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Reproduction session. Concluding remarks

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Concluding remarks

The majority of the communications presented at the oral and posters sessions could be grouped around two important problems concerned with the culture of carp and related species, namely, the effects of the environment and spawning induction. These problems were dealt with at both the practical and the theoretical levels. It was only natural to focus the round table discussion around these problems.

1. Effect of some environmental factors

All the relevant presentations have indicated that temperature is the main external factor affecting reproduction in cyprinids. Although the collection of papers presented at the symposium has not encompassed any paper on photoperiodic effects, there is enough evidence to show that the photoperiod does affect reproduction in some cyprinids like the goldfish, in which there is an interaction between photoperiod and temperature. However, carp, even maintained on a constant light or constant dark regimes, do respond, by gonadal recrudescence, to an increase in the temperature (Horvath).

The significance of the "amount of temperature" as related to carp reproduction was emphasized by Horvath. He recommended the determination of "degree days" to which the fish are exposed in order to predict the proper timing of spawning in carps. This term, which represents the multiplication of temperature by days, was challenged by Bieniarz and by Kime on the grounds that it does not fully reflect the complex situation of temperature effects on carp reproduction. The term may be useful only within the limits of optimal temperatures, especially when it implicates the definition of a critical temperature (Bieniarz). This is in agreement with the fact that gametogenesis does not occur in carp at a temperature below 15°C (Horvath). The possible role of daily fluctuation in the temperature was also discussed, especially in

relation to the daily cycle of hormones (Peter). Woynarovich summarized his experience in this subject and indicated that propagation of the common carp can take place all year round in tropical countries.

Other external factors affecting reproduction in cyprinids were pointed out: stress and stocking density, feeding (Peteri et al., Wiegand) and pollutants (Pathak).

2. Induced spawning and artificial fertilization

Under natural conditions, a cascade of glands and hormones "translate" environmental cues to gonadal growth and spawning. The various methods of spawning induction are, in fact, artificial interventions in one or another step of this cascade. Classical hypophysation, although suffering in most cases from the lack of standardization, is still a common practice among carp breeders (Toor; Dwivedi and Reddy). Introduction of a calibrated carp pituitary extract into hatchery practice will, probably, increase predictability of induced spawning by hypophysation (Yaron). Gonadotropins of mammalian origin (HCG), alone or in combination with carp pituitary extract, are not more effective than pituitary extract alone (Epler et al.). The report by Macintosh and Price does not conform, however, with this view. Nevertheless, several participants expressed the idea that the use of heterologous gonadotropins such as HCG for spawning induction may lead to the formation of antibodies in the recipient fish and interfere with their spawning in subsequent seasons.

The administration of gonadotropin-releasing hormones of hypothalamic origin (GnRH, LHRH) or their synthetic analogues seems to be a better substitution for the classical hypophysation technique. Their potency in raising endogenous gonadotropin levels and inducing ovulation in certain cyprinids was examined by using them alone (Kouril) or in combination with the dopamine antagonist pimozide (Kaul and Rishi; Sokolowska et al., Lin et al.). Moreover, Kaul and Rishi reported that pimozide, even given alone, was effective inducer of ovulation in the mrigal; in other cyprinids such effect was not observed. Nevertheless, Woynarovich complained that his experience with GnRH in characin, fish were not that successful. Pullin remarked that the cost of treatment with GnRH plus pimozide should be reduced in order to be acceptable in developing countries.

The possible toxicity of pimozide or its residues was raised by Fostier in relationship with the welfare of the recipient fish or the consumer. Unfortunately, no studies have yet been performed on the biological half-life of pimozide or its residues in the fish (Peter). The problem for the consumers does not seem to be that severe since females treated for spawning are kept usually as a broodstock in the hatcheries for subsequent seasons and are rarely marketed (Horvath). The paper presented by Lin et al. has indicated that reserpine, an inhibitor of dopamine secretion, can successfully substitute pimozide in carp induced to spawn by GnRH analogues. Nevertheless, the effects of both pimozide and reserpine on egg quality and fish health is yet to be studied.

Another aspect of spawning induction, whether by pituitary extracts or by GnRH, is the receptivity or suitability of the fish to the treatment. Palpation of fish abdomen for softness has been proven to be rather subjective and inaccurate. Ovarian biopsy and subsequent determination of the position of the germinal vesicle (GV) appears to be a more adequate way to determine the suitability of female carp for spawning induction. A high proportion (65 % or more) of oocytes in which the GV has already started migration (eccentric GV) may serve as an indication for a female ready for

treatment (Levavi-Zermonsky and Yaron). In fact, this simple method has been adopted already by certain hatcheries in Israel (Yaron).

However, the sensitivity of the oocytes to gonadotropin (GTH) may not be fully reflected by the position of the GV. A more accurate way would be the *in vitro* examination of ovarian response to the addition of GTH (Epler et al.). The sensitivity of the oocyte was found to fluctuate in a diurnal rhythm, coinciding with that of the circulating GTH. The daily peak of GTH occurs at 2 p.m. in Poland (Bieniarz) but it peaks in the "Dor-70" carp in Israel around 11 p.m. (Levavi Zermonsky and Yaron). There is not enough information to allow a conclusion whether this difference should be attributed to the difference in daylength or temperature between these countries at the time the observations were made, to genetic differences between the two carp populations, or other possible factors. The *in vitro* determination of ovarian sensitivity to gonadotropin still requires the proper application to the conditions and routine of the hatchery.

The technique of artificial fertilization in cyprinids using the milt extracted from one male to the spawn of one female seems, nowadays, rather unsophisticated. Research is now in progress to objectively assess sperm motility and viability (Gatty et al.). The amount of sperm produced and the volume of milt extractable after hypophysation have been determined together with the optimal amount of sperm required for successful fertilization in the common carp (Saad and Billard).

The last part of the discussion has dealt with the possible involvement of pheromones in carp reproduction. At high temperature, testicular tissue of the carp produce *in vitro* a large proportion of testosterone glucuronide. These results, taken together with the recent findings of Lambert's group in the catfish, raise the possibility that this or other steroid glucuronides, secreted in nature by the males, may act as pheromones that induce a surge of GTH in the surrounding females, leading to the final ovarian maturation, ovulation and spawning. If this will prove to be the case also in cyprinids, future research should be directed toward the discovery of the pheromones receptors in the olfactory system in the fish. Immunocytochemical studies in the goldfish have demonstrated the presence of GnRH in cells and tracts leading from the olfactory bulbs and nerves to the ventral telencephalon and the ventrolateral preoptic area of the hypothalamus (Kah). There is still a gap between the findings on carp testicular glucuronides and the putative olfactory receptors of male pheromones and GnRH containing brain structures in the females. However, one would wonder whether future research on reproduction may not lead to a new direction, the use of synthetic fish pheromones for the artificial propagation of carp and related species.

In summary, the well attended session and the vivid discussion have clarified the fact that the State of the Art in cyprinids reproduction leaves much to be desired as compared with the situation in salmonid fishes. The situation in cyprinids other than the goldfish and the common carp is even less satisfactory. Problems of special interest are the initiation of puberty in cultured cyprinids, especially in the temperature climate; the estimation of ovarian sensitivity to induced spawning; calibration of agents utilized in spawning induction; gamete management for optimal survival, and the study of early developmental stages.

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