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 β_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 α 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 spermato-

genetic tissue and the internal structure of the testis, not the spermatogenetic 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 extracellulair 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 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.

Under the relatively constant external conditions of experiments, in which isolated pairs were constantly kept at 25° C and at 12 hours artificial fluorescent light on which a varying amount of natural light was superimposed, no indications could be found for the existence of an endogenous seasonal periodicity. The number of spawnings per average pair during the different months of the year showed only small deviations from a mean value of 1.9 clutches per month. In individual pairs, successive spawnings may follow each other every 2 or 3 weeks for a continuous period of at least 250 days. The intervals between successive spawnings varied from 5 days up to 70 days and more. The highest frequencies are reached for intervals between 8 and 12 days. The frequencies for longer intervals decrease gradually. Intervals of less than 5 days were never found. The presence of young was found to lengthen the interval beyond the period needed for recuperation of the gonads. 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 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

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 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 β_1 cells, possibly regulating early sexual behaviour and contributing to "activation"; 2) hypophyseal secretory activity of β_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 α cells, playing a role with regard to parental care.

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