FLUCTUANT ASYMMETRY IN A *NEREIS DIVERSICOLOR* (ANNELIDA, POLYCHAETA) POPULATION FROM RIA DE VIGO (GALICIA, SPAIN)

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Abstract. The biometrical study of the mandibles of *Nereis diversicolor* from the Ria de Vigo (Galicia, Spain) revealed that both the mandibles and the teeth display a fluctuating asymmetry. The major factor responsible for inducing this fluctuating asymmetry is the rainfall level during the development period. Low precipitation during this time first leads to an increment of the asymmetry. The response of the mandible asymmetry to this factor is more prompt, while the teeth react after one-month latency.

Keywords: anatomy, fluctuating asymmetry, *Nereis diversicolor*.

Rezumat. Asimetria fluctuantă la o populaţie de *Nereis diversicolor* (Annelida, Polychaeta) din Ria de Vigo(Galiţia, Spania). Studiul biometric al mandibulelor la *Nereis diversicolor* din Ria de Vigo (Galiţia, Spania) a arătat că atât mandibulele cât şi dinţii acestora prezintă o asimetrie fluctuantă. Principalul factor responsabil pentru inducerea acestei asimetrii este nivelul precipitaţiilor din perioada dezvoltării individuale. Nivelul scăzut al precipitaţiilor din această perioadă determină o accentuare a asimetriei. În cazul mandibulelor, reacţia asimetriei este mai promptă decât în cazul dinţilor, care răspund după o de latenţă de o lună.

Cuvinte cheie: anatomie, asimetrie fluctuantă, *Nereis diversicolor*.

Introduction

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 (Selye, 1978). The identification of stressed populations can be carried out trough two types of characteristics. The first type characteristics are changes in community structure, diversity and species relative abundance and these changes occur relatively late after the environmental alteration (Clarke, 1993). The second type characteristics include vital parameters as survivorship, fecundity, reproduction success etc. All of these contribute to the fitness of the organism. Because the determination of these vital parameters is difficult and indeed 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 (Clarke, 1994).

A method that fulfils this necessity is the assessment of the developmental stability, which is the ability of an organism to isolate its development from stressing environmental perturbations and to produce an ideal form in a certain environment (Leary & Allendorf, 1989).

Developmental stability can be revealed, for example, by the individual variability, which refers to phenotypic differences among homologous structures, and fluctuating asymmetry – non-directional differences between the left and the right side of bilateral structures. The latter is extensively used to assess developmental stability (Thoday, 1955, 1958; Van Valen, 1962).

Although ideally symmetrical structures are scarce in the real world, they represent an important comparison term for deviations (Palmer et Strobeck, 1986), and therefore, provide a convenient method for the study of the influencing factors (Palmer, 1994). 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 (Waddington, 1942).

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 so-called developmental noise (Palmer & Strobeck, 1992). Benthic invertebrates are considered good indicator species because, being relatively immobile, they reflect both present and past environmental conditions (Reish, 1985, 1986; Leary & Allendorf, 1989). 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 filter-feeders. Because of these qualities, they are highly sensitive to various substances. This sensibility is variously reflected by alterations in reproduction, development, high mortality and toxic substance accumulation (Pocklington & Wells, 1992). For these reasons, many studies have focused on polychaetes: Briggs *et al.*, 1979; Kristensen, 1988; Riedel *et al.*, 1987, 1989; Esselink *et al.*, 1989; Tsutsumi, 1987; Tsutsumi *et al.*, 1990; Steimle *et al.*, 1990; Eriksen *et al.*, 1988, 1989, 1990; Bonsdorff *et al.*, 1990; Grant *et al.*, 1990; Miron & Kristensen, 1993; Davey & Watson, 1995; Fritzsche & Von-Oertzen, 1995; Batten & Bamber, 1996. Some similar works were carried out in Galicia (Spain) were our study area is situated: Villalba & Vieitez, 1985; Carral *et al.* 1995 a, b.

Given the above-mentioned aspects, the aim of this study is to reveal the fluctuating asymmetry of the mandibles in *Nereis diversicolor*, from the Ria de Vigo (Galicia, Spain) and to investigate other possibly connected anatomical patterns.

Material and Methods

Our study was carried out in Playa de La Portela from La Ria de Vigo – 42°15'N and 8°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 adult individuals of *Nereis diversicolor* was 346 distributed in 9 similar samples taken at different dates. 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. Each tooth was measured twice in order to assess the measurement error.

To validate the existence of the fluctuating asymmetry the data were submitted to a multiple-step analysis (Palmer & Strobeck, 2003). Outlier detection consisted in visual inspection of the scatter plots of the difference between replicate measurements and the one between sides, followed by the statistical confirmation (Grubb’s test and Bonferroni correction). Consequently, we checked if the asymmetries (the difference between right and left measurements) are significantly greater than the measurement errors through an ANOVA two-factor (sides and individuals) with replication (replicate measurements). Finally, we tested if the displayed asymmetry is fluctuating or of other type, by verifying the normal distribution of the asymmetries (visual inspection of frequency distribution plots, tests for skewness and kurtosis) and by verifying if the average asymmetry is 0 (one-sample t-test).

The possible directional asymmetries (DA) were compared with the value of FA4a index (Palmer & Strobeck, 2003) to see if they are largely due to developmental instability. The hetrogenity of the developmental instability was verified through an ANOVA two factor with replication on the absolute difference of the natural logarithms of the sides.

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

Results and Discussion

The asymmetry calculations were performed for the length of mandible and height of the teeth 2, 3, 4, and 5. The values of asymmetry for all the above-mentioned characters may be considered approximately normally distributed.

The discriminant analysis of the samples asymmetries showed that all the variables are correlated with the first factor axis (Fig. 1). 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.

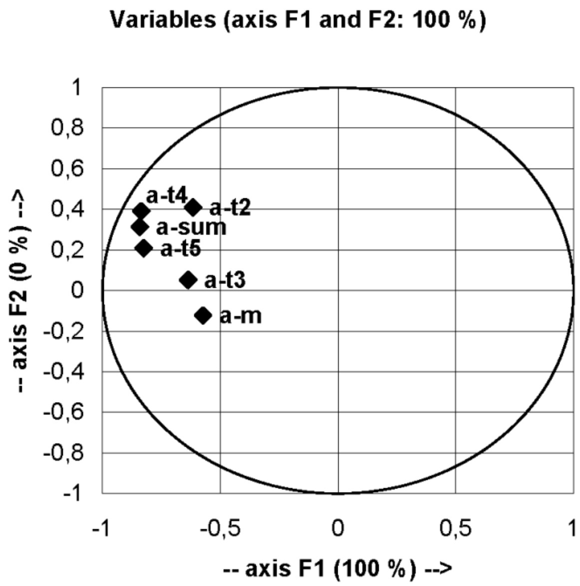

Figure 1. Correlation between trait fluctuating asymmetries and factors (m – mandible, t2-t5 – teeth, sum – sample asymmetry, a – asymmetry).

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 (Chambers & Milney, 1975) the specimens would reach sexual maturity after 18 months that is in August – October, December – February and April – June, respectively. The separation of the groups is evident (Fig. 2).

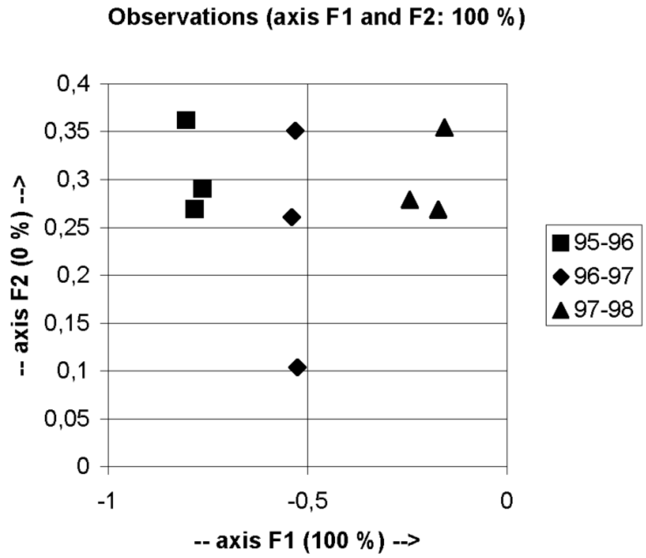

Figure 2. Observations on factor axes – Wilks' Lambda: 0,008, error rate: 0% (95-96 samples taken in October 1995, February 1996, May 1996, 96-97 – samples taken in September 1996, December 1996, June 1997, 97-98 – August 1997, January 1998 and April 1998).

The asymmetry of the mandibles augments if the precipitation level is low during the development period (Fig. 3).

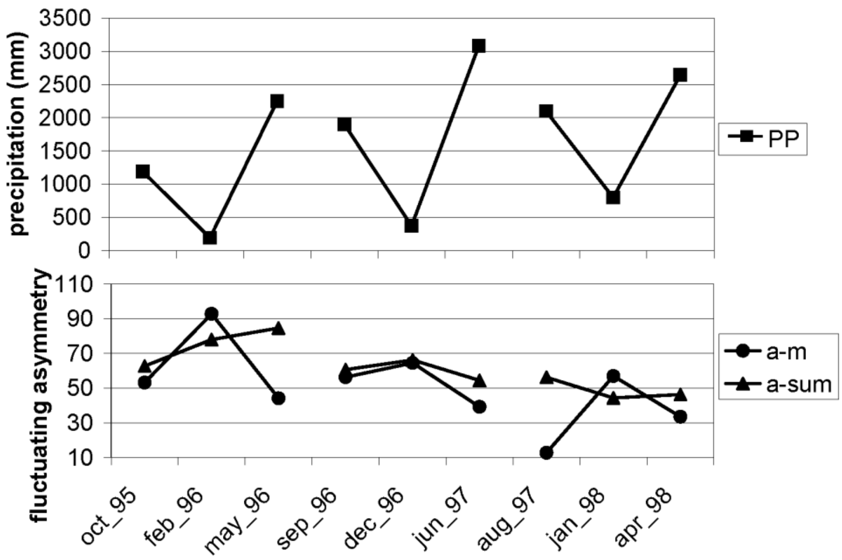

Figure 4. Variation of sample fluctuating asymmetry and precipitation (PP – precipitation in the period of development, a-m – fluctuating asymmetry of the mandible, a-sum – summed fluctuating asymmetry of all the teeth).

This relation appears more evident if we analyse the relation between seasonal rainfall level and the level of asymmetry (Fig. 4).

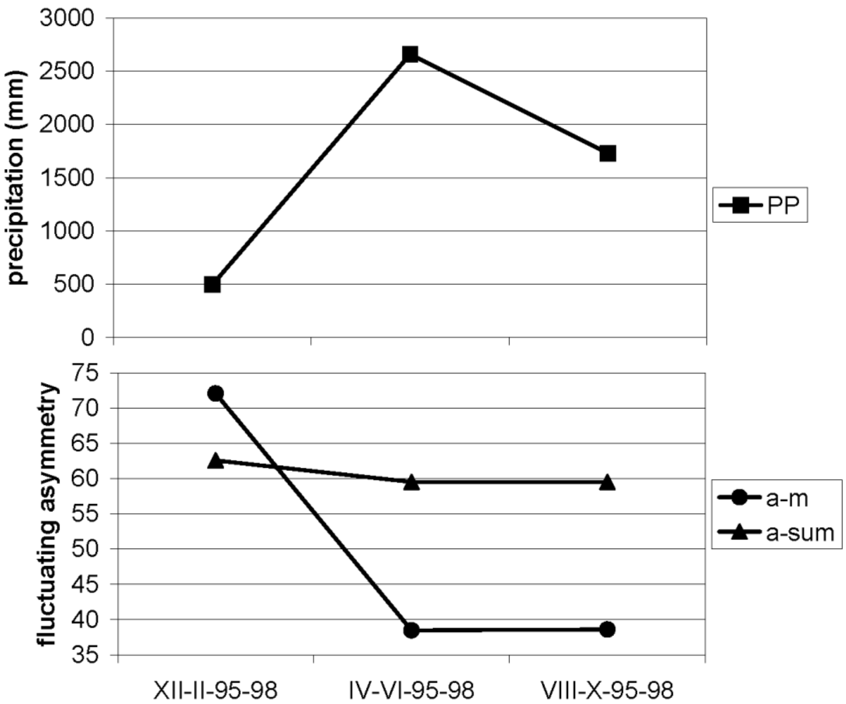

Figure 4. Variation of seasonal fluctuating asymmetry and precipitation (PP – precipitation in the period of development, a-m – fluctuating asymmetry of the mandible, a-sum – summed fluctuating asymmetry of all the teeth).

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 (Palmer, 1994). 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 teeth 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. Furthermore, this hypothesis is supported by the fact that it is highly improbable that directional asymmetry would affect just these 3 teeth out of all.

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 months (Chambers & Milne, 1975; Mettam, 1979; Mettam *et al.*, 1982; Gillet, 1990; Scaps *et al.*, 1993). 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 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. Among the studied environmental factors that could provoke fluctuating asymmetry variations (the concentration of Hg, Cd, Pb, Zn, Cu, ammonium sulphate and salinity) the only one associated with the asymmetry was the precipitation from the sampling area. We consider the precipitation as an important factor, given that *Nereis diversicolor* not only is able to survive in fresh waters (Bogucki, 1963) but also seems to prefer it to marine waters (Ibáñez, 1973). 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. 3). In the samples from 1995 – 1996 and 1997 – 1998, the sample asymmetry does not follow the correspondent 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 month later than expected. Thus, there may be a latency period in the reaction of teeth development to the asymmetry inducing factors.

If seasonal precipitation and asymmetry are plotted together (Fig. 4) 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.

Conclusions

The lengths of the mandibles and of its teeth in *Nereis diversicolor* display a fluctuating asymmetry.

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 months 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.

References

Batten, S.D., Bamber, R.N., 1996. The effects of acidified seawater on the polychaete *Nereis virens* SARS, 1835. *Marine Pollution Bulletin* 32 (3): 283–287.

Bogucki, M., 1963. The influence of salinity on the maduration of gametes of *Nereis diversicolor* O. F. Müller. *Polskie Archiwum Hydrobiologii* 11: 343–347.

Bonsdorff, E, Bakke, T, Pedersen, A., 1990. Colonization of amphipods and polychaetes to sediments experimentally exposed to oil hydrocarbons. *Marine Pollution Bulletin* 21 (7): 355–358.

Briggs, K.B., Tenore, K.R., Hanson, R.B., 1979. The role of microfauna in detrital utilization by the polychaete *Nereis succinea* (Frey y Leuckart). *Journal of experimental marine Biology and Ecology* 36: 225–234.

Carral, E., Puente, X., Villares, R., Carballeira, A., 1995a. Background heavy metal levels in estuarine sediments and organisms in Galicia (Northwest Spain) as determined by model analysis. *Science Total Environment* 172 (2 - 3): 175–188.

Carral, E., Villares, R., Puente, X., Carballeira, A., 1995b. Influence of watershed lithology on heavy metal levels in estuarine sediments and organisms in Galicia (Northwest Spain). *Marine Pollution Bulletin* 30 (9): 604–608.

Chambers, M.R., Milne, H., 1975. Life cycle and production of *Nereis diversicolor* O. F. Müller in the Ythan Estuary, Scotland. *Estuarine and Coastal Marine Science* 3: 133–144.

Clarke, G.M., 1993. Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. *Environmental Pollution* 82: 207–211.

Clarke, G.M., 1994. Developmental stability analysis: An early-warning system for biological monitoring of water quality. *Australian Biologist* 7: 94–104.

Davey, J.T, Watson, PG., 1995. The activity of *Nereis diversicolor (Polychaeta)* and its impact on nutrient fluxes in estuarine waters. *Ophelia* 41 (6): 57–70.

Eriksen, K.D.H, Deea, HL, Andersen, RA., 1988. Evidence of presence of heavy metal - binding proteins in polychaete species. *Comparative Biochemistry and Physiology* 91 c (2): 377–384.

Eriksen, K.D.H., Andersen, T., Gray, J.S., Stenersen, J., Andersen, R.A., 1989. Metal - binding in polychaetes: Quantitative and qualitative studies of five species. *Marine Environmental Research* 28: 167–171.

Eriksen, K.D.H., Andersen, T., Stenersen, J., Andersen, R.A., 1990. Cytosolic binding of Cd, Cu, Zn, and Ni in four polychaete species. *Comparative Biochemistry and Physiology* 95 c (1): 111–115.

Esselink, P., Van Belkum, J., Essink, K., 1989. The effect of organic pollution on local distribution of *Nereis diversicolor* and *Corophium volutator*. *Netherlands Journal of Sea Research* 23 (3): 323–332.

Fritzsche, D., Von Oertzen., JA., 1995. Metabolic responses to changing environmental conditions in the brackish water polychaetes *Marenzelleria viridis* and *Hediste diversicolor*. *Marine Biology* 12 (4): 693–699.

Gillet, P., 1990. Biomasse, production et dynamique des populations de *Nereis diversicolor (*annélide polychète) de l´estuaire de la Loire (France). *Oceanologica Acta* 13(3):361–371.

Grant, A., Hateley, J.G., Jones, N.V., 1990. Interpopoulation variation of life history and metal tolerance in *Nereis diversicolor*. In J. C. Aldrich (ed.). *Proceedings of Conference: Phenotypic Responses and Individuality in Aquatic Ectotherms*: 167 -175. Held at Trinity College. Dublin.

Ibáñez, M., 1973. *Contribución al estudio ecológico de los anélidos poliquetos de la Península Ibéric*. Tesis Doctoral. Universidad Complutense. Madrid. 125 p.

Kristensen, E., 1988. Factors influencing the distribution of Nereid polychaetes in Danish coastal waters. *Ophelia* 29(2):127–140.

Leary, R.F., Allendorf, FW., 1989. Fluctuating asymmetry as an indicator of stress : Implications for conservation biology. *Trends in Ecology and Evolution* 4(7):214–217.

Mettam, C., 1979. Seasonal changes in populations of *Nereis diversicolor* O. F. Müller from the Severn estuary. *Cys. Ph. In Mar Plants An* 7:123–130.

Mettam, C., Santhanam, V., Havard, M.S.C., 1982. The oogenic cycle of *Nereis diversicolor* under natural conditions. *Journal marine biology Association U.K.* 62: 637–645.

Miron, G., Kristensen, E., 1993. Factors influencing the distribution of Nereid polychaetes: the sulfide aspect. *Marine Ecology Progress Series* 93:143–153.

Palmer, A.R., 1994. Fluctuating asymmetry analysis: A primer in: Markow (ed), *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer, Dordrecht, Netherlands. 335 – 364.

Palmer, A.R., Strobeck, C., 1986. Fluctuating asymmetry: Measurement, analysis, patterns. *Annual Review of Ecology and Systematics* 17:392–421.

Palmer, A.R., Strobeck, C., 1992. Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. *Acta Zoológica Fennica* 191:57–72.

Palmer, A.R., Strobeck, C., 2003. Fluctuating Asymmetry Analyses Revisited. In Polak, M. (ed) *Developmental Instability Causes and Consequences*, Oxford University Press. p. 279–319.

Pocklington, P., Wells, P.G., 1992. Polychaetes, key taxa for marine environmental quality monitoring. *Marine Pollution Bulletin* 24(12):593–598.

Reish, D.J., 1985. The use of the polychaetous annelid *Neanthes arenaceodentata* as a laboratory experimental animal. *Tethys* 11:335–341.

Riedel, G.F., Sanders, J.G., Osman, R.W., 1987. The effect of biological and physical disturbances on the transport of arsenic from contaminated estuarine sediments. *Estuarine, Coastal and Shelf Science* 25:693–706.

Riedel, G.F., Sanders, J.G., Osman, R.W., 1989. The role of three species of benthic invertebrates in the transport of arsenic from contaminated estuarine sediment. *Journal of Experimental Marine Biology and Ecology* 134:143–155.

Scaps, P., Retière, C., Desrosiers, G., Miron, G., 1993. Effects de la ration alimentaire, de la densité intraspécifique et des relations entre individus sur la croissance des juvéniles de l´espèce *Nereis diversicolor (*Annelida: Polychaeta). *Canadian Journal of Zoology* 71(2):424–430.

Selye, H., 1978. *The stress of life, 2nd edition*. New York: McGraw – Hill. 516 p.

Steimle, F.W., Kinner, P., Howe, S., Leathem, W., 1990. Polychaete population dynamics and production in the New York Bight associated with variable levels of sediment contamination. *Ophelia* 31(2):105–123.

Thoday, J.M., 1955. Balance, heterozygosity and developmental stability. *Cold Spring Harbor Symposium on Quantitative Biology* 20:318–326.

Thoday, J.M., 1958. Homeostasis in a selection experiment. *Heredity* 16:125–142.

Tsutsumi, M., 1987. Population dynamics of *Capitella capitata* (Polychaeta Capitellidae) in an organicelly polluted core. *Marine Ecology Propress Series* 36:139–149.

Tsutsumi, M., Fukunaga, S., Fujita, N., Sumida, M., 1990. Relationship between growth of Capitella sp. and organic enrichment of the sediment. *Marine Ecology Progess series* 63:157–162.

Van Valen, L., 1962. A study of fluctuating asymmetry. *Evolution* 16:125–142.

Villalba, A., Vieitez, J.M., 1985. Estudio de la fauna de anélidos poliquetos del sustrato rocoso intermareal de una zona contaminada de la ría de Pontevedra (Galicia). *Cahiers de Biologie Marine* 26:359–377.

Waddington, C.H., 1942. Canalisation of development and the inheritance of acquired characters. *Nature* 150:563–565.