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A crossover design to assess feeding preferences in terrestrial isopods: A case study in a Mediterranean species

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Abstract: A crossover design was used to study food preferences and capability of nutritional acclimation to different food sources in terrestrial isopods, which live in xeric environments, by using *Armadillo officinalis* as an experimental model. The foods chosen for this experiment were three fresh foods with different content of water (potato, carrot, lettuce), and a dry food (leaf of plane tree). In order to quantify these preferences, two kinds of data able to provide complementary information were collected: number of droppings produced and food ingested per week. These data were used to fit some mixed effects models, in order to highlight statistically significant differences among the foods provided to the animals at a level of preferences. In addition, the buccal appendages of *A. officinalis* were observed and drawn in details, to provide further information at this level. Our results showed that *A. officinalis* seems not to have particular preferences between fresh foods with a moderate quantity of water and dry food, commonly eaten in its natural habitat. In contrast, foods with high quantity of water, like the lettuce, seem not to be instead particularly palatable for its taste, or its nutritional needs. Also, this study seems to have highlighted a better capability of digestion and absorption of the potato tuber compared to the leaf of plane tree for an equal quantity of ingested food. Anyway, this hypothesis needs further insights in order to be able to be verified.

Key words: *Armadillo officinalis*; biostatistics; behaviour; breeding; woodlice; diet

Introduction

Armadillo officinalis Duméril, 1816 is a species of terrestrial isopod (Crustacea: Isopoda: Oniscidea), belonging to the family Armadillidae, widely distributed in the Mediterranean basin and on the western coasts of the Black Sea (Schmalfuss 1996, 2003). Since its original description the species is well known for its pharmaceutical properties and its use (dried and pulverized) as a diuretic, and to facilitate digestion (Duméril 1816). From an ecological point of view, *A. officinalis* is considered as a xeric species, which shows mostly nocturnal habits (Vandel 1962). This species is able to reproduce more times throughout its life (iteroparous). In France its reproductive period mainly occurs in June with a possible extension until August (Vandel 1962); in Sicily it mainly occurs from May to July (Messina et al. 2011, 2012); in Israel it instead seems to mainly occur in October (Warburg 2013).

Armadillo officinalis can live on sandy, silty-clayey, or rocky substrates, and in environments populated by different plant communities (Messina et al. 2014). A possible use of these animals as indicators for the exposure to benzene was also demonstrated (Agodi et al. 2015).

Generally, terrestrial isopods are important members either of meso- or macro-fauna, with species-specific roles on decomposition of leaf litter (Zimmer et al. 2002; Abd El-Wakeil 2015). They occupy different levels within the trophic network of soil (Abd El-Wakeil, 2009), and nutrition can be achieved by means of different food sources (Udovic et al. 2009; Abd El-Wakeil 2011, 2015). Most terrestrial isopods are omnivorous, and may feed on detritus or plants and animals, both live and dead (Warburg 1993). Coprophagy was also demonstrated. It seems that microbial activity in faeces may increase their nutritive value (Hassal & Rushton 1982; Ullrich et al. 1991; Drobne 1995).

Feeding preferences of the taxon Oniscidea are quite well studied. In chapter V of his important review, Zimmer (2002) explained the mechanisms of nutritional requirements, coprophagy and feeding preferences of most species of oniscidean isopods, citing all the known studies. In particular this review describes in detail the anatomy of digestive system, gut microbiota, digestive processes, food sources and quality, and the impacts on ecosystem. On the other hand, foraging and the underlying mechanisms of food choice and food preferences are poorly studied. These animals likely have some chemoreceptors located on their antennal flagellum (Henke 1960; Gupta 1962; Mead et al. 1976; Seelinger 1977; Hoese & Schneider 1990; Zimmer et al. 1996) and on their mouthparts (Ábrahám & Wolsky 1930), which allow them to localize food. It was demonstrated that the food source affects the fitness of consumers determining individual longevity and reproductive success (Leather 1994).

Although several studies on terrestrial isopods dealt with many aspects of nutritional biology, different results or controversial conclusions were drawn. This might be due to different stages of adaptation to terrestrial habitat and to the corresponding food sources of the investigated species (Zimmer 2002).

Moreover, food preferences of *A. officinalis* have been poorly studied until now. In literature, there are only two papers on nutritional biology of this species, and both studies refer to results on body mass, rate of ingestion, and efficiency of absorption. The first paper reported growth rate and food conversion of specimens fed on leaves of blueberry tree (*Myoporum serratum*) and saffron thistle (*Carthamus lanatus*) (Nair & Fadiel 1991). The second one showed data from individuals fed on leaves of lemon (*Citrus limonia*) and pomegranate (*Punica granatum*) (Nair et al. 2003). Thus, no choice or preference has been really tested at present.

Many studies reported the use of carrots or potatoes (or other common vegetables) as preferred food for some species of terrestrial isopods, bred for different goals (e.g., Warburg & Bercovitz 1978a, b; Tuf et al. 2015). Anyway, it has never been demonstrated that the breeding of *A. officinalis* may be improved by using this kind of food.

Based on all the previous considerations, hence, the first goal of this study is to try to use a crossover design as a possible experimental design to be applied on studies of feeding preferences in terrestrial isopods. In such studies, the use of this species is quite simple, and this design is highly reproducible even if replicated with other species. Crossover designs have been widely used in agriculture and animal husbandry since the Thirties, but they are also used in clinical trials on human beings. A crossover design is a particular type of design for repeated measurements where each single subject receives all the different types of treatment in sequence in different time periods. This way, each treatment is compared within the same subject, which serves as his/her own matched control, by reducing the error variance thanks to the elimination of the variability between subjects. This aspect allows to use a lower number of subjects compared to a design with independent samples, with an equal study power, by highlighting even small differences in the effect size. In contrast, the main disadvantages of a crossover design are that repeated measurements are correlated, and the carryover effects cannot be estimated separately (Jones & Kenward 2014; Piantadosi 2005).

Another specific (and practical) goal of this study is to establish a correct way to feed *A. officinalis* in both laboratory conditions (e.g., choosing the right food that has to be offered, both qualitatively and quantitatively) and breeding in anthropic environment. Even though, nowadays, it is not used in pharmacy applications, this species might be bred for other purposes of interest. Effectively, as previously said, among the possible practical uses, it was recently demonstrated that this species might be used as biomarker of exposure to benzene (Agodi et al. 2015).

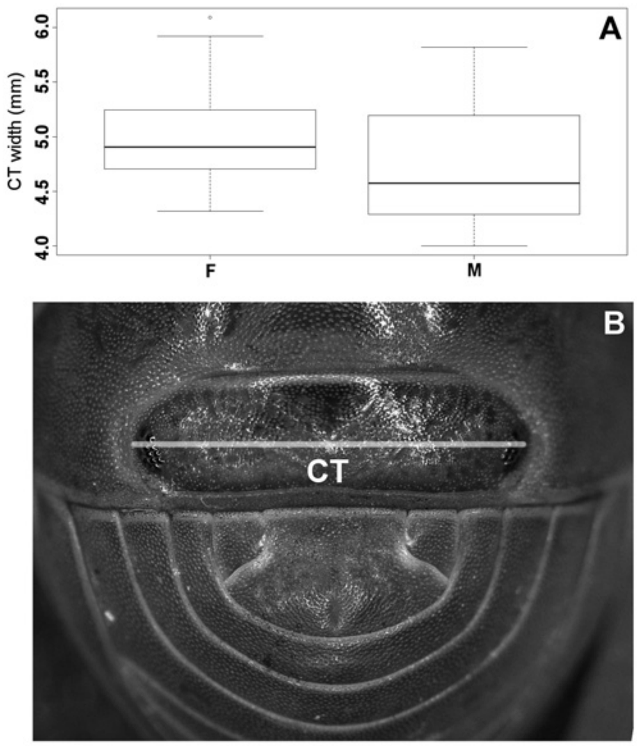

Fig. 1. A: Size distribution of the 24 animals used in the present study; CT – cephalothorax; F – females; M – males. B: Cephalothorax width of *Armadillo officinalis*.

Material and methods

Numerous specimens of *A. officinalis* were collected in Sicily (37°31′39″ N; 15°04′20″ E) in April 2015, and bred in Pisa (43°43′07″ N; 10°23′45″ E), in a climate room with 20°C and natural photoperiod, in order to be used in the present study. Since it was not easy to find a detailed description in literature, first, the buccal appendages of *A. officinalis* were observed and drawn, with the methods described in Montesanto (2015, 2016). In March 2016, a random sample of 24 animals (Fig. 1A) was extracted from our terrarium for the feeding experiments (see section below). March was chosen as the most appropriate month because it is far away enough from the reproduction period (see also Nair et al. 1989). The size (cephalothorax width) of each specimen (Fig. 1B) was determined by using a photographic method with ImageJ software (Rasband 1997) (see also Fig. 2); furthermore, the sex of all the specimens was annotated. Before starting the experiments, the animals were individually acclimated for one week inside Petri dishes (Falcon® 351029, 100 × 15 mm, with plaster of Paris substrate), and then kept fasting for 48 hours to empty the digestive tube.

Experimental design and procedure

A 4-period, 4-treatment crossover design was used. A total of 24 animals were randomly assigned to a particular food by means of a randomization plan based on all possible treatment orders. This design is balanced for the first-order carryover effect (no carryover effect in the first period), and

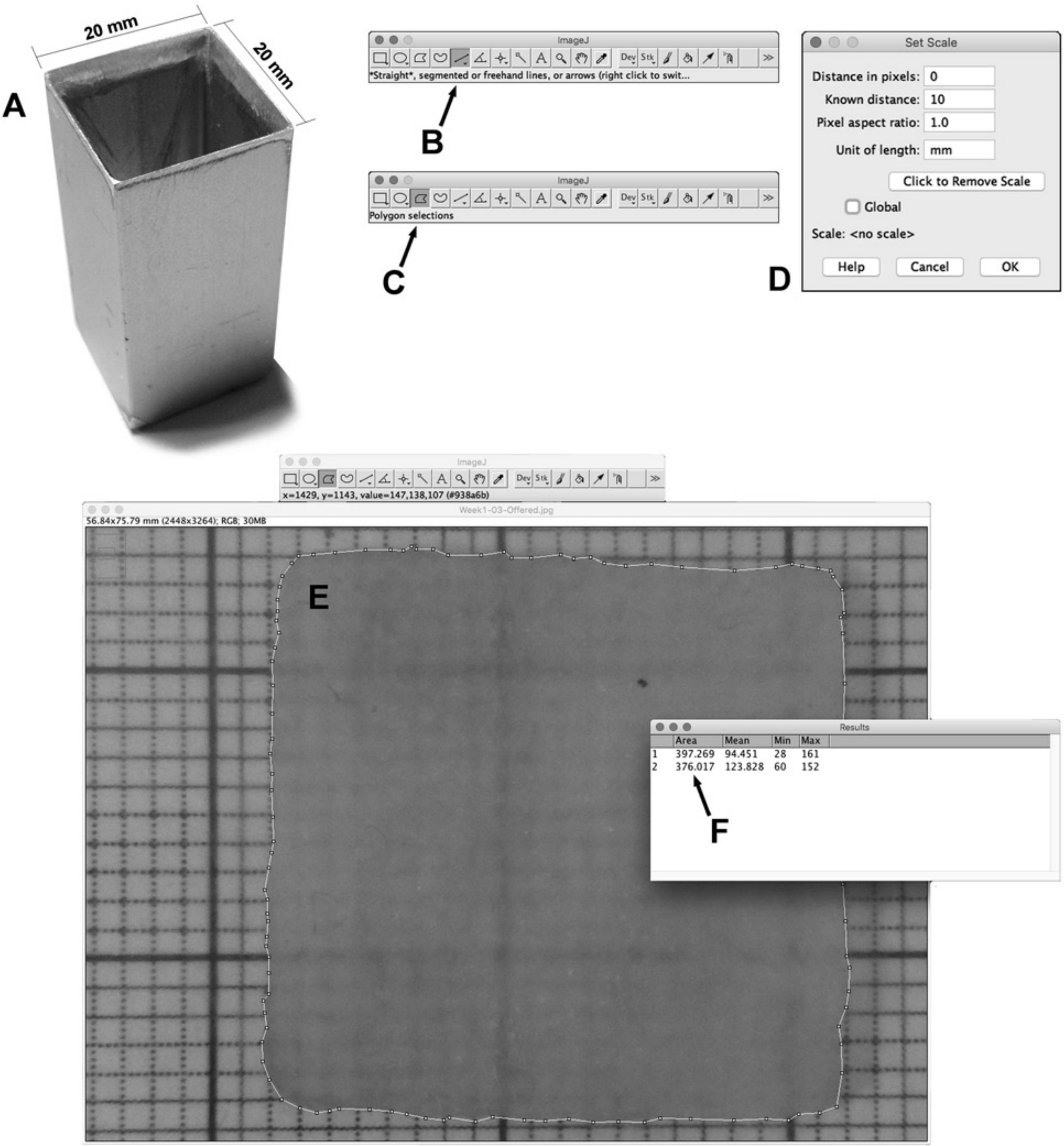

Fig. 2. A: Handmade cutter used in the present study. B–F: Commands used with the software ImageJ (Rasband 1997) to measure the differences of the food areas.

it is uniform within the periods, that is each treatment appears the same number of times within each period. A 66-hour washout period (where the animals were kept fasting) was applied between the administration of a type of food and the next one, in order to control the carryover effects (Fig. 3). This period of suspension of the 'treatment' also had the second aim to empty the animals' guts from the food previously given to avoid an overlap of faeces.

Four types of vegetable food were used: A – potato tubers (*Solanumtuberosum* L.); B – romaine lettuce (*Lactuca sativa* L. var. *longifolia*); C – carrots (*Daucus carota* L. subsp. *sativus*),D – leaves of plane tree (*Platanus occidentalis* L.). The four types of food were offered to the animals at the start of each week of the experiment. The first three were offered as fresh food, while the leaves of plane tree were picked up in the form of dry leaves at the Botanical Garden of Pisa. Before being introduced into the plates, each food was cut in slices of standardized size (19 × 19 × 1 mm) with an appropriate *ad-hoc* cutter (Fig. 2A).

Data retrieving

In this study, two types of data were considered: number of droppings and ingested food.

The droppings were counted more times per day, and also the photos of each Petri plate were taken day to day to check for the possible coprophagy. The ingested food was measured as difference between the area of the offered food and the area of the residual food (in mm^2). A micro-

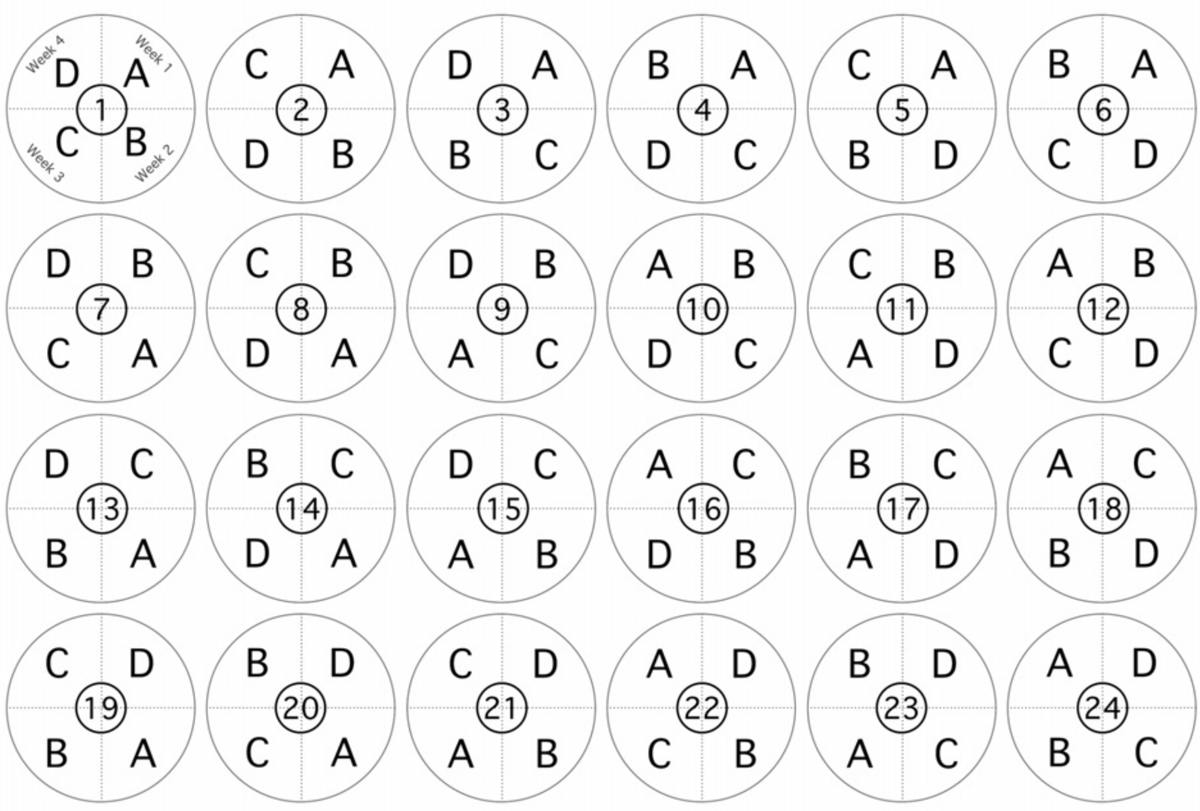

Fig. 3. Outline of a 4 period, 4-treatment crossover design, with all possible treatment orders (see text). The legend of the weeks is inside the first circle. The following scheme was applied each week per 4 weeks. Day 1 – food dosage (one type of food among A, B, C, or D, per week, based on the randomization table above); days 1–5, observation of the food preferences and evaluation of the outcome; days 6–7, washout period (48-hour fast, no food dosage). 1–24: specimens of *Armadillo officinalis* in Petri dishes; A – potato tubers (*Solanumtuberosum* L.); B – romaine lettuce (*Lactuca sativa* L. var. *longifolia*); C – carrots (*Daucus carota* L. subsp. *sativus*); D – leaves of plane tree (*Platanus occidentalis* L.).

photograph of each slice of food was taken at the start and at the end of each period. The measurements were taken using the ImageJ software (Rasband 1997), according to the following step-by-step instructions:

A. The slice of food was placed on a millimeter paper sheet (plasticized in order not to make it become moist), and covered with a square glass slide to eliminate eventual wrinkles. Subsequently, the pieces of food were photographed, and each digital photograph was opened into ImageJ.

B. First, the measuring scale was adjusted as follows. Select "Line tool" (Fig. 2B). Draw a line exactly equal to 10 mm (of millimeter paper). Select the "Analyze" menu, and then the "Set Scale" command. A new "Set Scale" window appears on the screen. Set "10" as "Known distance" and "mm" as "Unit of length", as shown in Fig. 2D.

C. The area of the food slice was measured selecting the "Polygon selection" tool (Fig. 2C). The view of each photo was magnified as much as possible. Clicking repeatedly on the left mouse button, the area's edge was selected by drawing a corresponding contour (Fig. 2E). The shape's area of the food was calculated by selecting again the "Analyze" menu, and then the "Measure" command. The software returns the area's value in another window called "Results", in the "Area" column (Fig. 2F).

The steps above were repeated for each photograph, at the start and at the end of each week.

Data analysis

The statistical analyses were carried out with the software SAS (version 9.4). In particular, the *proc mixed* was used in order to fit some mixed effects models for repeated measures. Since in a 4-treatment, 4-period crossover design, the carryover effect is not confounded with the sequence, a carryover term was calculated by using a SAS function, called lag(). The carryover term consists of the value of the treatment for the preceding period. The first period of treatment does not have any carryover. Subsequently, the *proc glm* was used to carry out a one-way ANOVA on the first-period data. The table of randomization, based on all possible treatment orders, was got with R (version 3.2.2) by using the *get.plan* function in the *crossdes* library. The calculation of the sample size always was carried out with R by using the *PowerTOST* library. Since the present study was the first one to try to assess food preferences in terrestrial isopods, we did not find useful information in literature to use to calculate the sample size. Hence, we speculated a coefficient of variation (CV) as ratio by 20%, with a power of the study equal to 80%. All the graphs were created with R *ggplot2* library.

Results

Figure 4 shows the morphology of the mouthparts of a specimen coming from the same population of the animals which were used as a model in the present study. Mouthparts (Fig. 4A) are identical in both females and males, and they consist of four couples of cephalothorax appendages: mandibles, maxilla, maxillula, and maxilli-

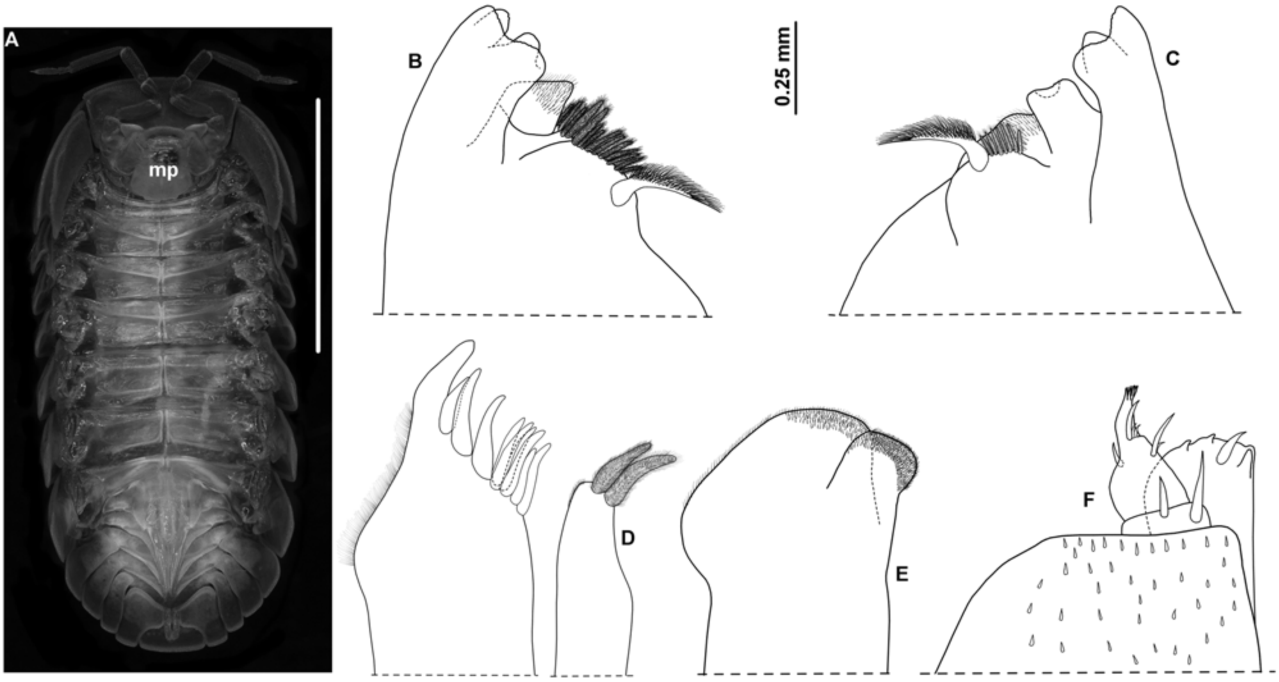

Fig. 4. A: *Armadillo officinalis* Duméril, 1816, male, ventral view, scale 1 cm.; B: left mandible; C: right mandible; D: maxillula; E: maxilla; F: maxilliped.

pede. Only mandibles are asymmetrical. Left and right mandibles bear a semi-dichotomized molar penicil and a line of several other penicils: 13–15 penicils on the hairy lobe of the left mandible (Fig. 4B) and 6–8 penicils on the hairy lobe of the right mandible (Fig. 4C). Maxillule outer lobe with 4 + 6 teeth; inner lobe with two subequal penicils (Fig. 4D). Maxilla apically setose, with subquadrangular inner lobe, much smaller than rounded outer lobe (Fig. 4E). Maxillipede with quadrangular endite bearing three short subtriangular setae on the distal margin and a longer subapical seta near the inner corner; basal article of palp with two long setae (Fig. 4F).

After each washout period (66 h) and the subsequent provision of the new food, it was observed that the first droppings appeared about 4–5 hours later the food ingestion. The events of coprophagy were monitored, and they were more frequent among the animals bred with the leaves of plane tree, whose droppings were quite dry. The ingested feces were counted together with the other ones. The food preferences of *A. officinalis* were assessed, thus, by using two dependent variables: the number of droppings produced by each animal at the end of each week, and the quantity of ingested food as difference between the area of the food provided at the start of each week and the area of the food remained at the end of the same week (in mm^2).

Throughout the experiment, one of the animals died in the second week, and other six animals moulted (one in the second week, four in the third week, and one in the fourth week). Because of the moult, these six animals might not to have eaten the food provided for that week as they would have likely done in normal conditions, and, at the end of the moult, they consumed their exuviae. This physiological event, although integral part of the biology of these animals, might have introduced, somehow, a potential source of bias at the level of carryover effect. Hence, we decided to create a binary indicator variable for the moult to be included in our mixed effects models to assess this possibility (no moult = 0, moult = 1). Both types of data were transformed in square root to get a distribution as similar as possible to a normal distribution. The deviations from the normality after transformation of the data were not so important to affect the validity of the analysis of the variance tests (Cramer-von Mises test: droppings, $P = 0.09$; Kolmogorov-Smirnov test: areas difference, $P = 0.042$). Below, we reported the best mixed effects models, got for each of the dependent variables of interest. In both models, 93 observations out of 96 were used because of the animal that died in the second week of experiment. Both models correctly met the convergence criteria, and the diagnostics on the model assumptions showed that the residuals were normally distributed (Shapiro-Wilk test: Model 1, droppings: $P = 0.87$; Model 2, areas difference: $P = 0.52$). A 0.05 significance level was used.

Model 1: Droppings

This first model highlighted a statistically not significant term of carryover ($P = 0.80$), and the same for sequence ($P = 0.36$) and interaction between food and period ($P = 0.07$). The direct effect of the period was not significant ($P = 0.17$), either, while the direct effect of the food was extremely significant ($P < 0.0001$). Moult, and interaction between moult and carryover term were not significant ($P = 0.08$ and $P = 0.81$, respectively) (Table 1). Based on the results above, then,

Table 1. Model 1, droppings. Type 3 Tests of Fixed Effects. The method of Kenward-Roger was used for computing the denominator degrees of freedom (Den DF). Num DF, numerator degrees of freedom.

Effect	Num DF	Den DF	F Value	$Pr > F$
Moult	1	47	3.29	0.0762
Sequence	23	47	1.12	0.3614
Carryover	3	47	0.34	0.7959
Food	3	47	11.74	< 0.0001
Period	2	47	1.86	0.1670
Moult*carryover	3	47	0.32	0.8074
Food*period	9	47	1.95	0.0677

Table 2. Model 2, difference of areas. Type 3 Tests of Fixed Effects. The method of Kenward-Roger was used for computing the denominator degrees of freedom (Den DF). Num DF, numerator degrees of freedom.

Effect	Num DF	Den DF	F Value	$Pr > F$
Moult	1	66.7	3.29	0.0742
Carryover	3	68.5	0.62	0.6064
Food	3	50.9	11.14	< 0.0001
Period	2	50.6	2.01	0.1443
Moult*carryover	3	69.4	0.56	0.6416
Food*period	9	69.5	2.95	0.0050

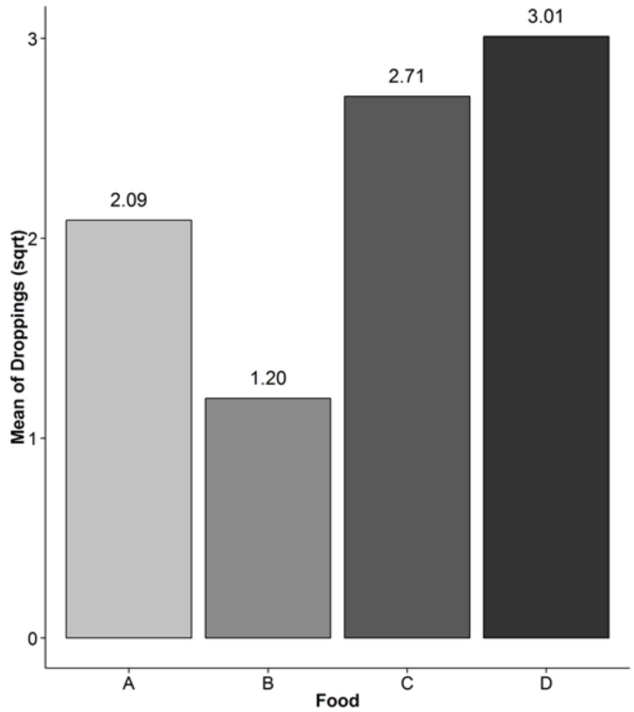

Fig. 5. Mean of droppings produced by the animals throughout the four weeks of experiment, by type of food. Data were transformed in square root.

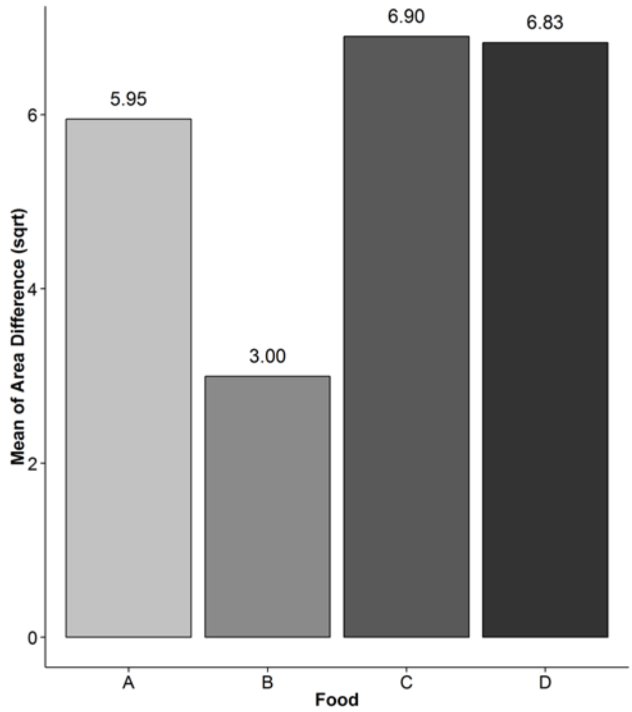

Fig. 6. Mean of the difference between the area of the food provided at the start of each week and the area of the food left at the end of the same week, throughout the four weeks of experiment, by type of food. Data were transformed in square root.

the main assumption for the validity of the results from a crossover design, that is, no carryover effect, was verified. Therefore, we reported the main differences between the types of food, adjusted with the Bonferroni correction, below. It was pointed out a significant difference, at a level of food preferences of these animals, between potato (A) and leaf of plane tree (D) (2.09 vs 3.01, $P = 0.0034$), lettuce (B) and carrot (C) (1.20 vs 2.71, $P = 0.0011$), and lettuce (B) and leaf of plane tree (D) (1.20 vs 3.01, $P < 0.0001$). No significant difference instead seemed to be present between potato (A) and lettuce (B) (2.09 vs 1.20, $P = 0.25$), potato (A) and carrot (C) (2.09 vs 2.71, $P = 0.31$), and carrot (C) and leaf of plane tree (D) (2.71 vs 3.01, $P = 0.52$) (Fig. 5).

Model 2: Ingested food (Difference of areas)

This second model showed, once again, that the carryover term was not statistically significant ($P = 0.61$), but, differently than before, the interaction between food and period was instead significant ($P = 0.005$). As far as concern the direct effects, the period was not significant ($P = 0.14$), while the food was extremely significant ($P < 0.0001$). Moult, and interaction between moult and carryover term were not significant ($P = 0.07$ and $P = 0.64$, respectively) (Table 2). The main differences between the types of food, adjusted with the Bonferroni correction, were as follows. At a level of food preferences of these animals, it was pointed out a significant difference, between potato (A) and lettuce (B) (5.95 vs 3.00, $P = 0.018$), lettuce (B) and carrot (C) (3.00 vs 6.90, $P = 0.0001$), and lettuce (B) and leaf of plane tree (D) (3.00 vs 6.83, $P < 0.0001$). No significant difference instead seemed to be present between potato (A) and carrot (C) (5.95 vs 6.90, $P = 0.75$), potato (A) and leaf of plane tree (D) (5.95 vs 6.83, $P = 0.13$), and carrot (C) and leaf of plane tree (D) (6.90 vs 6.83, $P = 1.00$) (Fig. 6).

ANOVA: Ingested food (Difference of areas, first-period data)
The mixed effects model for the ingested food yielded a significant interaction between food and period (Table 2), which might indicate the presence of a carryover effect, even though it seems unlikely enough (see discussion). Consequently, we decided to analyze only the first-period data, where there is no carryover effect, and there are no missing data (all 24 subjects were used). The ANOVA model used for this analysis was statistically significant (F-statistic $= 9.11$, $P = 0.0005$), and the main assumptions underlying the model were both verified (Shapiro-Wilk test, $P = 0.31$; Bartlett test, $P = 0.58$). Therefore, we carried out some multiple comparisons with the Bonferroni correction. The results obtained on the data of the first-period highlighted the following significant differences between the provided foods: potato (A) and lettuce (B) (10.29 vs. 3.25), carrot (C) and lettuce (B) (9.77 vs. 3.25), leave of plane tree (D) and lettuce (B) (10.89 vs. 3.25). No significant difference instead at the level of potato (A) and carrot (C) (10.29 vs. 9.77), potato (A) and leaf of plane tree (D) (10.29 vs. 10.89), and leaf of plan tree (D) and carrot (C) (10.89 vs. 9.77) (Fig. 7). The results from the ANOVA and the results from the model 2 were perfectly in concordance.

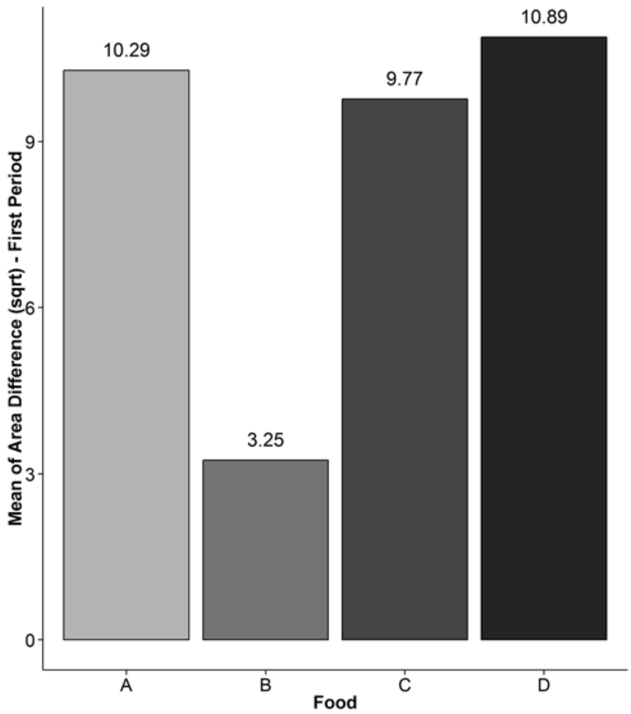

Fig. 7. Mean of the difference between the area of the food provided at the start of each week and the area of the food left at the end of the same week, on first-period data only, by type of food. Data were transformed in square root.

Discussion

The goal of this study was to assess the feeding preferences in *A. officinalis* by using a 4 × 4 crossover design, whose main feature is that each subject serves as his/her own matched control, by reducing this way the error variance with respect to a design with independent samples. This allows to use a lower number of statistical units with an equal study power, by highlighting even small differences in the effect size, which otherwise could be overwhelmed by the between-subjects variance. A total of twenty-four animals were randomized to receive four different types of food throughout four weeks of experiment, according to all possible treatment sequences. In the second week, an animal died, and other six animals moulted. This physiological event, although integral part of the biology of these animals, might have introduced, somehow, a potential source of bias at the level of carryover effect. Hence, in order to take into consideration this event, we created an indicator variable that was included into the models. The presence of missing data in a crossover design might have a greater impact on the estimates compared to a design with independent samples (Woods et al. 1989), but, in this study, we had only three missing data, and SAS *proc mixed* is rather robust with respect to this issue. Indeed, we were able to include even the only datum about the dead animal in the second week in all the three statistical analyses carried out.

Both mixed effects models reported a statistically not significant carryover term, although the model 2 showed a significant interaction between food and period. This latter might be an indication of the presence of a carryover effect, so we carried out a further analysis only on the data of the first period with a classical one-way ANOVA, as it is common practice in this case. Anyway, we have listed below several motivations in support of the fact that we believe that the carryover effect may be considered actually as not significant in this kind of experiment. A wash-out period of 66 hours of fasting between the administration of a type of food and the other one seems to be a reasonable time, from a biological point of view (Hryniewiecka-Szyfter & Storc 1986), to remove the carryover effect of the food given in the previous period, on the choice of the food given in the subsequent period. Unlike drugs, foods should not have physiological effects that are so extended in time, to affect the choice of another food for comparison with the previous one after almost three days of fasting. Even the stress linked to the Petri plate should be minimum because the animals were acclimatized before starting the experiment, and, anyway, the between-subjects variance is well controlled through the experimental design (Carefoot 1973; Dias & Hassall 2005; Abd El-Wakeil 2015). In addition, the animals were assigned the different foods in a random way. Based on all these considerations, it seems unlikely enough that the significant interaction between food and period in the second model can be due to an alias linked to a carryover effect. Furthermore, the same interaction was not statistically significant in the first model for the droppings, where the animals are necessarily the same ones. The analysis on the first-period data (ANOVA), where there are neither carryover nor missing data, is in perfect concordance with the results from the model 2. It is also plausible that the period effects (not significant in both models) do not affect the differences between treatments, because our crossover design is uniform within the periods, that is, each treatment appears the same number of times inside each period (Piantadosi 2005).

The comparison between the results provided by the models 1 and 2 (these latter are perfectly in agreement with the results from the ANOVA on the first-period data) highlighted that there is a difference at a level of statistical significance between potato (A) vs. leaf of plane tree (D), and potato (A) vs. lettuce (B). Indeed, in the model 1 with the number of droppings, the comparison between potato and leaf of plane tree is significant, but it becomes not significant in the model 2 with the difference between areas. Our hypothesis, at this level, is that this divergence probably may be due to the fact that the potato tuber is more assimilated compared to the leaf of plane tree by the digestive system of these animals. This might lead to the production of a lower number of droppings for a similar quantity of ingested food. More than a century ago, some enzymes able to digest fibrin, starch and amygdalin were identified in *A. officinalis* (Kobert 1903). At the level of starch digestion, then, it might be involved an enzyme of the α-amylase family. If so, we speculate that this enzyme may digest the starch present in the potato tuber in a more efficient way compared to the leaf of plane tree because of its higher concentration. As far as concern the comparison between potato and lettuce, the model 1 showed that there is no significant difference about the number of droppings produced by the animals. In contrast, the model 2 pointed out a significant difference about the quantity of ingested food. According to our previous hypothesis, even this datum might represent a further indication of the fact that the potato tuber is digested and assimilated very much, and so it produces a lower quantity of droppings. Anyway, our hypotheses need further insights in order to be able to be verified.

It seems that these animals have no particular preference among potato, carrot and leaf of plane tree, while it seems that the lettuce is not a particularly palatable food for their taste, or their nutritional needs. The latter food might be too rich in water for the feeding needs of this particular species of terrestrial crustacean, which mainly lives in dry environments, with a low rate of humidity. Our hypothesis at this level is that, in general, animals living in an arid environment have a more efficient system to get water from food, and retain it into the body, with respect to the other animals. In some terrestrial isopods, such as *Armadillidium vulgare* Latreille, 1804, some typhlosole channels which seem to have the function to recycle and retain fluids within the digestive system were identified. These channels are not present in marine isopods (Hames & Hopkin 1989). Furthermore, for equal preference among the other three types of food, the potato tuber might be more assimilated, and so provide a higher energetic support. According to our results, it seems that there is not any evidence showing a different consumption of dry food (such as leaves of plane tree) with respect to fresh food (such as potato or carrot) neither in quantitative terms nor as food preferences. In the only study present in literature, which reports data on the feeding preferences in *A. officinalis* (Nair et al. 2003), the authors declared that this species prevalently feeds on dead plant materials and dung of cattle, and, rarely, on remains of dead animals. More in general, terrestrial isopods are considered efficient digesters, but inefficient assimilators, even though food consumption varies according to species (Nair et al. 2003). Moreover, in the same study, Nair et al. (2003) reported that *A. officinalis* is considered a better assimilator compared to *Porcellio scaber* Latreille, 1804, which instead seems to be a better consumer. It was widely demonstrated that the species used in this study is well adapted to life in xeric habitats (Warburg & Bercowitz 1978b), and it is also able to resist to dehydration for a time twelve-fold higher than other species of terrestrial isopods (Warburg & Bercowitz 1978a). In fact, *A. officinalis* is commonly found even in the hottest months of the year in soils completely without humidity (Warburg et al. 1978; Montesanto, personal observation).

Conclusions

This study was the first to use a crossover design to try to assess food preferences in terrestrial isopods, by providing interesting information about the food habits of *Armadillo officinalis*, and, its general biology, which is not much known yet. For this reason, we think this design might be a useful tool to use even with other species of terrestrial isopods, given its versatility and power in detecting small differences by using relatively small sample sizes. Also, it is quite easy to set up this kind of experiment in a laboratory. Based on the results got in this study, we also think that it is possible to breed populations of *A. officinalis* for manifold purposes, even in anthropic environments, because these animals easily adapt to eat both fresh food and dry leaves.

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