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Effects of (D-Ala⁶, Pro⁹N ethylamide) – LHRH on plasma levels of gonadotropin, 17 α ,20 β -dihydroxy-4-pregnen-3-one and testosterone in male goldeye (*Hiodon alosoides* Rafineque)

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Abstract

Male goldeye were treated with (D-Ala⁶, Pro⁹-N ethylamide) – LHRH (LHRH-A) in saline or a silastic pellet (100 $\mu\text{g}\cdot\text{kg}^{-1}$ body weight) and changes in plasma gonadotropin (GtH), 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β P) and testosterone (T) levels were determined by radioimmunoassay. LHRH-A increased plasma GtH levels, with the response to LHRH-A in saline being of much greater magnitude and duration than the response to silastic pellet implants. Seasonal differences were found in the response to LHRH-A. Spermiated fish were the most responsive, recrudescing fish the least, and regressed fish displayed an intermediate response. Plasma 17 α ,20 β P levels were elevated in response to LHRHA in fish of all sexual stages although the magnitude of the increase was not related to the magnitude of the increase in GtH levels. Treatment with LHRH-A also resulted in a transitory increase in plasma T levels. The endocrine control of GtH and steroid secretion in goldeye is discussed in relation to studies in cyprinids and salmonids.

Introduction

Most of the studies investigating the effects of gonadotropin releasing hormones (GnRH) on gonadotropin (GtH) secretion in teleosts have been performed in species from only two families, the salmonidae (Weil *et al.* 1978; Crim *et al.* 1981, 1983; Hirose *et al.* 1983; Van Der Kraak *et al.* 1983, 1985; Weil and Crim 1983; Billard *et al.* 1985) and the cyprinidae (Peter 1980; Weil *et al.* 1980; Billard *et al.* 1983, 1984; Breton *et al.* 1983; Chang and Peter 1983; Sokolowska *et al.* 1984, 1985a, b; Peter *et al.* 1985). These studies have demonstrated that

the response to GnRH is dependent on several factors, including the mode of application, type and dose of GnRH, temperature, and the reproductive state.

In the present study, the effects of the potent mammalian GnRH analogue, (D-Ala⁶, Pro⁹-N ethylamide) – LHRH (LHRH-A) on GtH release in the goldeye were examined. Goldeye were chosen, as they are members of the Order Osteoglossiformes (Infradivision Osteoglossomorpha) which is phylogenetically distant from cyprinids and salmonids (Infradivision Euteleostei; Nelson 1984).

The steroidogenic response to changes in plasma GtH was monitored by measurement of plasma levels of 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,

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20 β P) and testosterone (T). In several teleost species, 17 α ,20 β P has been implicated in the induction of spermiation (Scott and Baynes 1982; Ueda *et al.* 1985) while T appears to be involved in stimulating the process of spermatogenesis (Fostier *et al.* 1983). The effects of changes in levels of plasma steroids in the goldeye on testes morphology and milt volumes were investigated.

In order to examine the influence of sexual stage on the endocrine response to LHRH-A, experiments were conducted on fish at several stages of sexual maturity.

Materials and methods

Male goldeye (360–670 g body weight) were captured from the North Saskatchewan River (Edmonton, Alberta) from May to October, by angling. Fish were tagged with numbered opercular tags (Jiffy Wing Bands, National Band and Tag Co.) for identification, transferred to aquaria within 3h of capture, and maintained at 15°C under simulated natural photoperiod. Fish were not fed, and used in experiments 24h to 1 week after capture. At the beginning of experiments, fish were anaesthetised in 0.05% 2-phenoxy ethanol (Syndel), weighed and given injections or implants as described below. During subsequent handling procedures, fish were immobilised by being placed ventral side up in a moistened foam rubber pad (Stacey *et al.* 1984).

LHRH-A (Syndel Laboratories) was dissolved in teleost saline (Burnstock, 1958) or mixed with Silastic 382 Medical Grade Elastomer (Dow Corning Corporation) to make solid silastic pellets. LHRH-A in saline was injected intraperitoneally while LHRH-A pellets were implanted in the peritoneal cavity via a 12G needle inserted through the body wall. Pellets (200 μ g.LHRH-A.cm⁻¹) were cut to length to give weight-specific doses (100 μ g.kg⁻¹ body weight for both methods of administration).

Spermiated fish captured in May (4 fish per group) and regressed fish captured in July and August (5 fish per group) were given an injection of LHRH-A and a blank silastic pellet, or a saline injection and a pellet containing LHRH-A. Controls received saline and a blank pellet. Recrudescent fish

captured in October (5 fish per group) received an injection of either LHRH-A or saline.

Reproductive stages of regressed and recrudescent fish were confirmed from measurement of gonadosomatic indices (GSI) and histological examination of testes. In regressed fish, the testis contained mainly primary spermatocytes and the GSI was less than 0.18%, while the mean GSI of recrudescent fish (where secondary spermatocytes predominated in the testis) was 0.43%. The GSI in male goldeye is low, with the GSI of sexually mature fish seldom being higher than 0.5% (unpublished data).

Fish were blood sampled by caudal puncture using heparinised 22G needles, at the time of injection, and at 6, 24, 48 and 72h after injection. Blood was centrifuged at 2000g (4°C, 10 min) and the plasma fraction frozen on dry ice and stored at -30°C. At the conclusion of experiments, fish were anaesthetised and killed by spinal transection. Pituitary glands were immediately removed and frozen on dry ice. The milt volume of spermiated males was determined at the 72h sample, by collecting expressible milt in calibrated glass capillary tubes. Samples of testes were fixed in Bouin. Tissue was dehydrated in an ethanol series, embedded in paraffin and sectioned at 10 μ m, prior to staining with hematoxylin and eosin (H & E).

Levels of GtH in plasma samples and pituitaries were measured by radioimmunoassay (RIA), using an antiserum directed against the β subunit of salmon GtH (details of sub-unit and antibody preparation appear in Breton 1981; Breton *et al.* 1984), and purified carp GtH (cGtH; prepared by B. Breton) for assay standards and tracer. The assay protocol was as described for cGtH (Peter *et al.*, 1984) except that 250 μ l of diluted antiserum was added to each assay tube. The salmon β -subunit antiserum was diluted 1:100,000 which gave 25–30% binding of added ¹²⁵I-cGtH in the absence of competitor. Minimum assay sensitivity was 0.16–0.32 ng.ml⁻¹ plasma. Interassay variability was determined by repeat measurement of GtH in a pooled plasma sample and was less than 3% (n = 3). Serial dilutions of goldeye plasma and pituitary extract (lyophilised pituitary homogenised in barbital buffer and diluted with assay buffer)

Fig. 1. Radioimmunoassay (logit-log) dose response curves for carp gonadotropin (cGtH, closed circles), and serial dilutions of goldeye plasma (open circles) and goldeye pituitary extract (open triangles). All points are the means of duplicate determinations.

produced inhibition curves that were parallel to that of the cGtH standards (t-test, $P > 0.05$; Fig. 1). The possibility that low molecular weight GtH fragments contributed to the measurement of plasma GtH levels was examined by chromatography of plasma samples prior to assay. Plasma samples obtained 6 and 72h after an injection of LHRH-A were chromatographed on a 40×1 cm column of G-50 (fine) Sephadex (Pharmacia) which had been conditioned with 1 ml of 5% BSA in barbital buffer. One millilitre fractions were collected, and assayed for immunoreactive GtH. There was only one immunoreactive peak (fraction 19) in both samples, with 80% of the immunoreactive material being eluted in fractions 17–22.

T and $17\alpha,20\beta$ P were measured in plasma samples as previously described (Pankhurst *et al.* 1985). Interassay variability assessed using a pooled sample of goldeye plasma was less than 19 and 11% for T and $17\alpha,20\beta$ P respectively ($n = 4$). Serial dilutions of plasma gave displacement curves parallel to those of hormone standards (t-test, $P > 0.05$, data not shown).

Fig. 2. Changes in plasma GtH, $17\alpha,20\beta$ P and T levels following treatment of spermated goldeye with saline + blank silastic pellet (control), saline + LHRH-A in a silastic pellet (LHRH-A pellet) or an injection of LHRH-A in saline + blank silastic pellet (LHRH-A injection). All LHRH-A doses $100 \mu\text{g}\cdot\text{kg}^{-1}$. Significantly different from control (closed diamond), significantly different from LHRH-A pellet (open diamond) $P < 0.05$, $n = 4$.

Differences in hormone levels between treatments were assessed using one-way ANOVA of log transformed data, and a student-Newman-Keuls test (Underwood 1981).

Results

The effects of LHRH-A on plasma GtH, $17\alpha,20\beta$ P and T levels in spermating fish are shown in Fig. 2.

Fig. 3. Changes in plasma GtH, $17\alpha,20\beta\text{P}$ and T levels following treatment of sexually regressed male goldeye with LHRH-A. Legend as for Fig. 2, $n = 5$.

Both routes of LHRH-A administration increased plasma GtH levels, however, an injection of LHRH-A resulted in a greater and more prolonged increase than a silastic implant containing LHRH-A. In contrast, both treatments had a similar effect on plasma $17\alpha,20\beta\text{P}$ levels. $17\alpha,20\beta\text{P}$ was significantly elevated by 6h and remained high for at least 72h. LHRH-A administration caused a transitory increase in plasma T levels which was greater in fish treated with an LHRH-A pellet than an injection of LHRH-A. Expressible milt volumes were higher in fish given an injection of LHRH-A than those given pelleted LHRH-A or controls (Table 1).

Table 1. Effect of LHRH-A on milt volumes in spermated goldeye

Treatment	N	Milt volume ($\mu\text{l.g}^{-1}$) ^b
Saline + blank pellet	4	88 ± 15
Saline + LHRH-A pellet ^a	3	188 ± 69
LHRH-A ^a in saline + blank pellet	3	$268 \pm 10^*$

Note: Values are mean $\pm$ SE

^a dose = $100 \mu\text{g.kg}^{-1}$ body weight

^b testes wet weight

* Significantly different from saline + blank pellet group ($P < 0.05$).

Regressed fish displayed a similar prolonged elevation of plasma GtH following LHRH-A injection, and pelleted LHRH-A again produced an increase of lesser magnitude than LHRH-A injection (Fig. 3). Treatment with LHRH-A significantly elevated plasma $17\alpha,20\beta\text{P}$ at 24h and 48h post-injection, while at 72h, only fish receiving an injection of LHRH-A had $17\alpha,20\beta\text{P}$ levels that were higher than controls (Fig. 3). At 48h, $17\alpha,20\beta\text{P}$ levels were lower in fish receiving a silastic implant of LHRH-A than in LHRH-A-injected fish. T levels were elevated in both LHRH-A treated groups at 6h post-injection but at all later sample times, were not significantly different from controls.

Recrudescent fish (Fig. 4) also displayed elevated plasma levels of GtH in response to an injection of LHRH-A, however; there was a longer latency of response, with GtH levels not increasing to high levels until 48h post-injection. The change in $17\alpha,20\beta\text{P}$ followed an identical temporal pattern to GtH, while T peaked at 6h after injection then fell to detection limits.

LHRH-A treatment did not have any effect on testes morphology at any stage of sexual development.

Discussion

The results of the present study show that LHRH-A is effective in elevating plasma levels of GtH and

Fig. 4. Changes in plasma GtH, $17\alpha,20\beta\text{P}$ and T levels following treatment of sexually recrudescing male goldfish with an injection of either saline or LHRH-A ($100\text{ }\mu\text{g.kg}^{-1}$). Significantly different from saline (closed diamond) $P < 0.05$, $n = 5$.

gonadal steroids in male goldfish. The response was found to vary depending on the route of administration of LHRH-A and the stage of the reproductive cycle of animals used in the experiments. An injection of LHRH-A was more ef-

fective in increasing plasma GtH levels than an implant of a silastic pellet containing LHRH-A. Sexually mature fish were more responsive to LHRH-A than regressed or recrudescing fish.

A single injection of LHRH-A elevated plasma levels of GtH for at least 72h in male goldfish. In female coho salmon held at 10°C , a single injection of LHRH-A ($200\text{ }\mu\text{g.kg}^{-1}$) elevated GtH levels for at least 96h (Van Der Kraak *et al.* 1983, 1985) while in female goldfish held at 12°C , LHRH-A ($100\text{ }\mu\text{g.kg}^{-1}$) increased GtH for at least 48h (Chang and Peter 1983; Sokolowska *et al.* 1984, 1985a, b). At 20°C in the goldfish, the increase was more rapid and had peaked 6–24h after injection (Sokolowska *et al.* 1985a, b). The temporal differences are probably due to the different temperatures at which the various experiments were conducted (affecting rates of uptake and clearance), and also possible species and sex differences in the dynamics of LHRH-A-receptor interaction in the pituitary. The results in the goldfish support the suggestion that LHRH analogues have a relatively long plasma half-life in teleosts (Van Der Kraak *et al.* 1983; Peter *et al.* 1985).

The lower effectiveness of LHRH-A in silastic pellets than LHRH-A injections in stimulating increases in plasma levels of GtH may reflect a slower rate of uptake of LHRH-A to the circulation from silastic than saline. Recent studies in goldfish demonstrated that uptake of 17β -estradiol (E_2) to the plasma occurred much more rapidly from an intraperitoneal injection of E_2 in saline than from an intraperitoneal implant of E_2 in a silastic pellet (Pankhurst *et al.* 1986). In Atlantic salmon, LHRH-A administered in a silastic pellet implant also caused only a small increase in plasma GtH levels (Weil and Crim 1983). Administration of LHRH analogues in cholesterol pellets produced a larger increase in plasma GtH (Crim *et al.* 1983). A similar effect has been found in the goldfish, where LHRH-A in a silastic pellet was ineffective in elevating serum GtH levels (Dr M. Sokolowska, personal communication) whereas cholesterol pellets containing des-Gly¹⁰(D-Trp⁶) - LHRH ethylamide increased GtH to high levels (Sokolowska *et al.* 1984). Relative to treatment with LHRH-A in saline or cholesterol, administration of

LHRH-A in a silastic pellet implant appears to be an ineffective means of inducing a sustained increase in plasma GtH levels.

Goldeye show seasonal variation in the response to LHRH-A. Spermiated fish displayed a rapid increase in plasma GtH to high levels, whereas regressed fish showed a slightly slower response of lesser magnitude. The response to LHRH-A was lowest in recrudescing fish, with the main phase of increase not occurring until 24h or more after treatment. There was no seasonal variation in pituitary GtH content of untreated fish (author's unpublished data), suggesting that the seasonal differences in responsiveness relate to differences in pituitary sensitivity to GnRH. The reason for the slower response to LHRH-A seen in recrudescing goldeye is not known but it may reflect receptor induction by circulating LHRH-A. Self-potential of stimulation of GtH release by LHRH-A has been demonstrated in the goldfish (Peter 1980; Chang and Peter 1983). In other species, the response to LHRH also varies seasonally. Female goldfish were found to be most responsive to LHRH-A during late recrudescence when a single injection ($100 \mu\text{g.kg}^{-1}$) resulted in serum GtH levels of over 100 ng.ml^{-1} . Regressed stages showed only a slight increase (less than 5 ng.ml^{-1} , Sokolowska *et al.* 1985a). Similarly, both sexes of common carp (Weil *et al.* 1975) and rainbow trout (Weil *et al.* 1978) were more responsive to LHRH during the spawning season, than at other times of the year.

Plasma levels of $17\alpha,20\beta\text{P}$ were elevated in response to increased GtH in male goldeye at all stages of sexual development. This finding is somewhat surprising in that increased $17\alpha,20\beta\text{P}$ production has been associated with the induction of spermiation in other teleosts (Scott and Baynes 1982; Ueda *et al.* 1984a, 1985). In addition, recent work suggests that 20β -hydroxysteroid dehydrogenase ($20\beta\text{HSD}$), the enzyme which mediates the conversion of 17α -hydroxyprogesterone to $17\alpha,20\beta\text{P}$, is located in spermatozoa in rainbow trout (Ueda *et al.* 1984a). If $20\beta\text{HSD}$ is associated with spermatozoa in the goldeye, then it must be present from at least the primary spermatocyte stage. The possibility that testicular tissues other than spermatozoa contain $20\beta\text{HSD}$ also cannot be discounted.

In the rainbow trout, Dépêche and Sire (1982) found that the testis was capable of synthesizing $17\alpha,20\beta\text{P}$ from tritiated precursors at all stages of the sexual cycle, although conversion rates were highest when large amounts of spermatozoa were present in the testis.

The present studies also suggest that factors in addition to $17\alpha,20\beta\text{P}$ are involved in the induction of spermiation. All sexually mature goldeye receiving LHRH-A displayed elevated plasma $17\alpha,20\beta\text{P}$ levels, but only fish receiving an injection of LHRH-A had increased milt volumes relative to controls. As fish receiving an injection of LHRH-A had much higher GtH levels than those receiving an implant of LHRH-A in a silastic pellet, it is possible that GtH has a direct role in spermiation.

Plasma T levels displayed only a transitory increase in response to treatment with LHRH-A. The magnitude of the increase was inversely related to the increase in plasma GtH, with higher T levels occurring in fish treated with LHRH-A in a silastic pellet than fish receiving an injection of LHRH-A. In contrast, $17\alpha,20\beta\text{P}$ levels were of similar magnitude in sexually mature fish treated with LHRH-A by either administration route. Other studies have shown that plasma androgens decrease while progestagens increase in male teleosts at spermiation (Scott and Baynes 1982; Scott *et al.* 1984; Ueda *et al.* 1984b). The manner in which steroid production is regulated by GtH remains unclear. Scott and Baynes (1982) have suggested that GtH inhibits the conversion of 17α -hydroxyprogesterone to androstenedione either directly, or indirectly via stimulation of conversion of 17α -hydroxyprogesterone to $17\alpha,20\beta\text{P}$. In addition, stimulation of 11β -hydroxysteroid dehydrogenase by GtH may increase the rate at which 11-keto testosterone (11KT) is synthesized from T, and thus prevent accumulation of T in the plasma. Increases in plasma 11KT concomitant with decreasing T levels have been found in male rainbow trout (Scott *et al.* 1980) and Atlantic salmon (Hunt *et al.* 1982), prior to spawning.

In summary, GtH secretion in the goldeye was shown to be stimulated by LHRH-A at all stages of the sexual cycle. The steroidogenic response of the goldeye to elevated plasma GtH was similar to that

described for other male teleosts, with the exception of the ability of the testes to synthesize $17\alpha,20\beta\text{P}$ at all sexual stags. Further study is required to determine whether other species are capable of synthesizing $17\alpha,20\beta\text{P}$ at relatively early stages of sexual maturity.

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References

- Billard, R., Alagarswami, K., Peter, R.E. and Breton, B. 1983. Potentialisation par le pimoïde des effets du LHRH-A sur la sécrétion gonadotrope hypophysaire, l'ovulation et la spermiation chez la carpe commune (*Cyprinus carpio*). C.R. Acad. Sci. Paris Ser III 296: 181–184.
- Billard, R., Bieniarz, K., Peter, R.E., Sokolowska, M., Weil, C. and Crim, L.W. 1984. Effects of LHRH and LHRH-A on plasma GtH levels and maturation/ovulation in the common carp, *Cyprinus carpio*, kept under various environmental conditions. Aquaculture 41: 245–254.
- Billard, R., Reinaud P., Hollebecq, M.G. and Breton, B. 1985. Advancement and synchronisation of spawning in *Salmo gairdneri* and *S. trutta* following administration of LHRH-A combined or not with pimoïde. Aquaculture 43: 341–350.
- Breton, B. 1981. A study of salmon (*Oncorhynchus tshawytscha*) pituitary gonadotropin dissociation. Gen. Comp. Endocrinol. 45: 147–152.
- Breton, B., Jalabert, B., Bieniarz, K., Sokolowska, M. and Epler, P. 1983. Effects of synthetic LH-RH and analog on plasma gonadotropin levels and maturational response to 17α -hydroxy- 20β -dihydro-progesterone. Aquaculture 32: 105–114.
- Breton, B., Zohar, Y. and Sambroni, E. 1984. Dosages radio-immunologiques hétérologues de gonadotropines de type glycoprotéiques maturantes de poissons téléostéens. Aquaculture 40: 307–323.
- Burnstock, G. 1958. Saline for fresh-water fish. J. Physiol. 141: 45–45.
- Chang, J.P. and Peter, R.E. 1983. Effects of pimoïde and Des Gly¹⁰, (D-Ala⁶) luteinizing hormone releasing hormone ethylamide on serum gonadotropin concentrations, germinal vesicle migration and ovulation in female goldfish, *Carassius auratus*. Gen. Comp. Endocrinol. 52: 30–47.
- Crim, L.W., Evans, D.M., Coy, D.H. and Schally, A.V. 1981. Control of gonadotrophic hormone release in trout: Influence of synthetic LH-RH and LH-RH analogues *in vivo* and *in vitro*. Life Sci. 28: 129–135.
- Crim, L.W., Evans, D.M. and Vickery, B.H. 1983. Manipulation of the seasonal reproductive cycle of the landlocked salmon (*Salmo salar*) with LHRH administration at various stages of gonadal development. Can. J. Fish. Aquat. Sci. 40: 61–67.
- Dépêche, J. and Sire, O. 1982. *In vitro* metabolism of progesterone and 17α -hydroxyprogesterone in the testis of the rainbow trout, *Salmo gairdneri* Rich., at different stages of spermatogenesis. Reprod. Nutr. Dévelop. 22: 427–438.
- Hirose, K., Nagahama, Y., Adachi, S. and Wakabayashi, K. 1983. Changes in serum concentrations of gonadotropin, 17α -hydroxy-progesterone and 17α - 20β -dihydroxy-4-pregnen-3-one during synthetic LH-RH-induced final oocyte maturation and ovulation in the ayu, *Plecoglossus altivelis*. Bull. Jpn. Soc. Sci. Fish. 49: 1165–1169.
- Hunt, S.M.V., Simpson, T.H. and Wright, R.S. 1982. Seasonal changes in the levels of 11-oxotestosterone and testosterone in the serum of salmon, *Salmo salar* L., and their relationship to growth and maturation cycle. J. Fish Biol. 20: 105–119.
- Nelson, J.J. 1984. Fishes of the World, 2nd edition. John Wiley and Sons, New York.
- Pankhurst, N.W., Van Der Kraak, G. and Peter, R.E. 1985. Effects of human chorionic gonadotropin, Des-Gly¹⁰ (D-Ala⁶) LHRH-ethylamide and pimoïde on final oocyte maturation, ovulation and levels of plasma sex steroids in the walleye (*Stizostedion vitreum*). Fish Physiol. Biochem. 1: 45–54.
- Pankhurst, N.W., Stacey, N.E. and Peter, R.E. 1986. An evaluation of techniques for the administration of 17β -estradiol to teleosts. Aquaculture (In press).
- Peter, R.E. 1980. Serum gonadotropin levels in mature male goldfish in response to luteinizing hormone releasing hormone (LH-RH) and des-Gly¹⁰-(D-Ala⁶) LH-RH ethylamide. Can. J. Zool. 58: 1100–1104.
- Peter, R.E., Nahorniak, C.S., Chang, J.P. and Crim, L.W. 1984. Gonadotropin release from the pars distalis of goldfish, *Carassius auratus*, transplanted beside the brain or into the brain ventricles: additional evidence for gonadotropin-release-inhibitory factor. Gen. Comp. Endocrinol. 55: 337–346.
- Peter, R.E., Nahorniak, C.S., Sokolowska, M., Chang, J.P., Rivier, J.E., Vale, W.W., King, J.A. and Millar, R.P. 1985. Structure-activity relationships of mammalian, chicken and salmon gonadotropin releasing hormones *in vivo* in goldfish. Gen. Comp. Endocrinol. (In press).
- Scott, A.P. and Baynes, S.M. 1982. Plasma levels of sex steroids in relation to ovulation and spermiation in rainbow trout (*Salmo gairdneri*). In: Reproductive Physiology of Fish pp. 103–106. Compiled by C.J.J. Richter and H.J.Th. Goos. Pudoc, Wageningen.
- Scott, A.P., Bye, V.H., Baynes, S.M. and Springate, J.R.C.

1980. Seasonal variations in plasma concentrations of 11-ketotestosterone and testosterone in male rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 17: 494–505.
- Scott, A.P., Mackenzie, D.S. and Stacey, N.E. 1984. Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*. II. Steroid hormones. Gen. Comp. Endocrinol. 56: 349–359.
- Sokolowska, M., Peter, R.E., and Nahorniak, C.S., Pan, C.H., Chang, J.P., Crim, L.W. and Weil, C. 1984. Induction of ovulation in goldfish, *Carassius auratus*, by pimozone and analogues of LH-RH. Aquaculture 36: 71–83.
- Sokolowska, M., Peter, R.E. and Nahorniak, C.S. 1985a. The effects of different doses of pimozone and (D-Ala⁶, Pro⁹-N ethylamide)-LHRH (LHRH-A) on gonadotropin release and ovulation in female goldfish. Can. J. Zool. 63: 824–833.
- Sokolowska, M., Peter, R.E., Nahorniak, C.S. and Chang, J.P. 1985b. Seasonal effects of pimozone and des Gly¹⁰ (D-Ala⁶) LHRH ethylamide on gonadotropin secretion in goldfish. Gen. Comp. Endocrinol. (In press).
- Stacey, N.E., Mackenzie, D.S., Marchant, T.A., Kyle, A.L. and Peter, R.E. 1984. Endocrine changes during natural spawning in the white sucker *Catostomus commersoni* I. Gonadotropin, growth hormone, and thyroid hormones. Gen. Comp. Endocrinol. 56: 333–348.
- Ueda, H., Kambegawa, A. and Nagahama, Y. 1984a. *In vitro* 11-ketotestosterone and 17 α ,20 β -dihydroxy-4-pregnen-3-one production by testicular fragments and isolated sperm of rainbow trout *Salmo gairdneri*. J. Exp. Zool. 231: 435–439.
- Ueda, H., Hiroi, O., Hara, A., Yamauchi, K. and Nagahama, Y. 1984b. Changes in serum concentrations of steroid hormones, thyroxine and vitellogenin during spawning migration of the chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 53: 203–211.
- Ueda, H., Kambegawa, A. and Nagahama, Y. 1985. Involvement of gonadotropin and steroid hormones in spermiation in the amago salmon *Oncorhynchus rhodurus* and the goldfish *Carassius auratus*. Gen. Comp. Endocrinol. (In press).
- Underwood, A.J. 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. Ann. Rev. 19: 513–605.
- Van Der Kraak, G., Lin, H.-R., Donaldson, E.M., Dye, H.M. and Hunter, G.A. 1983. Effects of LH-RH and des-Gly¹⁰ (D-Ala⁶) LH-RH-ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 49: 470–476.
- Van Der Kraak, G., Dye, H., Donaldson, E.M. and Hunter, G.A. 1985. Plasma gonadotropin, 17 β -estradiol and 17 α ,20 β dihydroxy-4-pregnen-3-one levels during LH-RH analogue and gonadotropin induced ovulation in coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. 63: 824–833.
- Weil, C. and Crim, L.W. 1983. Administration of LHRH analogues in various ways: Effect on the advancement of spermiation in prespawning landlocked salmon, *Salmo salar*. Aquaculture 35: 103–115.
- Weil, C., Billard, R., Breton, B. and Jalabert, B. 1978. Pituitary response to LH-RH at different stages of gametogenesis in the rainbow trout (*Salmo gairdneri*). Ann. Biol. Anim. Biochim. Biophys. 18: 863–869.
- Weil, C., Breton, B. and Reinaud, P. 1975. Etude de la réponse hypophysaire à l'administration de Gn-RH exogène au cours du cycle reproducteur annuel chez la carpe *Cyprinus carpio* L. C.R. Hebd. Seances Acad. Sci. Ser. D 280: 2469–2472.
- Weil, C., Fostier, A., Horvath, L., Marlot, S. and Berscenyi, M. 1980. Profiles of plasma gonadotropin and 17 β -estradiol in the common carp *Cyprinus carpio* L. as related to spawning induced by hypophysation of LH-RH treatment. Reprod. Nutr. Develop. 20: 1041–1050.