

# The amphipod as a bioindicator of human trampling on sandy beaches

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#### Accepted Manuscript

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1	The amphipod Talitrus saltator as a bioindicator of human
2	trampling on sandy beaches
3	
4	Alberto Ugolini <sup>a, *</sup> , Giuseppe Ungherese <sup>a</sup> , Silvia Somigli <sup>a</sup> , Giuditta
5	Galanti <sup>a</sup> , Davide Baroni <sup>b</sup> , Francesca Borghini <sup>b</sup> , Nicola Cipriani <sup>c</sup> ,
6	Massimo Nebbiai <sup>c</sup> , Maurizio Passaponti <sup>d</sup> , Silvano Focardi <sup>b</sup>
7	
8	<sup>a</sup> Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze, Via
9	Romana 17, 50125 Firenze, Italy
10	<sup>b</sup> Dipartimento di Scienze Ambientali, Università degli Studi di Siena, Via Mattioli 4,
11	53100 Siena, Italy
12	<sup>c</sup> Dipartimento di Scienze della Terra, Università degli Studi di Firenze, Via La Pira 4,
13	50121 Firenze, Italy
14	<sup>d</sup> Dipartimento di Chimica Organica, Università degli Studi di Firenze, Via della
15	Lastruccia 13, 50019 Sesto Fiorentino, Italy
16	
17	* Corresponding author: Tel.: +39 55 2288219, Fax: +39 55 222565
18	E-mail address: ugolini_alb@dbag.unifi.it
19	
20	Abstract
21	The present paper assesses the use of the supralittoral amphipod Talitrus saltator
22	as a bioindicator of the effects of human trampling on the supralittoral sandy band.
23	Samplings in delimited areas were carried out at sites subjected to different human
24	impact. The results showed a strong negative correlation between the number of
25	swimmers and the sandhopper population density, while there was no clear relationship
26	between sandhopper abundance and the other factors considered: granulometry,
27	compactness and organic carbon content of the sand, and trace metal contents in the
28	sand and sandhoppers. A field test of trampling conducted in a confined space showed
29	its direct negative effect on sandhopper survival. However, trace metal analysis

confirmed the ability of *T. saltator* to bioaccumulate some elements (Hg, Zn, Cu, Cd).

30

31	Our study demonstrates that <i>T. saltator</i> is a good bioindicator of human impact in the
32	supralittoral zone of sandy shores.
33	
34	Keywords: Human trampling, Heavy metals, Mediterranean Sandy shores, Beaches,
35	Bioindicator, Amphipod, Talitrus saltator.
36	
37	1. Introduction
38	
39	In the coastal zone, the input of materials and substances from terrestrial, fluvial
40	and marine sources is particularly important. In addition to elevated concentrations of
41	nutrients, there can be increased quantities of natural and anthropogenic contaminants.
42	Since the 1960s, European shores (particularly Italian ones) have seen a very strong
43	increase in the number of swimmers at beach resorts. Many studies on the submerged
44	and intertidal littoral environment of rocky and sandy shores have dealt with the use of
45	organisms as biomonitors of trace metal contamination. Similarly, many investigations
46	have attempted to evaluate the effects of human trampling and recreational uses on
47	natural populations and communities of the littoral zone of sandy shores (see Milazzo et
48	al., 2002 for a review). In contrast, the supralittoral band, an ecotonal environment of
49	great ecological importance in the economy of sandy shore ecosystems, has received
50	relatively little attention (see Brown and McLachlan, 1994; Schlacher et al. 2007).
51	Most research on the effects of trampling on natural communities concerns the
52	vegetation or macrofaunal components (especially birds) (Liddle, 1975; Brown and
53	McLachlan, 1994). Several studies have examined the effects of trampling on the
54	invertebrate fauna of rocky shores or of loose substrata of the intertidal zone (for
55	instance see Povey and Keough, 1991; Brosnan and Crumrine, 1994; Chandrasekara
56	and Frid, 1996; Casu et al., 2006; Dye, 2006; Johnson et al., 2007). Instead, there have
57	been very few investigations of the supralittoral fauna of sandy shores and the results of
58	these studies have been contradictory (Jaramillo et al., 1996; Moffet et al., 1998;
59	Weslawski et al., 2000b, c; Veloso et al., 2006). Recent studies have attempted to
60	establish the utility of supralittoral amphipods as indicators of the impact of tourist
61	activities on sandy shores (Fanini et al., 2005). However, because of their methods and

sampling sites, the investigations have not provided clear results.

62

63	In summer, the supralittoral zone is intensely frequented by swimmers but it is
64	also where the major consumers of stranded organic material, i.e. talitrid amphipods and
65	isopod, find shelter during the day, buried in the wet sand. In fact, most of the energy
66	flow deriving from the use of stranded material passes through supralittoral crustaceans
67	(Branch and Branch, 1981; Griffiths et al., 1983; Brown and McLachlan, 1994).
68	Sandhoppers occupy a trophic niche as detritivores and collector-gatherers. Hence they
69	feed on plant and animal material from both marine and terrestrial sources (Palluault,
70	1954; Wildish, 1988; Bergerard, 1989), integrating the two routes of contamination of
71	the supralittoral.
72	The aims of the present study were to examine the possibility of using the
73	amphipod T. saltator as a bioindicator of the effects of human trampling on the
74	supralittoral of sandy shores and to provide a simple low-cost monitoring tool. For this
75	reason the relationship between sandhoppers abundance and swimmers has been
76	considered, as well as the possible influence of some other factors such as granulometric
77	composition and sand compactness, organic carbon content in the sand and trace metal
78	contamination.
79	
80	2. Materials and methods
81	
82	2.1 Sandhopper sampling
83	
84	Samplings to determine the density of sandhoppers were carried out at eight sites on
85	the coast of Tuscany subjected to different tourist impact and either with or without
86	beach cleaning procedures. In the five sites inside the Regional Park of Migliarino, San
87	Rossore, Massaciuccoli (MSRM Park, Pisa), the sampling was performed once a month
88	from June to September 2004, 2005 and 2006:
89	O1) Oasi 1 (cleaned beach)
90	SM) Serchio Mare (seashore, not cleaned)
91	SF) Serchio Fiume (right bank of river mouth, not cleaned)
92	BV) Batteria Vecchia (not cleaned)
93	FM) Fiume Morto Vecchio (not cleaned)

94	At the three sites along the Feniglia sandbar (southern Tuscany, Grosseto), the
95	sampling was performed in July and September 2005:
96	FA) bathhouse (Ansedonia side, cleaned beach)
97	FB1) beach ca. 1 km from FA (cleaned)
98	FB2) beach ca. 2 km from FA (cleaned)
99	At each sampling, all the sandhoppers were captured (also disturbing the surface
100	layer to sample those in the sediment) and counted inside four areas (75 cm <sup>2</sup> each)
101	delimited by plastic containers inserted a few cm into the sand. Each container was
102	inserted along the sea-land axis of the beach at a progressively increasing distance
103	starting from the water line until the beginning of dry sand. At each site, the sampling
104	was carried out twice, a few meters apart, since the distribution of sandhoppers in the
105	band of wet sand does not seem to be uniform (Weslawski et al., 2000a).
106	At each sampling site, the people to the right and left of the shoreline, along a
107	150 m transect in the central hours of the day on the days with the highest tourist
108	presence, were also counted (Saturday and Sunday).
109	At some locations, the following factors were also analyzed to exclude
110	differences between the sampling sites: 1) contents of trace metals in the sand and in the
111	sandhoppers (Hg, Cr, Cd, Cu, Zn, Fe, Al, Pb); 2) compactness of the sand; 3)
112	granulometric composition of the sand; 4) organic carbon content of the sand.
113	
114	2.2 Trace metals
115	
116	The trace metal analysis was conducted on sand samples collected from the zone
117	frequented by the sandhoppers during the day and on adult individuals of <i>T. saltator</i>
118	collected at all the sites. The animals were transported to the laboratory in plastic
119	containers with sand and detritus from the sampling site and then sacrificed by freezing.
120	At each site, a sample of sand was collected in the zone frequented by the sandhoppers
121	during the day. We dehydrated the samples at 40°C until they reached a constant weight
122	and then were finely ground. Around 150 mg of each sample were mineralized in
123	Teflon vessels containing 3 ml of HNO <sub>3</sub> at 120°C for 8 h. After digestion, the solution
124	was brought to a volume of 10 ml by addition of deionized water. During each

125	mineralization, we performed several "blank trials", by putting only the reagents in the
126	Teflon containers, to verify the absence of contamination.
127	The analyses were performed by atomic absorption spectrometry. Cd, Cr, Cu
128	and Pb were determined by atomization with a graphite furnace and Zeeman
129	background corrector. The FIMS (Flow Injection Mercury System) was used for Hg,
130	while we determined Al, Fe and Zn by Inductively Coupled Plasma Atomic Emission
131	Spectrometry (ICP/EAS). Each analysis was carried out 3 times and the accuracy of the
132	analytical procedures was assessed by comparing the results of contemporary digestions
133	and analyses of Standard Reference Materials, SRMs): SRM 1566a "oyster tissue" from
134	the National Institute of Standards and Technology (NIST, Gaithersburg, USA). The
135	results of the determination were only accepted if the values measured for the SRMs
136	were within the certified concentration range; if not, we repeated the analyses. The
137	element concentrations (expressed as $\mu g/g$ ) were calculated by the method of "internal
138	additions". The coefficients of variation for the various elements were between 5 and
139	10%.
140	
141	2.3. Granulometric composition and sand compactness
142	
143	For the granulometric analysis, the sand samples were split to obtain samples of
144	approximately 10 grams (the choice of the representative volume was based on the
145	median of the sediment, as estimated by visual examination).
146	A statistically representative portion of the sample was placed in an oven at 80°C to
147	eliminate the humidity, after which we sifted the material through a series of
148	progressively finer sieves (2500 $\mu$ m, 1600 $\mu$ m, 1000 $\mu$ m, 630 $\mu$ m, 400 $\mu$ m, 250 $\mu$ m, 1600 $\mu$
149	$\mu m,100~\mu m,63~\mu m)$ for around 5 minutes with a Ro-Tap shaker. Since the samples
150	were prevalently sandy, it was not necessary to analyze the mud-clay fraction ( $< 63 \mu m$ )
151	of the sediment.
152	To assess the compactness of the sand in the zone frequented by sandhoppers
153	during the day, an aluminum pole was dropped from a height of 1 m and the distance
154	the pole penetrated into the sand was measured. The distance was transformed into
155	arbitrary units by assigning a value of $100\%$ compactness to $0~\mathrm{cm}$ and $50\%$
156	compactness to 10 cm.

157		
158	2.4. Organic carbon	
159		
160	The sand samples were collected at each site in July 2006. The organic carbon in	1
161	the sand samples was determined as the difference between the total carbon and the	
162	inorganic carbon. The sand was crushed to a fine powder so as to homogenize the	
163	sample. Total carbon was determined with a CHNS/O ANALYZER (mod. 2400 ser. II,	<b>b</b>
164	Perkin Elmer), while inorganic carbon was measured with a De Astis calcimeter.	
165		
166	2.5. Trampling test	
167		
168	The trampling test was carried out at Fiume Morto Vecchio (MSRM Park). Two	
169	plastic cylinders (110 cm diameter) were fixed in the sand in the wet band frequented by	y
170	sandhoppers during the day. All the individuals inside each of the delimited areas were	
171	captured, counted and then freed within the same area. After around 15 minutes	
172	(sufficient time for the sandhoppers to return to their refuges in the wet sand), two	
173	people performed 400 footsteps in 15 minutes inside one of the two cylinders. The	
174	number of footsteps was chosen on the basis of the footsteps recorded during a survey	
175	carried out from 11:00 to 12:00 on a Saturday in July 2006 on the Marina di Pietrasanta	
176	beach (a popular beach on the Tuscan coast). During this survey, a mean transit of 866	
177	swimmers per hour at a selected point on the shoreline was recorded. The sandhoppers	
178	within the other cylinder were left undisturbed. At the end of the trial, the sandhoppers	
179	still alive in both cylinders were captured, counted and transported to the laboratory in	
180	basins with wet sand. The numbers of individuals still alive 24 hours after the treatment	
181	was also recorded.	
182		
183	2.6. Statistical analysis	
184		
185	The granulometric data were analyzed with specific software (Dept. of Earth	
186	Sciences, Univ. of Florence) that provided parametric statistics indicative of the sorting	
187	of the samples, as well as the percentages of the granulometric classes for the purposes	
188	of textural classification (Shepard, 1954).	

189	The statistical analysis of sandhopper density was performed independently of
190	an analysis of natural population fluctuations (see Anastácio et al., 2003; Marques et al.,
191	2003) and/or a comparison of the population densities at different sites. Such analyses
192	were beyond the scope of our study and would not have provided information useful for
193	our aims. It is possible that sandhopper populations living in the same environment and
194	practically at the same latitude (ca. 200 km apart) present small differences in the
195	natural fluctuation of population density. However, since we performed relative
196	comparisons of the number of captured individuals among several localities of the same
197	zone, any differences in population density between different zones in the same period
198	of the year would not affect the interpretation of the results. The tests used are among
199	the most common ones in non-parametric statistics (Zar, 1984; Siegel and Castellan,
200	1989).
201	The possible influence of trace metal pollution on sandhopper density was
202	assessed with the Spearman rank-order correlation coefficient test (Siegel and Castellan,
203	1989). In the figure illustrating trace metal pollution, we arranged the sampling sites in
204	decreasing order of sandhopper density: the site with the highest sandhopper abundance
205	in July 2006 (FM, captured sandhoppers = 1013) was given the arbitrary value of 100.
206	The values for the other sites were expressed as a percentage of FM.
207	
208	3. Results
209	
210	3.1 Sandhopper density
211	
212	The frequency of T. saltator individuals collected at the MSRM Park sites was
213	negatively correlated with the frequency of swimmers (Fig. 1). Despite the small sample
214	sizes, statistical significance was reached for each of the four months (June-September)
215	(June and July, $r_s$ =- 1, $p$ = 0.01; August and September, $r_s$ = - 0.925, $n$ = 5, $p$ <0.05, $n$ =
216	5, Spearman rank-order correlation coefficient test, Fig. 1). This is also confirmed
217	considering the mean number of sandhoppers and swimmers (Table 1). In fact, the
218	Fiume Morto Vecchio site (FM) consistently had the lowest tourist impact and the
219	highest sandhopper abundance (Table 1). In contrast, no sandhoppers were found at the
220	site with the highest tourist impact, Oasi 1 (Table 1).

221	FIG. 1
222	
223	TABLE 1
224	The July vs. September comparison for the various sampling sites was
225	statistically significant for the localities in the MSRM Park (G = 114.5, df = 4, p<0.001,
226	fig. 1) but not for the sites on the Feniglia sandbar ( $G = 1.9$ , $df = 2$ , $p>0.1$ , $G$ test, Fig 3).
227	There was a significant monthly difference in sandhopper abundance even at a
228	single locality with very low tourist impact (Fiume Morto Vecchio, Table 1, Fig. 2, G =
229	664, df = 1, p<0.001, G test) due to the natural population fluctuation. However, these
230	differences did not affect the swimmers-sandhoppers relationship.
231	FIG. 2
232	
233	It was not possible to make statistical comparisons for the Feniglia sandbar (Fig.
234	3) because of the small number of sampling sites. Nevertheless, the number of
235	sandhoppers captured at the Feniglia sites clearly followed the same trend in relation to
236	tourist impact as the one recorded at the MSRM Park sites, i.e. localities with higher
237	tourist impact had lower T. saltator densities.
238	FIG. 3
239	
240	Regarding the possible influence of beach cleaning on sandhopper survival,
241	there was a significant difference between the only cleaned site $(O1, n = 0)$ and the total
242	of the other MSRM sites $(SM + SF + BV + FM, n = 7551; G = 10434, df = 1,$
243	P<0.0001, G test). However, there were also significant differences among the not
244	cleaned sites (SM, $n = 28$ ; SF, $n = 164$ ; BV, $n = 2160$ ; FM $n = 5$ 199; $G = 10$ 077, $df = 3$ ,
245	P<0.0001, G test). A similar result was obtained for the cleaned beaches of the Feniglia
246	sandbar (FA, n = 131; FB1, n = 41; FB2, n = 3; G = 164, df = 2, P<0.01, G test).
247	
248	3.2 Trace metals
249	
250	At the sites with sandhoppers, the Cu, Cd, Hg and Zn concentrations (Fig. 4)
251	were constantly higher in the sandhoppers than in the sand. In contrast, the Fe, Pb and
252	Al contents (Fig. 4) were higher in the sand than in the sandhoppers. For Cr, there was a

253	higher concentration in the sandhoppers at two sites and in the sand at the other two
254	sites.
255	There was no significant correlation between sandhopper abundance (in Fig. 4
256	abscissa, the sites are in decreasing order of abundance) and trace metal contents in the
257	individuals or in the sand ( $P = NS$ in each case, $n = 6$ , Spearman rank-order correlation
258	test).
259	FIG. 4
260	
<ul><li>261</li><li>262</li></ul>	3.3. Granulometric composition and sand compactness
263	The granulometric composition of the sand was fairly uniform among sites: all
264	samples were well classified and in the medium- to coarse-grained range (Table 2).
265	TABLE 2
266	THORE 2
267	For the MSRM Park sites, there was no correlation between mean sand grain
268	diameter and sandhopper density (Spearman rank-order correlation coefficient test, $r_s$ =
269	- 0.1, $n = 5$ , $p = NS$ , Fig. 5A). A qualitative evaluation also suggested a lack of
270	correlation between sandhopper abundance and mean sand grain diameter at the
271	Feniglia localities (Fig. 5B).
272	Sand compactness at the MSRM Park sites (Fig. 5C) was not significantly
273	correlated with the number of sandhoppers ( $r_s = -0.175$ ; $n = 5$ , $p=NS$ , Spearman rank-
274	order correlation coefficient test). Indeed the highest values occurred at site SF where
275	sandhoppers were present (Fig. 5C). For the Feniglia sites, a qualitative evaluation
276	indicated a negative correlation between sand compactness and sandhopper abundance
277	(Fig. 5D).
278	
279	3.4. Organic carbon
280	
281	For the MSRM Park sites, the organic carbon content of the sand was not
282	significantly correlated with sandhopper density ( $r_s = 0.7$ , $n = 5$ , $p = NS$ ; Spearman
283	rank-order correlation coefficient test, Fig. 5E). However, the localities along the
284	Feniglia sandbar showed a negative correlation between the two parameters (fig. 5F).

285	FIG. 5
286	
287	3.5. Trampling test
288	
289	The effect of trampling on T. saltator survival (Fig. 6) was evident immediately
290	after the treatment: the difference between the number of live individuals before and
291	after treatment was highly significant (G = 86, df = 1, p<0.001, G test). After 24 hours,
292	the percentage of surviving sandhoppers dropped almost to zero ( $G = 428$ , $df = 1$ ,
293	p<0.001, G test) while the survival of the control sandhoppers remained the same.
294	FIG. 6
295	
296	4. Discussion
297	
298	Of all the factors that might have negatively influenced the population density of
299	T. saltator, only the number of swimmers showed a significant correlation with the
300	number of captured sandhoppers. Although the effect of trampling in a confined test
301	space is probably not the same as in natural conditions (in which case the animals could
302	escape), our test results are the first direct proof that human trampling affects
303	sandhopper survival. This is likely an important factor at Mediterranean latitudes and
304	for species that burrow only slightly into the sand (ca. 3- 5 cm depth).
305	This does not mean that other factors are never influential, only that they are no
306	particularly important in the conditions encountered in this study. Sand compactness
307	probably has a negative effect on sandhopper abundance beyond a certain threshold: a
308	particularly compact substrate could hinder burrowing, thus reducing the probability of
309	survival. However, sites like BV and SF, where sandhoppers were present, had more
310	compact sand than O1, where sandhoppers were never found. The negative correlation
311	at the Feniglia sites probably depended on the fact that the increasing order of sand
312	compactness at the sites corresponded to an increasing order of tourist impact.
313	Furthermore, the sand compactness at O1, where sandhoppers were absent, was lower
314	than at FB2 and FB1 where sandhoppers were present. Therefore, sand compactness
315	does not seem to be a primary determinant of <i>T. saltator</i> population density.

316	There did not appear to be any relationship between sandhopper abundance and
317	sand grain size at the various sites. In fact, T. saltator lives on beaches with sand that
318	ranges from medium- to coarse-grained (i.e. from around 0.5 to 2 mm, Ugolini et al.,
319	1995). All the locations considered in this study fell within this range.
320	The results for organic carbon suggested a negative correlation with sandhopper
321	abundance at the Feniglia sandbar sites. However, as for sand compactness, the
322	increasing order of organic carbon content at the sites corresponded to an increasing
323	order of tourist impact. In addition, the FM site (MSRM Park) had a high sandhopper
324	abundance but a higher organic carbon content than at FA where there were very few
325	sandhoppers (but many swimmers).
326	The trace metal analysis confirmed T. saltator's good ability to bioaccumulate
327	Hg, Cu, Cd and Zn (Rainbow and Moore, 1986; Moore and Rainbow, 1987; Rainbow,
328	1998; Rainbow et al., 1998a, b; Marsden and Rainbow, 2004; Ugolini et al., 2004,
329	2005). Although there were some differences in trace metal contamination among sites,
330	there was no clear relationship with the abundance of sandhoppers. Hg accumulation
331	seemed to be negatively correlated with sandhopper abundance at the various sites (Fig.
332	4). Nevertheless, the Hg concentration in the sand was lowest at O1 where there were
333	no sandhoppers. Thus, the absence of sandhoppers at O1 and the low density at FA
334	cannot be attributed to Hg contamination. Furthermore, the trace metal concentrations in
335	both the sandhoppers and sand samples were much lower than those recorded
336	previously at heavily polluted localities where sandhoppers or beachflies were present
337	(Rainbow et al., 1998a, b; Fialkowski et al., 2000; Ugolini et al., 2004).
338	Since we did not evaluate the effects of beach cleaning separately from the
339	effects of trampling, we cannot exclude a possible influence of beach cleaning on
340	sandhopper density. Nevertheless, at the sampling sites not subjected to mechanical
341	beach cleaning, sandhopper abundance still varied in relation to the different tourist
342	impact. In fact, the sandhopper distributions along the Feniglia sandbar (where the
343	beach is regularly cleaned) and at Fiume Morto Vecchio and Batteria Vecchia (where
344	beach cleaning is not carried out) all presented the same pattern in relation to the
345	numbers of swimmers. Therefore, contrary to the hypothesis of Fanini et al. (2005),
346	trampling rather than beach cleaning has a negative effect on the survival of
347	sandhoppers. We can also add a qualitative datum: at site O1, the one with the highest

348	tourist impact in the MSRM Park, we never captured any sandhoppers during the
349	present research, whereas hundreds of sandhoppers were regularly collected around 25
350	years ago when the zone had no beach resorts and swimming was forbidden.
351	We studied populations living within 200 Km of each other and thus subject to
352	very similar general climatic and environmental conditions. Therefore, the natural
353	fluctuations of population density are probably synchronous. In any case, since the
354	proposed method is based on the comparison of densities recorded at the various sites in
355	the same time period, it is independent of natural population fluctuations and does not
356	require biodiversity estimates for the considered environment. Hence, the method is
357	simple and low-cost. Other recently proposed methods using sandhoppers to assess
358	human disturbance are based on quantification of the variability of spatial-temporal
359	habitat use (ElGtari et al., 2002; Nardi et al., 2003; Fanini et al., 2005; Scapini et al.,
360	2005) or on determination of the degree of genetic variability (Ketmaier et al., 2003).
361	However, T. saltator's high behavioral plasticity and remarkable ability to learn new
362	directions of the sea-land axis of the beach (see Ugolini and Macchi, 1988; Ugolini and
363	Scapini, 1988; Ugolini et al., 1988) prevent a reliable and rapid determination of the
364	reasons for variability in orientation behavior. The determination of genetic variability
365	is interesting but not easily performed. Therefore, since these methods require specific
366	expertise in various fields, sophisticated analytical techniques and complex statistical
367	analyses, their practical application appears to be problematic.
368	The effect of human trampling and heavy tourist use of beaches on sandhopper
369	populations raises the question of their conservation. T. saltator is a good osmoregulator
370	(Morritt, 1988; Calosi et al., 2007) and metapopulations of this species are also found
371	on the shores of retrodunal pools and on riverbanks near the mouth (Ugolini and
372	Macchi, 1988; Ugolini and Scapini, 1988; Ugolini et al., 1988). These places are usually
373	shunned by swimmers and if protected could be valid reserves for natural (spontaneous
374	migrations) or artificial sandhopper repopulation of the adjacent beaches.
375	In conclusion, 1) our method is reliable and low-cost, 2) T. saltator is a good
376	biomonitor of trace metal pollution (Cu, Hg, Zn, Cd), and 3) this species is a valid
377	bioindicator of the effects of human trampling and could be used for ecocompatible
378	management of the tourist impact on the supralittoral zone.

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381	
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386	
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506	Table captions
507	
508	Table 1. Captured sandhoppers and number of swimmers (mean $\pm$ SD; n = 3) for each
509	month of sampling
510	
511	Table 2. Results of the granulometric analysis for each sampling site. For further
512	explanations, see Figs. 1, 3 and text.
513	
514	
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517	Figure captions
518	
519	Fig. 1. Frequency (% of total numbers) of captured sandhoppers (black bars) and
520	swimmers (white bars) for each month of sampling. The order of the sampling sites is
521	based on the number of swimmers observed at each locality: FM = Fiume Morto; BV =
522	Batteria Vecchia; SF = Serchio Fiume; SM = Serchio Mare; O1 = Oasi 1. The total
523	numbers of sandhoppers and swimmers at all five sites are also given.
524	
525	Fig. 2. Monthly variation of sandhopper numbers (% of total sampled) at Fiume Morto
526	(FM). Total number of sandhoppers collected = 5199.
527	
528	Fig. 3. Frequency (% of total numbers) of captured sandhoppers (black bars) and
529	swimmers (white bars) along the Feniglia sandbar in July and September. The order of
530	the sampling sites is based on the number of swimmers observed at each locality: FA =
531	Feniglia bathhouse, $FB1 = 1$ km from bathhouse, $FB2 = 2$ km from bathhouse. The total
532	numbers of sandhoppers and swimmers at all three sites are also given.
533	
534	Fig. 4. Trace metal concentrations (µg g <sup>-1</sup> dry wt) in the sand (white bars) and in the
535	sandhoppers (black bars). For each element, the sampling sites are ordered according to
536	decreasing frequency of sandhoppers recorded in July 2006. For further explanations,
537	see Figs. 1, 3 and text.

538	
539	Fig. 5. A-B: mean sand grain diameter (white bars) and frequency of captured
540	sandhoppers (black bars; % of total numbers). The order of the sampling sites is based
541	on mean diameter. C-D: sand compactness (white bars) expressed in arbitrary units (a.
542	u.) and frequency of captured sandhoppers (black bars). The order of the sampling sites
543	is based on sand compactness. E-F: percentage of organic carbon in the sand (white
544	bars) and frequency of captured sandhoppers (black bars). The order of the sampling
545	sites is based on percentage of organic carbon. For further explanations, see Figs. 1 and
546	3.
547	
548	Fig. 6. Frequency of surviving sandhoppers subjected to the trampling test (black bars)
549	and controls (white bars). Before = before the test, After = after the test, 24h = 24 hours
550	after the beginning of the test. See text for further details.

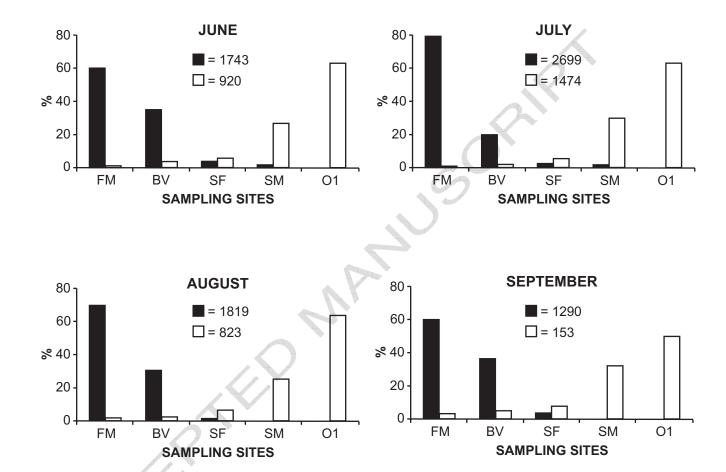
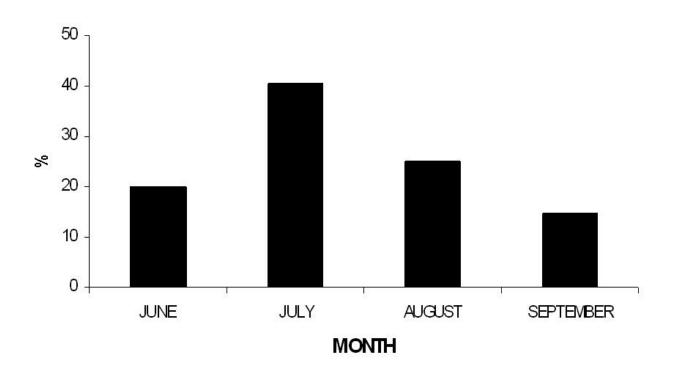


Figure 1



Figure

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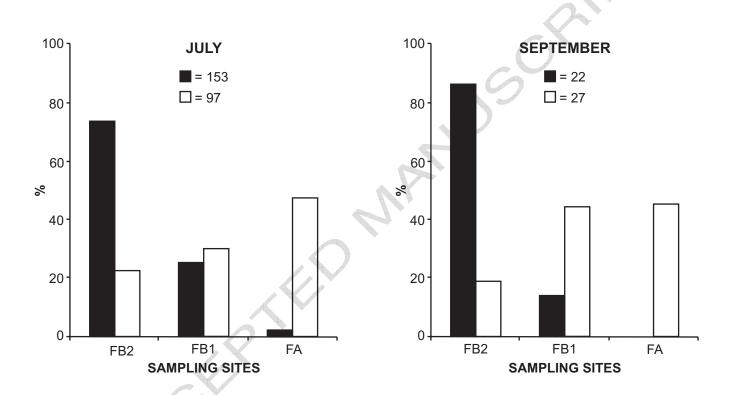


Figure 3

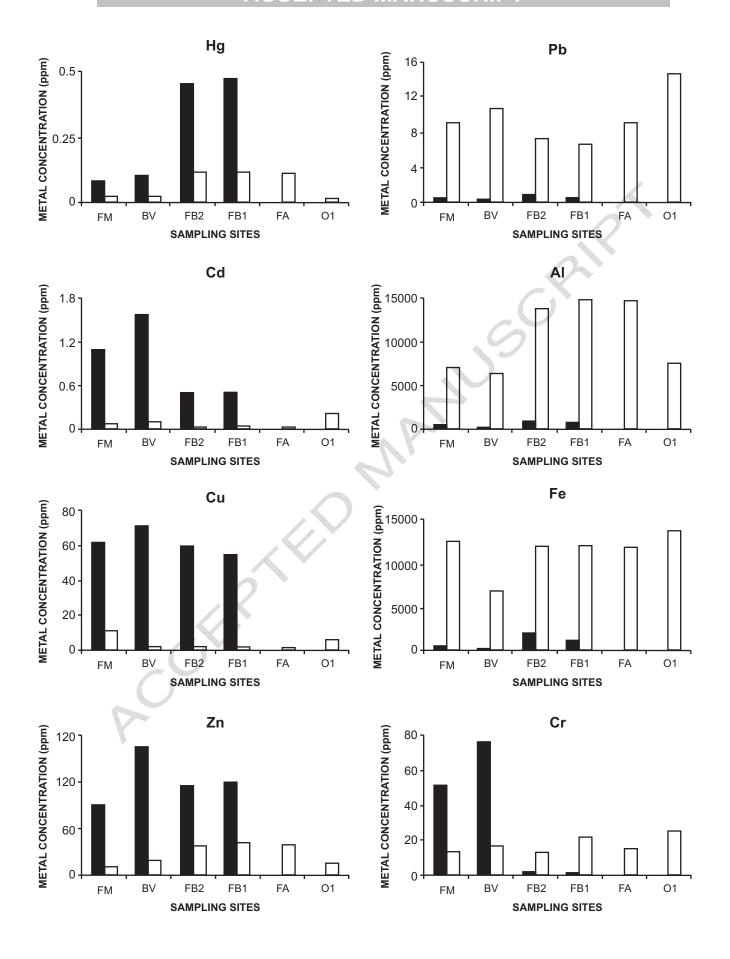
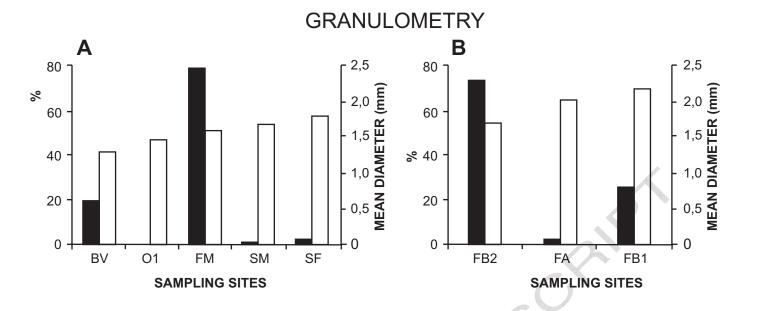
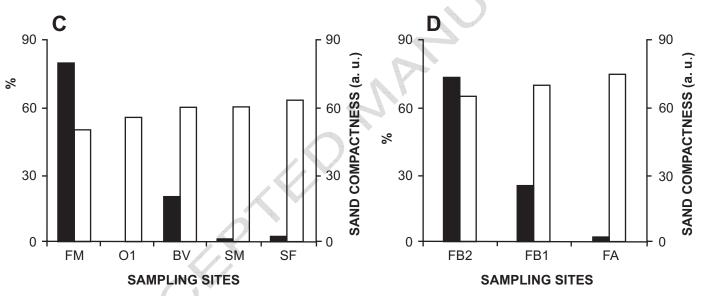


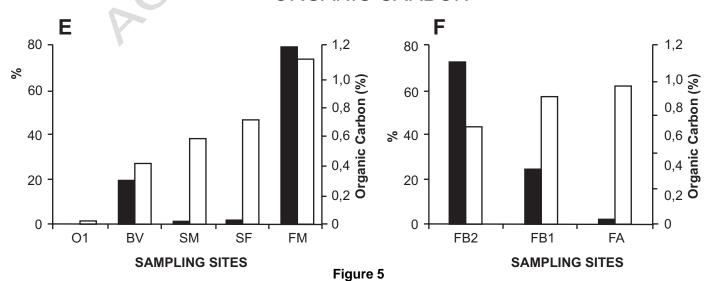
Figure 4



### SAND COMPACTNESS



#### **ORGANIC CARBON**



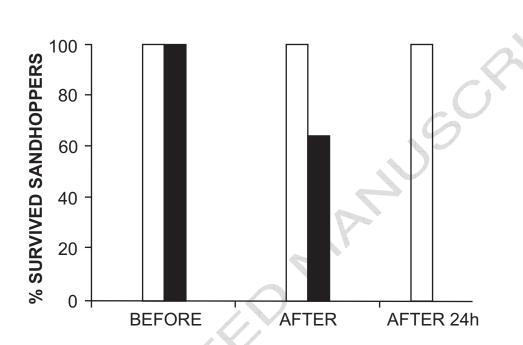


Figure 6

Sampling sites		June	July	August	September
FM	sandhoppers	348.3±59	709±332	438±466	256±325
	swimmers	1.3±1.5	4±2	3.6±4.4	1.66±1.5
BV	sandhoppers	204±85	174±40	184.6±104	158±192
	swimmers	10±13	6±2.6	6.6±9	1.3±1.5
SF	sandhoppers	21±31	15±15	2.3±4	16.3±18
	swimmers	18±19	25.6±15	18.6±12	4.3±5
SM	sandhoppers	8±12	1.33±1.5	0	0
	swimmers	82±70	146±137	70±21.2	16.6±13.5
01	sandhoppers	0	0	0	0
	swimmers	196±190	310±134	175±76	28.6±17.8
			Table		
		R			
	COX				
-					

Sampling sites	Mean sand grain diameter (mm)	Standard deviation	Skewness	Kurtosis
FM	1.605	0.471	-0.012	0.503
BV	1.324	0.507	0.247	0.494
SF	1.751	0.506	0.180	0.598
SM	1.669	0.469	0.051	0.582
01	1.484	0.486	0.170	0.543
FB2	1.738	0.436	0.234	0.631
FB1	2.166	0.435	-0.043	0.511
FA	2.032	0.489	0.065	0.552