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Evidence that elevated water temperature affects the reproductive physiology of the European bullhead *Cottus gobio*

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Abstract Climate change is predicted to increase the average water temperature and alter the ecology and physiology of several organisms including fish species. To examine the effects of increased water temperature on freshwater fish reproduction, adult European bullhead *Cottus gobio* of both genders were maintained under three temperature regimes (T1: 6–10, T2: 10–14 and T3: 14–18°C) and assessed for gonad development (gonadosomatic index—GSI and gonad histology), sex steroids (testosterone—T, 17β-estradiol—E2 and 11-ketotestosterone—11-KT) and vitellogenin (alkali-labile phosphoprotein phosphorus—ALP) dynamics in December, January, February and March. The results indicate that a 8°C rise in water temperature (T3) deeply disrupted the gonadal maturation in both genders. This observation was associated with the absence of GSI peak from January to March, and low levels of plasma sex steroids compared with T1-exposed fish. Nevertheless, exposure to an increasing temperature of 4°C (T2) appeared to accelerate oogenesis with an early peak value in GSI and level of plasma T recorded in January relative to T1-exposed females. In males, the low GSI, reduced level of plasma 11-KT and the absence of GSI increase from January to March support the deleterious effects of increasing water temperature on spermatogenesis. The findings of the present study suggest that exposure to elevated temperatures within the context of climate warming might affect the reproductive success of *C. gobio*. Specifically, a 4°C rise in water temperature affects gametogenesis by advancing the spawning, and a complete reproductive failure is observed at an elevated temperature of 8°C.

Keywords *Cottus gobio* · Global warming · Gonadogenesis · Reproduction · Sex steroids · Temperature

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Introduction

The Earth's climate has warmed by approximately 0.74°C over the past 100 years with two main periods

of warming, between 1910 and 1945 and from 1976 onwards (IPCC 2007). Current global warming models predict increases in mean air temperature of 1.1–6.4°C over the next 50–100 years (IPCC 2007), with similar rises in the temperature of aquatic environments. The first response of many animals to climate warming may be spatial shifts towards higher altitudes and higher latitudes, according to thermal preferences (Walther et al. 2002). In freshwater ecosystems that often have discrete physical boundaries, such large-scale movements may not be possible, but smaller-scale spatial variation in temperature, e.g. with depth, may allow aquatic animals to migrate to cooler areas during warming. However, climate change, which is not only responsible for increased thermal regime but also for reduced water flow during extended dry summer, with habitat partitioning as a corollary, will also affect the availability of suitable habitats (Rahel et al. 1996), and therefore exposure to increased temperature may be unavoidable. In such situations, physiological effects of climate warming are expected to occur and in turn potentially compromise the survival of aquatic species.

Freshwater fish are ectothermic, and therefore their metabolic rate and many of their physiological functions are fundamentally influenced by temperature. Thus, they are likely to be amongst those most affected by the long-term increases in environmental temperature predicted by models of global warming (Mohnen and Wang 1992). Despite the complexity of the different ways by which temperature can affect reproductive efficiency, there is a general tendency for an increase in the amount of energy allocated to reproduction at higher temperatures (Hirshfield 1980). Abnormally elevated temperature has the capacity to modify reproductive processes at multiple levels through the endocrine system (Pankhurst and Thomas 1998). Several studies on salmonids have shown that exposure to, or maintenance at, higher than normal temperature can change patterns of gonadotropin-releasing hormone (GnRH) activity, secretion, uptake and clearance of gonadotropin (GtH), testicular and ovarian steroidogenesis and metabolism, and plasma levels of reproductive steroids (reviewed by Pankhurst and King 2010; Van Der Kraak and Pankhurst 1997). Generally speaking, these modifying effects may lead to disorders of gametogenesis and gonad development, inhibition of ovulation, advance or delay in the timing of spawning and reduced embryonic survival in various species as for instance in the rainbow trout *Oncorhynchus mykiss*, the Atlantic salmon *Salmo salar* and the common wolffish *Anarhichas lupus* (King et al. 2003; Pankhurst et al. 1996; Pankhurst and Thomas 1998; Tveiten and Johnsen 1999; Tveiten et al. 2001). Recently, Geraudie et al. (2010) have reported that elevated temperature during winter time lead to an earlier gonad maturation of roach *Rutilus rutilus*. More generally, a review on the relative importance of external factors on the reproductive cycle in temperate fishes (including salmonids, percids and cyprinids) has shown that temperature is one of the main environmental cue driving the final stage of reproduction (Wang et al. 2010).

The European bullhead, a small bottom-dwelling freshwater cottid fish, has become endangered in several areas like Switzerland, Germany and the northern part of Belgium as a result of pollution and habitat destruction (Utzinger et al. 1998). Bullhead typically lives in well-oxygenated stream waters from 2 to 16.5°C (Andreasson 1971), whereas Elliott and Elliott (1995) found the critical thermal limits of bullhead to be 4.2 and 27.7°C. It has been selected in our study as a candidate sentinel species reflecting the biodiversity of headwater zones in river networks (Habitats Directive 2007). The current knowledge of *C. gobio* indicates that this species exhibits variability in its reproductive characteristics, and there is a strong environmental influence, especially from temperature and food availability, on bullhead life-history tactics (Fox 1978; Legalle et al. 2005). Bullheads spawn from February to June: typically once for females in upland stream and up to four times in warmer, more productive lowland streams (Fox 1978). In Samson River, a Belgian chalk stream, its reproduction is characterized by a short spawning season limited to a period of 2 weeks taking place in April when the water temperature is 9–10°C, and females usually produce one single batch of eggs (De Silva 1985). Within the context of climate warming, previous studies conducted on female bullhead have shown that an increase in temperature alters its reproduction and modifies its demographic strategy (Abdoli et al. 2005; Abdoli et al. 2007; Reyjol et al. 2009), although reproductive information on male remain largely unknown. Moreover, if some

disruption of reproductive function has been shown, no mechanistic studies on the endocrine regulation of gametogenesis under controlled conditions have been done. In order to extend our understanding, the current work was designed to examine the effects of global warming, in the range predicted by scientists, on some key aspects of the European bullhead reproduction. To address this question, morphological (gonadosomatic index), histological (oogenesis and spermatogenesis) and physio-biochemical (sex steroids and alkali-labile phosphoprotein phosphorus) variables were assayed in both genders following exposure to elevated temperatures in laboratory conditions. Understanding temperature effects on life-history characteristics of aquatic animals will make a significant contribution to current attempts to predict climate change effects at the population and community levels.

Materials and methods

Fish capture and maintenance

Adult European bullhead of both genders weighing 13.0 ± 3.6 g were caught by electrofishing in the Samson River (Belgium) in fall 2005. Fish were maintained under natural photoperiod and temperature conditions in outdoor tanks supplied with freshwater for 4 weeks before the experiment. Fish were fed daily to apparent satiation with frozen chironomid larvae (*Chironomus* sp.).

Experimental set-up

A total of 360 fish were transferred into 18 tanks (capacity 120 l) and exposed for 14 weeks (starting on 15 December 2005 and ending on 27 March 2006) to one of the three following thermal regimes: T1: 6–10°C, T2: 10–14°C or T3: 14–18°C, with a natural light–dark cycle. Each treatment included six replicate tanks, with 20 fish per tank. The lowest temperature (T1) was chosen to be as close as possible to the temperature *C. gobio* would have experienced in the wild during the mating period based on average temperature profiles recorded in the Samson River at the corresponding period. Temperature was measured in each tank and, if necessary, adjusted daily to simulate gradual temperature increase that is encountered in the wild. The number of degree-days for each thermal regime is depicted in Fig. 1.

Fish sampling

Samplings were conducted over a 6-day period approximately every 30 days (D1: 26th January, D2: 23rd February and D3: 22nd March), including the first day (D0: 15th December) of the experiment. At each sampling time, fish were selected randomly, weighed and measured for total length. Blood was sampled from the caudal vein, using a 1-ml heparinised syringe. Plasma was then collected after centrifugation at 3,500 rpm for 15 min and stored at −20°C prior to the analysis of sex steroid hormones and alkali-labile phosphorus (ALP) levels. Gonads were excised and weighed to calculate gonadosomatic index (GSI). A gonad portion was fixed in Bouin's solution (minimum 24 h) for histological examination.

Morphological and histological measurements

GSI

Gonads were weighed and GSI was calculated as: (gonad weight/body weight) × 100.

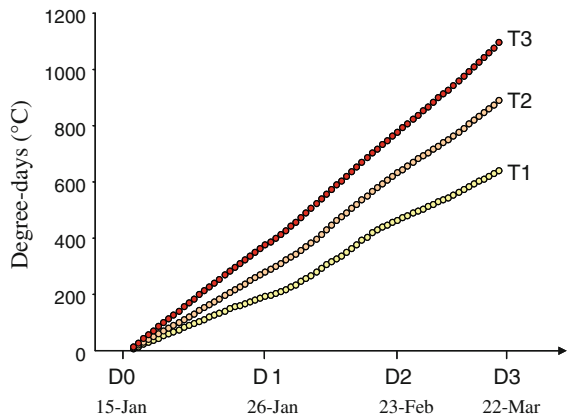

Fig. 1 Temperature regimes (degree-days) experienced by bullhead during the experiment (*T1:* 6–10°C, *T2:* 10–14°C, and *T3:* 14–18°C)

Gonad histology

Each sample was embedded with paraffin and cut at 4 µm for ovaries and 3 µm for testes. Ovaries were then stained with a trichrome hemaluin—phloxine—light green (Allied chemical—light green SF yellowish no. NA 0594 diluted 10 times in 1% acetified water), and testes were stained with a trichrome Regaud's haematoxylin (57°C) [(Merck 4305) 1 g, 10 ml absolute ethylic alcohol, 10 ml glycerine (Merck 4094), 80 ml distilled water]—red solution—light green. Testes were analysed qualitatively by recording the last stage of development (spermatogonia A or B, spermatocytes, spermatids and spermatozoa). In females, 200 oocytes were scored per ovary. Different maturation stages were determined according to Rinchard and Kestemont (1996) (protoplasmic oocytes, early cortical alveoli stage, late cortical alveoli stage, exogenous vitellogenesis and pre-ovulatory atresia), and their proportions were calculated. Mean diameter of the most advanced maturation stage was calculated (except for atretic oocytes) by measuring 15 oocytes of the corresponding stage. Only oocytes cut through the nucleus [except for the final maturation stage including nucleus migration (germinal vesicle) from a central to a peripheral position in the cell, and germinal vesicle breakdown (GVBD)] were considered in our study.

Biochemical measurements

Plasma vitellogenin assay

Plasma vitellogenin (Vtg) was evaluated by spectrophotometry as indirect endpoints of calcium and alkali-labile phosphorus (ALP), since levels of these ions were reported to be plausible indicators of the amount of vitellogenin (Verslycke et al. 2002). Plasma ALP assay was performed according to Mandiki et al. (2005). The plasma was thawed on ice. The proteins present in 30 µl plasma were obtained after precipitation with trichloroacetic acid, centrifugation, washing for lipid removal and drying 1 h in a desiccator under vacuum. The pellet containing proteins was then redissolved in NaOH and placed for 15 min in a warm water bath (100°C). After neutralization of the sample with HCl, the phosphates quantity was determined by the phosphomolybdenum method (Sigma 670-A). The absorbance was measured at 660 nm. Using the absorptions of the standard dilutions, the concentration of the sample can be calculated accordingly with recalculation factors related to dilution of the samples during the assay. All samples were assayed in duplicate.

Plasma sex steroid assays

Plasma steroid concentrations of testosterone (T), 17β-estradiol (E2) and 11-ketotestosterone (11-KT) were measured by radioimmunoassay (RIA) according to Fostier and Jalabert (1986). Samples and standards were assayed in duplicate. Radioactive hormones were purchased from Amersham Pharmacia (Buckinghamshire, England), the T and E2 antibodies were from the Laboratoire d'Hormonologie de Marloie (Belgium) and the anti-11-KT was provided by Dr. A. Fostier (INRA, Rennes France). The intra- and inter-assay coefficients of variation were 4.8 and 6.5, 5.1 and 7.8, and 4.2 and 7.3% for T, E2 and 11-KT, respectively. The detection limits of the assays were 55, 60 and 49 pg ml^{-1} for T, E2 and 11-KT, respectively.

Statistical analysis

Data were expressed as mean ± SEM. Statistical analyses were performed using Statistica 5.5 software (StatSoft, Tulsa, OK, USA). Individual fish was used as a statistical unit. The effects of temperature, sampling time and their interaction on gonadal parameters, plasma sex steroids levels and ALP values were tested using the two-way analysis of variance (ANOVA 2) followed by a multiple comparison Tukey's HSD test for unbalanced data (Spjotvoll/Stoline test). If a significant temperature–time interaction effect was observed, different letters mean significant differences between temperatures within a given sampling time. Data were log-transformed when the conditions of normality and/or homogeneity of variance were not fully filled, or arcsine-square-root-transformed if expressed in percentages. The level of significance used in all tests was $P < 0.05$.

Results

GSI and oocyte diameter variations

The dynamics of gonadosomatic index (GSI) changes in females and males are depicted in Fig. 2a, b, respectively. Temperature, sampling time and their interaction significantly ($P < 0.001$) influenced the GSI in females. Mean GSI values of 15.3 ± 1.6% were observed in D1 compared with 4.1 ± 0.7% in D2 in females held at the intermediate temperature T2. In this group, ovulation occurred from D1 to D2, and this was confirmed by visual observation of eggs in tanks. Females held at natural temperature T1 ovulated from D2 to D3 with mean GSI value of 22.5 ± 1.2% in D2. There was no ovulation in females held at the highest temperature T3 with decreasing GSI values (6.9 ± 1.0% in D1, 1.5 ± 0.2% in D2 and 1.3 ± 0.1% in D3) and absence of clear GSI peak. By contrast, no temperature–time interaction effect was observed in male GSI with values ranging between 0.1 and 2.9% (Fig. 2b). However, GSI values were significantly ($P < 0.001$) affected by temperature and sampling time independently. Both factors significantly reduced mean GSI values in an independent way.

Similarly, the oocyte diameter of the most advanced maturation stage significantly varied with temperature and sampling time ($P < 0.001$, Fig. 3). The average oocyte diameter was 0.80 ± 0.02 mm at the beginning of the experiment D0. Subsequently, a peak value in the oocyte diameter was measured in D1 in females held at the intermediate temperature T2 (1.14 ± 0.04 mm) and in D2 in T1-exposed group (1.40 ± 0.09 mm). The highest temperature T3 induced a decrease in the oocyte diameter from D0 to D3 (0.26 ± 0.01 mm).

Gonad developmental stages

The different oocyte developmental stages are shown in Fig. 4. The proportion of oocytes at the exogenous vitellogenesis stage, pre-ovulatory atresia stage and protoplasmic stage displayed significant interaction effects of temperature and sampling time ($P < 0.01$, Table 1). The proportion of oocytes at the exogenous vitellogenesis stage decreased with increasing temperatures in D1 (from 23 ± 3% at T1 to 3 ± 1% at T3) ($P < 0.001$). High proportion of this stage was also observed in T1-exposed group in D2, while it was null in females held at higher temperatures T2 and T3. Regarding the pre-ovulatory atresia stage, higher proportions were observed in females held at both elevated temperatures (T2 and T3) in D1

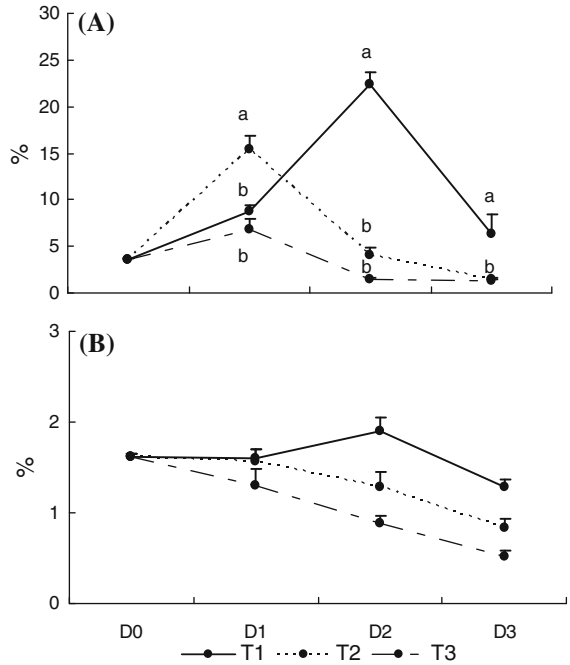

Fig. 2 Monthly changes in the gonadosomatic index (GSI) in bullhead females (**a**) and males (**b**) under different temperature regimes. Values are mean ± SEM ($n = 10$–17). If a significant temperature–time interaction effect was observed, different letters mean significant ($P < 0.05$) differences between temperatures within a given sampling time

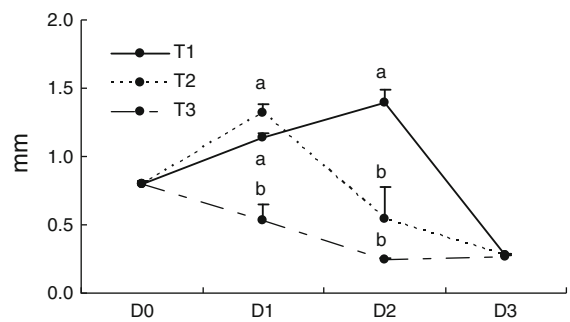

Fig. 3 Monthly changes in oocyte diameter in bullhead females under different temperature regimes. Values are mean ± SEM ($n = 8$–16). If a significant temperature–time interaction effect was observed, different letters mean significant ($P < 0.05$) differences between temperatures within a given sampling time

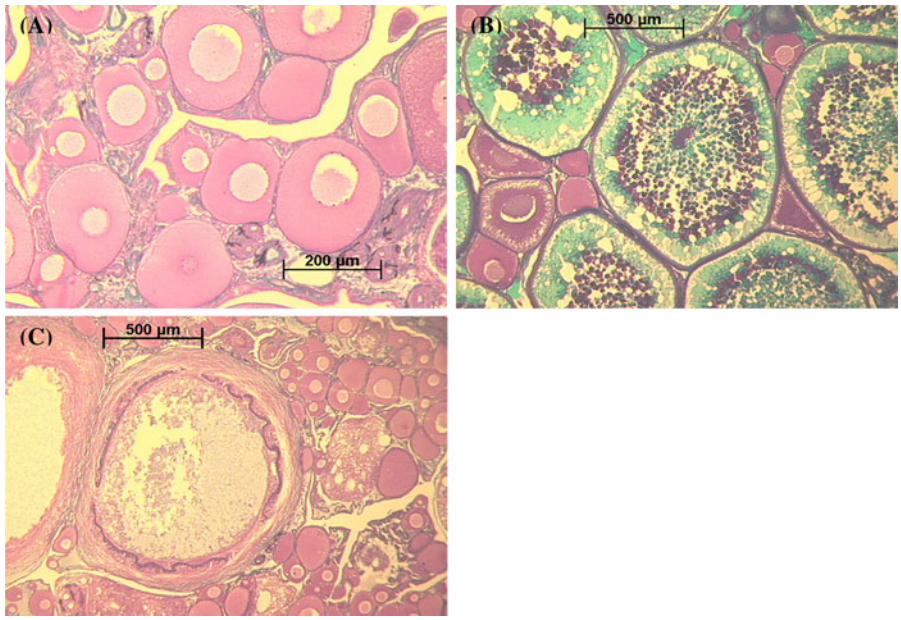

Fig. 4 Oocyte developmental stages obtained from bullhead females: protoplasmic stage (**a**), exogenous vitellogenesis stage (**b**) and pre-ovulatory atresia stage (**c**)

($P < 0.05$) and D2 ($P < 0.01$) than in females held at T1. No significant difference was observed between temperatures in D3. The proportion of the oocytes at the protoplasmic stage increased in females held at the highest temperature T3 from D2 and was maximal in fish held at T2 and T3 in D3. However, no significant difference was detected between temperatures at all sampling dates.

On a qualitative basis, bullhead males were all in advanced maturation stage as all fish displayed testis full of spermatozoa, whatever the temperature and the sampling time.

Plasma sex steroid and ALP variations

Temperature and sampling time, alone or in interaction, significantly affected sex steroid and ALP values in bullhead for both sexes (Table 2). In females, mean plasma E2 levels were 2.06 ± 0.13 ng ml^{-1} at the beginning of the experiment D0 (Fig. 5a). Subsequently, a peak of E2 level was detected in D1 in females held at the intermediate temperature T2 (3.99 ± 0.46 ng ml^{-1}), whereas the plasma concentration of this oestrogen continued to increase in females held at T1 until D2 (2.94 ± 0.18 ng ml^{-1}). Plasma E2 concentration in fish exposed to T3 remained unchanged over the sampling period with values ranging between 1.30 and 1.67 ng ml^{-1}. In females from all groups, the changes in plasma T levels exhibited the same pattern as E2 levels (Fig. 5b). *T* values peaked in D1 in females held at T2 (21.47 ± 3.26 ng ml^{-1}), and in D2 in fish held at T1 (13.54 ± 2.09 ng ml^{-1}) relative to D0 (5.57 ± 0.57 ng ml^{-1}). In females exposed to the highest temperature T3, plasma T level remained practically unchanged with values ranging between 1.23 and 1.73 ng ml^{-1}. Regarding ALP, plasma levels globally decreased from D1, whatever the temperature (Fig. 5c). In T3-exposed females, a sharp decrease was recorded along the tested periods (from 3.59 ± 0.45 in D1 to 0.41 ± 0.07 µg ml^{-1} in D3). Additionally, the levels recorded in females held at T3 were significantly lower than those measured in fish exposed to T1 in D2 and D3 ($P < 0.01$).

In males, mean plasma T and 11-KT levels were 5.40 ± 0.68 and 1.90 ± 0.40 ng ml^{-1} in D0, respectively (Fig. 6a, b). An early peak of *T* value (9.51 ± 1.07 ng ml^{-1}) was recorded in D1 in fish held at the intermediate temperature T2. Nevertheless, this peak *T* value was not significantly different from value recorded in T1-exposed group. Plasma levels of T and 11-KT peaked in D2 in males held at

Table 1 Mean percentage of oocyte developmental stages obtained from bullhead females under different temperature regimes

Sampling time	Temperature	Oocyte type		
		Protoplasmic	Exogenous vitellogenesis	Pre-ovulatory atresia
D1	T1	66 ± 3	23 ± 3[a]	5 ± 2[b]
	T2	67 ± 3	17 ± 2[a]	30 ± 7[a]
	T3	63 ± 6	3 ± 1[b]	31 ± 7[a]
D2	T1	63 ± 6	29 ± 6[a]	1 ± 0[b]
	T2	57 ± 8	0[b]	35 ± 8[a]
	T3	80 ± 4	0[b]	16 ± 4
D3	T1	64 ± 6	0	25 ± 6
	T2	85 ± 3	0	8 ± 1
	T3	81 ± 3	0	13 ± 2

Mean ± SEM

Mean values with different superscripts within the same column and sampling time are significantly ($P < 0.05$) different

T1 (11.52 ± 0.75 ng ml^{-1} for T; 243.89 ± 53.02 ng ml^{-1} for 11-KT). Plasma T and 11-KT concentrations in T3-exposed group remained practically unchanged along months.

Discussion

Temperature is one of the most ubiquitous environmental factors that influence aquatic wildlife. Thus, changing temperature profiles within rivers arising from climate change can influence the physiology and ecology of a variety of organisms including fish species. The temperatures applied in the present study were far below the upper limit of normal feeding or death for *C. gobio* which was found to be 27.7°C (Elliott and Elliott 1995). That being so, the current study showed that exposure to temperatures from 4 to 8°C higher than temperature *C. gobio* would have experienced in the wild during the recrudescence and mating periods significantly influenced its reproduction.

Seasonal changes in gonadal development of wild *C. gobio* have been described by several authors (De Silva 1985; Smyly 1957), and no clear anatomical and histological change is apparent until the end of autumn. Under natural temperature (T1), bullhead females exhibited a reproductive cycle similar to the one reported in the wild with GSI increasing from 3.6% in December to 22.5% in February followed by a decrease in March, reflecting ovulation. Indeed, De Silva (1985) reported that the mean GSI of bullhead females increased from 1.9% in November to 17.9% in March, and decreased to 1.7% in April after spawning. Consistent with these observations, a peak value in oocyte diameter, vitellogenesis stage frequency and levels of plasma testosterone and 17β-estradiol was recorded in February. We thus assume that bullhead females reared under natural temperature elevation achieve a complete oogenesis process. In males, a slight increase from 1.6% in December to 1.9% in February was observed, and a decrease to 1.3% in March. This observation was accompanied by an increased value of both plasma androgen levels in February followed by a decrease in March. In the study of De Silva (1985), the mean GSI increased from 1.2% in November to 1.9% in February, and decreased to 1.6% in March and further to 0.7% in April after spawning. Therefore, we speculate that the bullhead reproduction was not affected under environmentally controlled conditions, at least in case of exposure to natural temperature.

In a recent in situ study, Abdoli et al. (2005) hypothesized that a rise in the mean annual temperature of 2°C increases fecundity, egg diameter and reproductive effort (defined as the ratio of total egg production mass to body mass) of bullhead females, while a higher increase in temperature of 6°C may lead to local extinctions of bullheads because of reproductive failure. Later, Reyjol et al. (2009) showed that a constant elevated temperature had a strong negative effect on gonad fresh mass, fecundity, mean diameter of eggs and gonad triglyceride content of bullhead females under controlled laboratory conditions. In the present study, exposure to temperature of 8°C (T3) higher than temperature *C. gobio*

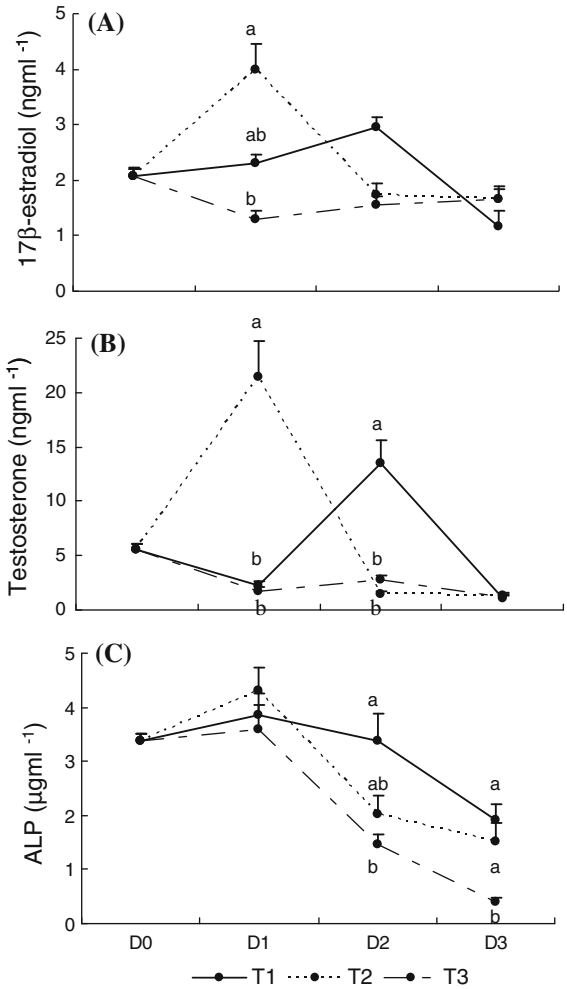

Fig. 5 Monthly changes in plasma 17β-estradiol (**a**), testosterone (**b**) and ALP (**c**) levels in bullhead females under different temperature regimes. Values are mean ± SEM ($n = 10$–16). If a significant temperature–time interaction effect was observed, different letters mean significant ($P < 0.05$) differences between temperatures within a given sampling time

would have normally experienced in the wild deeply disrupted the gonadal maturation in both genders. The low GSI, oocyte diameter, vitellogenesis stage frequency and the absence of GSI increase from January to March support the deleterious effects of such environmental context on the oogenesis process. Consistent with these observations, reduced levels of both plasma 17β-estradiol and its precursor testosterone were observed following exposure to this highest temperature. As the primary function of 17β-estradiol is the stimulation of hepatic synthesis and ovarian

Table 2 Two-way ANOVA performed for plasma steroids and ALP values measured in bullhead females and males, testing the effect of temperature treatment and sampling time

	Temperature	Sampling time	Temperature × Time
Female			
E_2 level	$P < 0.01$	$P < 0.001$	$P < 0.001$
T level	$P < 0.001$	$P < 0.001$	$P < 0.001$
ALP level	$P < 0.001$	$P < 0.001$	$P < 0.01$
Male			
T level	$P < 0.01$	$P < 0.001$	$P < 0.001$
11-KT level	$P < 0.001$	$P < 0.001$	$P < 0.001$

Given are P values of each variable

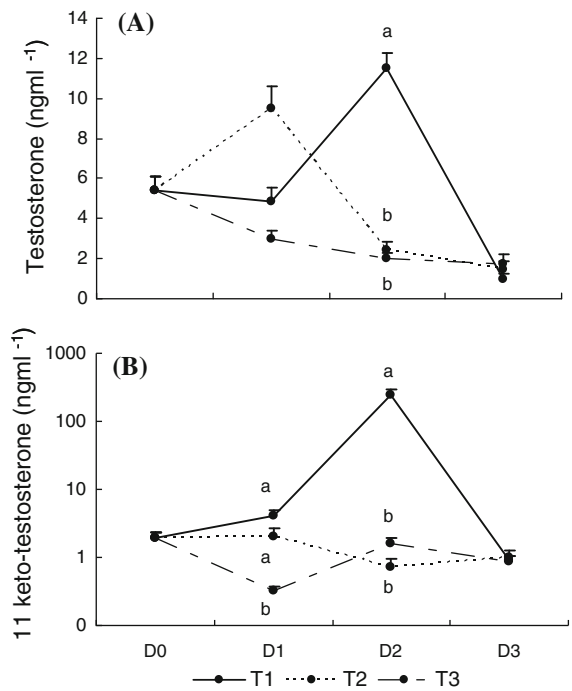

Fig. 6 Monthly changes in plasma testosterone (**a**) and 11 keto-testosterone (**b**) levels in bullhead males under different temperature regimes. Values are mean ± SEM ($n = 10$–14). If a significant temperature–time interaction effect was observed, different letters mean significant ($P < 0.05$) differences between temperatures within a given sampling time

sequestration of vitellogenin, the low levels of 17β-estradiol may explain the inhibition of gonadogenesis. In addition, the impairment of gonad development is strengthened by a high frequency of atretic

follicles observed from January. In the Atlantic salmon *Salmo salar*, elevated temperatures impaired maturation, ovulation and subsequent fertility in females through the disruption of endocrine processes as shown by the altered levels of plasma 17β-estradiol, vitellogenin and testosterone (King et al. 2003; Pankhurst and King 2010; Watts et al. 2004). Consequently, markedly elevated temperatures may stop oogenesis in a wide range of fish species because of suppression in circulating sex steroid levels. In addition, as shown in other fish species (e.g. Milla et al. 2009), we think that unsuitable thermal conditions possibly target the gonadotropin actions and subsequently the production of ovarian sex steroids.

In the present study, elevated water temperature of 8°C had also deleterious effects on both plasma androgen levels and gamete production in males. 11-Ketotestosterone and testosterone are known to be the main endocrine regulators of spermatogenesis (Schulz et al. 2010). In the current study, the plasma androgen depletions may thus be the cause of affected gametogenesis. The low and decreasing GSI from January to March may indicate a reduction in spermatozoa quantity and consequently a possibility of reduced fertilization success as water temperature increases. Increasing temperatures were reported to reduce both plasma testosterone and 11-ketotestosterone levels in vivo in male rainbow trout *Oncorhynchis mykiss* (Manning and Kime 1985). Nevertheless, Donelson et al. (2010) observed reduced spermatogenesis at high temperatures despite some increases in plasma 11-ketotestosterone levels in the coral reef damselfish *Acanthochromis polyacanthus*. So, we cannot rule out the possibility that, besides the androgens, other metabolic factors involved in the control of spermatogenesis are affected by exposure to such elevated temperatures.

In contrast to the impaired steroidogenic activity and inhibition of gametogenesis in both genders observed as a result of holding fish in increased temperature of 8°C, exposure to a slight increase in water temperature of 4°C (T2) appeared to accelerate oogenesis with an early peak GSI value of 15.3% recorded in January. This observation was accompanied by an increased value in the oocyte diameter of 1.31 mm in January followed by a decrease in February. Consistent with these observations, a high proportion of vitellogenic oocytes were recorded in January followed by their disappearance in February, since ovulation occurred between these two sampling dates (as evidenced by eggs observed in the fish tanks). In addition, ovaries from fish exposed to intermediate increase in water temperature displayed a higher proportion of atretic follicles in January than in females held at natural temperature. Given the role of 17β-estradiol in the process of vitellogenic oocyte growth, the peak level of 17β-estradiol measured in January could explain the early oogenesis. Nevertheless, the oocyte diameter, vitellogenin level and vitellogenesis stage frequency recorded in January were not significantly higher in females exposed to this increased temperature of 4°C than in fish held at natural temperature. The early onset of gonadal development and spawning may be explained by the early peak testosterone value measured in January. Testosterone as a precursor of 17β-estradiol synthesis is released into the plasma when no longer needed for aromatization and has a role during the final maturation/ovulation process, probably in the final stages of synchronization and in the regulation of GnRH/gonadotropin secretion (Nagahama et al. 1994; Nagahama and Yamashita 2008; Nagler and Idler 1992). The results of the present study are consistent with studies in other species showing accelerated gametogenesis and early spawning of roach *Rutilis rutilus* exposed to a slight increase in water temperature (+2 or 3°C) induced by thermal effluent from a nuclear power station in the River Meuse (Mattheeuws et al. 1981). In the same species, Gillet and Quetin (2006) also showed that an increase in water temperature of 1°C modified the life-history strategy of roach in Lake Geneva, by accelerating the development of gonads and affecting spawning date. Geraudie et al. (2010) also reported earlier gonad maturation in female and male roach due to elevated temperature recorded during winter time.

Contrary to the accelerated oogenesis and early spawning of females, a 4°C rise in water temperature negatively altered spermatogenesis in bullhead males. The low GSI, the GSI drop from January to March and the reduced levels of plasma 11-ketotestosterone support the deleterious effects of increasing water temperature on spermatogenesis. This result supports that bullhead reproduction may be compromised even in case of moderate temperature increase.

Global climate change appears to represent an additional stressor to fish populations together with

pollution, overfishing, recreational use of water bodies and the introduction of exotic species (Morgan et al. 2001). Assuming that reproduction in fish is only possible within a narrow range of temperatures that can be tolerated by adults (Van Der Kraak and Pankhurst 1997), elevated temperatures might alter reproductive success of bullhead. As a matter of fact, the present study has shown that increases in water temperature consistent with climate change predictions may affect the reproductive performance of bullhead, due to disruption of the endocrine regulation of vitellogenesis and changes in gonad maturation timing. Most obviously, a 4°C rise in water temperature affects gametogenesis by advancing the spawning, and a complete reproductive failure is observed at an elevated temperature of 8°C. The results of the present study, combined with previous data on reproductive and demographic traits of bullhead (Abdoli et al. 2005, 2007; Reyjol et al. 2009), suggest possible deleterious effects of warming on its population dynamics. According to Geraudie et al. (2010), reproduction is a key mechanism for the survival of a given species. The consequences on population and species survival could be severe unless development of phenotypic plasticity within a generation over multiple years or genetic adaptation over generations might produce individuals more tolerant to a warmer future. The potential for this to occur in species such as *Cottus gobio* is currently unknown, and long-term rearing experiments in relation to the plasticity or adaptability of life-history traits are now needed.

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