latter phenomenon only occurs in females which could see one or more conspecifics and which had their ovaries filled with oocytes of mature size (stage 14), a situation characteristic of all females that had spawned six days or more before.

b) (1) During the later pre-spawning phase, characterized by skimming, and during spawning there was found an activity of the \( \beta_2 \) cells, which apart from playing a role in the regulation of the advanced sexual behaviour, could have a function with regard to the causation of ovulation.

c) (2) During the later pre-spawning phase, characterized by skimming, there was also found an activity of the ganglion cells of the nucleus tuberis near the beginning of the hypothalamic-hypophyseal tract. This neurosecretory activity could possibly play a role in the regulation of the skimming-spawning movements.

d) During the parental phase, characterized by fanning, there was found an activity of the \( \alpha \) cells, which may have an influence with regard to parental behaviour.

In this way, the hypophyseal hormones could act as internal stimuli, among those mentioned in the second hypothesis of p. 352. Here a sufficient high level of gonadal hormones was considered to be necessary to enable the fishes to perform reproductive behaviour as a whole. However, it should be kept in mind that the results of the castration-experiments of Noble & Kumpf (1936), Aronson (1959) and Tavolga (1955), mentioned in the previous part of this chapter, have shown that at least in the species examined male courtship behaviour can be performed in absence of testicular hormone.

Conclusive evidence is urgently needed here.

7. SUMMARY

In this paper on the cichlid fish *Aequidens portalegrensis*, information is given on 1) the anatomy and histology of the gonads, 2) the functional anatomy of the accessory organs, 3) the occurrence of cyclical changes in the gonads, 4) the mutual behaviour of male and female under different conditions, 5) the temporal pattern of spawning, and 6) periodic changes in responsiveness of reproductive behaviour patterns to adequate external stimulation, in an attempt to contribute to a picture of the mechanism of reproduction in this species.

A description is given of the interstitial Leydig cells and "lipoid cells" in the testes. Contrary to the latter, the former never contained demonstrable amounts of sudanophil material. The spermatogenetic epithelium and the process of spermatogenesis is also described. Experimental inhibition of spawning influenced the amount of spermatogenetic...
genetic tissue and the internal structure of the testis, not the spermatogenic activity of the tissue. The question of the localization of hormone production in the testis is discussed on the basis of the literature. It is concluded that the “lipoid-cells” do not produce hormones, but must serve a trophic function.

In the ovary processes occurring in the oocytes during their period of growth, the formation of yolk vesicles and the accumulation of lipoid- and yolk globules are described and the role of the granulosa cells with regard to these processes is discussed. Ovulation was found to occur during a period in which the egg and the surrounding mucous substance, a secretion of the granulosa cells, swell as a result of an uptake of liquid. The way the post-ovulatory follicle material disappears is discussed.

An attempt has been made to estimate in one pair of ovaries the numbers of the oogonia and of the oocytes of the stages 2-14 which are distinguished. The regulation of the clutch-size is discussed. Inhibition of spawning resulted in atresia of the most advanced oocytes after 30 days or more. The process of resorption, following atresia, is described; the structures formed closely resemble those occurring in the Bitterling during the formation and development of the “pre-ovulatory corpora lutea”, as described in the literature. It is concluded that these structures can be excluded as sites of hormone production.

The anatomical structure of the genital papilla in males and females and the way it probably functions during sperm ejection and egg laying is described. The problem of the causation of motility of the spermatozoa is discussed. Experiments showed that in Aequidens portalegrensis motility is acquired after contact with a hypotonic solution. The way of attachment of the eggs to the substrate is discussed. It is concluded that the sticky mucous layer, a secretion of the granulosa cells which surrounds each egg, attaches the egg to the substrate. The fibers which run through the mucous layer and which are suggested to be thickened extracellular axes originally formed between adjacent granulosa cells, are considered to consolidate the mucous layer to the egg.

Four cases of sex-reversal are described; the process apparently starts at the caudal end of a degenerating ovary.

Pair formation, pre-spawning and spawning behaviour were studied 1) in communities with many males and females, 2) in communities of one male and eight females and 3) in couples in isolation. It is concluded that the female partner is selected (by the male) on its sex and physiological condition. The problems of the synchronization of the partners with regard to spawning and the maintenance of the pair bond are discussed. It is concluded that during motivational synchronization of both partners the endocrine mechanism of the female receives...
Reproduction in Aequidens portalegrensis

The spermatozoa are activated by the presence of hormones, but there are no data on their period of activity. The activation of the granulosa cells by the presence of follicle-stimulating hormone was found to be a result of an endogenous periodicity, the result of an ovulatory cycle.

Inhibition is not apparent in the granulosa cells, but is found in the ovaries of females which are being atretic. Inhibition is not apparent in the granulosa cells after the occurrence of atresia, which occurs as a result of the "preovulatory" phase of the "ovarian cycle." It is concluded that the ovulatory cycle is the production of the oocytes and the females are stimulated to lay eggs.

The question whether changes in daylength can cause seasonal fluctuations in the spawning frequency of Aequidens portalegrensis is discussed.

Experiments in which the pair bond was broken immediately after fertilization and a new sexually active female was made available showed that a male can successfully fertilize at intervals of 1 day or less. It was concluded that the minimum interval of 5 days, found between successive spawnings in isolated pairs, is determined by the sexual cycle of the female. Experiments in which the partners of isolated pairs were separated by a glass partition, just before skimming merged into pseudo-spawning, showed that the visual stimuli of the spawning female and of the clutch are unable to change the males' skimming into pseudo-spawning or spawning. In contrast a very short contact with eggs sufficed to evoke spawning suddenly (often within 4 seconds).

The presence of young was found to lengthen the interval beyond the period needed for recuperation of the gonads. The same effect also resulted from contact with the surface of a substrate touched shortly before by a pseudo-spawning female, which suggests that the ovarian mucous (which had been smeared over the substrate) contains a relevant stimulus for sperm ejection.

In isolated pairs presentation of a freshly laid clutch could evoke nipping off, quivering, skimming, pseudo-spawning, fanning, nibbling at eggs, calling young and eating eggs in both partners of the pairs. In a series of experiments fresh clutches were presented to isolated pairs during 1–4 days, 6–9 days and 10–90 days after the eggs which receive stimulation from the presence and the behaviour of the male until she is endocrinologically ready to spawn. The mutual binding of the parents to the offspring, which under normal conditions lasts several weeks, amply bridges the critical period during which a female is physiologically unable to spawn and to court a male, as a result of which continuance of the pair bond is a rule in this species.
had just been spawned were removed. The results showed that a fresh clutch can release all eight activities during each of these periods except during the first day after spawning, when the responses are restricted to fanning, nibbling at eggs and calling young. Quantitative differences in the incidence of the eight activities during the different periods lead to the conclusion that, when shortly after spawning the brood is lost, three largely incompatible tendencies can be distinguished in the parents: a tendency to care for offspring, a tendency to prepare a new spawning and a tendency to feed on eggs. The first tendency yields to both other tendencies as time passes. Presentation of fresh clutches to pairs fanning their own clutch, to pairs with wrigglers or to pairs guarding their school of small fry only elicited parental (fanning, nibbling) responses.

Experiments on transference of pairs to a new environment during different phases of the behavioural cycle showed that the spawning process cannot easily be upheld by a change in environment after ovulation has taken place.

Isolation was found to abolish spawning and all other reproductive behaviour in males and females. When kept with a mirror, isolated males only showed aggressive behaviour, but isolated females resumed reproductive behaviour, and spawning occurred at normal intervals. It was concluded that visual perception of a partner is essential for ovulation and spawning.

The early phase of the pre-spawning period, characterized by jerking and quivering cannot be recognized histologically; in both sexes the gonads did not show changes during that period. During the later phase of the pre-spawning period, characterized by skimming, the male gonads did not show changes, while in the female gonads the only change observed, swelling and ovulation, was found to occur when, shortly before spawning, skimming merges into pseudo-spawning. The next change, the removal of eggs from the ovaries and spermatozoa from the testes is closely related to spawning behaviour. It is immediately followed by a 5-days period of growth of a new batch of future eggs in the female and a refilling of the testes with spermatozoa, taking at least 2 days, in the male. The parental behaviour, which is normally shown by both sexes during these recuperation periods of the gonads, was found to be related to the external stimuli: eggs and young, rather than to a definite histological state of the gonads.

The ovaries continually show asynchronous oocyte development and the testes continuously show spermatogenetic activity. Features indicating a period of regression or decreased activity in the gonads have never been observed. The occurrence of differences in internal stimulation, as deduced from the results of the experiments on the presentation of fresh clutches, is considered indicative of the cause of spawning.

Comment: The causation of spawning in fishes is at present not adequately understood. At least in some species the spawning behaviour is considered indicative of the cause.

The cause of spawning probably involves a cyclical stimulation of the gonads by cells, possibly of the pituitary gland, which precipitate ovulation; 3 of the cells, probably the hypothalamus or the hypothalamic nuclei, are responsible for ovulation.

I am greatly indebted to Professor Dr. R. C. van der Poel for the opportunity to work under his guidance and for his interest in my work. I also wish to thank the editor of this journal for his kind criticism of the manuscript and Mr. L. K. for typing it.

Finally thanks to Dr. W. M. van den Bergh for his encouragement, to the English translation and Mr. L. K. for taking care of the manuscript.

---. 1951. Further biological studies of Tilapia macrocephala.
of fresh clutches during different phases of the reproductive cycle, is considered in relation to a possible influence of hormones.

Comments are made on the hormonal control of reproductive behaviour in fishes, in particular in *Aequidens portalegrensis*. It is suggested that, at least in males of this and some related species, reproductive behaviour is controlled by hypophyseal hormones, the testicular hormones playing a permissive role, a minor role, or no role at all.

The causation of ovulation is discussed; visual and proprioceptive stimulation are suggested to be of vital importance. The endocrine model is suggested to be composed of 1) hypophyseal secretory activity of \( \beta_1 \) cells, possibly regulating early sexual behaviour and contributing to "activation"; 2) hypophyseal secretory activity of \( \beta_2 \) cells, which possibly plays a role in the regulation of advanced sexual behaviour and which probably has a function with regard to the causation of ovulation; 3) neurosecretory activity possibly playing a role in the regulation of the spawning movements; 4) secretory activity of the hypophyseal \( \alpha \) cells, playing a role with regard to parental care.

**Acknowledgements**

I am greatly indebted to Professor Dr. G. P. Baerends for giving me the opportunity to work at his laboratory, for suggesting the problem, for his interest during this study and for his criticism and valuable suggestions during the preparation of the manuscript.

I also wish to express my sincere gratitude to Dr. B. Baggerman for her criticism and valuable suggestions for the improvement of this paper.

Thanks are further due to my colleagues for their critical discussions and kind help, in particular to Miss G. J. Blokzijl, for her assistance with the experimental work and to Mr. J. G. van Rhijn for his statistical advice.

Furthermore I want to thank Mrs. H. Lochorn-Hulsebos for typing the manuscript, Mrs. M. H. Beukema-Kampherbeek for correcting the English text, Mr. K. P. van Kempen for making the photographs and Mr. L. Hoekstra for preparing photographs and drawings.

Finally thanks are due to Mr. F. Bahlman and Mr. G. Bakker for taking care of the fishes and the aquaria.

**References**

