Aquaculture 376-379 (2013) 90–96

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of the thermal threshold and the timing of temperature reduction on the initiation and course of oocyte development in cultured female of Eurasian perch *Perca fluviatilis*

A. Abdulfatah [a], P. Fontaine [a,*], P. Kestemont [b], S. Milla [a], M. Marie [c,d]

[a] Université de Lorraine, Unité de Recherche Animal et Fonctionnalités des Produits Animaux, USC INRA 340, Vandoeuvre-lès-Nancy, F-54505, France
[b] Unité de Recherches en Biologie des Organismes, Facultés Universitaires Notre-Dame de la Paix, rue de Bruxelles 61, B-5000 Namur, Belgium
[c] Université de Lorraine, ENSAIA, B.P.172, F-54505 Vandœuvre-lès-Nancy, France
[d] INRA ASTER-Mirecourt, 88500 Mirecourt, France

ARTICLE INFO

Article history:
Received 1 June 2012
Received in revised form 28 September 2012
Accepted 10 November 2012
Available online 16 November 2012

Keywords:
Eurasian perch
Thermal threshold
Thermal timing
Reproductive cycle
Oogenesis

ABSTRACT

This study was designed to determine the influence of the thermal threshold and timing of temperature reduction on the initiation of the gonadogenesis under controlled conditions in the females of Eurasian perch. A set of 768 fish was distributed in 16 tanks (48 fish per tank). The photoperiod kinetics was identical in all the treatments (duration = 16 weeks, amplitude of photoperiod decrease = 8 h). The effects of 4 thermal thresholds (22, 18, 14 and 6 °C) in combination with two timing of temperature reduction (0 and 4 weeks between the photoperiodic and thermal reduction, respectively sudden — S or delayed — D), were investigated on the gonadosomatic index (GSI), oocyte diameter (OD), oocyte developmental stage or plasma oestradiol level (E2) during a 140 day period. The results show that all females underwent gonadogenesis whatever the thermal threshold and timing tested. Consequently the photoperiod changes alone allow the induction of reproductive cycle onset even under a high and constant temperature. However, at day 140, the GSI and OD were lower under 22 °C temperature in comparison with other thermal thresholds (GSI = 4%–4.3%) (OD = 577 μm–635 μm) and the oocytes did not exceed stage 3 (late cortical stage). A thermal threshold of 18 °C ensured a good gonadal development (GSI = 7.8%–8% and OD = 673 μm–689 μm) but the oocytes did not exceed stage 4 (early vitellogenesis). The highest GSI were obtained under the temperature of 14 °C whatever the timing of thermal reduction applied (GSI = 8.9%–9.4%; OD = 713 μm–753 μm) and 6 °C with temperature delay (GSI = 8.2%, OD = 727 μm). All the oocytes reached stage 5 (late vitellogenesis) in these treatments. From 22 °C to 14 °C, the thermal timing did not influence gonadogenesis contrary to what was observed at 6 °C, GSI and OD were 6.7% and 637 μm without temperature delay while the GSI and OD were 8.2% and 727 μm with delay. These results show that the temperature only plays a modulator role on the initiation of the gonadal development and suggest that the optimal temperature decrease is in the range of 14–6 °C and that the photoperiod is the signal factor that triggers the initiation of oogenesis.

1. Introduction

In the last few years, the European consumption of Eurasian perch *Perca fluviatilis* has raised in particular through the development of intensive methods using indoor water-recirculating systems (Fontaine et al., 2008). These methods need to control the environmental factors (photoperiod and temperature) for inducing the spawning, out of the natural reproductive season which occurs in mid spring (April).

In fish species with marked seasonality of breeding activity such as the Eurasian perch, the reproductive cycle is controlled and synchronized by seasonal environmental changes in relation to local climatic and feed availability conditions (Taranger et al., 2010). The photoperiod is considered as the main factor that synchronizes the reproductive cycle in some temperate species such as salmonids (Bromage et al., 2001; Migaud et al., 2010). In salmonids, the onset of gametogenesis (initiation) does not need the respect of specific thermal conditions such as in Atlantic salmon *Salmo salar* (Pankhurst and Porter, 2003; Taranger et al., 1999). By contrast, the temperature plays a very important role in the regulation of gametogenesis (achievement, timing) in many spring spawning species like in sea bass *Dicentrarchus labrax* (Prat et al., 1999; Zanuy et al., 1986), striped bass *Morone saxatilis* (Clark et al., 2005), gilthead seabream *Sparus aurata* (Kissil et al., 2001) and Senegalese sole *Solea senegalensis* (Oliveira et al., 2009). The temperature is considered as a key-factor in the achievement of gametogenesis in cyprinids (Peter and Yu, 1997). It may also be important in other fish families (percids, moronids) as evidenced by the effect of temperature decrease on the initiation of gonadogenesis and

* Corresponding author. Tel.: +33 3 83 68 56 00; fax: +33 3 83 68 55 98.
E-mail address: p.fontaine@univ-lorraine.fr (P. Fontaine).

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2012.11.010

oogenesis (Clark et al., 2005; Migaud et al., 2002; Prat et al., 1999; Wang et al., 2010). In particular, some spring spawners need specific thermal conditions for the onset of reproductive cycle such as in sea bass (Moretti et al., 1999) and in Eurasian perch in which the initial decrease of temperature amplitude was found to influence the initiation of gonadogenesis (Wang et al., 2006).

Contrary to the initiation, the vitellogenesis is affected by the exposure to inadequate temperatures in salmonids. For instance, a very cold temperature delays vitellogenesis, the growth of oocytes as well as the spawning in the rainbow trout *Oncorhynchus mykiss* (Mackay and Lazier, 1993). However, the effect of high temperature is highly variable and depends on species and reproductive stage (Kagawa et al., 1983; Khan et al., 1999) as well as on different regulatory factors according to developmental stage (Van Der Kraak and Pankhurst, 1997). For instance, King et al. (2003) showed that a high temperature decreases the plasma levels of oestradiol and vitellogenin in Atlantic salmon. In moronids and percids, the quality of vitellogenesis seems to be linked to temperature direction of change and temperature amplitude. In sea bass (Carrillo et al., 1995; Mañanós et al., 1997) and striped bass (Clark et al., 2005), the vitellogenesis does not restart until the temperature decreases. In yellow perch *Perca flavescens* most of data suggest that vitellogenesis is affected by the kinetics of temperature decrease (Ciereszko et al., 1997; Shewmon et al., 2007). In Eurasian perch, high temperature in Swedish and Lithuanian thermal effluent areas negatively influenced oogenesis, indicating a reduced reproductive capacity. Abnormally, oocyte atresia started during vitellogenesis in autumn, and was often followed by asynchronous oocyte development. Among other abnormalities, multi-nucleus oocytes and hermaphroditism were observed (Luksiene et al., 2000).

Within this context, we hypothesized that exposure to inadequate thermal threshold and timing of temperature reduction (delay between photoperiod and temperature decreases) at the onset of the reproductive cycle may impair oogenesis (initiation, vitellogenesis) in Eurasian perch. Therefore, this study was conducted to determine: (i) the effects of thermal threshold and high temperature (22 °C) on the onset of oogenesis under decreased photoperiod (duration 16 weeks, amplitude 8 h); (ii) the effects of the thermal timing reduction on this initiation.

2. Materials and methods

2.1. Fish

Eurasian perch fingerlings (10 g average body weight) were transferred on October 4th, 2006, from a Swiss fish farm (Percitech, Chavornay, Switzerland) to the experimental facilities of Lorraine University. Fish were reared up to the adult stage at 22 ± 2 °C under a constant photoperiod condition of 16 L: 8O and a light intensity of 200 Lx. Light was provided by fluorescent bulbs (daylight 2500 Lm, 36 W). Breeders (average weights = 160 g., standard deviation = 50 g.) were randomly transferred to the 500 L. experimental tanks on August 2nd, 2007 (48 fish per tank). They were acclimatized until September 26th, 2007. Fish were daily fed at 9AM to apparent satiation during the acclimation period and during the experiment (BioMar Bio-optimal type St 4.5, proteins 46%, lipids 11%, ash 8% and fiber 2.2%).

2.2. Experimental design and water quality management

The experiment started on September 26th, 2007 and was conducted in a closed recirculated water system (flow: 750 L h^{-1}) involving the use of biological filtration. Four thermal thresholds were applied (22, 18, 14, 6 °C) combined with two thermal timings (0 week or 4 weeks, delay between photoperiod and temperature decreases) in duplicates (16 tanks, Fig. 1a/b). The photothermal kinetics applied was 16 weeks and the light intensity was 200 Lx for induction of gonadogenesis (Abdulfatah et al., 2011). Light was provided by fluorescent bulbs (OSRAM L, 18 W). Heaters with a thermostat adjustable cane beach were used in each tank to control automatically the temperature (accuracy: around 0.5 °C).

In each tank, water quality was measured three times per week; pH was maintained between 7.0 and 7.5 by $NaCO_3$ additions. The dissolved oxygen was maintained over of 6 mg L^{-1}. The total ammonia and nitrite nitrogens were measured using a CARY I spectrophotometer and remained below 1 mg L^{-1}.

2.3. Organ and plasma sampling

Fish were sampled at days 0, 28, 70, 112 and 140 after the start of experiment (Fig. 1a/b). Ten fish per tank, both sexes confounded, were caught in order to obtain theoretically around 10 females per treatment (no evident sexual dimorphism, see Table 1 for number of sampled females). The first sampling occurred just before the initial photoperiod decrease. At each sampling date, fish were anesthetised into a 2-phenoxyethanol bath (0.5 mL/L, Sigma). Blood was removed from the caudal vein using a syringe, then stored on ice in heparinized microtubes until centrifuged at 4000 rpm for 25 min (Centrifuge Jouan C-412). Aliquots of plasma were stored at −20 °C until ELISA analysis was performed. Then, each fish was euthanatized by overanesthetizing in 2-phenoxyethanol and by a blow on the head, weighed and dissected for gonad weighing and calculation of the gonadosomatic index (GSI = 100 × gonad weight/total fish weight).

2.4. Histology

Samples of ovaries were stored in a Bouin–Holland solution for 7 days, washed once with running water, twice with 70% ethanol and stored in absolute ethanol. Then, fragments of gonads were dehydrated and embedded in paraplast for histological examination (Langeron,

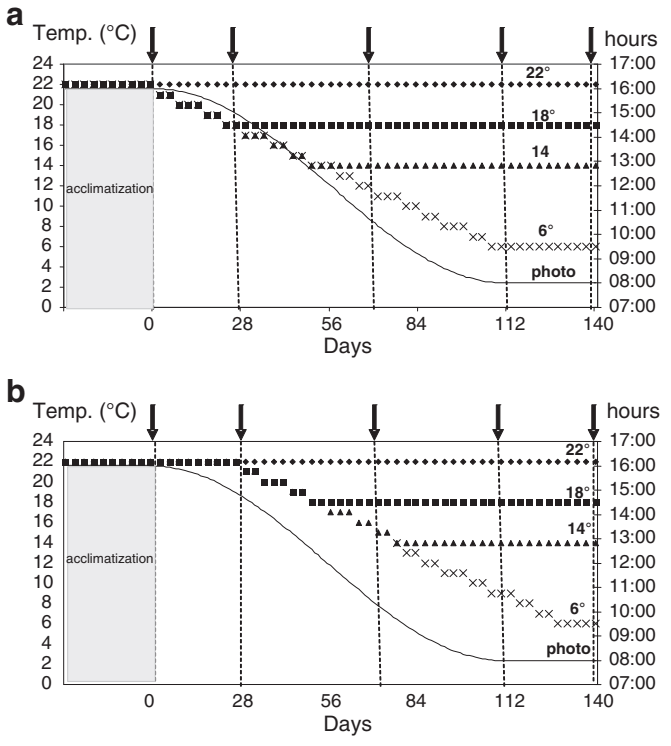

Fig. 1. Photo-thermal kinetics applied with four thermal thresholds (6, 14, 18 and 22 °C) in combination without (a) or with (b) a 4-week delay between photoperiod and temperature decreases. The arrows indicate sampling dates.