

EFFECT OF POLLUTION ON THE FLUCTUATING ASYMMETRY IN THE GRASS GOBY *ZOSTERISESSOR OPHIOCEPHALUS PALLAS*, 1811 AS CONTAMINATION INDICATOR OF EASTERN TUNISIA.

Research

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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ABSTRACT

In this work, we study the effect of habitat degradation and environmental stress on fluctuating asymmetry, a morphological parameter of the grass goby *Zosterisessor ophiocephalus* Pallas, 1811, sampled from eastern coasts of Tunisia. Ninety-six specimens of *Zosterisessor ophiocephalus* were collected during September-November 2012 from two sites: one polluted (Khniss) and one control (Chebba) in eastern littoral zone of Tunisia. Six bilateral metric and two meristic characters were used to assess fluctuating asymmetry. Statistical analysis showed that assessed asymmetries are fluctuating. FA of sub-orbital and post-orbital distances, orbital diameter, and pelvic and pectoral fin lengths, are higher in polluted site compared to control. *Chemical and organic pollution from sewage discharge seems to be responsible for this high FA*. Many actions should be taken to save the khniss ecosystem: control the quality of waste water, push the current treatment of certain plants to tertiary treatment, act on the current discharge to improve the conditions of his release in sea and move rejection seaward to a minimum depth of 15 m to prevent pollution reaching the coast and for better dilution and dispersion of pollutants

Keywords: Pollution; Fluctuating asymmetry; *Zosterisessor ophiocephalus*; sewage discharge

INTRODUCTION

Fishes are often used as sentinel species for monitoring aquatic environmental health (Franco *et al.* 2002) because they are greatly influenced by external environmental conditions especially demersal fish as our studied specie. Stress causes in fish a significant release of cortisol (Wendelaar 1997) which has negative effects on various physiological functions (reproduction, growth, immunity) if they are maintained over time (Pottinger & Pickering 1997).

Early detection of sub-lethal effects may permit biologists and resource managers to take remedial actions that could prevent further habitat degradation and population declines. Thus, there is great interest in understanding and monitoring sub-lethal biological responses that are faithful indicators of environmental quality (Allenbach 2011). One morphological trait that has been linked to habitat alteration and environmental stress is fluctuating asymmetry (FA). FA is defined as small, random deviations away from perfect bilateral symmetry. This deviation from perfection is thought to reflect the genetic and environmental pressures experienced throughout development, with greater pressures resulting in higher levels of asymmetry (Palmer & Strobeck 2003). Thus, significant increased levels of FA in a population may indicate that individuals have difficulty to maintain pre-

cise development, resulting in negative effects on the population over time (Markow 1995). Measurements of FA have become a popular technique for quickly and inexpensively examining sub-lethal stress and FA has been examined in a variety of taxa. In fish, stressors range from general (physical habitat changes, urban influence, undefined or mixed stressors) to specific (heavy metals, temperature, radiation, acidification, pesticides) (Allenbach 2011). Although many studies of fluctuating asymmetry in fishes have been conducted in several regions of the world, apart from the study by Mabrouk et al, 2014, no studies using this biomarker have been conducted up to now in Tunisia.

In the east of Tunisia, we analysed the fluctuating asymmetry in fish, and asked if anthropogenic disturbance, such as sewage discharge, increase FA compared to unpolluted area.

Materials and methods

Chosen species is the grass goby, *Zosterisessor ophiocephalus* (Pallas 1811), a demersal fish species belonging to the Gobiidae family. It is cavity nester species, living on soft-bottoms in shallow brackish water. The size of mature females and males was respectively 12.65 cm and 13.91 cm total length (TL) at 2.9 and 3.5 years (Hajji et al. 2013).

Ninety six specimens of *Zosterisessor ophiocephalus* were collected during September-November 2012 from the littoral zone using a long handled sweep net (40 cm x 55 cm with a 0.5 mm mesh). Sampling sites were selected in eastern coast of Tunisia (Fig. 1). The industrialized coast of Khniss (35°42'30"N, 10°49'30"E), (K), was located in Monastir Bay which is a semi-enclosed lagoon lying on the Eastern shore of Tunisia and does not exceed 3 m depth up to 2 km off the shoreline. This littoral fringe forms a receptacle of multiple wastewater effluents from urban zones. Previous geochemical studies have shown that this littoral is affected by heavy metal pollution (Sassi et al. 1998; Sahnoun 2000). Because of the weak hydrodynamics prevailing in the bay, nutrient-rich wastewaters discharged at numerous points along the shoreline have progressively led to eutrophication (Sassi et al. 1998).

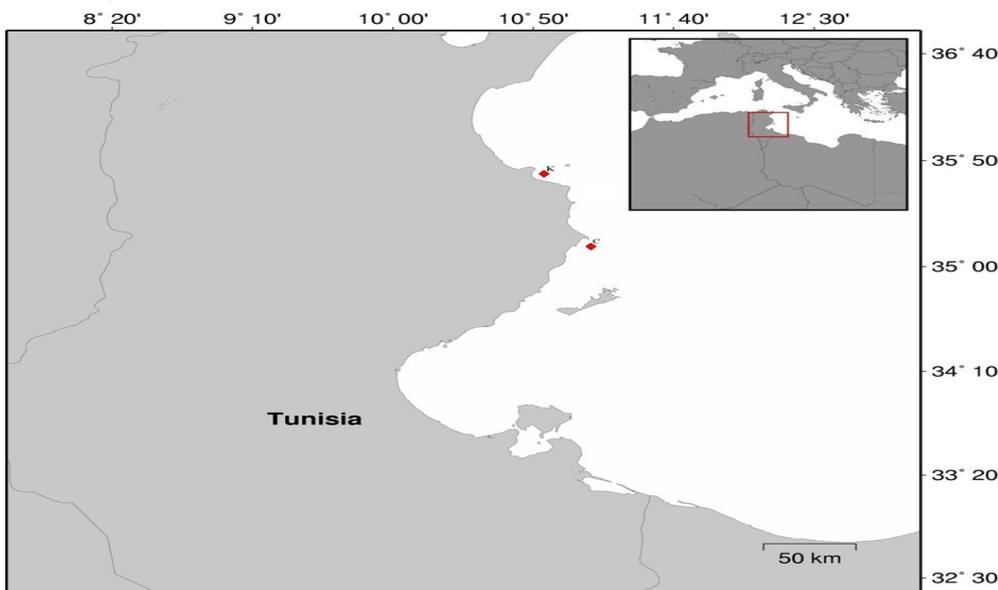


Figure 1. Map of the study area, showing the sampling stations: C, Chebba; K, Khniss.

The coast of Chebba (35°12'07"N; 11°08' 08"E) (C), located about 70 km south of Khniss, was used as a reference because of the absence of industrial and human activities

A total of 6 bilateral metric and 2 meristic characters were used to assess fluctuating asymmetry: as follows: (1) Length of the pre-orbital distance (labeled "PO"), measured from the mouth to the anterior edge of the orbit; (2) sub-orbital distance (labeled "SO"), measured from the Southern edge of the orbit to the end of the body; (3) orbital diameter (labeled "O"); (4) pre-pectoral distance from pectoral fin (labeled "PP"); (5) length of pelvic fin (labeled "LV"), the length of pectoral fin (labeled "LP"); (7) total number of pectoral fin rays (labeled "NRP"); and (8) total number of pelvic fin rays (labeled "NV") (Fig. 2). Most characters were counted and measured under a binocular dissecting microscope. For specimens too large to fit under a microscope, a magnifying glass was used. Characters were chosen from among those used in previous studies investigating fluctuating asymmetry in fish (Hechter et al. 2000; Lucentini et al. 2002).

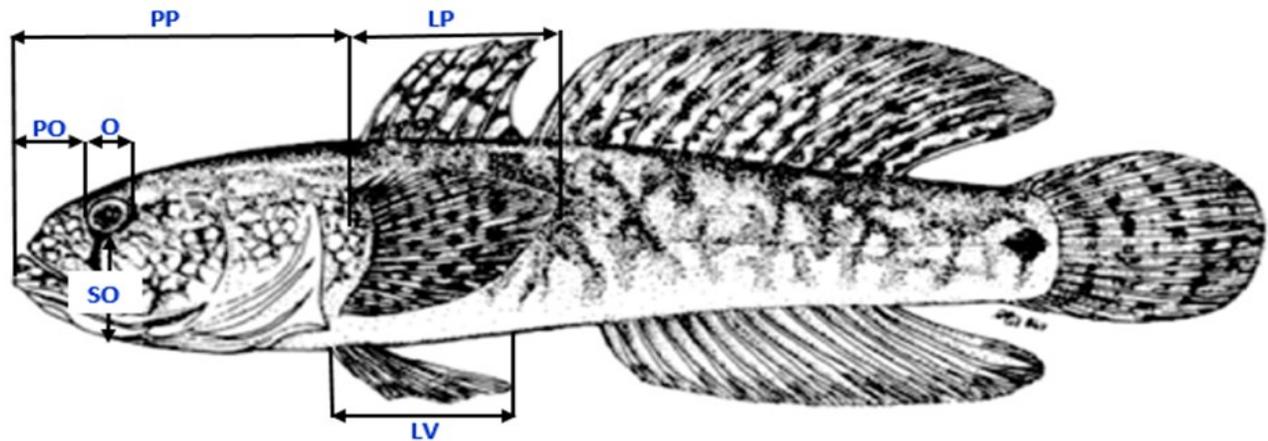


Figure 2. Metric traits used to assess the fluctuating asymmetry: PO: pre-orbital distance, SO: sub-orbital distance, O: orbital diameter, PP: pre-pectoral distance, LV: length of pelvic fin, LP: length of pectoral fin.

Like FA, measurement error is often small and normally distributed around a mean of zero (Merilä & Björklund 1995). Measurement error can artificially inflate estimates of fluctuating asymmetry or completely obscure its detection (Palmer *et al.* 1994) and so care was taken to control for it. All sets of measurements were made exclusively by one person to avoid possible inter-observer variability (Lee 1990; Kozlov *et al.*, 2016). All measurements were replicated ‘blind’ and averaged to the nearest 0.01 mm. Several days elapsed between each complete set of measurements on each fish as this has been shown to give the most reliable estimate of measurement error (Palmer 1994). To assess the relative contribution of measurement error each measurement was repeated two times on each side in 96 individuals. Two-way mixed model ANOVAs were carried out (Palmer & Strobeck 1986) for each trait with the factors ‘sides’ (S) and ‘repeat’ (R) as fixed and ‘individual’ (I) as random. This tests for whether variation between sides is significantly greater than the variation attributable to measurement error.

Data were also tested for alternative forms of asymmetry (directional and antisymmetry) and size dependency which can all inflate fluctuating asymmetry estimates (Palmer & Strobeck 2003). Directional asymmetry was tested for by a two-tailed one-sample *T*-test against a mean of zero. Antisymmetry was tested by examining evidence for platykurtic or bimodal curves in R (Right) – L (Left) scatter plots and running a Kolmogorov–Smirnov test which looks for departures from normality (Palmer & Strobeck 1986). Size dependency, variation of the magnitude of asymmetry due to the difference in trait size (Palmer 1994), was tested for by a Spearman rank correlation between absolute FA ($R - L$) and the average of both sides $[(R + L)/2]$. The FA-1 index defined by Palmer and Strobeck (1986) was applied and calculated as the absolute mean difference in length between right and left sides (mean $|R - L|$). As each pair of measurements was repeated twice, averages of the two estimates were used. FA-1 was calculated both for individuals within each of the populations and across all individuals.

We classified individuals into 2 subsamples according to their total length; great individuals group (g), fish with total length exceeding 13 cm size of matured individual (Hajji *et al.* 2013) and small individuals group (s), fish with a total length less than 13 cm. The FA indexes were compared among the two populations by means of Nested ANOVA test with site (two levels, fixed factor) and size (two levels, fixed factor nested to site).

Results and discussion

No directional asymmetry or antisymmetry was found in the data. The Kolmogorov–Smirnov test for normality revealed a significant departure from normality for LP. However, the distribution was leptokurtic (high, narrow peak), indicating little or no antisymmetry. Antisymmetry can be identified by a significant platykurtic (broad-peaked or bimodal) distribution (Palmer & Strobeck 1986). The leptokurtotic distribution that we observed was similar to that of other studies studying FA of pectoral fins in salmonid fishes (Palmer 1996; Leary *et al.* 1985). In traits PO, SO and LV, statistically significant size dependency (L–R increases by skull size) was detected and

the Spearman correlation a level just below 5%. When normalizing for size, this dependency disappeared and therefore it was decided to work with size normalized FA5 [(L-R)/(L/2+R/2)] in case of those traits in the further analysis. The two-way ANOVA, used to test whether between sides variation was significantly greater than variation due to measurement error, showed that, in most cases, results were significant ($P < 0.001$) suggesting traits to be repeatable.

Differences between the right and the left are distributed around zero for sampling individuals of Chebba (C) and Khniss (K).

High mean values of FA were detected in (K) site for O, PO, SO, LV and NV (fig. 3). Chebba population has a low fluctuating asymmetry. Fluctuating asymmetry detected in Chebba is probably due to minor inconsistencies during development such as the quality and quantity of food, extreme temperatures, parasites, diseases and behavioural stress imposed by interactions with conspecifics (Markow 1995).

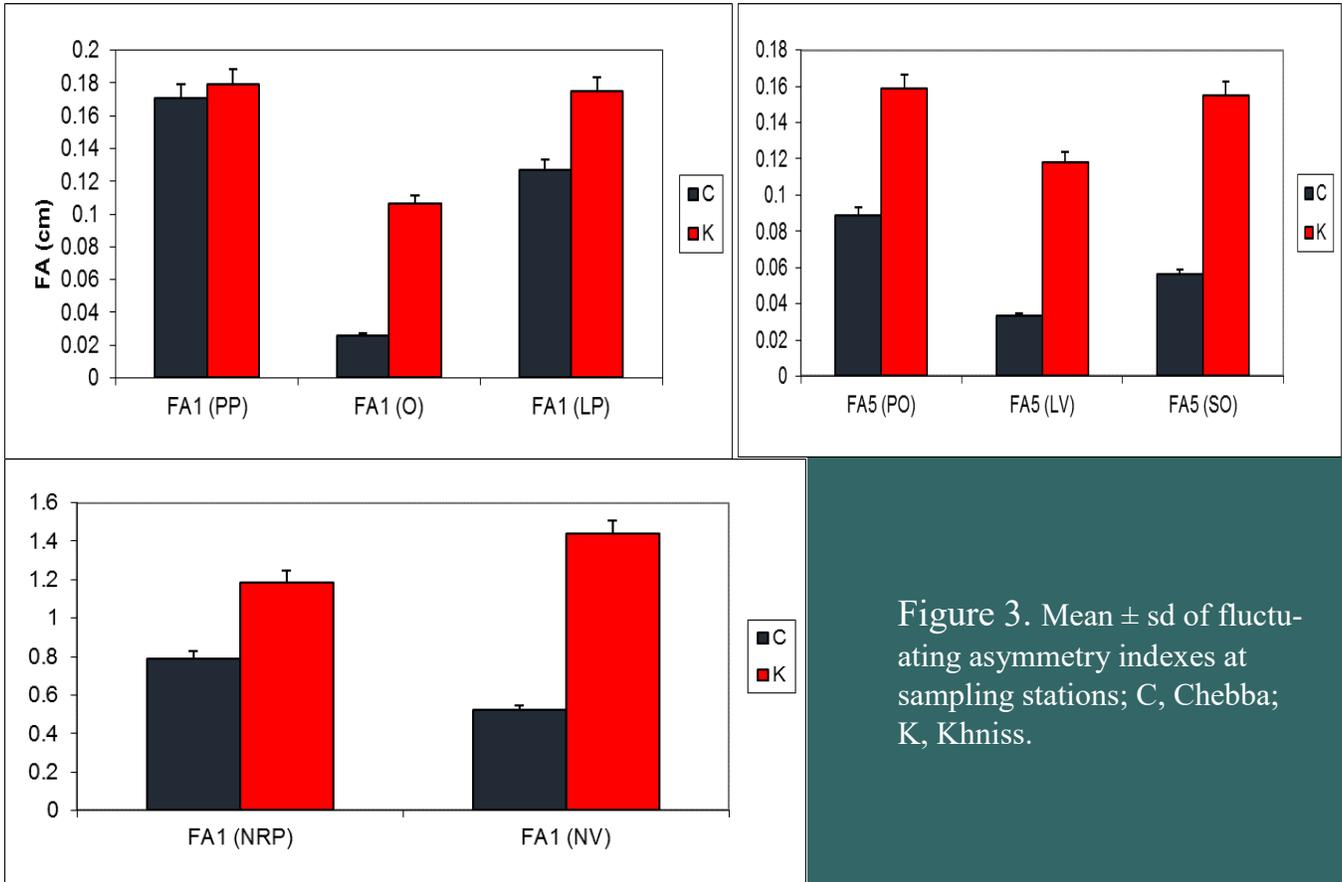


Figure 3. Mean \pm sd of fluctuating asymmetry indexes at sampling stations; C, Chebba; K, Khniss.

In all characters, mean asymmetry of *Z. ophiocephalus* from Khniss station were higher than those of Chebba except for prepectoral distance (PP) (Nested analysis of variance; table 1).

	Df	MS	F	p	Tukey test
FA1 PP					
Station	2	0.001	0.076	0.784	n. s.
Size (Station)	2	0.001	0.064	0.938	n. s.
Residuals	92	2.033			
FA5 PO					
Station	2	0.771	71.26	<0.001	K>C
Size (Station)	2	0.005	0.492	0.613	n. s.
Residuals	92	0.011			

FA1 (O)					
Station	2	0.287	65.52 3	<0.00 1	K>C
Size	2	0.018	4.057	0.020	s(C)=g(C)<s(K)
Residuals	92	0.004			
FA5 (SO)					
Station	2	0.647	62.49 1	<0.00 1	K>C
Size	2	0.026	2.473	0.090	n. s.
Residuals	92	0.010			
FA5 LV					
Station	2	0.358	78.67 0	<0.00 1	K>C
Size	2	0.007	1.632	0.201	n. s.
Residuals	92	0.005			
FA1 (LP)					
Station	2	1.108	75.13 5	<0.00 1	K>C
Size (Station)	2	0.204	13.85 7	<0.00 1	s(C)=g(C)=s(K) <g(K)
Residuals	92	0.015			
FA1 (NRP)					
Station	2	49.78 3	69.75 1	<0.00 1	K>C
Size	2	1.783	2.498	0.088	n. s.
Residuals	92	0.714			
FA1 (NV)					
Station	2	55.90 4	137.2 06	<0.00 1	K>C
Size	2	0.154	0.377	0.687	n. s.
Residuals	92	0.407			

Khmiss station is highly influenced by the industrial discharges from the industrial zone of Monastir and obvious sources of urban discharges (Dahmane 2000). Previous geochemical studies have shown that this littoral zone is affected by heavy metal as Cadmium and zinc (Sassi *et al.* 1998; Sahnoun 2000). As a consequence of the weak hydrodynamics prevailing in the bay, nutrients-rich wastewaters discharged in numerous points along the shoreline have progressively led to eutrophication (Sassi *et al.* 1998) and are probably responsible for the high fluctuating asymmetry. These results are consistent with several previous studies that showed that chemical pollution can lead to high levels of fluctuating asymmetry (Franco *et al.* 2002; Romanov & Kovalev 2004). These results are not surprising since the chemical and organic pollution causes developmental instability and the presence of heavy metals (Cadmium, zinc) can lead to significant morphological disturbances (Bengtsson *et al.* 1998).

We also detect significant variations for orbital diameter (O) length and pectoral fin length (LV) between small and great groups nested to sites (Nested ANOVA; Table 1); for Khmiss population, gobies exceeding 13 cm were more asymmetric than small individuals. For Chebba population, no significant differences between those groups for all traits (Tukey test, table 1). It seems that fluctuating asymmetry increases with age since for population of Khmiss site, great individuals (older) are more asymmetric than smaller individuals (younger). Two hypotheses may be issued to explain these results. The first one is that older individuals were exposed longer to environmental conditions (pollution in this case), as the animal grows, it loses its stability due to unfavourable conditions (Thiam 2004). The second one is that larger individuals are not just older, but also have grown faster (due to increased nutrients in the sewage of the polluted waters), their faster growth rate at the cost of developing

symmetrically (Morris et al. 2012).

Fish from Khniss coast are subjected to a different type of environmental stress, which can be detected by the FA, and is probably due essentially to the presence of continuous discharge from local industrial activities and from the wastewater (Sahnoun 2000; Jebali *et al.* 2011; Annabi *et al.* 2013). Based on the highest value of asymmetry for the orbital diameter, pelvic fin length and pectoral fin length of grass goby recorded in this study, those parameters could be used as an effective biomarker of stress in the environment. Prepectoral distance, Pre-orbital distance, total number of pelvic fin rays and total number of pectoral fin rays showed lower percentage of asymmetry. It could be assumed that those parameters are less sensitive to change of environmental variables, including pollution. Finally, it is urgent to propose the best management plans to save ecosystems in the Khniss since many deterioration has been detected for *Posidonia oceanica* meadows (Sghaier *et al.* 2006) and for animals (Annabi *et al.* 2013; this study). The first action should be to control the quality of wastewater. It must lead to significant decrease of the discharge of particulate matter and inorganic nutrients in coastal waters. The second solution is to push the current treatment of certain plants to tertiary treatment, which aims to eliminate nutrients (N and P) and heavy metals from water (UNEP/WHO 1996). The third solution is to act on the current discharge to improve the conditions of his release in sea. Move rejection seaward a minimum depth of 15 m to prevent pollution reaching the coast for better dilution and dispersion of pollutants (Quétin & DeRouville 1986). Further dilution of sewage can be obtained by increasing the water depth or the distance of the outfall site. Currently there are rearrangement works at Khniss station, which aims to discharge wastewater farther from the coast and deepen the place of discharge for a better mixing of pollutants. Are these actions sufficient to improve the living conditions of aquatic organisms? Only new research on the composition of water and the biological traits of the aquatic organisms (such as AF) that inhabit this vulnerable ecosystem can give us elements of response.

Conclusions

The developmental stability of an organism is reflected in its ability to produce an "ideal" form under specific conditions. A number of studies suggest that development stability would present an inexpensive, rapidly applicable and sensitive stress indicator. In addition, it could be used as an early warning signal or as a tool for regular monitoring of the impacts of human activity on animal or plant species. The most commonly used measure of development instability is fluctuating asymmetry (AF). This study shows that environmental changes may increase the fluctuating asymmetry, which is the result of a deterioration of developmental homeostasis in adult morphology. So that, fluctuating asymmetry can be used to evaluate the effects of environmental stress on animal organisms.

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