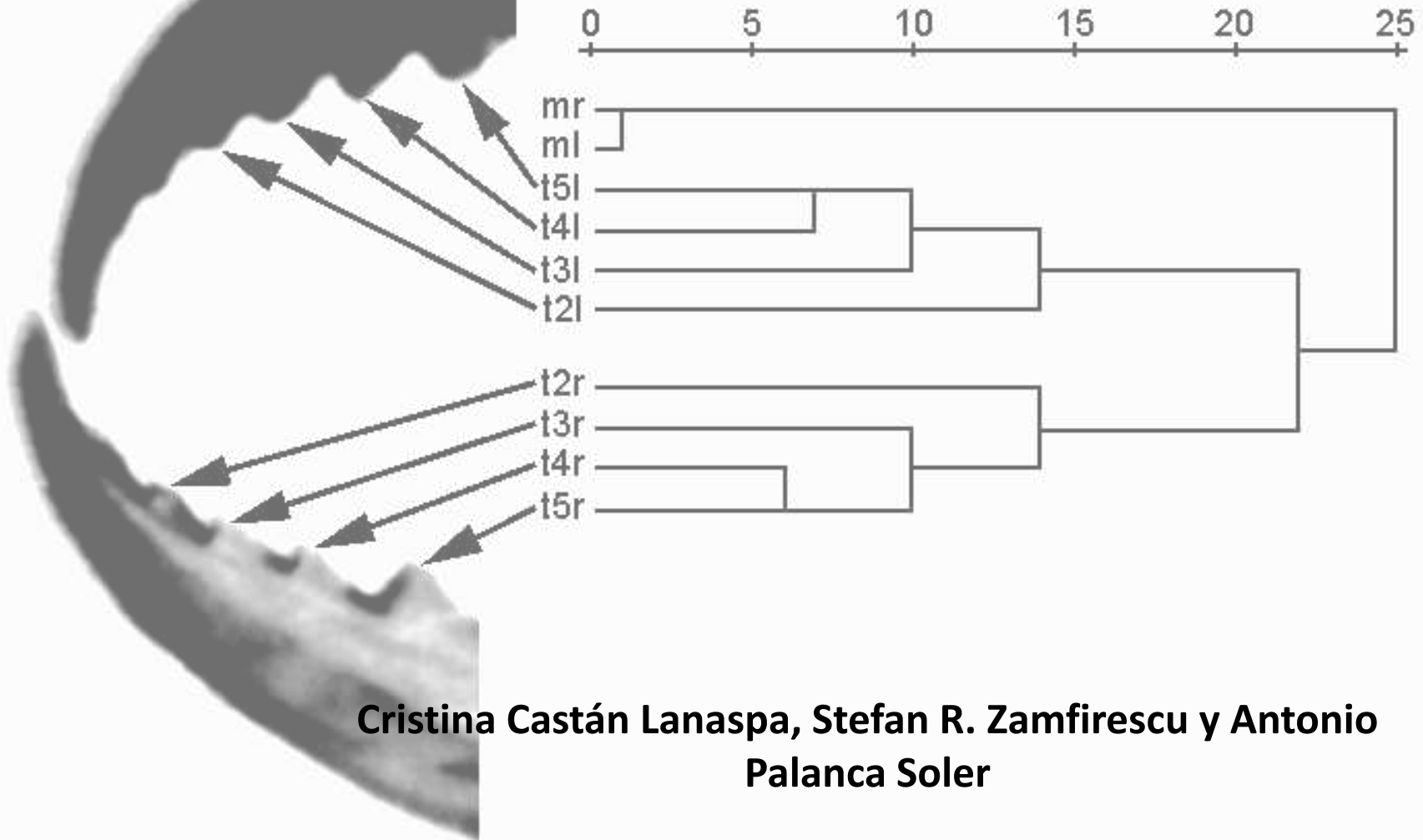# Concordant Anatomical Characters in *Nereis diversicolor* (Annelida Polychaeta) as a
New Factor in Fluctuating Asymmetry Research

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Concordant Anatomical Characters in *Nereis diversicolor* (Annelida Polychaeta) as a

New Factor in Fluctuating Asymmetry Research

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Abstract: The biometrical study of *Nereis diversicolor* from the Ria de Vigo (Galicia,

Spain) revealed that the mandibles and the teeth display a fluctuating asymmetry. The

inducing factor is the rainfall level during development. Low precipitation leads to an

increment of the asymmetry. The mandible responds more promptly to this factor,

while the teeth react after a one-moth latency. Due to the strong unilateral correlation,

the teeth behave like two distinct anatomical complexes. This pattern of concordant

anatomy can modify the individual asymmetry. The pattern of concordant anatomical

traits sheds a new light over the fluctuating asymmetry research.

The alteration of the environment may result in stressing conditions for the development of organisms. In addition, this may contribute to the extinction of many species. Stress is defined as any alteration of the normal environment that interrupts the steady state of an organism. Organism reactions to stress are known as stress general syndrome (*1*).

Stressed populations identification can be carried out trough two types of characteristics: First type characteristics refer to changes of community structure, diversity and species relative abundance. Unfortunately, these changes occur relatively late after the environmental alteration (*2*). Second type characteristics include vital parameters as survivorship, fecundity, reproduction success etc. All of them are responsible for the fitness of the organism. Because these vital characteristics determination is difficult and often impossible, there is a strong necessity to develop an indicator system that evidences the potential stressing alterations before they affect the fitness of the organisms (*3*).

A method that fulfils this necessity is the assessment of the development stability, which is the ability of an organism to isolate its development from stressing environmental perturbations and though to produce an ideal form in a certain environment (*4*).

Development stability can be revealed, among others, by the individual variability, which refers to phenotypic differences among homologous structures, and the fluctuating asymmetry – non-directional differences between the left and the right side of bilateral structures. The last is extensively used to assess the developmental stability (*5-7*).

Although, ideally symmetrical structures are scarce in the real world, they represent an important comparison term for deviations (*8*), and therefore, provide a convenient method for the study of the influencing factors.

Supposing that morphological structure development is genetically controlled, one can expect both sides to be identical because of the same genotype. Thus, the asymmetry of these structures would indicate a tendency to deviate from the genetically programmed result, during development. The differences between the two sides measurements must be environmentally induced, and reflect the altering factors effect upon development (*9*).

Among the "pure" forms of bilateral asymmetry – directional asymmetry, antiasymmetry, fluctuating asymmetry – the first two ones are genetically or developmentally directed while only the last one has an environmental origin, which means that it is the result of the so-called developmental noise (*10*).

The benthonic invertebrates are considered good indicator species because, being more or less immobile, they reflect both present and past environmental conditions (*11*). The use of Polychaeta in environmental quality control is supported by the fact that they are permanently in contact with the sediments, as well as with the water column. Additionally, many polychaetes are detritivores and filterers. Because of these qualities, they are highly sensible to various substances. This sensibility is variously reflected by alterations in reproduction, development, high mortality and toxic substance accumulation (*12*). For these reasons, many studies focused on polychaetes (*13-29*). Some simmilar works were carried out in Galicia (Spain) were our study area is situated (*30-32*).

Asymmetry of the mandibles. The asymmetry calculations were performed for the length of mandible and height of the teeth 2, 3, 4, and 5 (*33*). The values of asymmetry for all the above-mentioned characters may be considered approximately normally distributed (Table S1).

The descriptive statistics of mandible indicate that this trait manifests a fluctuating asymmetry – the asymmetry index has the mean and the skewness close to 0, the median 0, which means that the values are approximately normally distributed (*34*). The same applies to tooth 5. On the contrary, the asymmetry of the teeth 2, 3 and 4 seems to be directional which, in this case, means that the right teeth tend to be bigger than their left counterparts – the average asymmetry of these traits is greater than 0. Nevertheless, the median and the skweness values close to 0 suggest a fluctuating asymmetry. Thus, after a careful examination of the data, we observed that the teeth of the right mandible tend to be bigger in older specimens. An explanation of this fact could be that the second, third and forth tooth are worn out due to preferential usage. If this is true, then this case might be an example of wearing-induced directional asymmetry superimposed over a fluctuating one. Further more, this hypothesis is supported by the fact that it is highly improbable that directional asymmetry would affect just these 3 teeth out of all.

Concordance of traits. The next step was to investigate the relation, if there is any, among the dimensions of the teeth and the mandibles, and therefore, to see if the asymmetry of the teeth is connected to the asymmetry of the mandible, to which they belong. This analysis revealed that the mandibles are strongly correlated with each other, while the teeth fall into two groups that correspond to each mandible (Fig. 1). The most similar are tooth 4 and 5, and together form a cluster similar to tooth 3. Tooth 2 is the most dissimilar both in the right and the left group. Thus, it is about an

anatomical pattern that is independent of the asymmetry phenomenon. Each group acts as an anatomical complex. Therefore, the asymmetry of one component of these complexes, i. e. the asymmetry of a certain tooth, will be influenced by the other components, at individual level. Consequently, the assemblages of anatomical characteristics that follow the same anatomical pattern could be nominated as concordant anatomical traits. The "arrangement" of the teeth within a group (Fig. 1) reflects the manner in which mandibles grow – the newly formed, basal teeth are more similar to each other, while top ones are relatively different to each other and to the basal ones. This is also the result of the stressing factors that are chiefly effective at the beginning of the individual development.

Fluctuating asymmetry and rainfall. We investigated the fluctuating asymmetry at population level, trying to follow its temporal development.

The adult females full of eggs allowed the estimation of the time of year when the individuals were borne, given that *Nereis diversicolor* individuals achieve sexual maturity after 18 moths (*35-38*). Thus, we estimated the time of hatching for each sample from October 1995, February 1996, May 1996, September 1996, December 1996, June 1997, August 1997, January 1998 and April 1998.

The discriminant analysis of the samples asymmetries showed that all the variables are correlated with the first factor axis (Fig. 2). Among these, the asymmetry of tooth 4 (a-t4) and 5 (a-t5), and the sample asymmetry (a-sum) are the variables that best separate the groups of samples.

The groups include samples of individuals that emerged in the same year. As the clutches are mainly laid in February – April, June – August and October – December

(*39*) the specimens would reach sexual maturity after 18 months that is in August – October, December – February and April – June, respectively.

The evident separation of the groups (95-96, 96-97 and 97-98) endorses the idea that the level of asymmetry of each group is different (Fig. 3). One of the important stressing factors could be the precipitation in the studied area. We consider the precipitation as an important factor, given that *Nereis diversicolor* not only is able to survive in fresh water (*40*) but also seems to prefer it to marine water (*41*). Therefore, lack of rainfall can indirectly favour the fluctuating asymmetry through salinity augmentation. Consequently, the asymmetry of each sample was related to the precipitation levels during the development period.

The variation of the sample asymmetry in relation with the rainfall level is obvious only in the samples from September 1996, December 1996 and June 1997 (Fig. 4). In the samples from 1995 – 1996 and 1997 – 1998, the sample asymmetry does not follow the corespondent precipitation levels. The increment of asymmetry seems to be delayed with 1 month: the poor rainfall of a certain month during hatching induces the increase of asymmetry one moth later then expected. Thus, there may be a latency period in the reaction of teeth development to the asymmetry inducing factors.

This relation appears more evident if we analyse the relation between seasonal rainfall level and the level of asymmetry (Fig. 5). If seasonal precipitation and asymmetry are plotted together, the latency tends to become less evident.

At seasonal scale, the relation between precipitation and teeth asymmetry is more obvious – when the precipitation level is low, the asymmetry increases. The latency of the reaction to precipitation is reproduced as a slight increase of the asymmetries of mandibles and teeth from April – June 1995-1998 to August – October 1995-1998.

In conclusion, the lengths of the mandibles and of its teeth in *Nereis diversicolor* display a fluctuating asymmetry. The teeth from one mandible are stronger correlated to each other than to the correspondent ones from the other mandible.

The relation with one of the stressing factors also demonstrates the fact that the analysed traits display a fluctuating asymmetry. Thus, the low rainfall level during the development period (18 moths ago from the sampling moment) provokes an increment in the fluctuating asymmetry. This effect is not the same for all the traits – it seems that mandibles react more promptly, while teeth reaction occurs after a latency period.

Independent of asymmetry, the teeth dimensions display a concordant anatomical pattern of similarity, at individual level. The teeth from one mandible act like an anatomical complex, which can modify the asymmetry level. The similarity among the teeth within a group reflects the manner in which the stressing factors are effective chiefly at the beginning of the individual development.

The pattern of concordant anatomical traits casts off a new light over the fluctuating asymmetry concept, and therefore, must be taken into account for future research.

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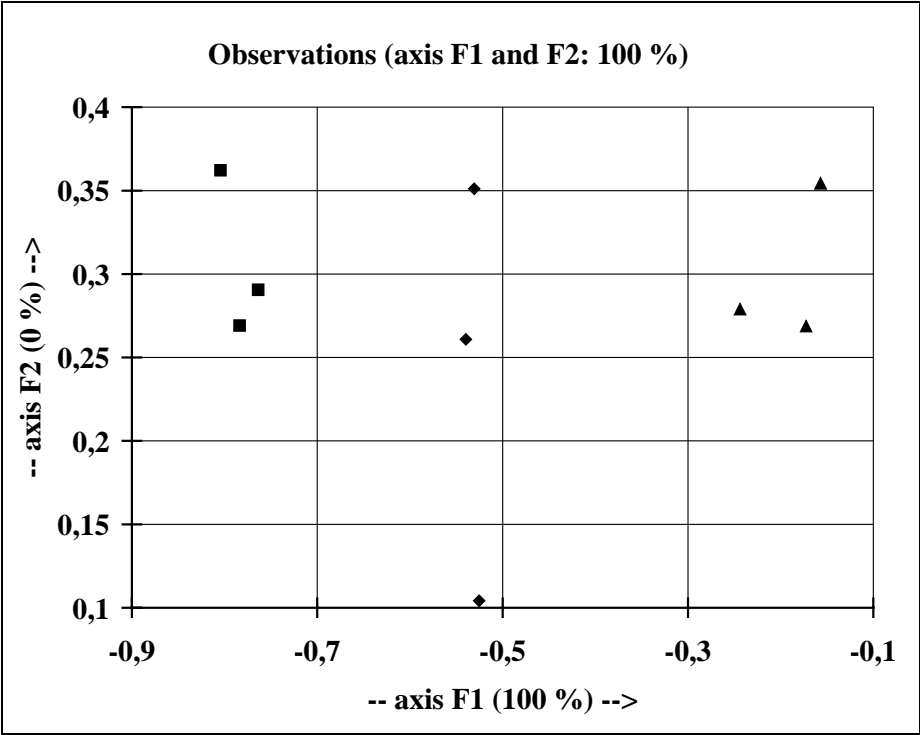
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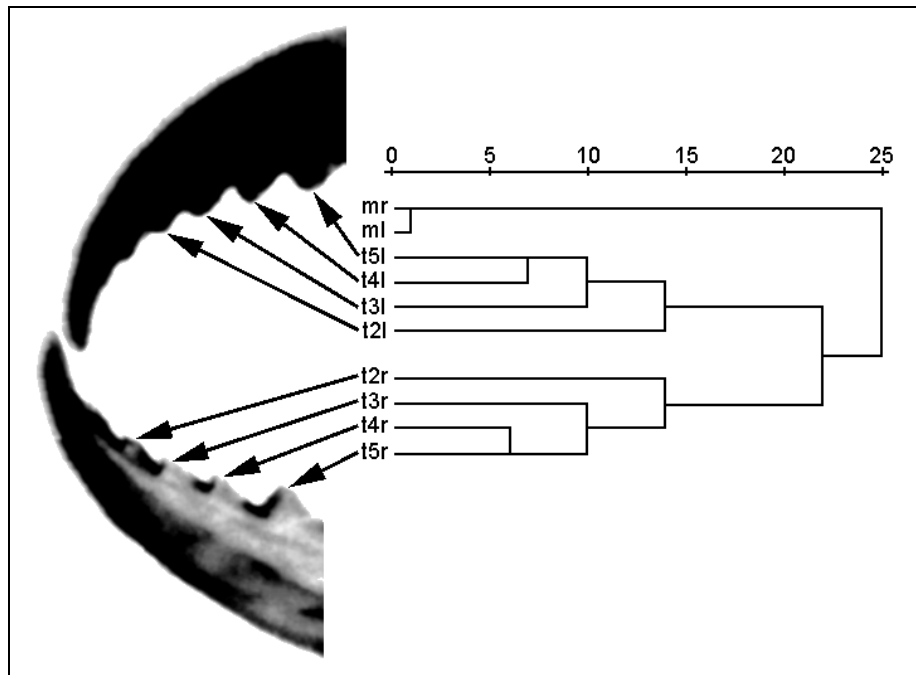
Fig. 1. Similarity cluster of the dimensions of mandibles and teeth (m – mandible, t2-t5 – teeth, r – right, l - left)
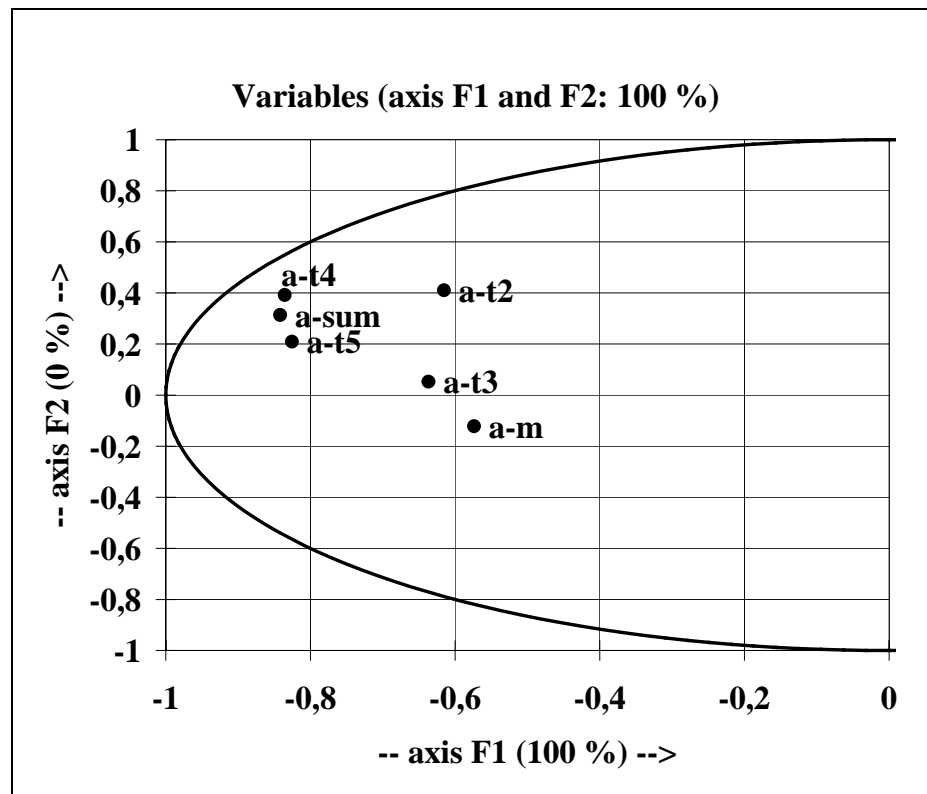
Fig. 2. Correlation between trait asymmetries and factors (m – mandible, t2-t5 – teeth, sum – sample asymmetry, a – asymmetry)

Fig. 3. Observations on factor axes – Wilks' Lambda: 0,008, error rate: 0% (■ – samples taken in October 1995, February 1996, May 1996, ◆ – samples taken in September 1996, December 1996, June 1997, ▲ – August 1997, January 1998 and April 1998)
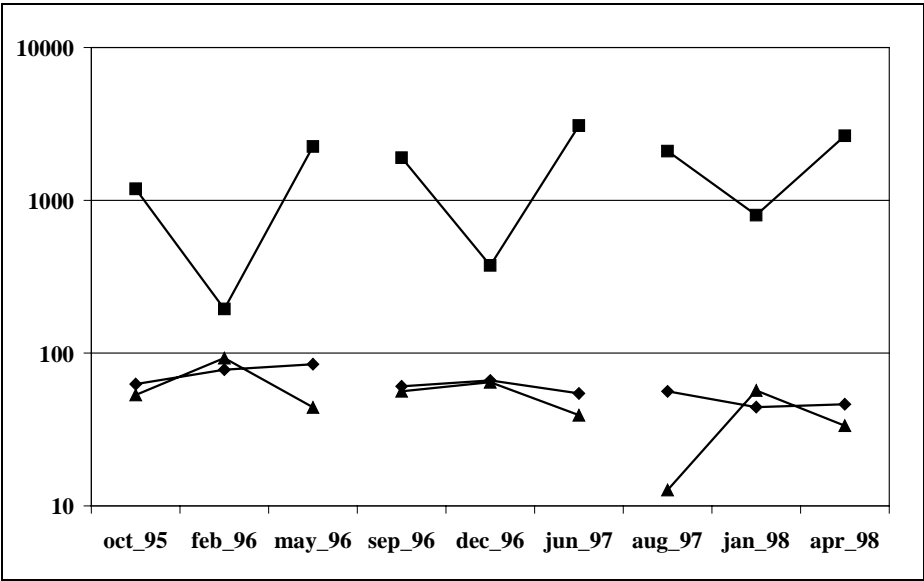
Fig. 4. Variation of samples asymmetry and precipitation (■ – precipitation in the period of development (mm), ▲ – asymmetry of the mandible (μm), ◆ – asymmetry of the teeth (μm))
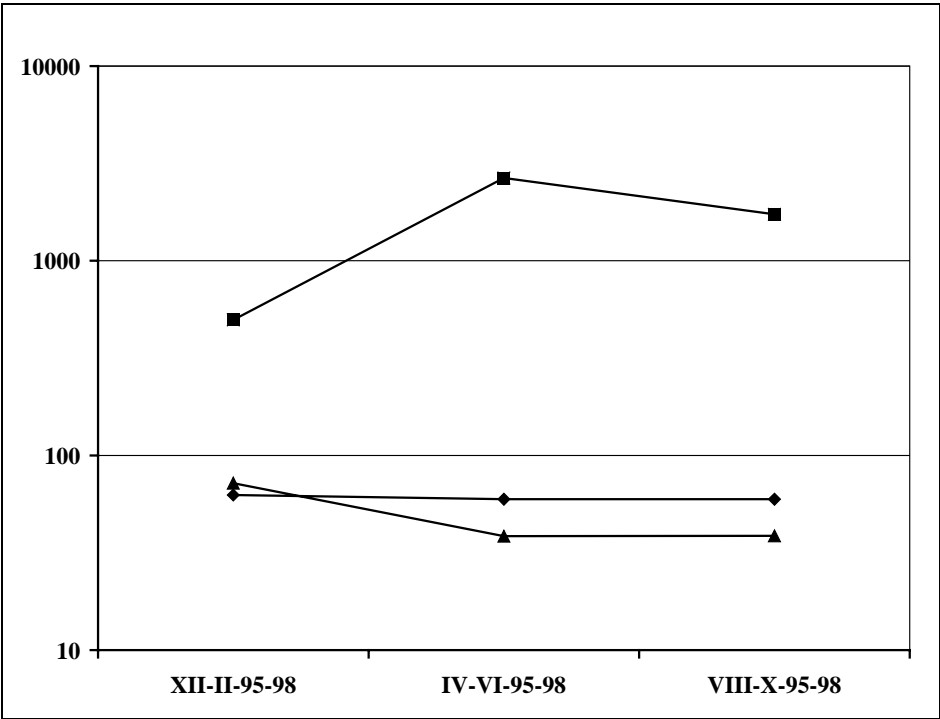
Fig. 5. Variation of seasonal asymmetry and precipitation (■– precipitation in the period of development (mm), ▲ – asymmetry of the mandible (μm), ◆ – asymmetry of the teeth (μm))

SUPPORTING ONLINE MATERIAL

Material and Methods

Our study was carried out in Playa de La Portela from La Ria de Vigo – $42^{o}15$'N and $8^{o}43$'W. La Ria de Vigo is an estuary from the Atlantic coast of Galicia (Spain), surrounded by mountains (200 – 600m altitude). The research was conducted from 1995 to 1999.

The total sampled number of individuals of *Nereis diversicolor* was 335. The material was kept in alcohol (75°). These specimens were dissected in order to obtain the mandibles and to verify if they contain eggs. Mandible and teeth measurements were used to assess the symmetry.

Mandible measurements (µm) were the followings: right mandible total length (mr), left mandible total length (ml), the distance between the apex of one tooth and the base of the following tooth for all the teeth from the right (t2r, t3r, t4r, t5r), and the left mandible (t2l t3l, t4l, t5l). The first and the sixth tooth could not be measured because they were naturally degraded.

For the asymmetry assessment we used the following indices (S1):

$$FA = \Sigma|R - L|/N$$

Though, the fluctuating asymmetry for one characteristic is given by the sum of the absolute difference between right (R) and left (L) for the whole sample (N).

$$A_i = \Sigma|R - L|$$

The asymmetry of an individual (A_i) is the sum of the absolute differences corresponding to each characteristic.

$$\Sigma F = \Sigma A_i/N$$

The sum of the individual based index (ΣF) gives the value for the whole sample (N).

The data processing consisted of a statistical description, a cluster analysis (cosine distance index, unweighted pair-group method average linkage), and a discriminant analysis.

Supporting tables

Table S1. Descriptive statistics of the asymmetry index

Descriptive statistic	mandible asymmetry	tooth 2 asymmetry	tooth 3 asymmetry	tooth 4 asymmetry	tooth 5 asymmetry
No of values used	335	335	335	335	335
Minimum (µm)	-265,487	-65,144	-62,839	-72,539	-75
1st quartile	-25,316	-6,999	-7,983	-8,41	-12,143
Median	0	0	0,673	0	-0,699
3rd quartile	24,563	12,41	13,278	12,538	11,23
Maximum (µm)	298,701	59,621	68,966	81,997	72,906
Range (µm)	564,188	124,765	131,805	154,536	147,906
Mean (µm)	0,505	2,334	1,715	1,553	7,82E-04
Kurtosis (Pearson)	4,05	1,126	0,954	2,198	0,912
Skewness (Pearson)	0,052	-0,112	-0,02	0,027	0,047
Estimated variance	4959,556	314,446	368,334	386,102	429,867

Estimated standard deviation	70,424	17,733	19,192	19,649	20,733
Standard deviation of the mean	3,848	0,969	1,049	1,074	1,133

Supporting reference

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