

Egg production of tench (*Tinca tinca* L.) kept in semi-intensive culture conditions

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ABSTRACT

Key-words:
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With the aim to evaluate the effect of different intervals of degrees-days on egg production of tench (*Tinca tinca* L.), 480 breeders were kept in 15 x 10 x 1 m bare concrete tanks from February until July and fed on commercial feed. Males and females were held together under natural photoperiod and temperature conditions. Ovulation was hormonally induced (LH-RHa) in three groups at different degrees-days (group I: 1090, group II: 1175 and group III: 1536). In the groups I and II, a mean of 88% of females was injected, obtaining a 98% of positive response, without significant differences in the egg production between both groups (10.7% body weight, b.w.). With 1536 degrees-days, percentages of injected and stripped females were significantly lower. First stripping yielded a total of 9332 g of eggs (8.3% b.w.). A second hormonal induction was evaluated in each group at 1900, 2047 and 2111 degrees-days respectively. A mean of 85% of the initial females of the three groups received a second hormonal treatment, obtaining a 92% of positive response, without significant differences in egg production among the groups. The second stripping yielded an egg production (598 g, 5.9% b.w.) significantly lower than the first one. Relating to males, semen was obtained without hormonal induction throughout 50 days and the positive response was around 90%. With the exception of eggs obtained in the first induction of group III (1536 degrees-days), where overripening process was detected, further mean fertilization (over 80%) and hatching rates (around 35%) support the good quality of eggs and sperm. This study showed that the advantages of the broodstock's maintenance in small concrete tanks are not only easiness of handling and possibilities of yield prediction, but also a good egg production at higher densities than in big earthen ponds.

RÉSUMÉ

Production d'œufs de tanche (*Tinca tinca* L.) en élevage semi-intensif

Mots-clés :
tanche, gestion
du stockage
des géniteurs,
reproduction
artificielle

Afin d'évaluer les effets d'intervalles de degrés-jours, sur la production d'œufs de tanche, 480 géniteurs ont été confinés dans des bassins de béton de février jusqu'à juillet et nourris avec des aliments composés pour truite. Les mâles et les femelles ont été maintenus ensemble sous des conditions de photopériode et de température naturelles. L'ovulation a été induite par des injections de LH-RHa à différents degrés-jours (groupe I : 1090, groupe II : 1175 et groupe III : 1536).

Dans les groupes I et II, une moyenne de 88 % de femelles a été injectée, avec 98 % de réponse positive, sans différence significative dans la production d'œufs entre les deux groupes (10,7 % de la masse corporelle). Dans le groupe à 1536 degrés-jours, les pourcentages de femelles injectées et strippées et la production d'œufs par femelle ont été plus faibles que dans les autres groupes. Le premier prélèvement d'œufs a produit un total de 9332 g d'œufs (8,3 % de la masse corporelle). Dans les trois groupes, une deuxième induction hormonale a été respectivement effectuée à 1900, 2047 et 2111 degrés-jours. En moyenne, 85 % des femelles des trois groupes ont reçu un deuxième traitement hormonal, induisant 92 % de réponse positive, sans conséquence sur la production d'œufs entre les groupes. La production dans le second prélèvement (5499 g d'œufs, 5,9 % de la masse corporelle) a été significativement plus petite que lors du premier. En ce qui concerne les mâles, du sperme a été obtenu sans induction hormonale pendant 50 jours et la réponse positive était d'environ 90 %. Excepté des œufs obtenus lors de la première induction du groupe III, les taux de fécondation moyen (plus de 80 %) et le taux d'éclosion (proche de 35 %) soutiennent la bonne qualité des œufs et du sperme. Notre étude confirme les avantages d'un stockage des géniteurs dans de petits étangs en béton (simplification du travail, prédiction possible des performances), mais montre aussi que ce type de structure permet des productions à plus haute densité que des grands étangs en pleine terre.

INTRODUCTION

Tench (*Tinca tinca* L.) is a freshwater fish endemic in Central and South Europe traditionally cultured in extensive or semi-extensive systems. Reproduction takes place under natural conditions in large earthen ponds with abundant vegetation, where females spawn various batches during the reproductive season (Morawska, 1984). There is an increasing interest in controlling the reproductive cycle for tench monoculture intensification. Artificial reproduction techniques are available, and synthetic analogues of gonadotropin releasing factors (GnRH) are employed for ovulation induction (Kouřil *et al.*, 1986; Fernández San Juan, 1995; Linhart *et al.*, 1995; Kouřil and Hamáčková, 1996; Barth *et al.*, 1997; Kouřil, 1998). Gonadal development and maturation take place in large earthen ponds or in the wild, where broodstock must be caught (Gela *et al.*, 2006) being a major limitation for control and management of tench reproduction.

Carral *et al.* (2003) proved that tench maintained in small concrete tanks and fed on artificial diets were able to reach maturity, but then only one stripping was performed and the effect of degrees-days accumulated was not evaluated. According to Breton *et al.* (1980b) and Horoszewicz (1983) under fluctuating temperature conditions tench breeders need around 1070 degrees-days to complete the prespawning period. Since artificial reproduction techniques are effective only when gonads of breeders have reached mature stage, a better knowledge of the effect of different time periods of gonadal development on egg production could provide useful information to improve the use of facilities on tench farms. Considering the different development degree of oocytes along the reproductive season (Epler *et al.*, 1981; Horoszewicz, 1981, 1983; Morawska, 1981, 1984; Pimpicka and Koryzno, 1995), successive induction treatments can be applied to obtain different batches of eggs and thus increase the amount of obtained eggs as well as spread the reproductive season in fish farms.

The aim of the present study was to determine the reproductive response of tench held in bare concrete ponds to two consecutive hormonal inductions after different periods of time, measured in degrees-days, in terms of egg production and fertilization and hatching rates.

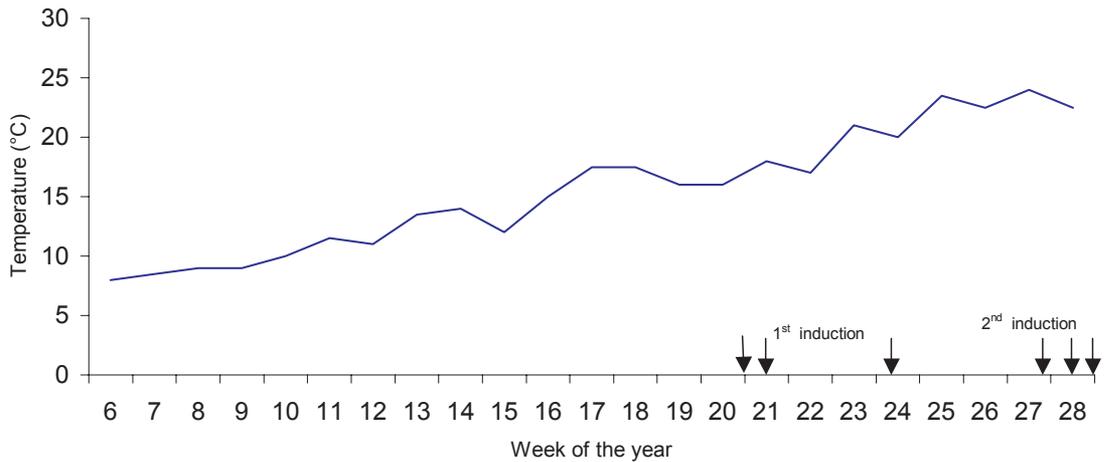


Figure 1
Variations of the weekly mean temperature during the experiment.

Figure 1

Variations de la température moyenne hebdomadaire de l'eau pendant la période expérimentale.

MATERIALS AND METHODS

The experiment was performed from February until July of 2002 in a tench farm located in the province of Zamora (Spain). On 4th February, 480 adult tench (237 females and 243 males) were stocked into three 15 x 10 x 1 m bare concrete tanks at an initial density of 0.3 kg.m⁻². Males and females were held together (sex ratio around 1:1) under natural photoperiod and temperature conditions.

Temperature was measured twice a day, in the morning and in the evening using maximum-minimum thermometers. Figure 1 shows the thermal regime during the experiment on weekly mean values (mean of 14 values, 2 per day). Water flow throughout was supplied at a rate of 0.17 L.s⁻¹. Oxygen concentration was daily measured with a portable Hach sensION6 oximeter (Hach Company) in two points of each pond (close to water inlet and close to water outlet) and values were always higher than 5 ppm. Commercial feed formulated for trout (Trouvit Europa 22[®], granule diameter: 2 mm; composition: crude protein 42%, crude fatty material 22%, crude ashes 9.5%, crude cellulose 1.6%, Vitamins: A 10 000 UI.kg⁻¹, D₃ 1500 UI.kg⁻¹, E 150 mg.kg⁻¹) was daily supplied, increasing from 0.25 to 4% of the initial body weight according to water temperature and food consumption (estimated by visualization of uneaten food at the bottom of ponds). Natural feed (mainly *Daphnia* spp.) was also available.

Taking into account that both spermatogenesis (Breton *et al.*, 1980a) and vitellogenesis (Breton *et al.*, 1980b; Epler *et al.*, 1981) begin when water temperature is higher than 10 °C, degrees-days count started when mean daily temperature reached values above 10 °C. In order to assess the effects of different periods of time on egg production response three intervals of degrees-days were established for the first hormonal induction (Table I).

Samples of fish (8 females and 8 males/pond) were weekly taken from the ponds starting after 950 degrees-days in group I, 1150 in group II and 1350 in group III in order to estimate the moment of the first induction. Production of semen was indicative of maturity in males, whereas external morphological characteristics (belly development and weakness) were indicative in females. As semen was easily obtained, males did not receive hormonal induction. Once established the date of induction in each interval of degrees-days, females with external signs of maturity were individually weighed and injected intramuscularly in the

Table I.*Broodstocks characteristics and intervals (degrees-days) selected for the first induction.*

Tableau I

Caractéristiques des lots de géniteurs et intervalles (degrés-jours) choisis pour la première induction.

Group	Females		Males		Degrees-days interval
	Number (Initial W ^(*) kg)	Mean W ^(*) (± MSE)	Number (Initial W ^(*) kg)	Mean W ^(*) (± MSE)	
I	57 (40.79)	0.716 ± 0.038	58 (17.85)	0.307 ± 0.013	950–1150
II	88 (49.34)	0.603 ± 0.031	90 (19.88)	0.219 ± 0.009	1150–1350
III	92 (32.98)	0.354 ± 0.018	95 (30.60)	0.321 ± 0.015	1350–1550

(*) Weight at the first hormonal induction.

dorsal area with a single dose (20 µg.kg⁻¹) of Gly¹⁰, [D-Ala⁶] LH-RH-Ethylamide (Sigma-Aldrich Química S.A., Madrid, Spain) after being anaesthetized with MS-222 (Ortoquímica S.L., Barcelona, Spain). Stripping was carried out after 36–39 h at 22 °C. Egg and semen production were considered as positive response. A second induction (Figure 1) was also evaluated. Between injections, animals were kept in the concrete tanks and fed the same commercial food. The same procedure described for the first injection was applied for the second.

The following parameters were determined: degrees-days until hormonal induction, rate of injected females related to the initial number, positive response (percentage of stripped females related to the injected ones), absolute egg weight (weight of stripped eggs per female) and relative egg weight (egg weight/female weight in percentage).

A sample randomly (210 g) of mixed eggs from 10 females of each group was fertilized with more than 1.5 mL of sperm from 4–7 males directly stripped. Water was used as fertilization solution and stickiness was eliminated with alcalase, according to Rodríguez *et al.* (2004) and Carral *et al.* (2006). Thereafter, eggs were distributed in three inverted Zug bottles (80 g/bottle) supplied with a flow of 2.5 L.min⁻¹ and incubated at 22 ± 1 °C. The fertilization rate was calculated as the percentage of eggs with 4 to 16 blastomeres in a sample (150 ± 10 eggs) taken from each bottle 1.5–2 h after fertilization. The hatched larvae were kept in hatchery trays. Numbers of larvae in each tray were estimated volumetrically after hatching. Hatching rates were calculated as the percentage of living larvae from the initial number of fertilized eggs.

Results were examined by analysis of variance (ANOVA) using STATISTICA 5.0 (StatSoft, Tulsa, OK, USA) software. Arc-sine transformation of percentages was made. Comparison of mean values was performed using the Newman-Keuls test. Significance level was $P < 0.05$.

RESULTS

The experiment lasted 161 days. Table II includes degrees-days until hormonal injection, rate of injected and stripped females, and egg production after both first and second hormonal inductions. Mortality during the experiment was around 2%.

The first hormonal induction was performed on 92.4% and 84.1% of females belonging to the groups I (1090 degrees-days) and II (1175 degrees-days), respectively. Positive response of females was high in both groups (95.9% and 100%), and egg production corresponded to was 10.3% and 11.0% of b.w., respectively, without significant differences. When induction was practiced after 1536 degrees-days (group III), rates of injected, stripped females, and

Table II. Degrees-days until LH-RHa injection, rates of injected and stripped females and egg production (absolute and relative egg weight) after the first and the second hormonal inductions, fertilization and hatching rates. Values given are mean \pm SME above and minimum and maximum below.

Tableau II

Degrés-jours jusqu'à l'injection de LH-RHa, taux de femelles injectées et strippées et production d'œufs (poids relatif et absolu) après la première et la seconde inductions hormonales, taux de fécondation et d'éclosion. Au dessus, moyenne \pm ESM et au dessous, minimum et maximum.

Induction	Group	Degrees-days	Injected/Initial females (%)	Positive response ^(*)	W of eggs (g) Mean (min - max)	Relative egg W (%)	Fertilization (%)	Hatching (%)
First	I	1090	92.4	95.9	69.5 \pm 6.9 ^a 23.4 - 329.3	10.3 \pm 0.4 ^a 5.1 - 19.0	95.2 \pm 0.6 ^a	38.9 \pm 1.0 ^a
	II	1175	84.1	100	74.7 \pm 4.62 12.6 - 206.6	11.0 \pm 0.4 ^a 2.7 - 16.4	95.4 \pm 0.8 ^a	41.6 \pm 0.7 ^a
	III	1536	67.4	74.2	18.2 \pm 2.4 ^b 0.5 - 72.8	3.56 \pm 0.3 ^b 0.5 - 9.4	47.2 \pm 2.6 ^b	18.7 \pm 0.8 ^b
Second	I	1900	94.1	93.6	43.3 \pm 5.2 ^c 4.1 - 204.6	6.2 \pm 0.4 ^c 0.7 - 12.9	81.8 \pm 5.2 ^c	31.1 \pm 0.8 ^c
	II	2047	93.0	91.0	38.5 \pm 3.1 ^c 0.76 - 149.3	5.9 \pm 0.3 ^c 0.2 - 13.4	83.0 \pm 1.7 ^c	30.4 \pm 0.6 ^c
	III	2111	68.7	90.9	31.1 \pm 3.5 ^c 3.4 - 88.2	5.5 \pm 0.5 ^c 1.9 - 16.2	85.1 \pm 3.9 ^c	29.5 \pm 0.5 ^c

(*) Positive response: % of stripped/injected females.

Values followed by the same superscript are not significantly different ($P < 0.05$ in a given column for first and second hormonal induction respectively).

egg production were significantly lower. Concerning the males, semen was easily obtained and the positive response was around 90%.

When the second induction was performed, females had accumulated 1900 (group I), 2047 (group II) and 2111 (group III) degrees-days. The rate of injected females was lower in the group III. Positive response was over 90% in the three groups without significant difference in egg production. Positive male response was 90%.

As Table II shows, there were not significant differences neither in the fertilization (around 95%) nor in the hatching rates of eggs obtained after first hormonal induction of groups I and II which were significantly higher than those obtained in the group III and those corresponding to the second induction. With respect to the second hormonal induction, there were not significant differences in egg fertilization and hatching rates between the three groups (Table II).

DISCUSSION

In aquaculture, it is generally accepted that artificial conditions induce stress that can generate strongly harmful effects on the reproductive processes. This is increased in the tench, being an elusive, not domesticated species, extremely sensible to the stress caused by handling (Billard and Flajshans, 1995; Pérez-Regadera and Velasco, 1995). We have recorded repeatedly the failure of the ovarian development under intensive confinement in plastic basins (Rodríguez, 2003). In the present study, a good development was reached in small and bare concrete ponds, and the degrees-days needed to reach the maturity (1090) have been similar to those recorded in large earthen ponds, 1063 by Horoszewicz *et al.* (1981) and 1077 degrees-days by Breton *et al.* (1980b). This fact provides evidence that the artificial confinement conditions of our experiment did not prevent the animals from entering the maturation process and the high egg production obtained may be a good indicator of reproductive success. In this way and with the exception of the first hormonal induction in group III, mean fertilization rates were over 80% and, after the artificial incubation, the estimated mean hatching rates (around 35%) were higher than the 30% suggested as being good by Kokurewicz (1981) and in the range of those obtained by Linhart and Kvasnicka (1992), Aguilera *et al.* (2005), Rodríguez *et al.* (2004) and Carral *et al.* (2006), with small quantities of eggs in the laboratory. This supports that the quality of the eggs and sperm obtained in the present experiment may be considered normal.

Positive response to hormonal induction takes only place in fully ripe females which were in the groups with a temperature accumulation of 1090 and 1175 degrees-days. However, a pre-spawning period of 1536 degrees-days, resulted in a lower positive response and egg production, probably due to the eggs have reached the final ripening process before the hormonal injection. Once the ovulation process starts the eggs must either be spawned or stripped (Woynarovich and Horváth, 1980). So spontaneous spawning due to a high amount of accumulated degrees-days and helped by male presence (Horváth *et al.*, 1992) could explain the poor egg production of group III. In addition, fertilization and hatching rates were significantly lower in this group suggesting also that eggs obtained are over ripped and thus with scarce possibilities to be fertilized.

Under the artificial conditions of our trial, rate of stripped females has been higher than the maximum of 80% (Barth *et al.*, 1997) and the average of 87% (Kouřil, 1998) recorded in females maintained in extensive ponds. In previous works (Carral *et al.*, 2003), we obtained a positive response between 64.7% and 90.9% with females held in concrete tanks at a higher density (2 kg.m⁻²). Concerning egg production, a mean of 8.3% b.w. was reached in the first stripping, with a maximum of 11% b.w. yielded by the group injected at 1175 degrees-days. Both values are higher than those reported up to date (around 6% b.w.).

Moreover, the present study provides the first data on the feasibility of the use of the same breeders for a second stripping. Although the egg production was lower, it is not negligible since it was similar to the mean values of 6.75% and 5.61% reported for a single induction

by Kouřil (1998) and Carral *et al.* (2003), respectively. The good fertilization (around 83%) and hatching rates (around 30%) obtained in the three groups also support the interest of a second induction under culture conditions.

In big ponds, Vachta *et al.* (1992) assert that hormonal induction efficiency in females depends on the density of animals, since they registered greater response (93%) and egg production (5.39% b.w.) with 121 kg.ha⁻¹ than with 417 kg.ha⁻¹ (densities of 0.012 kg.m⁻² and 0.042 kg.m⁻², respectively). In the present experiment, with a density 33 times greater than 0.012 kg.m⁻², a higher production was obtained from females after the first induction and even after the second one.

Regarding the application possibilities of these results in tench farms, the advantages of the broodstock maintenance in small concrete ponds are not only easiness of handling, but also the good results that can be obtained with greater densities than in big earthen ponds. In addition, data obtained can be useful to estimate the number of breeders needed for a predetermined egg production. As artificial reproduction techniques are effective only when ovarian eggs reach the ripe stage, successful hormonal induction and further female's stripping can be performed with guarantee after 1090 degrees-day and henceforth up to at least 1200 degrees-days. A period around 1500 degrees-days seems to be too long leading to both spontaneous spawning and egg overripening. The same females can be stripped two times with an interval around 850 degrees-days. The period of egg production applying two hormonal inductions can be extended up to at least 50 days. In the first stripping, egg production can reach 11% b.w. In the second, egg production can reach more than 50% of the first one. During the same period, sperm can be obtained without hormonal induction.

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