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Effects of temperature on biological and biochemical indicators of the life-history strategy of bullhead *Cottus gobio*

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The biological and biochemical effects of temperature on life-history strategy of female bullhead *Cottus gobio* were investigated. Fish from two populations (Bez Basin, south-east France) experiencing contrasted thermal environments (*i.e.* more or less stable) were reared during 4 months at three distinct temperatures (7, 9 or 12° C). Both somatic (soma fresh mass and muscle triglyceride content) and reproductive (gonad fresh mass, fecundity, mean diameter of eggs and gonad triglyceride content) indicators were examined. Mixed models indicated that an increasing temperature had significant negative effects on all life-history indicators except for soma fresh mass. Differences in life-history strategy with regard to muscle and gonad triglyceride contents, however, suggest that populations experiencing more variable thermal environments may be better adapted than others to cope with an increasing temperature. These findings may have important implications for *C. gobio* populations, within the context of climate warming.

Key words: experimental ecology; freshwater fish; phenotypic plasticity; triglycerides.

INTRODUCTION

Because of its large influence on metabolic reactions and physiological processes (*e.g.* digestive and assimilation efficiencies, oxygen consumption, growth rate and reproduction), temperature is a keystone environmental variable for ectotherms such as fishes (Hochachka & Somero, 2002). Considering reproduction, the most common effects of temperature is a modulation in the timing of spawning and hatching (Pepin *et al.*, 1997). It has also been shown to influence a variety of reproductive life-history features, among which fecundity (Portner *et al.*, 2001), egg survival (Sandström *et al.*, 1997) and egg diameter (King *et al.*, 2003) are the most documented. Within the context of climate warming, it has recently been shown that

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an increase in water temperature of 1° C could modify the life-history strategy of the species, by accelerating the development of gonads and affecting spawning date for roach *Rutilus rutilus* (L.) in Lake Geneva (Gillet & Quélin, 2006). In rivers, a variety of studies have shown that both recruitment and year-class strength are affected by density-independent mechanisms involving temperature and hydrology (Lobón-Cerviá & Mortensen, 2005), which could in turn have significant effects on fish population dynamics and life-history strategies (Cattaneo *et al.*, 2001; Daufresne & Renault, 2006). Climate warming could have significant effects on fish populations and communities in rivers by affecting both thermal and hydrological regimes (Parry *et al.*, 2007). An increase in warmwater species abundances parallel to a decrease in coldwater species abundances has been predicted (Mohseni *et al.*, 2003) and recently documented (Daufresne & Boët, 2007), indicating that the consequences of climate warming on riverine fish communities are becoming increasingly obvious (Parry *et al.*, 2007). Besides, recent studies emphasized that the viability of some fish populations with temperature-dependent sex determination can be compromised through alterations in their sex ratios, as a response to temperature fluctuations of the magnitude predicted by climate change (Ospina-Álvarez & Piferrer, 2008).

Lipid contents are known to be dependent on temperature conditions (Hochachka & Somero, 2002; Kamler, 2008), and the absorption rate of yolk lipids typically increases with temperature (Jaworski & Kamler, 2002). More precisely, yolk lipids act as the principal source of metabolic organic energy for egg development and participate in embryonic tissue formation in freshwater fishes (Kamler, 2008). The importance of free amino acids as a significant energy substrate in metabolism and during embryogenesis in marine fishes has been suggested by many studies (Kamler, 2008), but neutral lipids (mainly triglycerides) are the dominant endogenous energy source during embryonic development in freshwater species, and in numerous marine ones (*e.g.* 80–90% in gilthead seabream *Sparus aurata* L. yolk-feeding larvae: Ronnestad *et al.*, 1994). In addition, triglycerides are the major lipid class for energy storage in freshwater fishes (Weber *et al.*, 2003), and their dynamics are particularly important for fish condition and future population success (Adams, 1999). They are known to play a role as energy reserves (especially during non-feeding and reproductive periods) and as regulators of body density, cellular metabolism, detoxication, behaviour and reproduction (Morris & Culkin, 2000). Triglycerides deficiency has previously been shown to negatively affect gonad development and fecundity, and inadequate lipid reserves have been related to reduced reproductive effort for several fish species, primarily through reduced fecundity and in altered quality of eggs and larvae (Weber *et al.*, 2003).

Bullheads (Cottidae, Scorpaeniformes) comprise a family of small benthic fishes found throughout Europe, in high-order streams with cold oxygen-rich water (Reyjol *et al.*, 2007), where it is usually associated with brown trout *Salmo trutta* L. (Maitland, 2000). In France, bullhead *Cottus gobio* L. mainly dwell in areas with mean annual air temperatures of *c.* 11° C (Pont *et al.*, 2005; Pont 2006). Because of its low mobility and high fidelity to sampling site and its coldwater preferences, and also because it does not benefit from massive restocking operations in contrast to *S. trutta*, *C. gobio* is a very reliable target species for investigating the potential effects of climate warming on French coldwater fish populations. The present study was conducted on female *C. gobio* to (1) document the effects of temperature on biological (soma fresh

mass, gonad fresh mass and egg number and diameter) and biochemical (muscle and gonad triglyceride content) indicators of life-history strategy, (2) determine whether related changes in these indicators were affected by natal-stream thermal regimes and (3) investigate whether or not trade-offs were apparent among life-history indicators. Indeed, a trade-off between growth and reproduction, or between fecundity and egg diameter, has been repeatedly documented in the study of life-history theory (Stearns, 1992) and it was therefore interesting to test if these classical forms of trade-offs were identifiable in this experimental work. Moreover, it was also interesting to investigate whether or not a trade-off could be identified between biochemical life-history indicators (*i.e.* muscle and gonad triglyceride content), as, as far as it is known, it has never been documented before for freshwater fishes and could provide a finer resolution of the analysis compared with classical biological indicators. The first hypothesis was that growth and reproduction would be optimal at natal-stream temperatures. The second hypothesis was that a population originating from a more variable thermal environment would be better adapted to an increase in water temperature than a population originating from a relatively stable thermal environment. The third hypothesis was that a trade-off between reproductive and growth features, either biological or biochemical, or between different facets of reproduction (*i.e.* fecundity and egg diameter) should be apparent here.

MATERIALS AND METHODS

TARGET SPECIES, STUDY AREA AND SAMPLING SITES

The study was carried out in the Bez River basin (France) [Fig. 1(a)]. This basin drains 275 km² of relatively undisturbed forest and brush land, ranging in elevation from 479 to 1781 m (mean annual flow 4.5 m³ s⁻¹). Two brooks were considered for the present work: Borne and Nonnières [Fig. 1(a)]. These brooks were chosen because they are known to shelter abundant densities of *C. gobio* (Borne: 2.2–3.8 individuals m² and Nonnières 1.0–2.7 individuals m²; Abdoli *et al.*, 2005). Habitat conditions are very close in Borne and Nonnières; the microhabitat characteristics (in terms of water depth, water velocity and substratum composition) are typical of the preferential habitat of *C. gobio*; reaches with relatively steep slopes and coarse substratum, mainly cobbles and large stones (Maitland, 2000) (Table I). The mean temperature was similar for Borne (8.78° C) and Nonnières (8.46° C), but due to the complex hydrology of the Bez Basin (*i.e.* karstic water input for Nonnières), water temperature was more stable in Nonnières (mean daily temperature: 4.5–11.5° C, c.v., = 0.21) than in Borne (mean daily temperature: 0.5–19.2° C, c.v. = 0.48) [Fig. 1(b)]. The levels of nutritional resources (macro-invertebrates) were close for the two brooks (Table I). Based on the whole of these habitat characteristics, the assumption was made that the variability in thermal regimes constituted the main difference between the Borne and Nonnières Brooks.

EXPERIMENTAL DESIGN

Cottus gobio were collected at two sampling sites [one in each brook; Fig. 1(a)] using electrofishing (DEKA 3000 Lord apparatus; www.efko-gmbh.de), from 17 to 28 November, 2003. Only sexually mature fish were collected (total length, L_T , ≥ 71 mm; Abdoli *et al.*, 2005). During the transport to the laboratory, fish were put into two plastic tanks (one for each population) filled with natural water and kept artificially oxygenated. In the laboratory, fish were dispatched into three rows of six plastic tanks (80 × 50 × 50 cm). Within each row, each

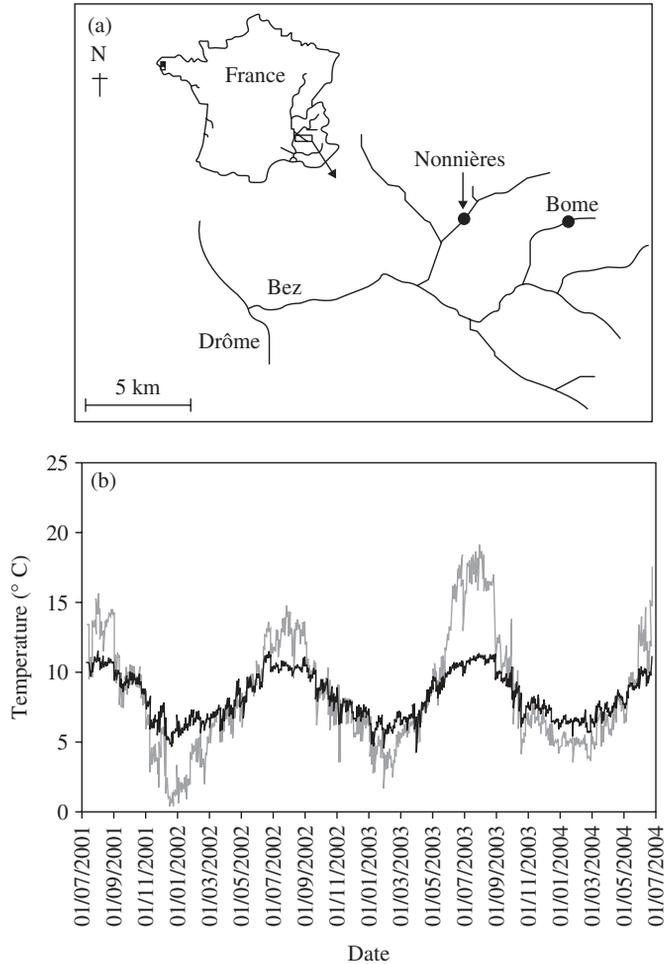


FIG. 1. (a) Study area ((●), sampling sites). (b) Illustration of the thermal regime time in the Borne (●) and Nonnières (●) Brooks.

tank was alternately filled with fish from one of the two populations (Borne, PopB; Nonnières, PopN). Eleven fish were placed in each tank after an acclimatization period of 10 days. A total of 198 fish (11 fish, six tanks and three rearing temperatures) were thus used for the experiments. Tank densities were higher than the densities observed in the brooks, but ensured a reasonable number of samples for statistical analyses. Some plastic half-cylinders (length = 12 cm; diameter = 8 cm) were added in each tank to mimic shelters. More shelters than fish were provided (13 *v.* 11), so that competition for space was negligible. This was verified by daily observation at different times of day, during which no aggressive behaviour among *C. gobio* was observed. *Ad libitum* feeding using defrosted chironomids *Chironomus* sp. was provided daily, following recommendations made by Jeevanthi De Silva (1985) for the species. Both the L_T and the mass (M , g) were randomly distributed among tanks (one-way ANOVA, both L_T and M : $F_{17,180}$, $P > 0.05$). A cooling machine was connected to each row to keep water temperature constant. One row was maintained at 7° C (T_1), one at 9° C (T_2) and one at 12° C (T_3). The lowest temperature (T_1) was chosen to be as close as possible to the mean temperature *C. gobio* would have experienced in the brooks during the rearing

TABLE I. Summary of the main habitat variables for *Cottus gobio* from the two sampling sites: Borne and Nonnières Brooks

Habitat variable	Borne	Nonnières
Site length (km)	2.3	1.5
Mean elevation (m)	862	679
Temperature [mean \pm s.d. ($^{\circ}$ C)]*	8.78 \pm 4.21	8.46 \pm 1.79
Temperature (c.v.)	0.48	0.21
Substratum (D ₁₀)	21.4	19.9
Substratum (D ₅₀)	66.1	58.7
Substratum (D ₉₀)	208.3	188.8
Mean water depth at low flow (cm)	10.2	12.5
Mean river width at low flow (m)	3.5	3.7
Mean river slope (m km ⁻¹)	4.3	3.4
Mean water velocity at low flow (cm s ⁻¹)	43.3	43.1
Food resource (benthos) (individuals m ⁻²)	8600	9100

*From 1 July 2001 to 30 October 2003 (before fish sampling). c.v., coefficient of variation; D₁₀, D₅₀ and D₉₀, the 10, 50 and 90% quantiles of the grain size distribution.

period, *i.e.* 6.6 $^{\circ}$ C. The water temperature was checked every hour using MINILOG[®] data loggers (VEMCO; www.vemco.com). Each row of tanks worked as a water recirculation system, so that water did not need to be replaced during the whole study duration. Tests of physical and chemical characteristics of the water (conductivity, pH, DOC, nitrates and phosphates) were made weekly to ensure that no significant variation in quality occurred. JBL[®] tests (<http://www.jbl.de/factmanger/index.php>) were used for nitrates and phosphates concentrations. As tanks were situated in a greenhouse, a natural cycle of illumination was applied.

QUANTIFICATION OF BIOLOGICAL AND BIOCHEMICAL INDICATORS

At the end of the rearing period (20 April), all fish were killed. The killing procedure took place tank by tank: all fish from a given tank were sampled and placed for 5 min in a 0.5 g l⁻¹ tricaine methane sulphonate solution (Sandoz MS-222; www.sandoz.com) before dissection. Fish were then weighed, measured and dissected. The two gonads as well as a percutaneous biopsy (50–100 mg) of dorsal muscles (twitch fibres) were taken from each female. One entire gonad (always the left one) was used for counting the eggs, while the other one (always the right one) was used for assays of triglycerides. Both the gonads and muscle tissues used for triglyceride assays were immediately stored in capped vials at -75 $^{\circ}$ C until the assay. The second gonad was placed in a vial with Gilson fluid in order to harden eggs and dissolve ovarian membranes (Abdoli *et al.*, 2005). After 1 month in the Gilson fluid, eggs were poured into a Petri dish and maintained in a small volume of water to prevent desiccation. Diameter was determined using a binocular microscope fitted with an integrated ruler. Triglycerides were extracted according to Hervant *et al.* (2001) and assayed using specific test combinations (Boehringer-Mannheim, Roche Applied science; www.roche-applied-science.com). Assays were performed in a recording spectrophotometer (Beckman DU-6; www.beckman.com) at 25 $^{\circ}$ C. The accuracy of each analysis was tested by assaying samples with and without an added internal standard. Triglyceride content was expressed in mg of triglycerides g⁻¹ of fresh mass (mg g⁻¹).

STATISTICAL TREATMENT

ANCOVA was used to examine the effects of temperature (three modalities: 7, 9 or 12° C) and population (two modalities: PopB or PopN) on allometric indicators of growth and reproduction for female *C. gobio*. For growth, either the soma fresh mass (M_S ; the fish mass before dissection) or the muscle triglyceride content was used as dependent variables, while L_T was used as adjustment covariate. This procedure enabled the removal of the effect of fish size on allometric indicators (Glazier, 1999). For reproduction, the gonad fresh mass (M_G), the number (N_E) and mean diameter (D_E) of eggs and the gonad triglyceride content were used as dependent variables. Following Glazier (1999), the M_S was used as adjustment covariate when the M_G and N_E were predicted, while the muscle triglyceride content was used as adjustment covariate when the relative triglyceride allocation to gonads was examined. The N_E was used as adjustment covariate when mean D_E was predicted.

Mixed models were applied. For this purpose, the residual variance was allowed to vary according to both temperature and population. The possible correlation of the dependent variable among animals from the same tank was taken into account by introducing the tank as a random effect on the mean value of the dependant variables. All models were structured as follows:

$$y_{ij} = \beta_0 + \sum_{f=1}^p \beta_f x_{fij} + (\mu_{0j} + e_{kij}), \quad (1)$$

where y_{ij} was the value of the dependent variable for the i -th fish in the j -th tank, x the explicative fixed effects (*i.e.* temperature, population, adjustment covariate and all possible interactions), β_0 the intercept, β_f the fixed effects associated to the i -th fish in the j -th tank, μ_{0j} the random effect of the tank j on the mean value of y_{ij} conditioned to the fixed effects and e_{kij} the residual error term specific to the group k (one population at one rearing temperature). To improve variance homogeneity, all continuous variables were ln-transformed prior to analysis. For easier interpretation, they were also centred on their grand mean.

For each dependent variable, the highest order model was built, *i.e.* with all interaction terms between fixed effects and all variance–covariance statistics. Heteroscedasticity according to temperature and population was tested using likelihood ratio tests (LRT), based on the likelihood estimation of variance statistics (Goldstein, 2003). When non-significant, variance statistics were removed from the model. Restricted maximum likelihood estimates were used to test the significance of the fixed effects. All terms were successively removed from the model, and the final model was selected based on the Akaike information criterion (AIC) and Bayesian information criterion (BIC). Because AIC and BIC gave congruent results in the present case, only models selected using BIC are presented here (Burnham & Anderson, 2002). The significance of each explicative term that remained in the final model was examined using non-sequential F -tests. The denominator d.f. was computed using the Kenward–Roger method. This method is similar to the Satterthwaite estimation, and it uses the covariance statistic to adjust the d.f. according to the level of dependency among observations made on same tank (Littell *et al.*, 2006). Responses observed in one tank were often found to be uncorrelated (*i.e.* null or not positive

covariance). In such cases, it follows that the denominator d.f. simplify to the residual d.f. When an interaction between the adjustment covariate and temperature or population was detected, local tests (*i.e.* at the first, second and third quartile of the adjustment covariate) were performed, to examine the effect of the factor with regard to the level of the adjustment covariate. Tests for linear trends in the response of the dependent variables to increasing temperature were also conducted, using orthogonal polynomials to describe the linear and quadratic components in the responses.

Statistical analyses were carried out using SAS (www.sas.com).

RESULTS

At the end of the rearing phase, 169 *C. gobio* of the initial total of 198 were alive. The *post mortem* determination of sex indicated that, among these 169 fish, 84 were females and 85 were males. Because mortality during experiment was high for two tanks of PopB at T₂ due to an unidentified reason (no obvious diseases, problem with water quality or problem with the water recirculation system), only females from T₁ and T₃ were used to compare the effects of temperature between populations ($n = 53$). For the same reason, the analyses investigating the effect of temperature alone were performed on fish from PopN only ($n = 49$). The total number of females considered for statistical treatment was 76. It was not possible to use all 84 females alive at the end of the experiment because some samples (eight) were unusable for triglycerides assays. The total number of females used (76) does not match the sum of the number of females used in the two separate analyses ($53 + 49 = 102$), simply because some females were used in both analysis (those belonging to PopN and reared at T₁ and T₃).

EFFECTS OF TEMPERATURE ALONE (P_oPN)

Soma fresh mass

Temperature did not alter the mean value of M_S but significantly altered the allometric relationship between M_S and L_T , indicating a different effect of temperature on body condition according to size [Table II and Fig. 2(a)]. Contrast analyses revealed that as temperature increased significantly, female M_S decreased proportionally as the size of the female increased (tests at the first, second and third quartile of the adjustment covariate for a linear component and a quadratic one: $F_{1,49}$, $P > 0.05$; $F_{1,49}$, $P < 0.01$; $F_{1,49}$, $P < 0.01$ and $F_{1,49}$, $P > 0.05$; $F_{1,49}$, $P > 0.05$; $F_{1,49}$, $P > 0.05$).

Muscle triglyceride content

Muscle triglyceride content varied significantly more at T₂ and T₃ than at T₁ (LRT for a model allowing respectively variance heterogeneity among T₁, T₂ and T₃, or variance heterogeneity between T₁ and both T₂ and T₃: χ^2 , d.f. = 2, $P > 0.05$; χ^2 , d.f. = 1, $P < 0.05$).

The muscle triglyceride content did not vary with L_T , but was significantly altered by temperature (Table II). Contrast analysis indicated a non-linear reduction of muscle triglyceride content with increasing temperature [Table II and Fig. 2(b)]: there was a significant difference between T₃ and T₂ ($F_{1,9,19}$, $P < 0.001$), but not between T₁ and T₂ ($F_{1,14,4}$, $P > 0.05$).

TABLE II. Summary of the models predicting different indicators of the relative somatic investment by *Cottus gobio* as a function of rearing temperature alone

	M_S			Muscle TG		
	d.f. _n , d.f. _d	F	P	d.f. _n , d.f. _d	F	P
BIC		-112.1			-40.4	
Covariate	1, 49	722.01	<0.001			
Temperature	2, 49	1.27	>0.05	2, 12.2	36.63	<0.001
Covariate \times temperature	2, 49	4.23	<0.05			
Linear polynomial	1, 49	1.55	>0.05	1, 16	33.21	<0.001
Quadratic polynomial	1, 49	0.82	>0.05	1, 12.2	19.93	<0.001

M_S , fresh soma mass; BIC, Bayesian information criterion; TG, triglycerides; d.f._n, d.f. for the numerator; d.f._d, d.f. for the denominator.

Gonad fresh mass

The relative mass allocation to gonads varied significantly more at T_2 and T_3 than at T_1 (LRT for a model allowing respectively variance heterogeneity among T_1 , T_2 and T_3 , or variance heterogeneity between T_1 and both T_2 and T_3 : χ^2 , d.f. = 2, $P < 0.01$; χ^2 , d.f. = 1, $P < 0.01$).

Temperature significantly altered the relative mass allocation to gonads (Table III). Contrast analysis indicated that the relative mass allocation to gonads decreased as temperature increased. This trend, however, was non-linear: the reduction was higher between T_2 and T_3 than T_1 and T_2 [Table III and Fig. 3(a)].

Number of eggs

The allometric relationship between N_E and M_S was significantly more heterogeneous at T_2 and T_3 than at T_1 (LRT for a model allowing respectively variance heterogeneity among T_1 , T_2 and T_3 , or variance heterogeneity between T_1 and both T_2 and T_3 : χ^2 , d.f. = 2, $P < 0.001$; χ^2 , d.f. = 1, $P < 0.001$).

Temperature significantly altered the allometric relationship between N_E and M_S : N_E decreased as temperature increased but this effect decreased as M_S increased [Table III and Fig. 3(b)]. This effect partly resulted from higher variance heterogeneity for lighter females according to temperature (*i.e.* variance heterogeneity resulting from the level of the adjustment covariate which was not taken into account in the model).

Mean egg diameter

Mean D_E was significantly more variable at T_3 than at T_1 or T_2 (LRT for a model allowing respectively variance heterogeneity among T_1 , T_2 and T_3 , or variance heterogeneity between T_3 and both T_1 and T_2 : χ^2 , d.f. = 2, $P < 0.001$; χ^2 , d.f. = 1, $P < 0.001$).

The analysis indicated that mean D_E controlled for the N_E was significantly reduced as temperature increased (Table III). Contrast analysis indicated a significant linear trend in the reduction of mean D_E with an increasing temperature [Table III and Fig. 3(c)].

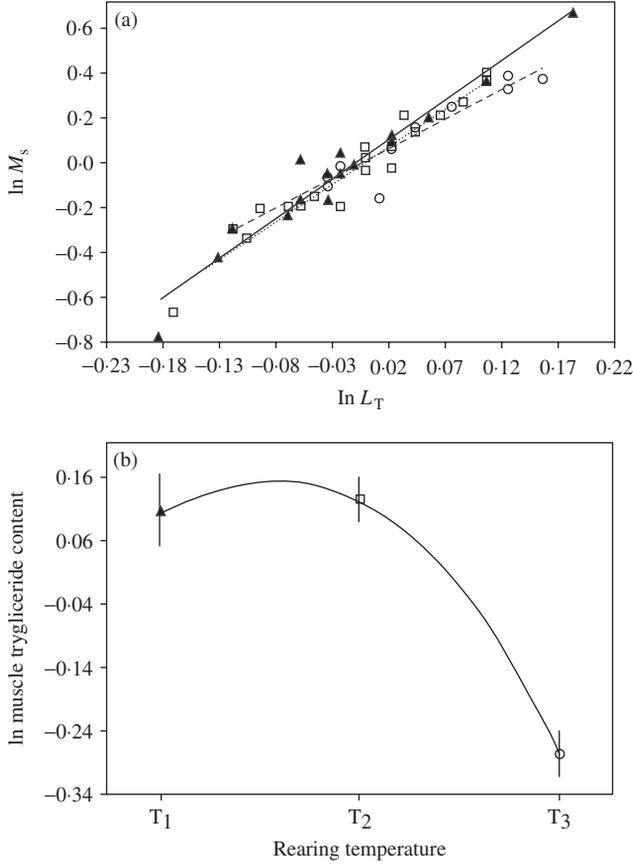


FIG. 2. Illustrations of the effect of rearing temperature [\bullet , T_1 (7° C); \square , T_2 (9° C); \circ , T_3 (12° C)] alone on the indicators of relative somatic allocation: (a) fresh soma mass (M_S) and (b) muscle triglyceride content (mean \pm s.d.) for the Nonnières Brook population. The dependent variable is either directly represented as a function of rearing temperature (b) or as a function of the adjustment covariate if significant (a) [allometric relationship between M_S and total length (L_T)] (\circ , T_1 ; \square , T_2 ; \blacktriangle).

Gonad triglyceride content

The gonad triglyceride content varied significantly more at T_2 and T_3 than at T_1 (LRT for a model allowing respectively variance heterogeneity among T_1 , T_2 and T_3 , or variance heterogeneity between T_1 and both T_2 and T_3 : χ^2 , d.f. = 2, $P > 0.05$; χ^2 , d.f. = 1, $P < 0.05$).

The analysis failed to reveal a relationship between the triglyceride contents of gonads and muscle. Temperature change, however, significantly decreased gonad triglyceride content (Table III). Contrast analysis indicated a linear reduction of the gonad triglyceride content with increasing temperature [Table III and Fig. 3(d)].

EFFECTS OF TEMPERATURE AND POPULATION

The M_S , M_G , N_E and mean D_E did not vary according to population. The effects of both temperature and the adjustment covariate were similar to those observed

TABLE III. Summary of the models predicting different indicators of the relative reproductive investment by *Cottus gobio* as a function of rearing temperature alone

	M_G			N_E			D_E			Gonad TG		
	d.f., d.f.-n,	F	P	d.f., d.f.-n,	F	P	d.f., d.f.-n,	F	P	d.f., d.f.-n,	F	P
BIC		110.1			134							
Covariate	1, 21.2	69.7	<0.001	1, 33.5	18.52	<0.001	1, 33.1	5.6	<0.001			
Temperature	2, 41.9	33.76	<0.001	2, 35.2	8.41	0.001	2, 24.9	73.5	<0.01	2, 39.1	11.84	<0.001
Covariate \times temperature				2, 34.2	4.13	<0.05						
Linear polynomial	1, 44.3	66.58	<0.001	1, 34.7	14.93	<0.001		7.27	<0.05	1, 45.8	23.82	<0.001
Quadratic polynomial	1, 36.6	5.07	<0.05	1, 33.5	0.76	>0.05		2.27	>0.05	1, 39.7	0.8	>0.05

M_G , gonad mass; N_E , number of eggs; D_E , mean egg diameter; BIC, Bayesian information criterion; TG, triglycerides; d.f., d.f. for the numerator; d.f.-n, d.f. for the denominator.

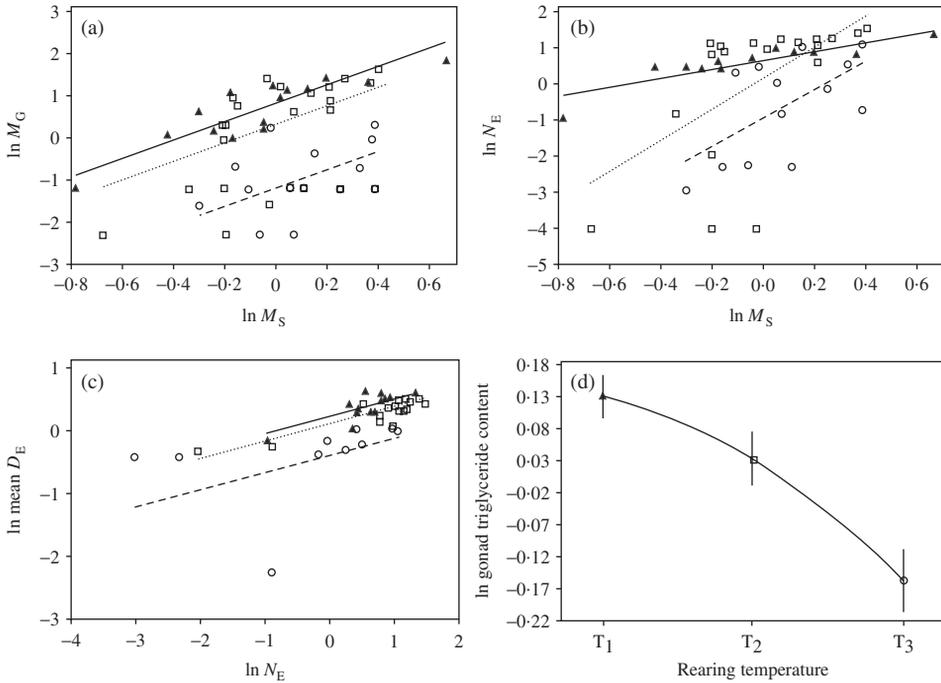


FIG. 3. Illustrations of the effect of rearing temperature [T_1 (7°C), T_2 (9°C) and T_3 (12°C)] alone on the indicators of relative reproductive allocation: (a) gonad mass (M_G), (b) number of eggs (N_E), (c) mean egg diameter (D_E) and (d) gonad triglyceride content (mean \pm s.d.) for the Nonnières Brook population of *Cottus gobio*. The dependent variable is either directly represented as a function of rearing temperature (d) or as a function of the adjustment covariate if significant [allometric relationship between M_G and fresh soma mass (M_S) (a), N_E and M_S (b) and D_E and N_E (c)].

in the analysis with temperature alone, and there were no significant interactions between these variables and the population of origin (Tables IV and V). For clarity, the corresponding illustrations were not represented, but the tendencies are similar to those obtained for temperature alone (Figs 2 and 3).

In contrast, muscle triglyceride content and gonad triglyceride content significantly varied with population (Tables IV and V). For muscles, it was higher for PopB than for PopN [Fig. 4(a)]. For gonads, it was higher for PopN than for PopB [Fig. 4(b)].

DISCUSSION

TEMPERATURE EFFECTS ALONE

Normal feeding for *C. gobio* occurs for a temperature range of $0\text{--}26^\circ\text{C}$, and the upper lethal temperature has been shown to be $>30^\circ\text{C}$ (Elliott & Elliott, 1995). Even if phenotypic plasticity was necessarily present between the populations studied by Elliott & Elliott (1995) and those studied here, there is a high probability that the temperatures applied here were far below the upper limit of normal feeding or death for the species. That being so, the present study showed that an increasing

TABLE IV. Summary of the models predicting different indicators of the relative somatic investment of *Cottus gobio* as a function of both rearing temperature and population of origin

	M_S			Muscle TG		
	d.f. _n , d.f. _d	F	P	d.f. _n , d.f. _d	F	P
BIC		-112.1			-22.9	
Covariate	1, 53	733.46	<0.001	1, 50	4.54	<0.05
Temperature	2, 53	2.75	>0.05	1, 50	104.53	<0.001
Population				1, 50	32.41	<0.001
Covariate \times temperature	2, 53	6.45	<0.05	1, 50	0.02	>0.05
Covariate \times population				1, 50	5.50	<0.05
Temperature \times population				1, 50	3.03	>0.05
Covariate \times temperature \times population				1, 50	7.41	<0.01

M_S , soma mass; BIC, Bayesian information criterion; TG, triglycerides; d.f._n, d.f. for the numerator; d.f._d, d.f. for the denominator.

temperature had a strong negative effect on all indicators of somatic and reproductive allocation except for soma fresh mass. The lowest rearing temperature applied was close to the temperature the fish would have experienced in the brooks for the same period (*i.e.* 6.6° C). Thus, the results agreed with those of Haugen & Vollestad (2000), who showed that populations of grayling *Thymallus thymallus* (L.) performed better in terms of early life-history indicators (*i.e.* growth rate and survival during the period of first feeding) at the temperature they normally experience in nature. The present results also parallel those by McManus & Travis (1998), who showed that distinct temperatures (*i.e.* 23 or 29° C) did not affect body mass of male sailfin molly *Poecilia latipinna* (Lesueur), for four populations raised under controlled laboratory conditions. A causal explanation to this phenomenon remains unclear, but in the present case a hypothesis could be that the duration of the experiment was too short for changes in body mass to become apparent. Numerous studies have examined the effect of temperature on metabolic rates and tissue and organ physiology within lethal limits, showing that performance is usually maximized near the thermal optimum, but drastically decreases with decreasing or increasing temperatures, parallel to an important decrease in enzymatic activity rates (Hochachka & Somero, 2002). The relationship between body temperature and metabolism can be described by an asymmetric function, where metabolic rate is maximized at an intermediate temperature: the thermal optimum (Angilletta *et al.*, 2002). From an ecological standpoint, this bell-shaped curve can be explained by the principle of allocation (Huey & Slatkin, 1976): there is a trade-off between the maximum and the range of the performance. An individual that performs well (relatively to other individuals) in its optimal temperature zone should perform relatively poorly at non-optimum temperatures (Huey & Hertz, 1984). As *C. gobio* is known to be a coldwater species, it is very probable that the lowest temperature applied here (7° C) was close to the thermal optimum for fish from these populations, and that the higher temperatures (9 and 12° C) corresponded with non-optimum temperatures (right side of the bell-shaped curve), reducing metabolism and negatively affecting growth and reproduction.

TABLE V. Summary of the models predicting different indicators of the relative reproductive investment of *Cottus gobio* as a function of both rearing temperature and population of origin

	M_G			N_E			D_E			Gonad TG		
	d.f. _n , d.f. _d	F	P	d.f. _n , d.f. _d	F	P	d.f. _n , d.f. _d	F	P	d.f. _n , d.f. _d	F	P
BIC		120.00			93.80			20.80			-33.30	
Covariate	1, 27.8	69.13	<0.001	1, 26.8	33.38	<0.001						
Temperature	1, 28	82.98	<0.001	1, 26.1	45.99	<0.001	1, 17.6	5.02	<0.05	1, 50	36.89	<0.001
Population										1, 50	50.08	<0.001
Covariate × temperature				1, 26.1	10.46	<0.01	1, 33	48.73	<0.001			
Covariate × population												
Temperature × population												
Covariate × temperature × population												

M_G , gonad mass; N_E , number of eggs; D_E , mean egg diameter; BIC, Bayesian information criterion; TG, triglycerides; d.f._n, d.f. for the numerator; d.f._d, d.f. for the denominator.

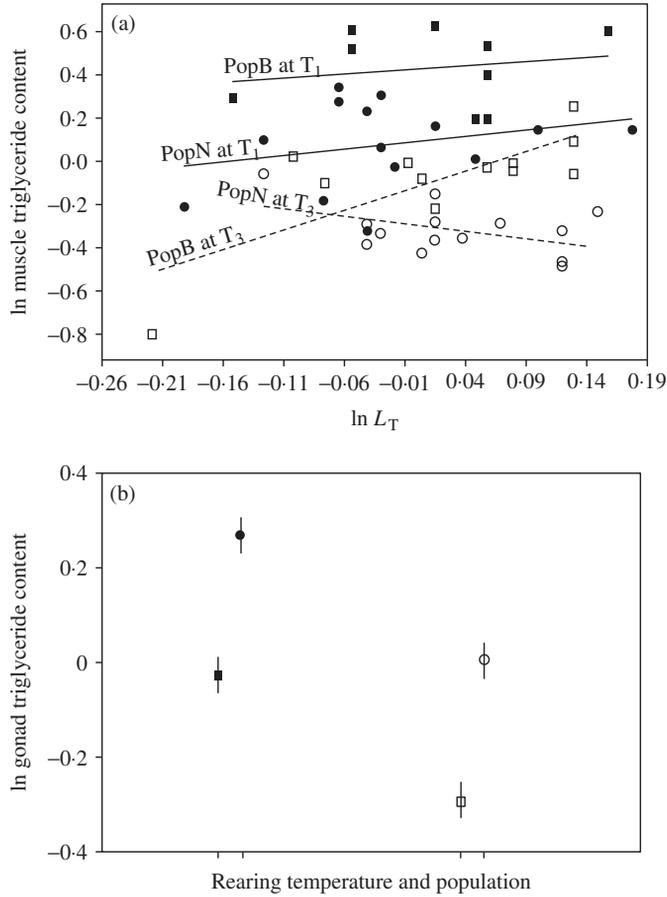


FIG. 4. Illustrations of the effect on *Cottus gobio* of both temperature [T_1 (7°C) and T_3 (12°C)] and population [Borne (PopB) and Nonnières (PopN)] on biochemical indicators: triglyceride content of (a) muscle and (b) gonad (mean \pm S.D.). The dependent variable is either directly represented as a function of rearing temperature (b) or as a function of the adjustment covariate if significant [allometric relationship between muscle triglyceride content and total length (L_T)] (a). ((●), PopN at T_1 ; (○), PopN at T_3 ; (■), PopB at T_1 ; (□), PopB at T_3).

POPULATION EFFECTS

As far as is known, there are very few studies that have focused on the effects of both temperature and population on ectotherms' life-history strategies, and that include biochemical indicators. One of them is the study by McManus & Travis (1998), who investigated the effects of temperature and salinity on the lipid storage and reproductive allocation of four natural populations of *P. latipinna* (male individuals). They found the amount of stored lipids to be altered by temperature variation but the direction and magnitude of the effect substantially varying among populations (a significant interaction between temperature and population was detected). In the present study, no interaction between temperature and population was observed, implying that the two populations reacted similarly to an increase in temperature.

The muscle triglyceride content, however, was higher for the individuals originating from the population of Borne than for those originating from the population of Nonnières, while the opposite was true for gonads (significant effect of the population term but not of the interaction term). In natural conditions, spatial variation in life-history strategy of riverine fish populations has previously been reported for length at maturity (Lobón-Cerviá *et al.*, 1997 for *S. trutta*), fecundity (Jonsson & Jonsson, 1999 for *S. trutta*) and egg diameter (Heins & Baker, 1987 for blacktail shiner *Notropis venustus* Girard), but not for lipid contents. As shown in the methods section, the main difference between the habitat characteristics of Borne and Nonnières was the higher variability of the thermal regime in Borne. As fish from Borne live in a more unstable thermal environment than fish from Nonnières, this result agreed with life-history predictions that fishes living in unpredictable environments should be characterized by reproductive traits corresponding to 'pioneering' species, among which low fecundity, small eggs (Winemiller & Rose, 1992), and, plausibly, low egg quality (*i.e.* with low energy stores). As far as is known, there are very few studies that have focused on the effects of temperature stability on energy (mainly lipids) allocation. Nevertheless, Hervant *et al.* (2001) and Hervant & Renault (2002) proposed a general adaptive strategy for groundwater organisms, including a reduced metabolism, low energetic requirements, low reproductive allocation and large body stores (energy allocation in organs such as muscles, liver in vertebrates). These adaptive responses, favouring investment in somatic energy stores instead of gonadic allocation, might be proposed for numerous organisms as an efficient strategy in harsh and unpredictable environments (Hochachka & Somero, 2002), and could be considered as a security trade-off allowing to withstand hazardous conditions. The present study clearly showed that muscle and gonad triglyceride contents significantly varied with population and temperature stability. As fish from Borne live in a more unstable thermal environment than fish from Nonnières, the results agreed with this metabolic hypothesis: *C. gobio* living in unpredictable environments (Borne) should be characterized by both large somatic triglyceride stores (mainly in muscles) and low gonad triglyceride contents.

ABSENCE OF TRADE-OFFS

No trade-off between growth (as measured by soma fresh mass or muscle triglyceride content) and reproduction (as measured by gonad fresh mass, fecundity, mean egg diameter or gonad triglyceride content) was observed. It is likely that under particularly favourable environmental conditions (*e.g.* *ad libitum* food and no competition for space or food, as in the present study), trade-offs disappear, because a major limiting factor is no longer present (Stearns, 1992). Besides, even though negative correlation between growth and fecundity has been repeatedly documented, this is usually in reference to situations in which the effects of reproduction on later growth are measured, a different form of trade-off to the one studied here. This study can be termed an analysis of income trade-off because it balances investment in growth and reproduction during the same season, while other studies can more aptly be termed capital trade-off, balancing investment in one season against investment in a future season (Lardner & Loman, 2003). Similarly, no consistent trade-off between egg number and egg size was observed, despite that such trade-offs have often been reported in both natural and experimental studies for various taxa (*e.g.*

snakes, fishes and mammals), and usually serve as reference in life-history theory (Stearns, 1992). In natural environments, variations in egg number and egg size among and within populations of freshwater fishes have been shown to be regulated by local environmental variables, and by latitude and combinations of its covarying factors (Lobón-Cerviá *et al.*, 1996, 1997). Because a trade-off between egg number and mean egg diameter was observed in the field for the two populations studied, the absence of trade-off in this study is probably due to the absence of limitation of food and space.

IMPLICATIONS FOR *COTTUS GOBIO* POPULATIONS OF CLIMATE WARMING

The lowest rearing temperature was close to the temperature *C. gobio* would have experienced in the brooks for the same period. Therefore, the results suggested that an increase in water temperature in response to climate warming may negatively affect *C. gobio* populations of Borne and Nonnières Brooks, as well as other populations from the Bez Basin experiencing similar thermal conditions. For a mean air warming of 3.7° C in France (Scenario ARPEGE METEO France at 100 years; <http://www.cnrm.meteo.fr/gmgec/arpege/arpege.html>), a warming in the Drôme catchment (where the two studied brooks are located) of 2.0–2.5° C during winter and 4.0–4.5° C during summer has been predicted (Pont, 2006). According to the present results, this range of increase in air temperature and subsequent corresponding increase in water temperature is enough to negatively affect the *C. gobio* populations living in these brooks, notably for the Borne Brook where temperature regulation *via* karstic water inputs does not occur. This phenomenon should be all the more pronounced as *C. gobio* has restricted movement capacities (Knaepkens *et al.*, 2005) and great difficulties moving upstream of river obstructions such as dams or weirs, even with a low heights (18–20 cm) (Utzinger *et al.*, 1998). The examination of biochemical indicators, however, suggested that populations living in a more variable thermal environment (*e.g.* the Borne Brook) may be better fitted to cope with increasing temperatures. A monitoring programme is now needed to determine whether or not these slight differences in life-history strategy will allow local populations to maintain themselves.

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