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Morgane Novak, Asghar Abdoli, Didier Pont, Pierre Sagnes. Otolith asymmetry as a proxy of thermal stress in cold water fish: do observations on natural populations of *Cottus gobio* meet experimental results?. *Cybium : Revue Internationale d'Ichtyologie*, 2013, 37 (4), pp.267-280. halsde-00924856

HAL Id: halsde-00924856

<https://hal.science/halsde-00924856v1>

Submitted on 13 Nov 2024

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Otolith asymmetry as a proxy of thermal stress in cold water fish: do observations on natural populations of *Cottus gobio* meet experimental results?

by

Morgane NOVAK* (1), Asghar ABDOLI (2), Didier PONT (3) & Pierre SAGNES (1)

Abstract. – An increase of water temperature due to global warming is expected to be stressful for many aquatic species such as cold-water fishes. Otolith asymmetry is known to be a proxy of fish stress. However, *Cottus gobio* Linnaeus, 1758 inhabiting potentially stressing (i.e. warm) thermal conditions did not present a higher level of asymmetry in their otoliths than other ones. Local adaptations to thermal stress of the different populations studied or a weak *a priori* definition of the thermal tolerance of this species are discussed.

Résumé. – L'asymétrie des otolithes comme marqueur de stress thermique chez les poissons d'eau froide : les observations sur des populations naturelles de *Cottus gobio* concordent-elles avec les résultats expérimentaux ?

Une augmentation de la température de l'eau, due par exemple au réchauffement climatique, devrait être stressante pour de nombreuses espèces aquatiques telles que les poissons d'eau froide. Une asymétrie au niveau des otolithes est connue pour être un marqueur du stress chez les poissons. Cependant, les individus de *Cottus gobio* Linnaeus, 1758, vivant dans des conditions potentiellement stressantes (chaudes) ne présentent pas de plus forts niveaux d'asymétrie sur leurs otolithes que les autres. Ces résultats peuvent être expliqués par des adaptations locales des différentes populations étudiées au stress thermique ou par une mauvaise définition de la tolérance thermique de cette espèce.

Key words

Cottidae

Cottus gobio

Temperature

Global warming

Fish stress

Fluctuating asymmetry

Streams and rivers are relatively shallow and well-mixed systems; they easily exchange heat with the atmosphere. Therefore, global warming will have direct impacts on

aquatic habitats, such as an elevation of some river temperatures (i.e. average and/or maximum temperatures), which will obviously modify metabolic and physiological processes of poikilotherm species. A direct consequence is that global warming currently threatens some species, such as cold-water fish. The ability to detect stresses (e.g. thermal ones) before they seriously affect threatened populations could enhance the effectiveness of conservation programs, and recent studies have shown that within-individual morphological variability may provide a valuable early indicator of environmental stress (e.g. Leary and Allendorf, 1989). Among morphological characters, fish otoliths were described to be more asymmetric under high stress conditions (Somarakis *et al.*, 1997). Although Le Moal (2007) stated that “*stress is almost impossible to define, at least in simple terms*”, Iwama (1998) suggested to characterize fish stress as “*the response of the cell, or organism, to any*

demand placed on it such that it causes an extension of a physiological state beyond the normal resting state”. We will hereafter refer to this definition of stress.

Bullhead *Cottus gobio* Linnaeus, 1758, is usually defined as a “stenotherm species preferring cold waters” (Blanck *et al.*, 2007). It is often defined as a “sentinel” species (Dorts *et al.*, 2011), as it is very sensitive to various environmental stresses. For instance, *C. gobio* is a morphologically variable species (Koli, 1969), and a change or a strong deviation from its optimum developmental conditions (e.g. a thermal stress) is expected to produce an asymmetry of some morphological or anatomical characters (Downhower *et al.*, 1990; Allenbach, 2010). *C. gobio* populations are generally supposed to live in water temperatures ranging from 1 to 16°C (Adamicka, 1991), with a maximum tolerate temperature of 20°C (i.e. maximum temperature that the species can withstand during a few days exposure, see Leuven *et al.*, 2011). In 3 closely related species, Brown (1989) experimentally showed that the routine metabolic rate rapidly increased [i.e. stress increased *sensu* Iwama (1998), see above] between 15 and 20°C. The temperature at which feeding stops and the lethal temperature for *C. gobio* have been experimentally

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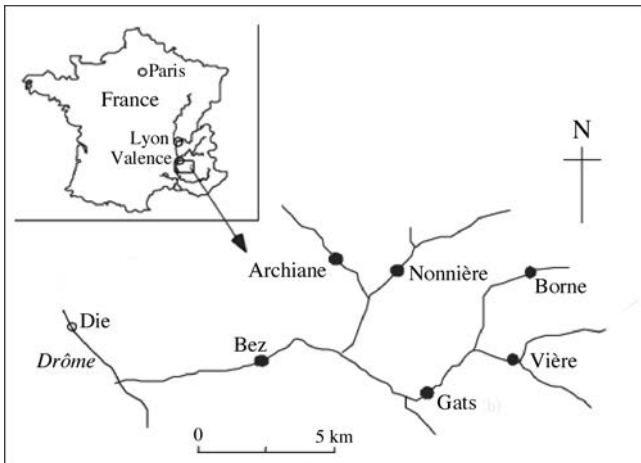


Figure 1. - Localization of the Bez River network (Rhône drainage basin) and the 6 sampling sites (points).

shown to be close to 26°C and 30°C, respectively (Elliott and Elliott, 1995). Moreover, a rapid increase of water temperature was also previously shown to induce fish stress [e.g. 1°C/30 min (Elliott and Elliott, 1995)].

Therefore, we hypothesized that *C. gobio* specimens living in thermal conditions (a) exceeding 16°C (and, *a fortiori* approaching 20°C and lethal values) or (b) presenting punctual swift increases of water temperature should be stressed and should present more asymmetries than others on their otoliths [see examples in other species in Somarakis *et al.* (1997) and Albusaidi *et al.* (2010)]. If this hypothesis was verified, asymmetry in otoliths could be defined as a proxy of thermal stress for this species, and could be used in future investigations about global warming effects on such threatened species.

MATERIAL & METHODS

The study was carried out in the Bez River network (France), a tributary of the Drôme River, in the south-western French Alps (Fig. 1). The 6 sampling sites were chosen for three main reasons. First, they represent typical habitat for *C. gobio*, nearly undisturbed by human activities. Second, hydraulic and trophic conditions were similar between sites and brown trout *Salmo trutta* Linnaeus, 1758 was the only species caught in association with *C. gobio* at all sites (Abdoli, 2005); this let us presume that *C. gobio* specimens inhabit-

ing the different sites were not differently stressed regarding these factors. Third, the different tributaries showed a strong thermal heterogeneity and the temperature regime varied between sites (Abdoli *et al.*, 2007). These different temperature regimes were previously shown to affect growth parameters of *C. gobio* between the sampling sites (Kielbassa *et al.*, 2010). Moreover, previous results (unpublished yet) showed a genetic segregation between the different populations of this small river network. Therefore, *C. gobio* specimens born at a given site are expected to inhabit this site, and subsequently to withstand the associated thermal condition during their whole life.

Water temperature was recorded continuously at each site (one measurement every 2 hours) from 13th July 2001 to 2nd July 2002. These data allowed to classify the 6 sampling sites from their thermal characteristics (Tab. I): (1) “cold” sites, with daily temperatures always lower than 16°C and low temperature increasing rates (Archiane and Nonnière): we hypothesized that this situation should be the less stressful for *C. gobio* specimens, because extreme thermal values were far from lethal ones (i.e. specimens from these sites should present less asymmetries than specimens from other sites); (2) “warm” sites, with daily temperatures which often exceeded 16°C, sometimes exceeded 20°C and relatively high temperature increasing rates (Bez and Gâts): we hypothesized that this situation should be the more stressful for *C. gobio* specimens (i.e. specimens from these sites should present more asymmetries than specimens from other sites); (3) “intermediate” sites, with annual temperatures which never reached 20°C and intermediate temperature increasing rates (Vièrè and Borne): we hypothesized that this situation should present intermediate stressing conditions for *C. gobio* specimens, as daily thermal values sometimes exceeded 16°C (but less often than in case 2).

In September 2002, a battery-powered electrofishing gear (DEKA 3000) was used to capture 343 *C. gobio* specimens (cold sites: N = 108, intermediate sites: N = 92, warm

Table I. - Temperature characteristics of the six sampling sites (July 2001 to July 2002, Bez River network). Standard deviations in brackets.

	Archiane	Nonnière	Borne	Vièrè	Gâts	Bez
Min (°C)	5.2	-0.1	0.0	-0.1	0.0	3.2
Max (°C)	9.9	15.7	19.2	16.8	20.2	20.2
Mean summer 2001 (°C)	8.2(0.6)	10.5(2.0)	11.6(2.7)	11.7(2.2)	12.9(2.6)	12.7(2.0)
Mean autumn 2001 (°C)	7.0(0.6)	5.7(2.7)	5.8(3.2)	5.8(3.3)	6.5(3.6)	8.0(2.0)
Mean winter 2001 (°C)	6.6(0.6)	3.4(1.8)	3.3(2.0)	2.7(1.8)	3.7(2.2)	6.5(1.2)
Mean spring 2002 (°C)	7.3(0.5)	7.4(1.8)	7.9(2.3)	7.9(2.3)	9.2(2.7)	9.7(2.4)
Number of hours $\geq 16^\circ\text{C}$ (cumulated)	0.0	0.0	130.0	14.0	338.0	250.0
Number of hours $\geq 20^\circ\text{C}$ (cumulated)	0.0	0.0	0.0	0.0	6.0	2.0
Maximum temperature increasing rate (°C/30 min)	0.3	0.5	0.6	0.6	1.2 (once > 1)	1.0

Table II. - Mean values (in mm) and standard deviations (in brackets) of otolith measurements, direct asymmetry ($R_i - L_i$, mm) and relative asymmetry ($|(R_i - L_i) * 100 / (R_i + L_i)|$) (see text) for 343 bullhead specimens inhabiting 3 different thermal regimes (Bez River network) and distributed in three size classes (large “L”, intermediate “I”, small “S”, see text).

	Groups		Raw values			Direct asymmetry			Relative asymmetry		
	Size class	N	Area	Length	Width	Area	Length	Width	Area	Length	Width
Cold sites	L	39	1.78(0.16)	3.05(0.28)	1.32(0.14)	0.00(0.04)	0.00(0.10)	0.00(0.03)	0.01(0.99)	0.05(1.64)	-0.02(1.26)
	I	62	1.29(0.13)	2.24(0.26)	0.94(0.09)	0.01(0.01)	0.02(0.05)	0.01(0.02)	0.29(0.85)	0.38(1.56)	0.19(1.25)
	S	7	0.55(0.05)	0.82(0.09)	0.48(0.03)	0.00(0.00)	0.01(0.02)	0.00(0.02)	0.27(0.52)	0.27(1.44)	0.29(1.71)
Intermediate sites	L	51	1.89(0.22)	3.30(0.34)	1.38(0.19)	0.01(0.04)	0.01(0.11)	0.01(0.04)	0.33(1.11)	0.31(1.62)	0.34(1.96)
	I	36	1.33(0.11)	2.35(0.22)	0.96(0.08)	0.01(0.03)	0.01(0.07)	0.00(0.03)	0.18(0.99)	0.28(1.63)	0.08(1.71)
	S	5	0.58(0.05)	0.90(0.10)	0.48(0.04)	-0.01(0.01)	-0.01(0.03)	-0.01(0.02)	-0.67(0.45)	-0.57(1.29)	-0.78(1.86)
Warm sites	L	35	1.79(0.17)	3.16(0.28)	1.30(0.14)	-0.01(0.03)	0.01(0.09)	-0.02(0.04)	-0.26(0.79)	0.14(1.50)	-0.66(1.44)
	I	96	1.48(0.10)	2.62(0.18)	1.07(0.08)	0.01(0.04)	0.04(0.08)	-0.01(0.04)	-0.06(1.03)	0.32(1.54)	-0.44(1.65)
	S	12	0.75(0.12)	1.25(0.21)	0.58(0.09)	0.00(0.00)	0.01(0.03)	-0.01(0.02)	0.05(0.34)	0.46(1.13)	-0.35(1.05)

sites: N = 143), which were immediately anaesthetized to death. The right and left sagittae (i.e. the biggest otoliths) were removed from each specimen just after fish sampling and immersed in glycerin. Later, in laboratory, otoliths were photographed under a binocular microscope and their length (main axis), width (second axis) and area were measured using a free image analysis software (ImageJ, available at: <http://rsb.info.nih.gov/ij/>). In order to avoid any size effect in the results, differences in the data values between right and left otoliths were standardized before subsequent analyses. The square roots of areas were calculated, and all parameters were transformed using the following equation (Downhower *et al*, 1990):

$$|(R_i - L_i) * 100 / (R_i + L_i)|$$

with R_i and L_i = values of parameter i measured on the right and left otolith, respectively.

Considering that, for a given species, otolith morphometry can vary between specimens of different lengths (Lombarte and Leonart, 1993), three length groups were made for subsequent comparative analyses: small fish [< 50 mm total length (Lt)], intermediate fish (50-80 mm Lt) and large fish (> 80 mm Lt) (see number of fish in each group in table II).

When data were numerous enough, test for normality and homogeneity of variance (Kolmogorov-Smirnov and Bartlett tests) were performed. As these values were not normally distributed ($p < 1.22 * 10^{-09}$) and did not conform to variance homogeneity except for length ($p < 0.003$ and $p = 0.77$), Kruskal-Wallis tests were used to assess potential asymmetries in the otoliths of specimens of the different length groups inhabiting the different thermal regimes. Bonferroni corrections were applied. Statistical analyses were carried out using R (v 2.12.1, R Development Core Team, 2010).

RESULTS AND DISCUSSION

None of the tests showed significant results considering the Bonferroni correction (Tab. III), i.e. there were no significant differences of symmetry (Tab. II) between specimens inhabiting the three different thermal regimes, for any of the three length groups, neither on the length, the width nor on the area of otoliths.

Therefore, *C. gobio* specimens inhabiting potentially stressing thermal conditions did not present higher levels of asymmetry in their otoliths than other ones, although some specimens had to withstand thermal conditions that were previously described as stressful for this species (in the Gâts site for instance, water temperatures used to exceed 20°C and 16°C during 6 and 338 hours a year, respectively). This result suggests two hypotheses.

First, specimens inhabiting “warm” habitats were actually stressed without showing any significant asymmetry of their otoliths. As stated by Rasmuson (2002), a low fluctuating asymmetry is not the “*unambiguous measure of well being and good genes that has been claimed*”. Homeostasis and canalization are two mechanisms for overcoming fluctuations during development that could decrease phenotypic variation and subsequently limit asymmetry of “stressed” specimens (Rasmuson, 2002). In this case, other proxies of

Table III. - Results of Kruskal-Wallis tests (H, df, p value) used to assess potential asymmetries on otolith area, length and width, for three size classes of bullheads (large “L”, intermediate “I”, small “S”, see text) inhabiting three different thermal regimes.

Length class	Area			Length			Width		
	H	df	p value	H	df	p value	H	df	p value
L	0.24	2	0.88	2.30	2	0.32	0.78	2	0.68
I	0.62	2	0.73	1.12	2	0.57	1.40	2	0.50
S	7.64	2	0.02	1.27	2	0.53	2.00	2	0.51

C. gobio stress have to be defined in the context of global warming.

Second, specimens inhabiting “warm” habitats were not stressed at all. This would suggest that the stressing thermal ranges given in the literature (e.g. defined from maximum tolerate temperature, temperature increasing rate, lethal temperature and temperature at which feeding stops) could be underestimated. One explanation is that they were experimentally determined and, perhaps, could not be directly considered in natural population studies. In the Bez River network, we observed that fish acclimatization is usually slower than shown in Elliott and Elliott (1995): the increasing rate of temperature was only once higher than 1°C/30 min (Gâts site). Moreover, this increasing rate was observed between 8.6°C and 13.2°C (i.e. a temperature range far below the potential stressing value of 16°C). Finally, as *C. gobio* is usually considered as a stationary fish species (Smyly, 1957), the populations studied could be adapted to local thermal regimes since several generations [see Reyjol *et al.* (2009) for an example of adaptations of *C. gobio* life-history indicators in the same river network]. This was partly confirmed by genetic data (unpublished results), showing a segregation between the different populations of this small river network. In this case, the threatening effects of global warming on *C. gobio* populations (especially those from “cold” sites) should depend on the speed of warming and the adaptive abilities of local populations.

Acknowledgments. – We wish to thank P. Bady, G. Grenouillet, S. Mérigoux, J.M. Olivier, N. Roset and P. Tedesco for assistance in the field, and N. Péru for the help in statistical analyses. We also thank an anonymous reviewer for his helpful comments, which have significantly improved the manuscript.

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