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16 October 2026

Can fluctuating asymmetry in *Talitrus saltator* (Montagu, 1808) (Crustacea, Amphipoda) populations be used as a bioindicator of stress on sandy beach ecosystems?

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Abstract

This study focused on verifying the fluctuating asymmetry hypothesis in the crustacean *Talitrus saltator*, which lives in sandy beaches. We analysed three populations, one from an unpolluted Tuscan beach relatively free of tourism, and two from Sicilian beaches, which have been increasingly used for tourism and have been exposed to hydrocarbon/pesticide pollution. Results confirmed the sexual dimorphism in the second antennae flagella, which in the Tuscan population presented directional asymmetry. This population had a significant level of fluctuating asymmetry in the P6 and P3 meri. The results showed the importance of the developmental stage during which environmental mechanical stresses act.

1. Introduction

The aim of our research was to verify the fluctuating asymmetry hypothesis (Møller & Swaddle 1997) in the supralittoral amphipod *Talitrus saltator* (Montagu 1808) in order to postulate this population characteristic (in the case of a verification of the hypothesis) as an indicator of environmental stress on sandy beach ecosystems. Fluctuating asymmetry is defined at population level as a random slight deviation of a morphological character

The complete text of the paper is available at http://www.iopan.gda.pl/oceanologia/

from perfect bilateral symmetry. Body asymmetry is measured through the differences between the right and left elements of bilateral characters of an organism in a population sample. It is usually the result of the different development of the two body sides of an individual. From a statistical point of view, fluctuating asymmetry follows two rules: the values of the differences between the right (R) and left (L) body sides are (1) normally distributed, and (2) have the mean around zero (as a certain number of individuals have a character more developed on one side of the body and the same number of individuals have the same character more developed on the other side). The variance of the frequency distribution characterises the asymmetry level of each character. The analysis of statistical properties makes it possible to distinguish fluctuating asymmetry from other kinds of asymmetry, that is, directional asymmetry and antisymmetry (Figure 1, Palmer 1994). Directional asymmetry is observed when one side tends to be more developed than the other one. In this case it is possible to predict which side of the character will be greater before its development. In directional asymmetry the values of the differences between the right and left sides of the body are normally distributed, but with the mean significantly different from zero. In directional asymmetry development path deviations are oriented towards a specific direction and genetically coded. Antisymmetry occurs when one side of a character is larger than the other one, but there is no preference for one side. Therefore, it is not possible to predict which side will be larger. An example in crustaceans is the male fiddler crab (the genus *Uca*). These crabs begin their life with two large signal claws, similarly developed. Developmental asymmetry is determined by which of the two claws will first be damaged or lost. The damaged or lost claw will grow again, but will be small, like those of females. This will happen in a random way, with 50% occurring on the right side and 50% on the left; then there is a typical bimodal distribution (in the same sample there are two groups of individuals which tend to develop claws in opposite ways) or a platycurtic (broad-peaked) distribution of the R-L difference

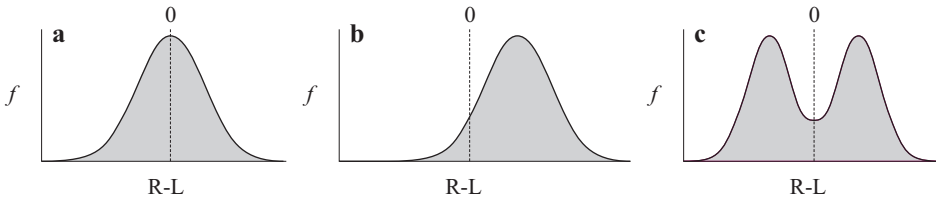

Figure 1. Three common frequency distributions of R-L (difference values between right and left body sides) in bilateral organisms: a) fluctuating asymmetry; b) directional asymmetry; c) antisymmetry (from Palmer 1994, modified)

values around a mean of zero (Palmer & Strobeck 1986, Møller & Swaddle 1997). Møller & Swaddle's (1997) hypothesis asserts that whenever, during development, there are genetic or environmental stresses, asymmetries of the bilateral structure of an organism will occur. Among genetic stresses we count mutations, a high degree of homozygosity, hybridisation that divides coadapted gene sets, inbreeding, intense periods of directional selection; all these phenomena interfere with the organism's genomic balance, reducing its ability to buffer random developmental mistakes that will eventually result in body asymmetries. Environmental stresses, e.g. adverse temperatures, chemical pollution or food shortage, can further destabilise organisms during sensitive phases of their development. Therefore, populations showing different levels of fluctuating asymmetry are very likely subjected to different degrees of stress. The bilateral characters of an individual are distinguished in: 1) meristic characters, which are in a discrete quantity and can be counted; asymmetry of these characters is usually suggestive of extremely high levels of stress; 2) metric characters, i.e. distance measures between two points of the body; metric characters are more sensitive indicators of the developmental stability of an organism (Møller & Swaddle 1997).

We used the supralittoral amphipod *Talitrus saltator*, a common species of sandy beach communities in the north-eastern Atlantic, southern Baltic and Mediterranean, to test for the possible relation between fluctuating asymmetry and the degree of environmental stress acting on beach ecosystems. The species has a number of characteristics that make it a reliable model organism for this kind of research. First, its morphology (bilateral symmetry and metameric organisation) is particularly well suited to morphometric analyses (Barca-Bravo et al. 2008). Second, the species is relatively widespread with abundant populations (Scapini & Morgan 2002). Third, this species is robust enough to be found also in relatively polluted and/or disturbed beach ecosystems; for this reason it cannot be considered an indicator species of impact for its presence/absence, but differences between local populations may reflect differences between beaches (Scapini & Morgan 2002). Nevertheless, a reduction and/or disappearance of *T. saltator* populations was documented from beaches where tourist pressure was high (Węsławski et al. 2000). It was suggested that the dynamics of *T. saltator* populations could be used as a bioindicator of environmental stress caused by activities linked to tourism (Jędrzejczak 2004, Fanini et al. 2005). More importantly, *T. saltator* has been the subject of extensive studies, which has led to a detailed knowledge of the species' population dynamics (Marques et al. 2003), behavioural adaptations (a review in Scapini 2006) and population genetic structure (De Matthaeis et al. 2000, Ketmaier et al. 2005). The genetic homeostasis hypothesis

(Lerner 1954) received support from interdisciplinary researches based on the simultaneous analysis of molecular markers and orientation behaviour related to shoreline stability (Scapini et al. 1995, Ketmaier et al. 2010).

The present study focused on morphometry. For each individual, we examined one meristic bilateral character (the number of articles of the second antennae flagellum) and three metric bilateral characters, which are associated with different periods of the development of individuals (length of the merus of the P3, P6 and P7 pereiopods). In addition, we measured the length of the cephalic capsule as a proxy of body size (Marques et al. 2003). We studied three populations: two from south-eastern Sicily and one from Tuscany. The Sicilian populations have been exposed to hydrocarbon (this is a zone of marine oil drilling) and pesticide pollution (from greenhouses). Furthermore, in recent years these beaches have been increasingly used for tourism. The Tuscan population came from a natural park, the Maremma Regional Park, an unpolluted location relatively free from tourism (Fanini et al. 2005). We evaluated the degree of fluctuating asymmetry in the three populations to study possible relations with the impacts of activities linked to tourism and oil or pesticide pollution of water and sediments.

2. Material and methods

2.1. Study site description and sampling

We sampled three populations from the following sandy beaches (Figure 2 and Table 1):

1. Italy – southern Tuscany – Maremma Regional Park (province of Grosseto) – Collelungo (42°38′10.14″N – 11°04′07.95″E).
2. Italy – south-eastern Sicily – Malta Channel (province of Ragusa) – Maganuco (36°43′01.00″N – 14°49′ 13.62″E).
3. Italy – south-eastern Sicily – Malta Channel (province of Ragusa) – Bruca (36°44′07.65″N – 14°40′41.75″E).

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. Talitrids were then transferred to a glass container containing moist sand from the same beach and with perforations 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.

Table 1. Characteristics of the study sites

Beach	Collelungo (Tuscany)	Maganuco (Sicily)	Bruca (Sicily)
length	4400 m	890 m	1630 m
maximum width (diurnal low tide)	32.73 m	80 m	27.50 m
width at the capture point (diurnal low tide)	32.73 m	45.90 m	27.50 m
mean orientation (perpendicular to the shoreline)	211°	174°	234°
dune vegetation	yes	yes	yes (small cottages behind the dunes)
lateral boundaries	rocks on one side, gravel and rocks on the other side	rocks on both sides	rocks on one side, gravel and rocks on the other side
access points	2	6, of which 3 frequently used	3, of which 2 are adjoining and frequently used
stream or drain	yes, left bank of the Ombrone River, 5051 m from the sampling point	no	yes, a drain from greenhouses located behind flows into the sea, 678 m from the sampling point
protected area	yes	no	no
tourist presence	yes, especially in April and May	yes, especially in August	yes, especially in August
sand cleaning	no	yes, mechanical, in August	no
presence of talitrids	everywhere on the beach	in the eastern part of the beach (not very frequented by tourists)	everywhere, except in the 380 m around the main access points
sampling dates	3 October 2008	21–23 August 2008	25 August 2008
area in which we collected about 50 talitrids	10 m^2	17 m^2	21 m^2
number of collected talitrids	54	50	50
density (individuals m^{-2})	5.4	2.9	2.4
presence of talitrids after one year	yes, more abundant	yes	yes, but with lower density, the beach was slightly narrower

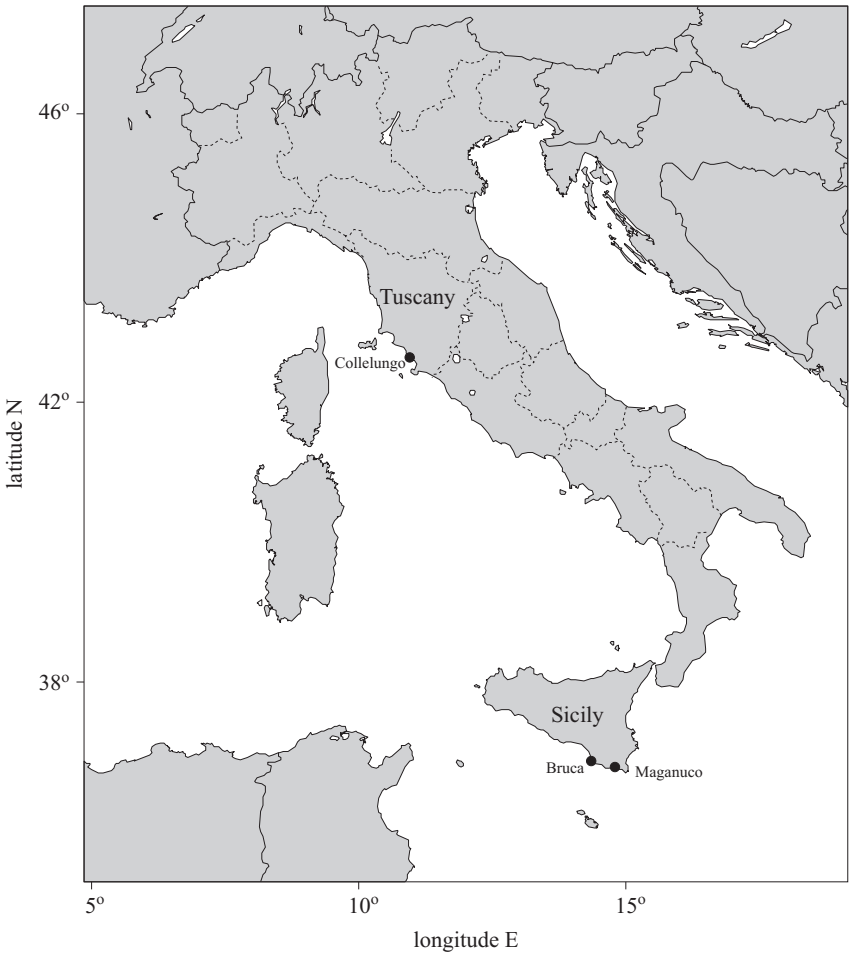

Figure 2. Location of the study sites

2.2. Morphometric measurements

The first basic criterion for the choice of 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, as we took the measures directly by the micrometer eyepiece of the stereomicroscope, without taking photos and performing image analyses. Finally, we did not measure two or more segments of the same appendage, e.g. the merus and carpus, as they correlate (personal observation). In view of the anamorphic

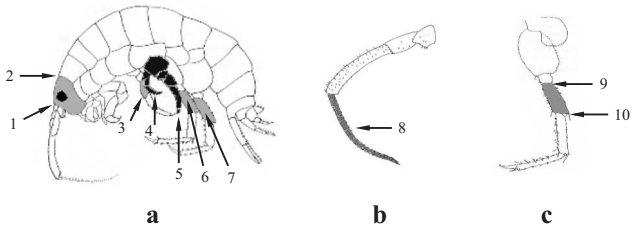

Figure 3. Diagram illustrating: a) *Talitrus saltator*. In black – the appendages that will be used for genetic analyses, in grey – the segments used for morphometric analyses, 1. distal landmark of the cephalic capsule, base of the first antenna, 2. proximal landmark, posterior end of the cephalic capsule, 3. merus of the P3 pereiopod, 4. P4 pereiopod, 5. P5 pereiopod, 6. merus of the P6 pereiopod, 7. merus of the P7 pereiopod; b) The second antenna, 8. articles of the flagellum (in grey); c) P6 pereiopod, the merus is shown in grey, 9. proximal landmark, light notch with the ischiopodite, 10. distal landmark, apex of the knee (disregarding the spine)

Table 2. Segments measured and landmarks used

	Cephalic capsule	Right and left second antennae (A2)	Right and left P3 pereiopods	Right and left P6 pereiopods
segment		flagellum	merus	merus
face			outer	inner
side	right		anterior	posterior
proximal landmark	posterior end of the capsule		light notch with ischiopodite	light notch with ischiopodite
distal landmark	base of the first antenna		apex of the knee (disregarding the spine)	apex of the knee (disregarding the spine)

	Right and left P7 pereiopods
segment	merus
face	inner
side	posterior
proximal landmark	light notch with ischiopodite
distal landmark	apex of the knee (disregarding the spine)

development of crustaceans (first the anterior part of the body, then the posterior), the P3, P4, P5, P6 and P7 pereiopods were suitable for our aims. We decided to use the right and left P4 and P5 pereiopods for genetic

analyses, as it is possible to extract a sufficient quantity of DNA from them (study in progress), while the right and left P3, P6 and P7 pereiopods were shown to be 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 fluctuating asymmetry it is important that the units used are not too large in relation to the actual asymmetry. 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. Figure 3 and Table 2 summarise the segments and appendages chosen for the genetic and morphometric analyses and the landmarks.

Below we give the protocol we prepared to ensure the best conditions for the morphometric and genetic analyses of each individual, to avoid breakage or confusion of appendages, and finally, to reduce the impact of human measurement error. Each individual was placed in a little glass sink filled with absolute ethanol and was observed under a stereomicroscope. From each sandhopper we took the right and left P4 and P5 pereiopods by means of sterilised dissecting forceps and transferred these appendages into an Eppendorf tube filled with absolute ethanol for subsequent genetic analyses. For each individual we prepared two histology slides, one for the right appendages and the other for the left. On the short sides of the slides we wrote 'Head' and 'Telson', on the longer ones we wrote 'Proximal' and 'Distal' as reference points to ensure all the segments were properly placed and to reduce the risk of confusion. Then we took the right P7, P6 and P3 pereiopods and placed them on the 'Right' histology slide; we did the same for the left appendages. Using 50x magnification, we counted the number of flagella articles of the right and left second antennae. Then, using morphological keys (Karaman 1993, Ruffo (ed.) 1993) we identified the species the sandhopper under observation belonged to. All the individuals captured were *Talitrus saltator*, but two, both from Bruca beach, had broken third uropods, so they could not be definitively identified as *T. saltator*. Therefore the total number of individuals for Bruca decreased to 48. For each sandhopper we defined the sex by recording, in males, the two penes, which appear as two small opposite tubes in the ventral face of the individual at the seventh mesosomite level. We recognised females from the oostegites, two controlateral lines of thin foils visible in the ventral face of the sandhoppers from the second to the fifth mesosomite. For

each female, we determined whether it was immature (small oostegites), fully developed (small setae on the oostegites, which, interlacing with the opposite ones, create a bag, the marsupium) or with eggs/hatched juveniles in the marsupium; in the last case we counted the number of eggs or hatched juveniles. Using 25x magnification, we detached the cephalic capsule and placed it to show its right side; then we measured its length from the base of the right first antenna to the posterior end of the capsule. We stored the remains of the sandhopper in its original Eppendorf tube. Successively, putting the 'Right' and 'Left' histology slides under the stereomicroscope one at a time, we disarticulated the meri of the P7, P6 and P3 pereiopods from the rest of each appendage. This procedure allowed for a better identification of the landmarks. The P7 and P6 meri showed their inner face, while the P3 merus showed the outer one. To achieve a better contrast we used a black background under the slides. Using 50x magnification, we performed two measurements of the posterior sides of the P7 and P6 meri and of the anterior side of the P3 meri. We carried out 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 outcomes.

The metric measures obtained by the stereomicroscope were converted to micrometers by suitable conversion calculations. The samplings and measurements of sandhoppers were both performed by the same person to reduce the error due to different observers.

2.3. Statistical analysis

The analysis of fluctuating asymmetry (FA) was performed as recommended by Palmer & Strobeck (1986) and Palmer (1994) using Excel and S-Plus 6 for Windows. The significance level was 5%.

Outliers are a common source of skew or leptokurtosis in studies of fluctuating asymmetry. The difference values between the right and left sides of the body (R-L) in a few individuals in a large and otherwise normally distributed sample, may deviate unusually far from zero. Sandhoppers that were lacking in one of the bilateral appendages were outliers, as this strong difference between the values of the two sides of their body would have seriously affected the fluctuating asymmetry index. We excluded outliers from the analyses also because their values may be related to physical damage or severe deformities, and these events are not developmental noise (Palmer 1994). Thus, before performing the tests for measurement error, box plots of the right and left sides and of the cephalic capsule were made and screened for outliers. If one sandhopper was an outlier, e.g. for the

second antennae, we excluded it only from the statistical analysis of the second antennae, but not from that of other traits. If one sandhopper was an outlier for any of the pereiopods we excluded it from the statistical analysis of all pereiopods. Table 3 reports the numbers of sandhoppers collected and those actually subjected to further statistical analyses for each trait.

Table 3. Number of sandhoppers collected and statistically analysed for each trait in each population after the exclusion of outliers by box plots

Population	Collected sandhoppers	Cephalic capsule	Number of articles of the second antenna flagellum	P7, P6 and P3 merus
Collelungo	54	52	46	46
Maganuco	50	49	41	43
Bruca	48	48	43	46

The 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 trait, we divided the population samples according to sex to check whether there was sexual dimorphism. When this was absent, males and females were put together 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 fluctuating asymmetry levels were analysed with the index FA1 = Mean |R-L| (as defined by Palmer 1994). One-way ANOVAs were used to test differences among populations for each of the traits and for fluctuating asymmetry levels.

3. Results

The sex ratios (males/females) of the samples were significantly female biased in the Collelungo population (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 population females were more abundant than males but the sex ratio was not significantly different from the expected balance of 1:1 (Table 4).

Comparing the three samples we observed that in Collelungo and Maganuco there were the highest percentages of outliers for all the traits measured, while the Bruca population had the lowest ones (Figure 4).

Table 4. Sex ratio in the three populations

Population	Collelungo	Maganuco	Bruca
n	54	50	48
n males	18	19	35
n females	36	31	13
sex ratio	0.50	0.61	2.69
χ^2-test	$p < 0.05$	n. s.	$p < 0.01$

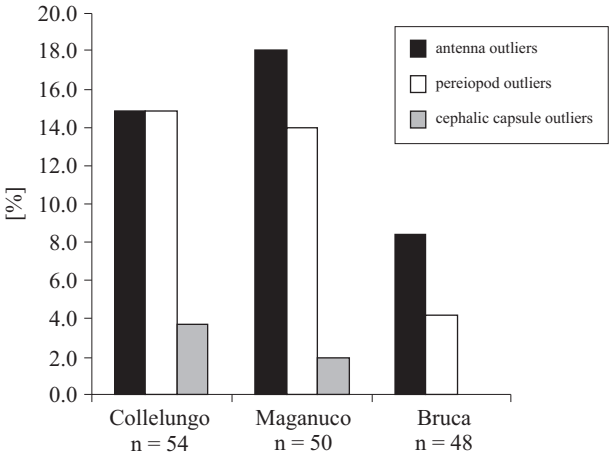

Figure 4. Percentage of outliers for the traits measured in the three populations

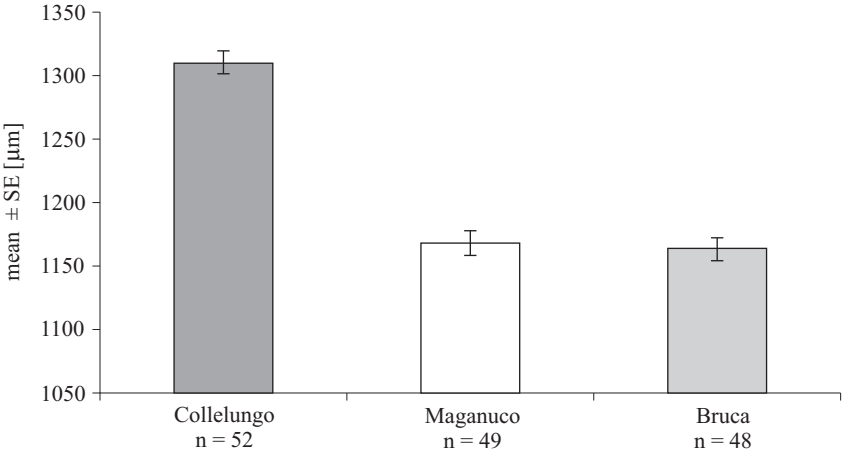

Figure 5. Mean length $\pm$ SE [μm] of the cephalic capsule in the three populations

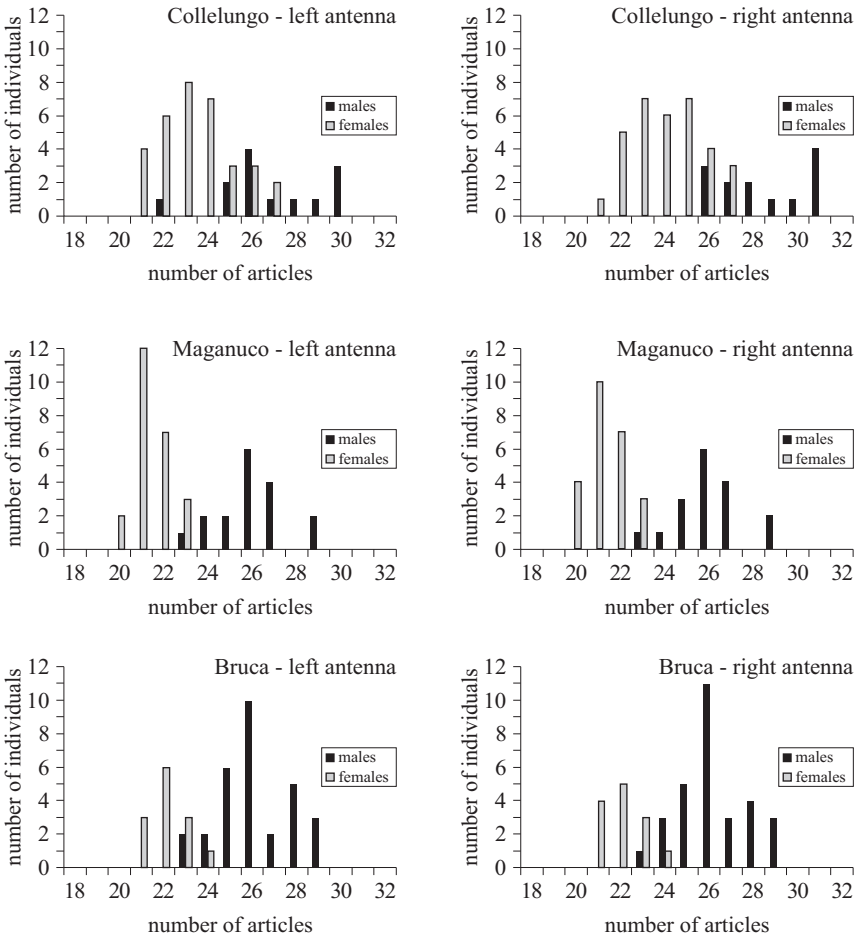

Figure 6. Sexual dimorphism of the number of articles of the second antennae flagella

Table 5. Cephalic capsule length [μm]: comparison between sexes by F and t tests

Population	Collelungo	Maganuco	Bruca
male mean $\pm$ SE	1302.35 ± 19.07	1181.47 ± 17.40	1157.94 ± 10.56
female mean $\pm$ SE	1314.86 ± 10.36	1160.00 ± 11.05	1178.46 ± 19.47
F-test	n. s.	n. s.	n. s.
t-test	n. s.	n. s.	n. s.

Sandhoppers from Collelungo had significantly (ANOVA: $p < 0.001$) larger cephalic capsules than those of the two Sicilian populations, which did not differ from each other (Figure 5). We checked whether there was any

sexual dimorphism of the cephalic capsules, but there were no significant differences (Table 5).

As regards the number of articles of the second antennae flagella, considering males and females together, graphically the data were not normally distributed in either of the three populations. Therefore, we divided each sample according to sex and obtained normally distributed data for each sex. In each population, we found significant sexual dimorphism, with males having a greater number of articles than females, on both the right and left antennae (*t*-test: $p < 0.001$) (Figures 6, 7 and Table 6).

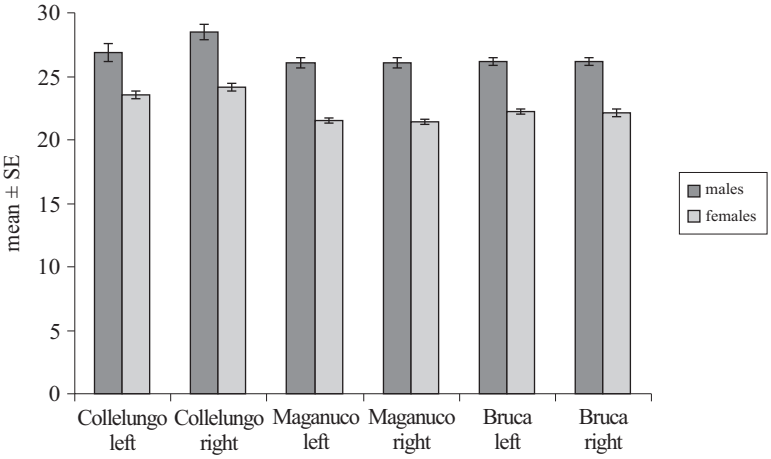

Figure 7. Sexual dimorphism: mean number ± SE of articles in the second antennae flagella

Table 6. Number of articles of the second antennae flagella: comparisons between males and females

Population		Side	Males	Females	*F*-test	*t*-test
Collelungo	n		13	33		
Collelungo	mean ± SE	left	26.9 ± 0.7	23.5 ± 0.3	n. s.	$p < 0.001$
Collelungo	mean ± SE	right	28.5 ± 0.6	24.1 ± 0.3	n. s.	$p < 0.001$
Maganuco	n		17	24		
Maganuco	mean ± SE	left	26.1 ± 0.4	21.5 ± 0.2	$p < 0.05$	$p < 0.001$
Maganuco	mean ± SE	right	26.1 ± 0.4	21.4 ± 0.2	$p < 0.05$	$p < 0.001$
Bruca	n		30	13		
Bruca	mean ± SE	left	26.2 ± 0.3	22.2 ± 0.2	$p < 0.05$	$p < 0.001$
Bruca	mean ± SE	right	26.2 ± 0.3	22.1 ± 0.3	n. s.	$p < 0.001$

In each sex, we tested the right and left antennae for the presence of directional asymmetry, analysing the values of the differences between the right and left sides (R-L). In the Collelungo population both males and females had a significantly (one-sample *t*-test: $p < 0.05$) greater number of articles in the right antenna flagellum than in the left one. In the Maganuco and Bruca sandhoppers, there was no significant difference between the right and left antennae in either sex. Therefore, right directional asymmetry may be present in the Collelungo population (Figures 8 and 9).

For each of the P7, P6 and P3 pereiopod meri, we compared the first and second measures of the same side of a segment using F and t tests. We did

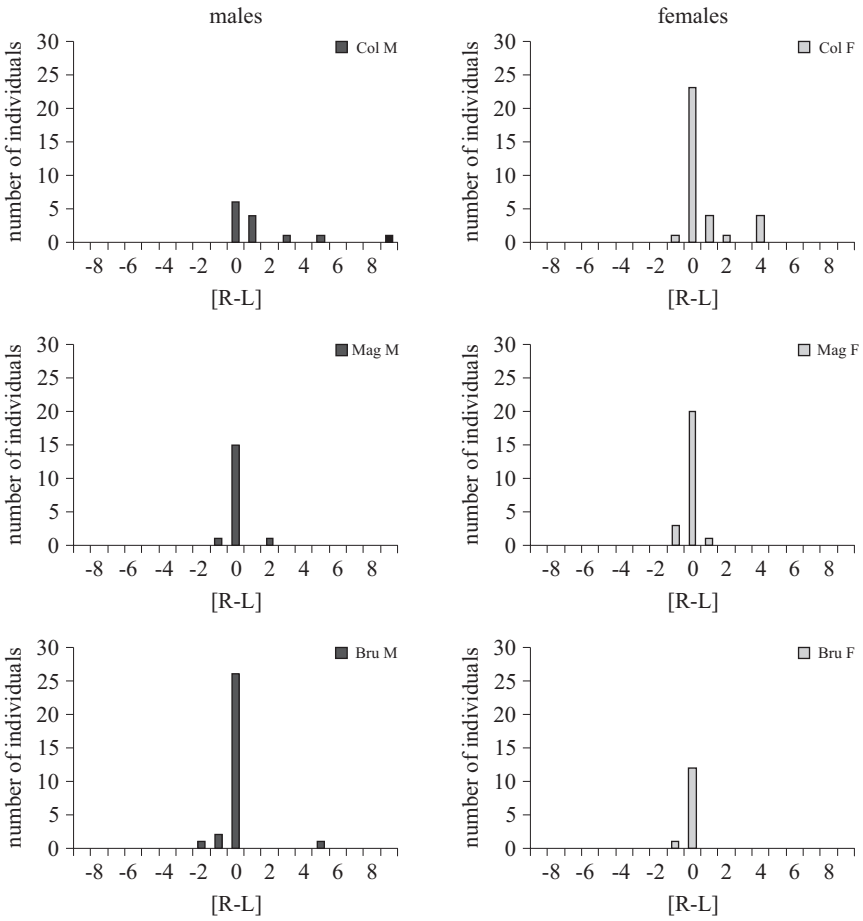

Figure 8. Distributions of the difference values between the number of articles of the right and left second antennae flagella (R-L) in each sex in the three populations. Col = Collelungo; Mag = Maganuco; Bru = Bruca; M = Males; F = Females

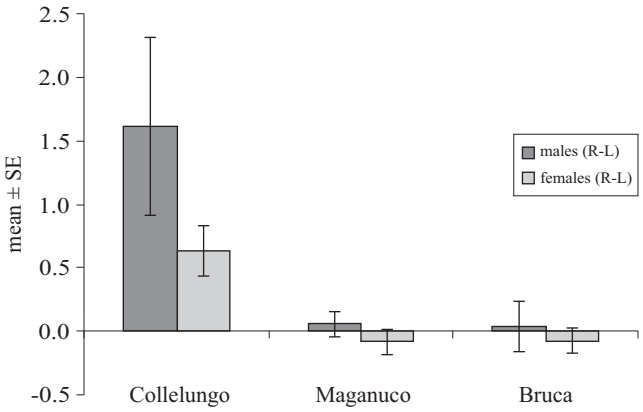

Figure 9. Means (±SE) of the differences between the number of articles of the right and left second antennae flagella (R-L) in each sex in the three populations

not find any significant difference between the first and the second measures in any populations for any meri, for either the right or left sides. Therefore, having ruled out any significant contribution of human measurement errors, the two replica measurements of each side were averaged for further analysis. To check whether there was any sexual dimorphism of the meri, we divided each population according to sex. In the Collelungo and Bruca 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

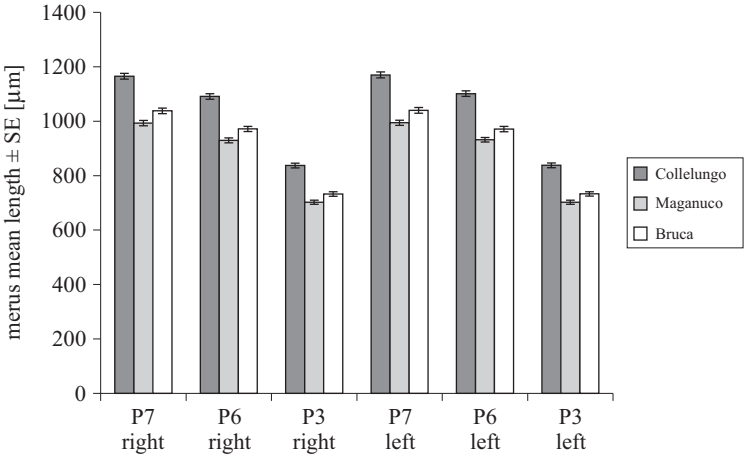

Figure 10. Mean length ± SE [μm] of the right and left meri of the P7, P6 and P3 pereiopods in the Collelungo, Maganuco and Bruca populations

Table 7. Mean length ± SE [μm] of the right and left P7, P6, P3 meri in males and females in each population: comparisons between sexes by F and t tests

Population	Pereiopods	Side		Males	Females	F-test	t-test
Collelungo			n	14	32		
Collelungo	P7	left	mean ± SE	1189.93 ± 20.96	1161.31 ± 13.11	n. s.	n. s.
Collelungo	P7	right	mean ± SE	1189.00 ± 19.98	1154.84 ± 12.63	n. s.	n. s.
Collelungo	P6	left	mean ± SE	1126.71 ± 19.64	1090.09 ± 11.54	n. s.	n. s.
Collelungo	P6	right	mean ± SE	1112.00 ± 19.32	1081.97 ± 11.79	n. s.	n. s.
Collelungo	P3	left	mean ± SE	853.79 ± 18.41	831.03 ± 9.76	n. s.	n. s.
Collelungo	P3	right	mean ± SE	854.93 ± 18.61	829.53 ± 9.18	n. s.	n. s.
Maganuco			n	16	27		
Maganuco	P7	left	mean ± SE	1016.88 ± 18.24	980.74 ± 10.13	n. s.	n. s.
Maganuco	P7	right	mean ± SE	1015.56 ± 19.74	979.96 ± 10.16	n. s.	n. s.
Maganuco	P6	left	mean ± SE	940.63 ± 14.99	926.52 ± 9.45	n. s.	n. s.
Maganuco	P6	right	mean ± SE	936.75 ± 18.00	925.19 ± 9.78	n. s.	n. s.
Maganuco	P3	left	mean ± SE	722.25 ± 15.58	690.67 ± 7.47	$p < 0.05$	n. s.
Maganuco	P3	right	mean ± SE	720.25 ± 14.59	691.89 ± 7.81	n. s.	n. s.
Bruca			n	34	12		
Bruca	P7	left	mean ± SE	1051.21 ± 12.05	1006.92 ± 20.54	n. s.	n. s.
Bruca	P7	right	mean ± SE	1048.00 ± 12.20	1009.50 ± 18.94	n. s.	n. s.
Bruca	P6	left	mean ± SE	977.94 ± 11.45	951.67 ± 17.85	n. s.	n. s.
Bruca	P6	right	mean ± SE	980.15 ± 11.18	947.33 ± 17.78	n. s.	n. s.
Bruca	P3	left	mean ± SE	740.76 ± 9.61	710.75 ± 13.04	n. s.	n. s.
Bruca	P3	right	mean ± SE	739.79 ± 9.59	712.08 ± 13.41	n. s.	n. s.

pereiopods; yet, as in all other comparisons, the *t*-test was not significant (Table 7). Thus, we considered the two sexes together.

Using one-way ANOVA, we compared the length of the meri of each pereiopod in the three populations. In the Collelungo sandhoppers the meri of the right and left P7, P6 and P3 pereiopods were significantly longer than in the Sicilian populations ($p < 0.001$). Moreover, in the Bruca talitrids the meri of the right and left P7, P6 and P3 pereiopods were significantly longer than in the Maganuco population ($p < 0.01$) (Figure 10).

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), so we excluded

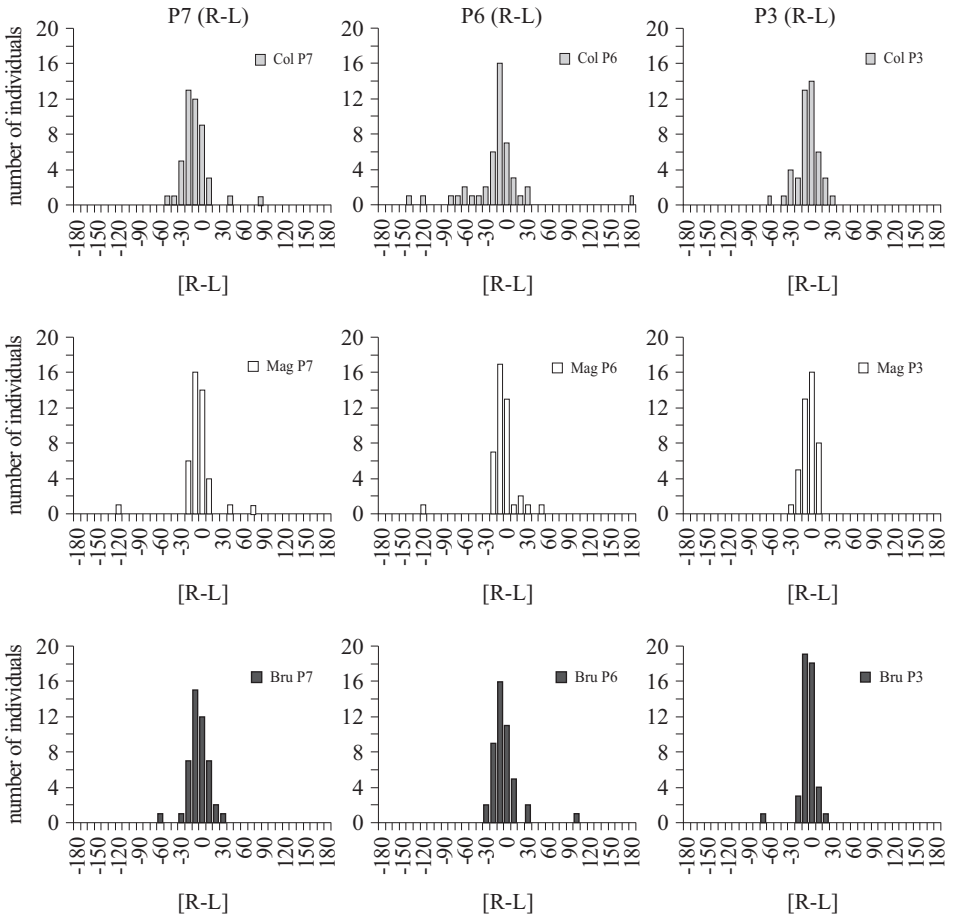

Figure 11. Distributions of the difference values (R-L) between the right (R) and left (L) meri of the P7, P6 and P3 pereiopods in the three populations. Col = Collelungo; Mag = Maganuco; Bru = Bruca

directional asymmetry (Figure 11). The plots showed normality, thus antisymmetry was discarded.

The fluctuating asymmetry levels of the three populations were analysed by the FA1 = Mean |R-L| index (Palmer 1994), and the differences among the populations were tested by one-way ANOVA. The Collelungo sandhoppers had a significantly greater level of fluctuating asymmetry in the P6 meri and in the P3 meri than the Sicilian populations ($p < 0.05$). The fluctuating asymmetry levels of the Maganuco and Bruca populations did not significantly differ from each other. No significant difference among the three populations was found for the P7 merus, which is the longest of the three meri measured and develops later (Figure 12).

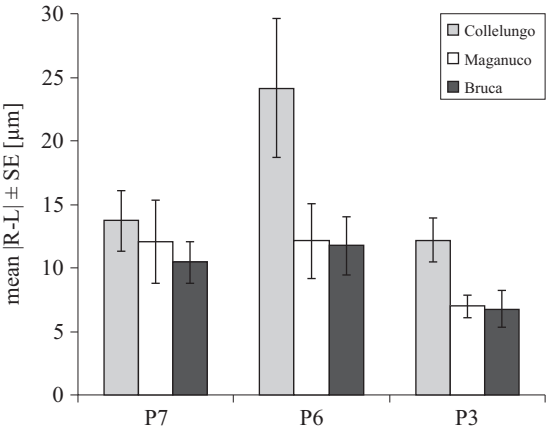

Figure 12. Fluctuating asymmetry values (FA1 index = Mean |R-L| ± SE) for the meri of the P7, P6 and P3 pereiopods in the three populations

4. Discussion

We may assume that the three samples analysed were representative of the respective populations, which were abundant and contained ovigerous females and juveniles (personal observations); for the purposes of this study we sampled only adults. As the sandhoppers were captured by the same person using the same method, we assumed that the sex ratios recorded in the samples reflected those actually existing in the populations at the moment of sampling. As regards outliers for the measured traits, we inferred that those percentages were real and not to be ascribed to the method of capture. The use of entomological aspirators to capture small arthropods is generally assumed to be a non-invasive method.

The study confirmed the sexual dimorphism of the second antennae flagella in *Talitrus saltator* (Ruffo (ed.) 1993). Sexual dimorphism in this

species seems to be limited to this trait and does not extend to the cephalic capsule or to the meri of the P3, P6 and P7 pereiopods. Yet we think this result, especially for the meri, merits further analysis.

Right directional asymmetry was observed in the second antenna flagellum in the Collelungo population. The sensory function of the antennae and their role in closing the burrow in the sand are known (Schellenberg 1942, pp. 139–140, and personal observations). Directional asymmetry is more likely in bilateral sensory organs (Møller & Swaddle 1997). However, the right directional asymmetry in the Collelungo sandhoppers, but not in the Maganuco and Bruca sandhoppers, remains to be explained.

In the merus of the P3 and P6 pereiopods, the Collelungo sandhoppers showed a higher fluctuating asymmetry level than the Sicilian populations. The absence of fluctuating asymmetry in the merus of the P7 pereiopod, the longest one, may be linked to its functional importance for the balance of a laterally compressed body (Schellenberg 1942, pp. 139–140). According to the same line of thinking, we suggest that the merus of the P3 pereiopod has the highest level of fluctuating asymmetry because it is not used for balancing, but for crawling on the sand. Even though it is located within a nature reserve, the Collelungo beach has a relatively high presence of visitors (sometimes on horseback, personal observations) during April and May, which is the period of sandhopper development; during the summer months, for fire prevention, the nature reserve is closed to visitors, so it is possible to reach the beach only on foot from an entrance 5 km away (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. In this month, in Maganuco we noticed the disappearance of sandhoppers from the western part of the beach, in the vicinity of a holiday village and the main access points, where the tourist flow is greatest. Similarly, sandhoppers were found everywhere on the Bruca beach, except in the 380 m around the two main access points. It must be stressed that in southern Sicily in August the sandhoppers born in spring are already developed and a new generation develops in the following autumn months (personal observations). Taking into account the developmental stage in which environmental stresses act, fluctuating asymmetry levels of the meri of the P6 pereiopod and especially the P3 pereiopod may be postulated as a bioindicator of mechanical stress (e.g. direct trampling) on sandy beach ecosystems. The use of *T. saltator* populations as bioindicators has the advantage that comparisons can be drawn over a wide geographical area (the species distribution) and throughout the year (mean life cycle of the species). This has been suggested by several authors for different traits:

population structure (Fanini et al. 2005), genetic variability (Ugolini et al. 2004), abundance and orientation behaviour (Fanini et al. 2007). Here we suggest the fluctuating asymmetry level, which is a quantitative trait of development.

Another possible explanation of the higher fluctuating asymmetry level observed in the Collelungo sandhoppers as compared with the Sicilian populations analysed could be the latitudinal difference. Weather stress, e.g. storms during spring and early summer, may negatively influence sandhopper development. This hypothesis could be tested by evaluating the fluctuating asymmetry level in populations from higher latitudes (this study is under preparation).

A further use of the fluctuating asymmetry level as a bioindicator of environmental stress could be its relation with pollution levels. In south-eastern Sicily oil drilling occurs in the coastal sea, and it is common to find tarry residues from oil wells or the illegal flushing of oil tankers in the sand of beaches (included those we examined, personal observations). Besides, at Bruca beach drainage waters containing pesticides from greenhouses backing the beach are discharged into the sea. However, we did not find any difference between the two Sicilian populations analysed, and their fluctuating asymmetry levels were lower than those of the Collelungo population. Also Barca-Bravo et al. (2008) obtained a negative result regarding the possible use of the fluctuating asymmetry level in *T. saltator* populations as a bioindicator of environmental stress due to oil pollution. These authors compared three populations from Spanish beaches differently impacted by the Prestige oil spill in 2002. They found a higher level of fluctuating asymmetry in the population from the beach most subject to activities linked to tourism and industry than in the populations from oil polluted beaches. The absence of a significant level of fluctuating asymmetry in the *T. saltator* populations exposed to oil (Spain and Sicily) and pesticide (Sicily) pollution of water and sediments may reflect high levels of tolerance.

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