose macerated bovine pineal injection	1	18.2	0.5580	++	+	++	+	+	0	0	++	-
	2	18.5	0.2950	+	+	+++	0	++	-	-	-	-
	3	13.7	0.0636	+++	+++	++++	+	+	0	-	-	-
	4	15.7	0.8702	++	++	+++	0	+	0	+	++	-
	5	22.1	1.2929	++	+	0	0	+	0	+++	++	-
	6	18.5	1.3228	0	++	0	0	-	++	0	+	+++
	7	21.3	0.2107	++	+	++	++	0	+	++	-	-
	8	21.5	0.9709	+	+	++	0	++	+	0	+++	0
	9	19.1	0.9986	0	+	0	0	+	+	++	+	0
	10	17.0	0.1179	+++	++	++++	+	0	0	+	-	-
	11	16.7	0.5876	+	+	++	0	+++	+	0	++	+
	12	13.2	0.8328	+++	++	+++	+	0	-	-	-	-
	13	19.3	0.6800	++	++	+++	+	+	+	+	++	-
	14	15.4	0.3744	++	+	++	+	+++	0	-	-	-
	15	20.0	1.0852	+	+	+	+	++	+	+	+++	0
	16	16.8	1.1213	0	++	0	+	0	+	+++	++	0
	17	20.5	0.5189	+	+	++	0	+	0	0	++	0
	18	20.0	1.2000	0	0	+	0	++	+	0	+++	0
	19	21.8	1.0154	++	++	+	+	++	+++	0	+	-
	20	16.1	1.0632	+	++	+++	+	++	+	++	+	0
	21	19.5	1.0910	+	+	0	0	+	++	+	++	-
	22	12.2	0.8804	+	++	+++	+	0	+	++	-	-
	23	25.4	1.1490	+	+	++	+	+	+	+	++	0

Bovine pineal dissection method (Geater, personal communication)

The pineal gland used in this study were obtained from adult Thai bulls (Bos indicus) from a local slaughter house in Hat Yai, Songkhla province. Bulls weighing approximately 400 kg and were slaughtered by common carotid artery section. The animals were decapitated within 30 minutes of slaughter. A manual or low-speed electric drill filled with a 5.5 cm outside-diameter circular "key hole" bit was used to make a circular cut in the temporal and parietal bones dorsal to, and including the dorsal edge of, the foramen magnum. In cases, where the drill cut did not penetrate the bone completely, a fine cold chisel was used to complete the cut and the circular disc of bone withdrawn by using heavy forceps, thereby exposing the meninges. From this point on, sterile technique was employed. The exposed meningeal membranes were removed to reveal the posterio-dorsal surface of the cerebellum. A tea-spoon was inserted dorsal to the cerebellum and extending to the region of the culmen and used to retract the cerebellum posterio-ventrally and expose the rostral colliculi, anterior to which the pineal was situated, extending posterio-dorsally from the subpineal fossa. Cerebrospinal fluid was sucked out using a syringe. The pineal stalk was transected using fine angled scissors and the pineal removed with forceps, placed in a sterile bottle and kept under ice until transfer to a refrigerator at - 10 C.