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# FLUCTUATING ASYMMETRY AS AN INDICATOR OF ENVIRONMENTAL STRESS IN *TRACHURUS TRACHURUS* L. 1758.*

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## ABSTRACT

Fluctuating asymmetry (FA) occurs when slight non-directional differences appear between sides of a bilaterally symmetric structure or between paired structures on both sides of the body among individuals in a population. Fluctuating asymmetry (FA) has been used as a measurement of developmental stability and an indicator of stress in *Trachurus trachurus* populations. We are interested in the study of the variations of the fluctuating asymmetry levels between samples. We noted an increase in fluctuating asymmetry values with the growth of fish. We observed that males and females are respectively more stressed than immatures in the study of sexual variations of fluctuating asymmetry. The study also showed us that the individuals collected in the Mediterranean are more stressed than those collected in La Ria de Vigo. And that individuals harvested in winter and summer are respectively more stressed than those harvested in spring. Temperature is an important environmental determinant for *Trachurus trachurus*. This fish operates optimally within a very narrow temperature range and deviations from this could impose stress during development.

**KEYWORDS**: *Trachurus trachurus*; fluctuating asymmetry; stress;

developmental stability

**ABBREVIATIONS**: fluctuating asymmetry (FA), directional asymmetry (DA), right (R), left (L), right minus left (R-L), right more left (R+L).

## INTRODUCTION

In the late 1970s and early 1980s, many hydrobiologists published work in which high frequencies of abnormal individuals belonging to different zoological groups (insect larvae, fish and amphibians) were recorded. In most of this work, a relationship has been established between the appearance of deformed individuals and their presence in contaminated environments. This provoked and favored, at the level of the scientific community (Parsons 1961, 1962, 1990, 1992; Van Valen 1962; Palmer and Strobeck 1986; Leary and Allendorf 1989; Markow 1995; Clarke and Mckenzie 1997; Imasheva et al .. 1997; Roy and Stanton 1999; Kark et al. 2001; Mpho et al. 2002; Velickovic 2004), the use of fluctuating asymmetry for detect the disturbances which take place in ecosystems. Thus the analysis of fluctuating asymmetry levels as an indicator of environmental Stress and a measure of developmental instability has achieved great popularity (Waddington 1957; Zakharov 1989; Palmer 1994).

Fluctuating asymmetry (FA) occurs when slight non-directional differences appear between sides of a bilaterally symmetric structure or between paired structures on both sides of the body among individuals in a population. This phenomenon is caused by stress of genetic or environmental origin and constitutes a form of individual adaptation (Leary and Allendorf 1989; Parsons 1990; Alados et al. 1993; Van Dogen and Lens 2000; Lens et al. 2002). .The use of measuring fluctuating asymmetry as an instrument for detecting the degree of stress to which populations are subjected in aquatic ecosystems is promising (Palmer 1986; Eber and Brandl 1992; Kozhara 1994; Mitton 1995) .. In a study Lubchenko et al. (1991) showed that an ideal biomonitoring method should be able to detect a problem before it has a major impact in the ecosystem. The response of individuals to stress is easier to detect than the response of populations, communities or ecosystems since stress first

destabilizes the physiological processes and development of individuals. This has repercussions on its final morphology. These effects occur before other parameters indicating alteration at a higher level of integration (fecundity, abundance). Thus many scientists approve the use of fish as bioindicators for the assessment of environmental quality (Leary and Allendorf 1989; Graham et al. 1993a; Clarcke 1994; Vermeulen 1995). Today, fish are essential models in fundamental biology, as demonstrated by the use of certain species such as zebra fish, medaka and fugu in developmental biology. Ectothermal (for the most part) and incredibly plastic, fish have established and diversified all the adaptive bases both anatomical and physiological. This means that they are currently commonly used to detect environmental disturbances (Bruslé and Quignard 2001). Adaptation or developmental stability refers to the ability of an organism to combat environmental and genetic disturbances experienced during its ontogeny (Waddington 1942; Zakharov 1992; Clarcke 1998). Pollution is an abusive form of appropriation of the landscape, whatever the level of damage: local, regional, continental or global. The medium-term repercussions of human activities on the entire biosphere are made evident by a few shocking examples. One of the most dramatic is the link between the recent but widespread use of sprays and holes in the ozone layer. Chlorofluorocarbons have been used industrially since the 1950s. They are found today in the Mediterranean waters emerging from the Strait of Gibraltar, which remain identifiable in Atlantic waters thanks to their content of these molecules (Tenore and González 1975; López-Jamar 1981 ). *Trachurus trachurus* is an important commercial species. It is currently one of the three most important pelagic species in the European fish industry (Abaunza et al. 2003). It presents a great plasticity in its growth and has a great geographical distribution. In order to know the fluctuating asymmetry levels and to carry out comparative studies, La Ria de Vigo (Atlantic) and Murcia (Mediterranean) were chosen as sampling locations.

## MATERIAL AND METHODS

Study site description and sampling (Fig.1)

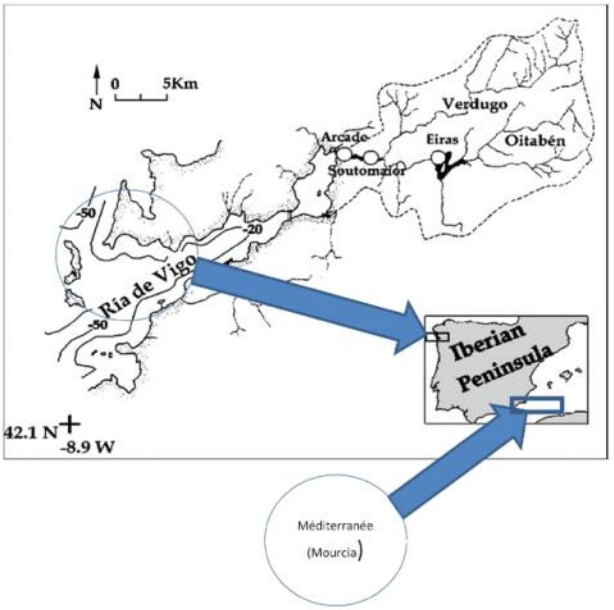
The fish were harvested in two different sites: La Ria de Vigo, located on the atlantic continental margin to the north-west of the Iberian peninsula and Murcia located to the south of the Iberian peninsula at the level of the Mediterranean sea (Fig.1).

Located between 8° 37' 00" and 8° 54' 00" west longitude and 42° 21' 00" and 42° 09' 00" north latitude, La Ria de Vigo covers an area of 176 $km^2$ where the climate characterizes the region. This humid Atlantic climate is characterized by mild summers and winters. Winter temperatures average between 5 and 9°C. Summer temperatures do not exceed 22°C (Fig. 1) (Maldonado and Zamarreno 1983). Due to the intense aquaculture activity due to its biological richness, the hydrographic network of La Ria de Vigo has been the subject of numerous studies (Tenore and González 1975; Lopez-jamar 1978 a, b, 1981, 1982, 1992; Blanton et al. 1984) over recent decades. La Ria de Vigo is under the considerable influence of the ocean waters of the Atlantic. A series of drowned valleys called Rias are located along the Galician coast (north/west of Spain) where most of the regional population is located due to the commercial importance of fishing (Blanton et al. 1984). In the past, only small towns located in the Rias, near certain beaches, concentrated most of the tourist activity. Today, rapid and uncontrolled urban and industrial development in certain areas is causing serious concern due to negative impacts on the environment.

In their work, certain authors (Hiecke et al. 1989; Voutsinou and Varmavas 1993) showed a horizontal distribution of heavy metals in the sediments of La Ria de Vigo. The greatest concentrations are recorded near places where urban and industrial effluents are discharged. Which constitutes a syntomy of contributions of anthropogenic origin.

The Mediterranean is located between 7° 40' and 39° 25' west longitude and 30° 16' and 47° 18' north latitude. It covers an area of 2510,000 $km^2$ (Fig.1). It is an almost closed sea, communicating only with the oceanic mass through the Strait of Gibraltar and connected to the Black Sea through the Bosphorus. Between temperate and semi-arid climatic zones, the Mediterranean records, due to evaporation, a loss of water equal to 3,130 $km^3$/year (Vanney 1991). Temperatures vary according

to latitude with winter falls more marked in the north (Adriatic, Gulf of Genes, Gulf of Lion) with an amplitude of 11-22° C, than in an average zone corresponding to the Spanish and Italian coasts. While in the waters of the Peloponnese, Tauride and Africa which are in the southern zone, the amplitude is 17 to 28 °C (Béthemont 2003). The entry of water from the Atlantic and the Black Sea makes these thermal variations more complex. The minimum surface water temperature is 13°C. The Mediterranean is an oligotrophic sea, poor in nutrients due to a deficiency of phosphorus and phosphates as well as poor water mixing. On the other hand, photosynthesis takes place there up to a depth of more than a hundred meters due to the great transparency of the water (Carsin and Chassard-Bouchaud 1998). Intense human activity, such as coastal development, agricultural pesticides, and the discharge of urban and industrial effluents, is the cause of multiple pollution denounced both by the presence of heavy metals in bivalves and by the proliferation of algae. anaerobes in summer. The approaches to the tourist coasts between the Spanish Levant and the Italian Riviera, the coastal ponds and the lagoons are the places most affected by these dystrophic phenomena (Béthémont 2003).




145.

146. Fig. 1: Geographic situation of La Ria de Vigo and the Mediterranean

147. Measurement protocol

148. A total of 258 individuals were collected including 207 at the level of La
149. Ria de Vigo and 51 at the level of Murcia at the rate of three samples
150. for each station. The numbers are 20, 147 and 40 for La Ria de Vigo
151. and 06, 25 and 20 for the Mediterranean respectively. The time limit
152. forced us to carry out for each station one sampling in winter, one in
153. spring and one in summer 2018.
154. Five traits were selected for monitoring of individuals asymmetries
155. (Fig.2): the máximum length of the supracleitrum (2a), the máximum
156. length of the scapular (1b), the máximum width of the scapular (2b), the
157. máximum length of the coracoid (1c) and the máximum width of the
158. coracoid (2c).
159. The characters were observed at 40x magnification, using a Nikon
160. Eclipse 80 microscope equipped with a digital camera (resolution: 12
161. Mpixels in total). Line size was quantified using an image analysis
162. system (Nikon EclipseNet 1.20 for Windows). Each trait was measured

twice and all measurements were sent to a database. This allowed us to avoid remembering the first measurement when taking the second measurement.

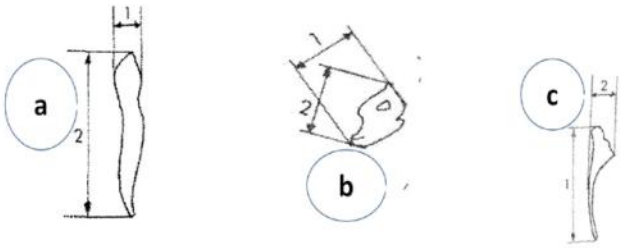



Fig. 2: The characters chosen at the level of the pectoral girdle: the máximum length of the supracleitrum (2a), the máximum length of the scapular (1b), the máximum width of the scapular (2b), the máximum length of the coracoid (1c) and the máximum width of the coracoid (2c).

## Statistical analysis

Analysis of FA data was performed as recommended by Palmer and Strobeck (1986) and Palmer (1994). Values were considered statistically significant at the 5% level. When using multiple analyses for testing the same hypothesis sequential Bonferroni correction was used to adjust the observed probability values. All statistical analyses were performed using SPSS for Windows version 16.0. and Microsoft Office Excel

## Measurement error

Relative to the size of the trait measured, fluctuating asymmetry are small and measurements must be replicated in order to distinguish true asymmetry from measurement error. Measurement error was assessed using a two-way ANOVA with side (fixed) and individual (random) as the two factors. The interaction term of the two-way ANOVA, wich contains information about FA, was tested against error, reflecting

measurement error. In all cases, the analyzes of variance in the two-way ANOVA (side individual) show that the variance due to the measurement error is significantly smaller than the variances due to the side factor and the side x individual interaction factor. Fluctuating asymmetry was significantly larger than measurement error.

Size dependence of fluctuating asymmetry

The individual fluctuating asymmetry of each trait was estimated as the absolute difference ( | R- L | ) between sides, corresponding to the index FA1. Biased results may be obtained if FA levels show any dependence on trait size. A possible source of error in the interpretation of FA levels is the dependence of these levels on the size of the analyzed trait. In order to detect this dependence, we performed a correlation analysis between the absolute values of the asymmetry of each trait (| R-L |) or FA1 and their size ((R+L)/2) using Spearman's correlation. The correlation should not be significantly different from zero. The analysis shows the existence of significant but very weak correlation only for the máximum width of the scapular (2b). We can therefore consider that there is no association between FA1 and the size of the traits. This confers to our study all the necessary characteristics that justify the use of these traits.

Tests for directional asymmetry and antisymmetry

The distributions of the values of the differences between the right side and the left side (R-L) of each trait were tested to verify their normality and their mean equal to zero, in order to detect the presence of a directional asymmetry (DA) or an antisymmetry. As with an authentically normal distribution, the skewness values of the traits are estimated to be zero or very close to zero. However, we note that the skewness value is significantly different from zero in the máximum width of the scapular (2b) (skewness =-14,882± 0,153). All the traits therefore present a leptocurtic distribution (positive curtosis values). We will verify

up to which level the observed directional asymmetries lie to avoid that it can interfere in the interpretation of the fluctuating asymmetry variations. The variable concerned is the máximum width of the scapular (2b). There is a very good method of comparing the FA4a index with the average of the relative fluctuating asymmetry or directional asymmetry (DA).

FA4a = 0.798 × the square root of the variance (R-L).

DA = Average (R-L)/2

For the máximum width of the scapular (2b) we have:

FA4a = 0,798 ×√258831092= 405,9859802

DA=-0,011

DA< FA4a

DA is less than FA4a, so the variation in directional asymmetry is between 10 and 20% of the average directional asymmetry. And deviations from the mean directional asymmetry are mainly due to developmental instability. This directional asymmetry can be seen as a fluctuating asymmetry.

FA was normally distributed, based on skew and kurtosis statistics for (R-L).

## RESULTS

Distribution of individuals harvested according to the standard length

The individuals studied are divided into three classes according to the standard length. The first class (1) consists of individuals of sizes between 5 and 11cm, they are all immature. The second class (2) consists of individuals of sizes between 13 and 20 cm: 69 immature, 4 males and 5 females. And finally a third class (3) of sizes between 20 and 29 cm, they are all mature: 42 males and 44 females. The smallest mature individuals have a standard length of 19.1cm in females and 19.2cm in males. The largest individuals have a standard length of 28.5 cm in males and 28.6 cm in females. In total we have 46 males, 49 females and 162 immature individuals.

Variations of the fluctuating asymmetry values according to the standard length

The analysis of FIg. 3 shows that the highest fluctuating asymmetry values are observed in individuals of class 3 for all the traits studied. While class 1 individuals have the lowest fluctuating asymmetry values for all the traits studied except for the maximum length of the coracoids (1c) where the lowest value of fluctuating asymmetry is observed in individuals of class 2. At the level of all the other characters, individuals of class 2 present intermediate values. We can therefore say that individuals in class 3 and 2 are respectively more stressed than those in class 1. We note an increase in the values of fluctuating asymmetry with the growth of the individuals.

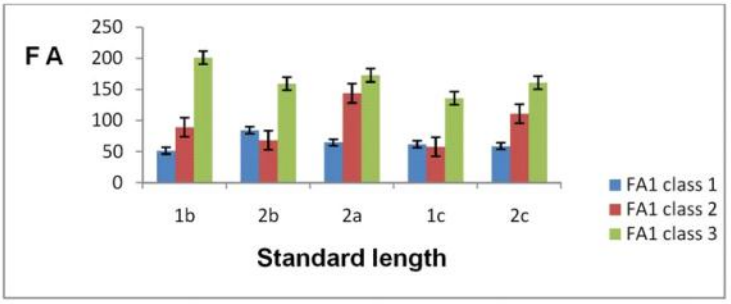



Fig. 3: Variations of the fluctuating asymmetry values according to the standard length: the máximum length of the supracleitrum (2a), the máximum length of the scapular (1b), the máximum width of the scapular (2b), the máximum length of the coracoid (1c) and the máximum width of the coracoid (2c), the máximum width of the scapular (2b).

| | FA1 class 1 | FA1 class 2 | FA1 class 3 |
|---|---|---|---|
| 1b | 51,663 | 89,25 | 201,073 |
| 2b | 84,363 | 68,141 | 159,35 |
| 2a | 64,742 | 143,706 | 172,777 |
| 1c | 61,741 | 57,639 | 135,885 |
| 2c | 59,05 | 111,085 | 160,881 |

Data Fig 3:

Sexual variations in fluctuating asymmetry values

The analysis of Fig. 4 shows that the highest fluctuating asymmetry values are observed in males for certain traits such as the máximum length of the scapular (1b), the máximum length of the coracoid (1c). While the lowest fluctuating asymmetry values are observed in immatures for all the traits studied. In females the highest fluctuating asymmetry values are observed at the level of the máximum length of the scapular (1b), the máximum length of the supracleitrum (2a), and the máximum width of the coracoid (2c). In general we can say that males and females are respectively more stressed than immatures.

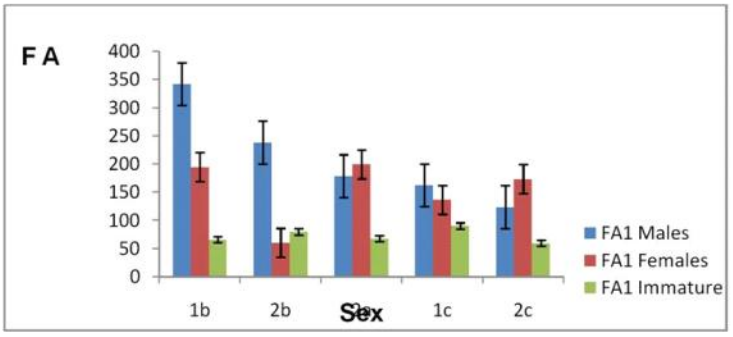



Fig. 4: Sexual variations in fluctuating asymmetry values: the maximum length of the supracleitrum (2a), the máximum length of the scapular (1b), the máximum width of the scapular (2b), the máximum length of the coracoid (1c) and the máximum width of the coracoid (2c).

| | FA1 Males | FA1 Females | FA1 Immature |
|---|---|---|---|
| 1b | 341,986 | 194,9851 | 66,12 |
| 2b | 238,62 | 60,4624 | 79,87 |
| 2a | 178,74 | 199,7096 | 67,76 |
| 1c | 162,42 | 136,7064 | 90,4 |
| 2c | 123,73 | 173,6873 | 59,85 |

Data Fig. 4

Spatial variations in fluctuating asymmetry values

The analysis of Fig. 5 shows that the highest fluctuating asymmetry values are observed in individuals collected in Murcia (Mediterranean)

for all the traits studied. While the lowest fluctuating asymmetry values are observed in individuals collected in La Ria de Vigo. We can therefore say that the individuals collected in Murcia are more stressed than those collected in La Ria de Vigo. In other words, we can say that the Mediterranean is more polluted than La Ria de Vigo.

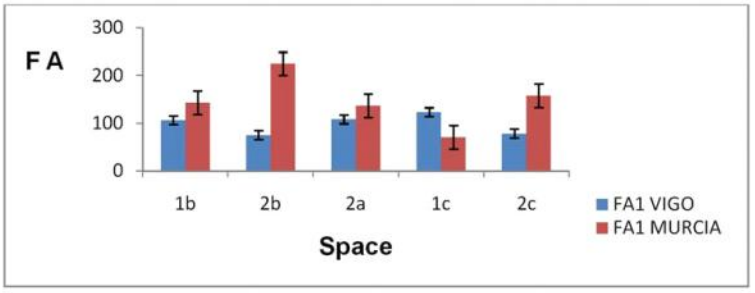



Fig. 5: Spatial variations in fluctuating asymmetry values: the máximum length of the supracleitrum (2a), the máximum length of the scapular (1b), the máximum width of the scapular (2b), the máximum length of the coracoid (1c) and the máximum width of the coracoid (2c).

| | FA1 VIGO | FA1 MURCIA |
|---|---|---|
| 1b | 105,72 | 142,33 |
| 2b | 74,93 | 223,96 |
| 2a | 107,62 | 136,21 |
| 1c | 122,77 | 69,99 |
| 2c | 77,72 | 156,87 |

Data Fig. 5

Temporal or seasonal variations in fluctuating asymmetry values

The analysis of Fig. 6 shows that the highest fluctuating asymmetry values are observed in individuals collected in winter at the level of such characters as the máximum width of the scapular (2b) and the máximum width of the coracoid (2c). While the lowest fluctuating asymmetry values are observed in individuals harvested in the spring for all the traits studied. In summer the highest fluctuating asymmetry values are observed at the level of such characters as the máximum length of the scapular (1b), the máximum length of the supracleitrum

(2a), and the máximum length of the coracoid (1c). We can therefore say that individuals harvested in winter and summer are respectively more stressed than those harvested in spring.

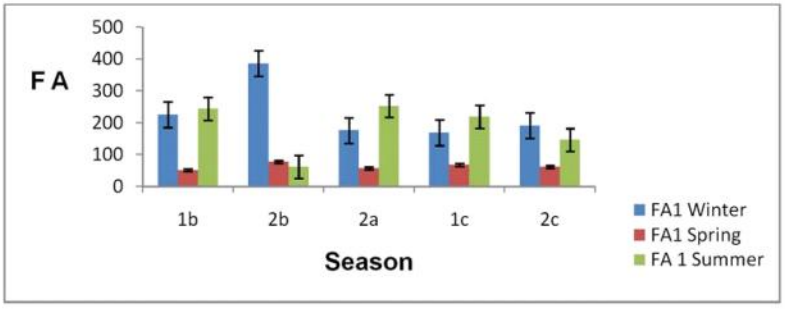



Fig. 6: Temporal or seasonal variations in fluctuating asymmetry values : the máximum length of the supracleitrum (2a), the máximum length of the scapular (1b), the máximum width of the scapular (2b), the máximum length of the coracoid (1c) and the máximum width of the coracoid (2c).

| | FA1 Winter | FA1 Spring | FA Summer 1 |
|---|---|---|---|
| 1b | 225,13 | 51,1 | 243,98 |
| 2b | 385,58 | 76,69 | 61,73 |
| 2a | 175,65 | 56,17 | 252,34 |
| 1c | 168,54 | 67,32 | 218,63 |
| 2c | 190,68 | 60,81 | 145,67 |

Data Fig. 6

## DISCUSSION

### Measurement error

Some authors such as Bailey and Byrnes (1990) mentioned that measurement error can be defined as the variability of repeated measurements of a given trait taken on the same individual, compared to its variability between individuals. The evaluation of measurement error is essential in studies of fluctuating asymmetry (Palmer and Strobeck 1986). Measurement error and measurement repetition are

not new concepts, but very few studies explicitly assess trait-specific measurement error (Lee, 1982, 1990, Allenbach 2011). A meaningful estimate of the measurement error in any trait can only be made by repeatedly measuring the traits of each of the individuals sampled in the study population. This is important (Lajus et al. 2003b), because high measurement error can bias the interpretation of results in studies of fluctuating asymmetry. Some authors (Bailey and Byrnes 1990; Lee 1990; Lougheed et al. 1991) argue that little is known about the generality of character-specific measurement error across taxa, or about the effect of experience of the researcher or statistical comparison, although there is a good methodology for assessing measurement error. Evaluating these effects would make it easier to design study protocols and choose traits.

## Size dependence of fluctuating asymmetry

Evaluating the relationship between the magnitude of fluctuating asymmetry and character size is an essential aspect to correctly analyze the levels of fluctuating asymmetry in individuals in the population studied (Bader 1965; Ames et al. 1979; Angus and Schultz 1983). The method of reducing or eliminating the dependence of the levels of fluctuating asymmetry on the size of the characters (if this dependence exists), by calculating the variance of the values of the characters does not make it possible to eliminate or reduce this dependence of the levels of fluctuating asymmetry (Palmer and Strobeck 1986). The dependence of fluctuating asymmetry levels on character size can distort the analysis of differences in fluctuating asymmetry between populations or between characters. The existence of a correlation between asymmetry levels and two factors such as fitness and growth does not allow obtaining all the information necessary for a good interpretation of the data. Since levels of fluctuating asymmetry and heterozygosity can be correlated with fitness (Leary et al. 1984; Leary et al. 1985a,b; Leary et al. 1985; Dobzhansky and Wallace 1953; Dobzhansky and Levene 1955; Dobzhansky 1970;

Lewontin 1974). Larger individuals in species with indeterminate growth constitute a risk that can bias the data analysis.

Tests for directional asymmetry and antisymmetry

When assessing fluctuating asymmetry some authors such as Graham et al. (1998); Polak and Trivers (1994) suggested taking into account other forms of asymmetry, namely directional asymmetry and antisymmetry. Directional Asymmetry is present in structures in which the development of one side is, generally speaking, greater than the other, and this side is always the same in all individuals of the species . Antisymmetry, for its part, refers to distributions for which symmetrical individuals are relatively rare; there is a tendency for one side to be larger than the other, but half of the individuals are larger on the left side and half on the right side. Directional asymmetry and antisymmetry constitute forms of adaptation and that structures exhibiting such forms of symmetry also contain fluctuating asymmetry (Lajus et al. 2019). In studies of fluctuating asymmetry carried out in sticklebacks, some authors (Reimchen and Bergstrom 2009; Lajus et al. 2019) have shown the existence of two forms of asymmetry, namely fluctuating asymmetry and directional asymmetry. Hardersen and Frampton (1999) accepted the use of data with leptocurtic distributions . Leung and Forbes (1997) obtained leptocurtic distributions by applying models designed to generate fluctuating asymmetry. Variations in the degree of developmental instability between individuals (Van Dongen 1998; Gangestad and Thornhill 1999; Palmer and Strobeck 2003) and outliers due to character attrition or size-dependent variation (Palmer and Strobeck 2003) are at the origin of leptokurtic distributions. The existence of a platykurtic distribution is the most appropriate condition to indicate the presence of antisymmetry (Palmer and Strobeck 1986; Palmer 1994).

Variations of the fluctuating asymmetry values according to the

standard length

In our study we noted that the levels of fluctuating asymmetry are respectively higher in individuals of classes 3 and 2 than in individuals of class 1. Which means that the levels of fluctuating asymmetry increase with the growth of the fish. Large individuals are more stressed than small individuals. Contrary to what Lajus et al. (2019) showed fluctuating levels of asymmetry in sticklebacks in a study. They mentioned that the smaller the fish, the more asymmetrical they are.

Sexual variations in fluctuating asymmetry values

In a study of fluctuating asymmetry carried out on individuals belonging to the species *Trisopterus luscus* Thiam (2004) showed that levels of fluctuating asymmetry are higher in juveniles than in mature individuals. These results are different from those we obtained since in our study, juveniles were less stressed than females and males. We also noted that males are more stressed than females. Levels of fluctuating asymmetry are higher in males than females in *Trachurus trachurus*. The fact that juveniles are less stressed than mature individuals may be due to the fact that the former occupy biotopes where environmental factors are favorable to allow normal development while mature individuals are faced with certain environmental constraints such as research. for food and sexual partners while simultaneously trying to avoid large predators. Levels of fluctuating asymmetry in adult organisms reflect the effects of all environmental and genetic factors during development (Palmer and Strobeck 1986; Parsons 1990). Only in-depth studies can allow us to have precise information on the interactions between genes and environmental factors on variations in the levels of fluctuating asymmetry. Developmental instability is generated by the vulnerability of organisms to environmental factors or low genetic resistance (Kowner and Thornhill 1999). The reaction of individuals in a population when subjected to environmental stress

varies by sex. In haplo-diploid organisms, males exhibit more unstable development than females. Clarke (1997).attributed this to behavioral or physiological differences between the sexes differentially affecting patterns of exposure or responses to environmental variations, resulting in one sex being more exposed to stress than the other. The armyworm (*Pseudaletia unipuncta*) is a nocturnal lepidopteran that has been the subject of numerous studies of fluctuating asymmetry (Svard and Wiklund 1986; Rutowski et al. 1987; Royer and McNeil). 1993). In this species, the reproductive success of females depends not only on the number of matings, but above all on the quality of the male with which they mate. Some studies (Møller 1990; Thornhill 1992a, b; Swaddle et al. 1994; Eggert et al. 1994) have demonstrated that in this species females use the symmetry of males as an index of their quality in choosing a sexual partner. Other studies (Møller 1990, 1996; Liggett et al. 1993; Moodie and Moodie 1996) have correlated low fluctuating asymmetry with high reproductive success. In these cases, fluctuating asymmetry levels can provide females with valuable information about the quality of the males courting them. On the other hand, some studies have shown that fluctuating asymmetry is negatively correlated with reproductive success (Bakker et al. 2006) and levels of heterozygosity (Mazzi et al. 2002). Waddington (1957) showed that heterozygosity is a factor which increases the diversity and stability of ontogeny and allows the organism to develop normally despite the genetic and environmental stresses to which it is subjected. Mitton (1995) and Britten (1996) showed in their studies a very weak relationship between levels of fluctuating asymmetry and homozygosity. They believe this is because homozygosity increases the organism's vulnerability to sources of genetic and environmental disturbance. Inbreeding increases levels of homozygosity and fluctuating asymmetry in some species (Soule and Cuzin-Roudy 1982; Parsons 1990; Clarke et al. 1986; Alados et al. 1995; Lacy and Horner 1996) but has no effect in other species (Fowler and Whitlock 1994). In four Bedouin tribes where the rate of inbreeding is approximately 10 times higher than that of most European populations, studies (Ben David et al. 1989; Markow and

Martin 1993) have shown fluctuating levels of asymmetry in the teeth. higher than in two large populations of whites and blacks. Graham and Felley (1985) and Leary et al. (1985) showed in their studies that in interspecific hybrids the fluctuating asymmetry values are higher than in the two parental species even if the hybrids are more heterozygous than their parents. In a study carried out in the German scorpion fly, Thornhill and Sauer (1992) showed that fluctuating asymmetry is hereditary. If this is the case, sexual selection of individuals exhibiting traits with low fluctuating asymmetry would reduce its prevalence (Møller and Eriksson, 1995). A meta-analysis of 34 studies covering 17 species was carried out by Møller and Thornhill (1997a). These authors showed that the overall effect size of the heritabilities of individual fluctuating asymmetries is equal to 0.19. But it is unclear whether this is related to genetic variation in developmental mechanism, disease resistance, or other unknown aspects of viability. Loehr et al. (2012) showed in their study that fluctuating asymmetry is hereditary, while other authors such as Hermida et al. (2002) and Aguirre et al. (2004) showed that it was not. Developmental stability and fluctuating asymmetry are influenced by the introduction of new mutants into an organism's genome. Increased levels of fluctuating asymmetry following the introduction of a new advantageous mutant into Australian blowflies resulted in greater resistance to insecticides (Clarke and Mckenzie 1987). But after two decades of adaptation, the flies returned to initial FA levels and retained the increase in fitness due to greater resistance.

Spatial variations in fluctuating asymmetry values

Thiam (2004) carried out a study of the spatial variation of the fluctuating asymmetry of *Trisopterus luscus* from La Ria de Vigo, Andalusia and the Mediterranean and was able to demonstrate that individuals from the Mediterranean presented asymmetry values fluctuating higher than those of Andalusia and La Ria de Vigo. On this point, our results confirmed their work. Since we observed that the fluctuating asymmetry values are higher in individuals from the

Mediterranean than in individuals from La Ria de Vigo. We can therefore say that individuals collected in the Mediterranean are more stressed than individuals collected in La Ria de Vigo. This is due to the fact that the Mediterranean is more polluted than La Ria de Vigo. Many studies (Parsons 1990; Clarke and McKenzie 1992; Markow 1995) have shown that increased fluctuating asymmetry is caused by environmental or genetic stress. And that this increase in fluctuating asymmetry is seen as an indication of developmental instability or reduced developmental homeostasis. Some authors (Clarke 1993 a, b; Hardersen and Wratten 1998) have established in their studies a correlation between the degree of chemical stress of anthropogenic origin (fertilizers, industrial chemicals, pesticides) or natural substances such as deposits of metals and fluctuating asymmetry levels. Some animals such as dipteran insects (Mckenzie and Yen 1995; Freebairn et al. 1996; Imasheva et al. 1997), fish (Campbell et al. 1998) and isopod crustaceans (Savage and Hogarth 1999) live in a range of temperatures to which the organism is well adapted. Any change in temperature from the temperature range to which the organism is adapted leads to an increase in the levels of fluctuating asymmetry and therefore a disruption of developmental stability in these animals. Some authors (Prieto et al. 2005); van Dongen et al. 2009; Loehr et al. 2013) studied the relationship between fluctuating asymmetry and abiotic factors. And they showed a geographic diversity of fluctuating asymmetry levels without indicating the existence of a relationship between fluctuating asymmetry levels and a well-determined environmental factor. Bergstrom and Reimchen (2002, 2003) showed in their studies that levels of fluctuating asymmetry decreased with increasing water clarity, altitude and pH. In a study, Moodie (1977) showed that variations in the levels of fluctuating asymmetry, between organisms that live in rivers and organisms that live in lakes, depend on the trait studied. In a study of fluctuating asymmetry carried out in sticklebacks, Trokovic et al. (2012) showed that levels of fluctuating asymmetry are higher in freshwater individuals than in individuals from marine environments. Studies carried out by Bergstrom and Reimchen

(2002) and Reimchen and Bergstrom (2005) showed the existence of a positive correlation between fluctuating asymmetry and parasitic infection. This means that individuals who are less physically fit have lower developmental stability and less resistance to parasites. In a study of geographic and temporal variations in fluctuating asymmetry in sticklebacks, Prieto et al. (2005) showed the existence of a negative correlation between fluctuating asymmetry and the parasite load of *Eustrongylides sp*. In a study of virulence, parasitic transmission, and fluctuating host asymmetry, Agnew and Koella (1997) showed that the wings of parasite-infected mosquitoes had higher levels of fluctuating asymmetry than the wings of control mosquitoes. These authors also showed higher levels of fluctuating asymmetry in horizontally transmitted parasites than in vertically transmitted parasites. They believe this is because vertical transmission links the reproductive interests of both parties during co-host-pathogen-evolution. Folstad et al. (1996) showed that antiparasitic treatment in naturally infected female reindeer affected the development of antler symmetry, but not antler length. In three studies carried out in sticklebacks (Moodie 1977; Bergstrom and Reimchen 2003, 2005) to establish a relationship between high levels of fluctuating asymmetry in individuals with reduced fitness and vulnerability to predators, only two Studies have shown that levels of fluctuating asymmetry are lower in the presence of predators. In a study in sticklebacks, Robinson and Wardrop (2002) showed increased levels of fluctuating asymmetry at higher water temperatures and in the presence of a richer diet, resulting in faster growth. In a study, Van Dongen et al. (2009) showed that directional selection and evolutionary change increase the strength of the relationship between fluctuating asymmetry and genetic variation at the population level. Lescak et al. (2013) showed in their study that levels of fluctuating asymmetry increase with increasing pelvic expression, without interpreting their results in terms of relationships between fluctuating asymmetry and fitness.

Temporal or seasonal variations in fluctuating asymmetry values

Our study showed that the highest fluctuating asymmetry values are respectively observed in individuals harvested in winter and summer than in individuals harvested in spring. And that the fluctuating asymmetry values are higher in individuals collected in winter than in individuals collected in summer. It is therefore among individuals collected in spring that we observe the lowest fluctuating asymmetry values. Which means they are the least stressed. Extreme levels of seasonal temperature variations are observed in winter and summer. Too low or too high a temperature can affect fluctuating asymmetry during development. This explains why the highest levels of fluctuating asymmetry are observed in winter and summer. Studies (Parsons 1987, 1989; Imasheva et al. 1997) have shown that many organisms are well adapted to a very narrow temperature range and deviations from this can cause stress. during development. *Trachurus trachurus* is one of them. In a study of egg clutch embryos of two species of sand lizards at four constant temperatures, Zakharov (1989) showed that variation in levels of fluctuating asymmetry was minimal at 25°C, whereas at – above and below this temperature, it increased. *Trachurus trachurus* is a temperature-dependent species. The species' migrations are guided by water temperature and prey availability. In spring, schools of *Trachurus trachurus* disperse and migrate north as water temperatures increase. The minimum water temperature limit is 8ºC for *Trachurus trachurus*. At temperatures below 9°C, the species stops feeding (Polonsky 1965; Chuksin and Nazarov 1989; Abaunza et al 2003). This may explain the low fluctuating asymmetry values observed in spring. Imasheva et al. (1997) mentioned in their studies that at the molecular level heat stress directly affects biological molecules such as enzymes, which are crucial for the survival of the organism, while at the population level it can lead to chronic effects within the genetic architecture of the population. Temperature affects growth rates, as with any biological process. However, this influence can be exerted differentially on various parts of the body. This sometimes results, in addition to variations in overall size (notably in the final size of the

individual), variations in shape depending on the temperatures encountered during development. In the temperature range compatible with life, the speed of biological processes increases with temperature. A biological or biochemical activity is completely canceled above a thermal limit specific to it (Parsons 1961). The slowdown and disappearance of activity are most often due to an alteration of proteins and enzymes by excessively high temperatures. In fact, each organic activity is under the complex dependence of a double system of chemical and enzymatic reactions: one which accelerates it, the other which slows it down.

## CONCLUSION

A living population is always evolving, it is never immutable. The ecosystem in which it lives is subject to exogenous and endogenous disturbances, to which it responds with structural and functional adaptations and subsequent evolutions. The factors responsible for developmental instability measured in terms of fluctuating asymmetry are very varied. They are genetic (mutations, hybridization), environmental (thermal or haline shocks), trophic (nutritional abundance or deficiencies), parasitic (myxosporidia, etc.) and above all chemical (numerous chemical pollutants involving a great diversity of heavy metals, hydrocarbons, pesticides, surfactants). But fish are vertebrates having established and diversified all the adaptive bases, both anatomical and physiological, preparing the emergence of tetrapod vertebrates. Fish have become essential models in developmental biology. Variations in fluctuating asymmetry between traits are generally attributed to different levels of developmental stability. These levels of developmental stability vary depending on the role of the traits, the mode of selection, or the degree of stress that affects the traits during their formation. However, the relationship between fluctuating asymmetry and stress is not well defined. The analysis of variations in fluctuating asymmetry as a function of standard length showed that individuals in classes 3 and 2 are respectively more stressed than

individuals in class 1. Individuals in class 3 are more stressed than those in class 1. class 2. This indicates an increase in fluctuating asymmetry values with the growth of the fish. In our study, we found that juveniles are less stressed than females and males. We also noticed that males are more stressed than females.

We observed that individuals collected in the Mediterranean are more stressed than those collected in La Ria de Vigo. This is due to the fact that the Mediterranean is more polluted than La Ria de Vigo. We also observed that individuals harvested in winter and summer are respectively more stressed than those harvested in spring. Individuals harvested in summer are less stressed than those harvested in winter. It is therefore among individuals collected in spring that we observe the lowest fluctuating asymmetry values. Which means they are the least stressed.

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## ETHICAL APPROVAL RESEARCH

Ref. : CER/UCAD/AD/MSN/008/2023 Subject: Opinion 0002/2023/CER/UCAD relating to the publication of an Article entitled: "FLUCTUATING ASYMMETRY AS AN INDICATOR OF ENVIRONMENTAL STRESS IN *TRACHURUS TRACHURUS* L. 1758"

The content of your article was examined according to the rules laid down by the National Ethics Committee for Health Research (CNERS) of Senegal and in accordance with the procedures established by the Cheikh Anta DIOP University of Dakar (UCAD) for ethical approval of all research involving human and animal participants. The UCAD Research Ethics Committee (CER) considers that ethical standards have been respected and therefore approves its submission for publication. (See attached file).

**The President, Professor Alioune DIEYE Dakar, 27 – 10 – 2023.**

## CONFLICT OF INTEREST DECLARATION

This work does not raise any ethical concerns or conflicts of interest.

## DATA AVAILABILITY

The data used to write this article are presented in the manuscript in the form of tables.