The possible use of sandhoppers as bioindicators of environmental stress on sandy beaches

Posibilidades de utilización de talítridos como bioindicadores de estrés ambiental en playas de arena

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Palabras claves: talítridos, playas de arena, bioindicadores, asimetría fluctuante.

ABSTRACT

Several studies have addressed the biology and behavioural adaptations of sandhoppers, common and widespread inhabitants of sandy beaches in temperate areas. The increasing stress (both natural and anthropogenic) to which beaches are subject around the world has posed the question of the choice of good bioindicators to monitor changes in sandy beach ecosystems and propose management options for their conservation. Sandhoppers carry out their whole life cycle on the same beach throughout the year, so they may integrate the effects of environmental changes over a yearly time scale in population features. Moreover, throughout their life cycle, sandhoppers occupy various zones of the beach, from the intertidal zone up and behind the dunes, integrating the local spatial scale that is of interest for the management of sandy beaches. Comparisons among populations from beaches subject to different stressful factors have been conducted over a wide geographical scale (that of the species distribution) and a large base of data exists. Population characteristics, such as seasonality of the life cycle, age structure, sex ratio and genetic variation, as well as behavioural and physiological traits, have shown a potential for their use as suitable bioindicators of environmental stress on beaches. In this study, following the hypothesis of Møller and Swaddle we propose the level of fluctuating asymmetry as a bioindicator of mechanical stress during the critic phases of development.

RESUMEN

Muchos estudios previos han tenido como objetivo la biología y las adaptaciones comportamentales de talítridos, que son comunes y ampliamente difundidos en playas de arena de áreas templadas. El aumento de estrés (natural o de origen humano), al que las playas están expuestas a nivel mundial, ha hecho necesaria la selección de bioindicadores adecuados para el monitoreo de cambios que afectan los ecosistemas de las playas y la proposición de prácticas de manejo para su conservación. Los talítridos desarrollan su ciclo vital completo en la misma playa de origen, durante todo el año, así que en las características de las poblaciones se pueden integrar los efectos de las variaciones ambientales a lo largo de una escala temporal de un año. Además, durante su desarrollo, los talítridos ocupan diferentes zonas de la playa, de la zona intermareal hacia las dunas, cubriendo, a escala local, la zona completa de interés para el manejo de playas. Se han comparado poblaciones de playas afectadas por diferentes factores de estrés, en una amplia escala geográfica (coincidente con la de la distribución de la especie), recopilando una base de datos importante. Las características de la población, como estacionalidad del ciclo vital, clases de edad, sex ratio y variación genética, así como componentes comportamentales y fisiológicas, han resultado potenciales bioindicadores de estrés ambiental sobre playas de arena. En este estudio proponemos la asimetría fluctuante, según la hipótesis de Møller y Swaddle, como bioindicador de estrés mecánico durante las fases criticas del desarrollo de los individuos.

INTRODUCTION

Sandhoppers, e.g. the amphipod talitrids *Talitrus saltator* and *Talorchestia spp.*, are widespread and common inhabitants of sandy beaches in temperate areas and represent an important trophic link (scavengers) between the land and the sea. A rich literature exists on these species, on its zonation and distribution (e.g. Pavesi *et al.*, 2009), life cycle (e.g. Marques *et al.*, 2003), physiological (e.g. Morritt & Spicer, 1998) and behavioural (review in Scapini, 2006) adaptation over a range of beaches. Sandhoppers carry out their whole life cycle on the same beach and move from the intertidal to the supratidal zones and back by adjusting their zonation to the current substrate and trophic features as well as weather conditions and tidal phases (Scapini *et al.*, 1992). The wide and sound baseline of data on the same species in sandy beaches that are subject to increasing pressures and threats (Defeo *et al.*, 2009; Dugan *et al.*, 2010) makes sandhoppers good candidates as bioindicators of environmental stress. The use of talitrids as bioindicators has the advantage to allow comparisons across a wide geographic range (that of the distribution of the species) and throughout years (broods are produced each month, with two or three generations a year, and individuals may live for about one year). Moreover, being the species relatively robust against impacts, differences among populations may be used as early warning indicators of stress, before the populations disappear completely under severe environmental pressures (Scapini, 2002).

The sandhoppers have been repeatedly proposed as bioindicators of the pressure of human trampling on sandy beaches (e.g. Weslawski *et al*., 2000a, 2000b, 2000c; Fanini *et al*., 2005; Ugolini *et al*., 2008). Trampling is an increasing pressure on sandy beaches due to their increasing use for recreational activities. To estimate the carrying capacity of these ecosystems under pressure, it is important to define an early warning bioindicator of trampling, whose estimates would not depend on the sampling efforts and the abundance of the population at that particular time of the year.

A good candidate for early warning of environmental stress is the level of fluctuating asymmetry (FA) as proposed by Møller and Swaddle (1997) for bilateral organisms. The factor of interest is the correct development of the bilateral symmetry in the individuals of a particular population. The mean level of fluctuating asymmetry found in populations subject to different levels of impact would then represent the bioindicator of environmental stress. The development of an organism depends on the interactions, throughout time, of its genes and the environment where development occurs. FA is a population phenomenon defined by random slight deviations of one morphological character from a perfect bilateral symmetry. FA is measured by the differences between the right and left sides of bilateral characters in the individuals of a population. Statistically, FA follows two rules: the differences between right (R) and left (L) body sides are normally distributed and have a mean around zero, as under ideal conditions a certain number of individuals have a character more developed on one side of the body and the same number of individuals has the same character more developed on the other side. The variance of the frequency distribution characterizes the asymmetry level of each character. According to the hypothesis suggested by Møller and Swaddle (1997), each time that genetic or environmental stresses are acting during development, asymmetries may derive in the bilateral structure of the organisms. Stressful factors of genetic origin may be mutations, high levels of homozygosity, hybridation that disrupts coadaptated genes and/or selection pressures. In all of these cases, the capacity of the organism to buffer environmental stress is affected. Environmental stressful factors may be: extreme temperatures and humidities, chemical pollution, decreased food availability, etc. Therefore, populations that show relatively high levels of fluctuating asymmetry may have been subject to stressful factors.

MATERIAL AND METHODS

Study sites and sampling

We sampled four populations of *Talitrus saltator* (Montagu, 1808) from the following sandy beaches (Fig. 1):

1. Maganuco – southeastern Sicily – Malta Channel (province of Ragusa, Italy) – 36°43'01.00"N 14°49' 13.62"E, sampled the 21/08/2008 and 23/08/2008;
2. Bruca – southeastern Sicily – Malta Channel (province of Ragusa, Italy) – 36°44'07.65"N 14°40'41.75"E, sampled the 25/08/2008;

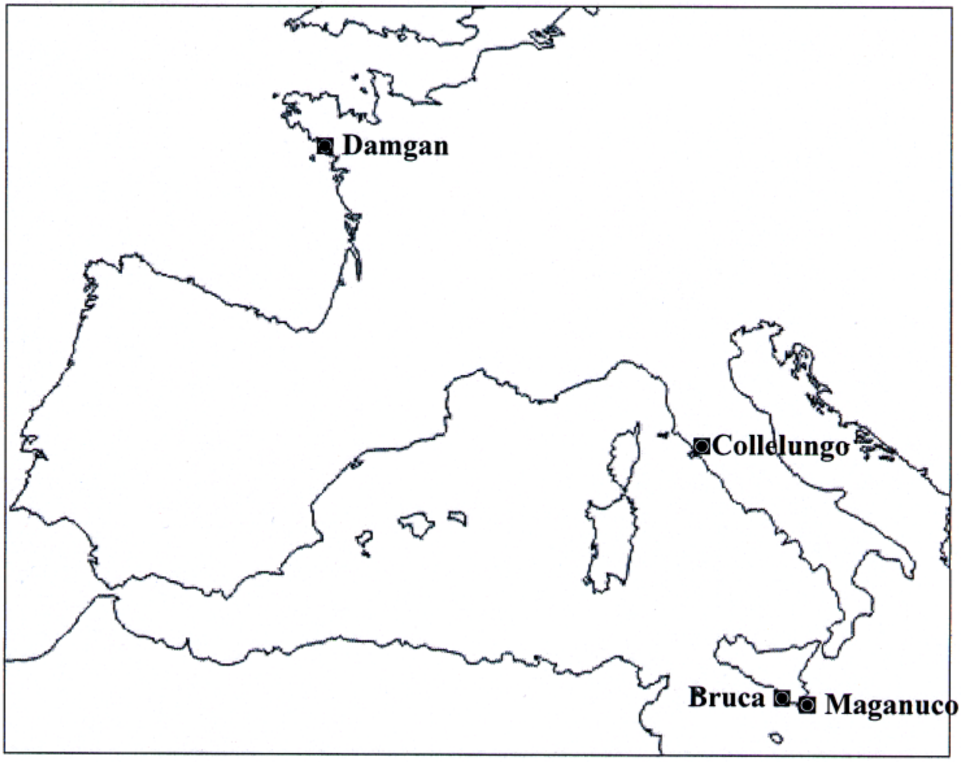


Fig. 1.—Location of the study sites with U.T.M. coordinates. Damgan (30T531633.08 E, 5262681.39 N); Collelungo (32T669625.22 E, 4722485.92 N); Bruca (33S471274.31 E, 4065574.73 N); Maganuco (33S483965.40 E, 40633487.86 N).
Fig. 1.—Localización de las estaciones de studio con coordenadas UTM. Damgan (30T531633.08 E, 5262681.39 N); Collelungo (32T669625.22 E, 4722485.92 N); Bruca (33S471274.31 E, 4065574.73 N); Maganuco (33S483965.40 E, 40633487.86 N).

3. Collelungo – southern Tuscany – Maremma Regional Park (province of Grosseto, Italy) – 42°38’10.14”N 11°04’07.95”E, sampled the 03/10/2008 and 21/4/2010;
4. Damgan - Brittany (Department of Morbihan, France) – 47°31’00.45”N 02°34’47.45”, W, sampled the 29/08/2009.

We chose these sampling sites to compare the levels of FA of populations exposed to increasing tourism impact (Maganuco, Bruca and Damgan), to a chronic pressure of oil pollution (Maganuco and Bruca), and to pesticide pollution (Bruca), with the FA of the Collelungo population that lives within a relatively non-impacted natural park. The Atlantic (Damgan) population was chosen to compare the effects of more severe tidal and climatic pressures. We also analysed eventual seasonal effects on FA by comparing samples of the same population (Collelungo) collected in spring and autumn.

After moving the superficial layer of sand, we captured talitrids using an entomological aspirator. We collected only adult talitrids and the samples consisted of about 50 individuals for each population. Then we measured the sampling area to quantify the sampling effort and calculate the density of each population. Talitrids were then transferred to a plastic boox containing moist sand from the same beach and with perforations on the top to ensure aeration. On the same day of the sampling, or on the following day, the talitrids were placed singly into Eppendorf tubes filled with absolute ethanol (99.9%), and thereafter stored at −20°C.

Morphometric measurements

The first basic criterion for the choice of the bilateral segments to measure was the clarity and constancy of landmarks; the second was to avoid linking the measure of one trait to another (for example, the use of a spine as the landmark of a segment would give a biased measure, by proceeding from the point where the spine develops and depending on its length and orientation); the third principle was to analyse rectilinear segments; finally, we did not measure two or more segments of the same appendage, e.g. the merus and carpus of a pereiopod, as they may correlate (Ottaviano & Scapini, 2010). In consideration of the anamorphic development of crustaceans, the right and left P3, P6 and P7 (P=pereiopod) were the best candidates for the morphometric analyses. In these appendages the merus is the best segment with regard to landmarks and ease of measuring at the magnification of 50X. When studying FA it is important that the units used are not too large in relation to the actual measure. According to our observations, the posterior side was the most suitable for the P6 and P7 meri; for both segments, the distal landmark was the apex of the knee (disregarding the spine), while the proximal one was the light notch with the ischiopodite, best observed looking from the inner face of the segment. For the P3 merus, the anterior side was the most suitable, with the same landmarks as for the P6 and P7 meri, but best observed from the external face of the segment. For a better identification of the landmarks we disarticulated the meri of the P3, P6 and P7 from the rest of each appendage. We performed two measurements of the anterior side of the P3 meri and of the posterior sides of the P6 and P7 meri; we carried out the two measurements of the same merus of each sandhopper on the same day, with a time lapse of at least three hours. Between the two measurements of the same merus, we analysed other samples, so that the observer was not conditioned by the previous measures. The metric measures obtained by the stereomicroscope were converted to micrometers by suitable conversion calculations. For each specimen we identified the species and defined the sex. The samplings and measurements of sandhoppers were both performed by the same person to reduce the eventual bias due to different observers.

Statistical analysis

The analysis of FA was performed as recommended by Palmer and Strobeck (1986) and Palmer (1994); we used Excel and S-Plus 6 for Windows; the significance level was 5%. Sandhoppers that were lacking one of the bilateral appendages were discarded from the analysis as this strong difference between the values of the two sides of their body would have seriously affected the FA index. We excluded outliers from the analyses also because their values may be related to physical damage or severe deformities, and these events can not be considered developmental noise (Palmer, 1994). Thus, before performing the tests for measurement errors, box plots of the right and left sides were made and screened for outliers. If a sandhopper was an outlier for any of the pereiopods we excluded it from the statistical analysis of all pereiopods. To check whether there was any sexual dimorphism of the meri, we divided each population according to sex. In the Bruca and Collelungo populations no significant difference between sexes was found in any pereiopods. In the Maganuco population the F-test showed a significant difference ($p<0.05$) when males and females were compared for the left merus of the P3; yet, as in all other comparisons, the t-test was not significant. Thus, we considered the two sexes together. Chi-square test was used to test the sex ratio of the sample of each population. For metric traits we used the F- and t-tests to compare the first and the second measures of a segment. Having ruled out significant contributions of human measurement error, the two replica measurements were averaged for further analysis.

For each bilateral trait the presence of directional asymmetry was tested by one-sample *t*-test. Normality was tested using graphical techniques. The FA levels were analysed using the index FA1=Mean |R-L| (as defined by Palmer, 1994; R=length of the right merus, L=length of the left merus). One-way ANOVAs were used to test differences among populations for each of the traits and for FA levels.

RESULTS

The sampled populations had different densities (individuals/m²): Maganuco 2.9, Bruca 3.8, Collelungo 11.4 in October 2008 and 6.8 in April 2010; Damgan 5.5.

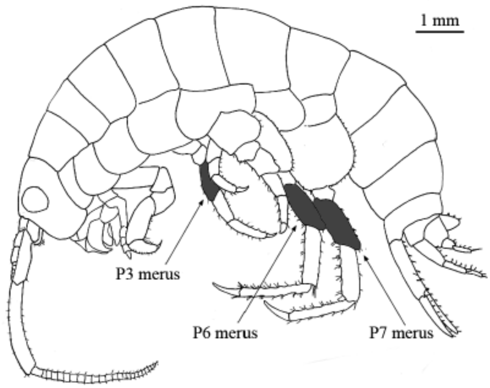
The sex ratios (males/females) of the samples were significantly female biased in the Collelungo population in October 2008 (0.50, χ^2-test: $p<0.05$), but significantly male biased in the Bruca population (2.69, χ^2-test: $p<0.01$); in the Maganuco and Damgan populations females were more abundant than males, but the sex ratio was not significantly different from the expected balance of 1:1.

Considering all the pereiopods together, we observed the highest percentages of outliers in Collelungo 2008 (14.8%), while the Bruca population had the lowest one (4.2%).

Using one-way ANOVA, we compared the length of the meri of each pereiopod in the populations. We found a significant difference among them (ANOVA: $p<0.001$). In the Collelungo April population the meri of the right and left P6 and P7 were longer than in the other populations. The Damgan population had the longest P3. The Maganuco population had the shortest P3, P6 and P7.

In all populations and in all meri the values of the differences between the right and left sides (R-L) were normally distributed and their means were not significantly different from zero (one-sample *t*-test), except for the P3 in the Damgan population that showed a right directional asymmetry. We excluded directional asymmetry for all the other cases. The plots showed normality, thus antisymmetry was also discarded.

The Collelungo October sandhoppers had significantly higher levels of fluctuating asymmetry in the P3 ($p<0.001$), P6 ($p<0.001$) and P7 ($p<0.05$) meri than the other populations (Fig. 2).



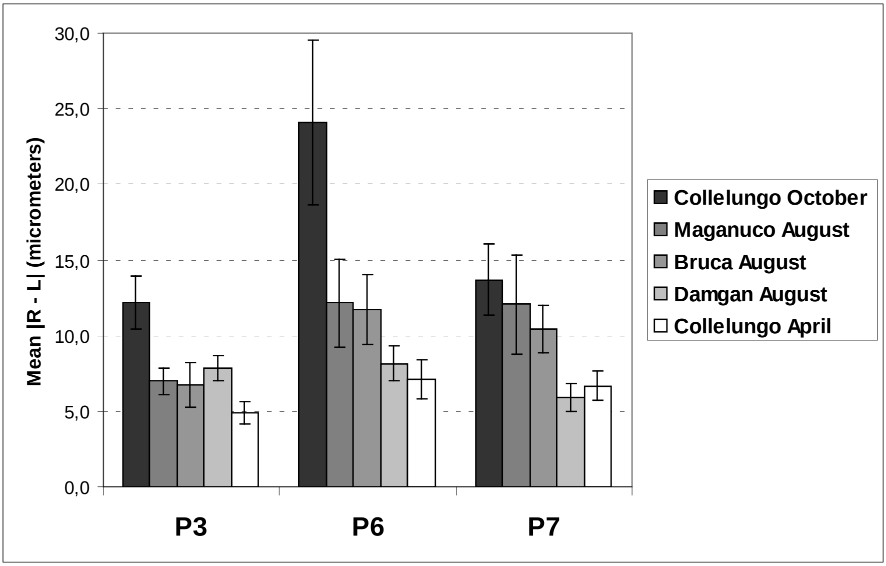


Fig. 2.—A: *Talitrus saltator*. In grey – the segments used for morphometric analyses (meri of P3, P6 and P7); P=pereiopod. (From Ruffo, 1993, modified.). B: Fluctuating asymmetry values (FA1 index =Mean |R-L| ± SE; Palmer, 1994) for the meri of the P3, P6 and P7 in the populations of Collelungo October (sampled on 3/10/2008), Maganuco (sampled on 21 and 23/08/2008), Bruca (sampled on 25/08/2008), Damgan (sampled on 29/08/2009) and Collelungo April (sampled on 21/04/2010). P=pereiopod; FA=fluctuating asymmetry; R=length of the right merus; L=length of the left merus; SE=standard error.

Fig. 2.—A: *Talitrus saltator*. En gris los segmentos usados para los análisis morfométricos (mero de P3, P6 y P7); P=pereiópodo (modificado de Ruffo, 1993). B: Valores de asimetría (FA1 índice = Media |R-L| ± SE; Palmer, 1994) para el mero de P3, P6 and P7 en las poblaciones de Collelungo octubre (muestreado el 3/10/2008), Maganuco (muestreado el 21 y 23/08/2008), Bruca (muestreado el 25/08/2008), Damgan (muestreado el 29/08/2009) y Collelungo abril (muestreado el 21/04/2010). P=pereiópodo; FA=Asimetría fluctuante; R=Longitud del mero derecho; L=Longitud del mero izquierdo; SE=Error estándar.

DISCUSSION

When we planned the sampling strategy, we expected higher levels of FA and lower densities in the two Sicilian populations (Maganuco and Bruca) in comparison to the Collelungo one, in consideration of the chemical pollution and the increasing (in the last fifteen years) tourism impact in this area. The two Sicilian populations are subject to chronic oil pollution, as documented by tarry residues on the beach, as well as to pesticide discharge in the Bruca beach.

However, our analyses did not highlight any difference between the two Sicilian populations and their FA levels were significantly lower than that of Collelungo October population. Also Barca-Bravo *et al*. (2008) did not observe increasing levels of FA in Galician (Spain) populations of *T. saltator* that had been subject to pulse stress from the oil spill of Prestige shipwreck (2002) in comparison to populations impacted by chronic pressures of activities linked to tourism and industry. The absence of high levels of FA following chemical pollution may be attributed to high levels of tolerance expressed by talitrid populations. Thus, FA levels in *T. saltator* populations can not be used as bioindicators of chemical stress on sandy beaches. However, other elements of the ecosystems may be affected.

In contrast, the Collelungo population showed a significantly higher level of FA in October. This beach is not chemically polluted, being located within a natural park. In the surroundings of the park biological agriculture is the rule and the drainage waters are controlled for chemicals (Scapini & Nardi, 2007). Nevertheless, the Collelungo beach has a relatively high presence of visitors during April and May, the period of development of *T. saltator* (Marques *et al*., 2003), while during the summer months the park is closed to visitors for fire prevention, so it is possible to reach the beach only on foot from an entrance 5 km away from the sampling site (Fanini *et al*., 2005). In contrast, the two Sicilian beaches are not frequented during the period of sandhopper development, whereas there is a relatively high presence of tourists during August. It must be stressed that in August the sandhoppers born in spring are already developed.

The latitudinal difference between the Tuscan (Collelungo) and Sicilian (Bruca and Maganuco) sampling sites may affect the FA levels, by disturbing development during spring. For this reason we analysed the Damgan population, located at a higher latitude on the Atlantic coast and thus subject to tidal and climate conditions different from the Mediterranean ones. Also the comparison between the samplings performed at the same locality (Collelungo) in spring and autumn may clarify this point. The low levels of fluctuating asymmetry observed in both the Collelungo April and the Damgan populations excludes a climate impact on meri bilateral development.

The sandhoppers collected in April at Collelungo were older than those collected in summer and autumn (Marques *et al*., 2003). This may explain the low level of fluctuating asymmetry observed in Collelungo April. In older populations asymmetric sandhoppers may have died or may have compensated asymmetries throughout moults.

The current dynamics of the shoreline of Collelungo, subject to erosion at one end of the beach (the side of the Ombrone River mouth) and accretion at the other one, may cause a stress to the *T. saltator* population, which was observed in the genetic structure and orientation behaviour (Ketmaier *et al.,* 2010). However, these morphodynamic factors cannot explain the relatively high levels of FA observed in October in the Collelungo population and the low ones observed in April in the same population. The difference is better explained by the population dynamics (Marques *et al*., 2003).

Regarding densities, we found lower densities in the two Sicilian populations as expected from the impacts acting on these beaches. However, the differences among populations were not marked and the order of magnitude was similar for all the populations sampled. The lower densities of the Sicilian populations in comparison with the other ones may be attributed to the impact of trampling in the sampling period. However, a different sampling strategy, conducted in different times of the year and including also juveniles, would be necessary to derive conclusions on this point (Fanini *et al*., 2009). It is interesting that the FA levels do not appear linked to density levels.

An interesting and unexpected finding of this study was the low FA level in the meri of P7. This is the longest pereiopod and, according to Møller and Swaddle (1997), higher levels of FA are to be expected in longer structures in comparison with shorter ones, except when a bilateral symmetry is physiologically useful and thus subject to a stabilising selection. In his basic monograph, Schellenberg (1942, pp. 139-140) described the movements of *T. saltator* and stressed the importance of a balanced use of P7 during crawling and hopping. In contrast, the merus of P3 pereiopod showed the highest levels of FA.

CONCLUSIONS

Ecosystems are among the highest levels of organization of living matter. Therefore, the assessment of the ecosystem quality should be made through parameters of a high level of integration. These are population characteristics (e.g. seasonality of the life cycle, age structure, sex ratio and genetic variation, as well as behavioural and physiological traits) or community features (e.g. presence of allochthonous species and biodiversity level). Some of these parameters, however, may depend on the sampling efforts and the abundance of the populations at that particular time of the year. Apparently FA levels in sandhopper populations avoid these constraints and are sensitive enough to mechanical factors (e.g. trampling). Following the present study we propose the level of FA of *T. saltator* as an early warning bioindicator of mechanical stress of sandy beach ecosystems. We also recommend to estimate FA levels of the meri of P3 and P6 because they are shorter and thus less subject to measurements errors. Considering the season of development of *T. saltator*, the FA levels should be related both to the life cycle of the population and the seasonality of the mechanical stressful factors, particularly the seasonality of the activities linked to tourism.

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