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ECOLOGICAL MEDIATORS FOR THE GREGARIOUS BEHAVIOUR OF *ACHATINA FULICA* (MOLLUSCA; ACHATINIDAE)

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Abstract *The Giant African Land snail,* Achatina fulica *Bowdich 1822, which is regarded as one of the 100 worst invasive species in the world, has evolved on the edges of tropical forests, where it has developed its general characteristics showing an ability to adapt to a variety of environments. The objective of this study has been to evaluate the ecological mediators of aggregation, both among wild and captive animals. The sample consisted of 3623 specimens that had shown a preference for isolation illustrating the fact that aggregation is not a set pattern for this species, but rather, a strategy that varies, depending on the site, the season, maturity of the animal and the substrate. This suggests that there is a link between aggregation and factors such as protection, reproduction and overpopulation. Homing and aggregation with the same co-specifics were found to be common since both the co-specifics and the substrate can be employed as a means of orientation through the environment and are a way of locating the areas used for feeding, reproduction and shelter. The data from this study provides evidence that the gregarious behaviour of* A. fulica *is a complex mechanism mediated by a wide range of ecological factors. The behaviour is a strategy used for its protection and adaptation to different substrates and environmental conditions and can thus maximize its invasive potential.*

Key words *Chemoreception; homing; intraspecific interactions; substrate selection.*

INTRODUCTION

The giant African land snail, *Achatina fulica* Bowdich 1822 is regarded as one of the hundred worst invasive species in the world because of the harmful effects it has on the environment, economy and public health in different countries (Raut & Barker, 2002). The species evolved on the edges of tropical forests and shows an ability to adapt to a variety of environments owing to mechanisms such as protandric hermaphroditism, aestivation, gregarious behaviour and homing (Kekauoha, 1966; Raut & Barker, 2002; Fischer, 2009).

Achatina fulica makes use of chemical senses to act as a mediator of social behaviour, feeding and homing (Croll, 1983). The different features of its chemical communication are known as follows: morphology of receptors (Chase 1981, 1982; Chase & Croll, 1981; Chase & Rieling, 1986; Chase & Tolloczko, 1986); reaction to the mucus of conspecific animals (Chase, Pryer, Baker & Madison, 1978; Chase & Boulonger, 1978); aggregation behaviour (Chase *et al*., 1980); homing (Tomiyama, 1992) and the memory of plant odours (Croll & Chase, 1977, 1980).

Aggregation, homing and territoriality are mechanisms used for the acquisition of resources and the distribution of the molluscs (Tomiyama, 1992). Aggregation is a key element in the defense against dehydration and predation, as well as serving to bring about reproductive encounters (Chase, 1982; Chase *et al*., 1980; D'Avila, Dias & Bessa, 2006). Although in the case of many animals aggregation represents an individual reaction to particular external factors, Chase *et al.* (1980) believed that *A. fulica* adopts a social behaviour that is mediated by chemoreception (Chase, 1982; Chase, Croll & Zeichner, 1980). Thus, homing behaviour can be characterized as a pattern of activity that is centered around shelter and is one of the mechanisms linked to aggregation since it is based on communication between individuals (Croll & Chase, 1977; Chelazzi, 1990; Tomiyama, 1992). For this reason, a determining cause involves memorizing the site through odour cues that are released through

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its faeces and mucus or the odours of other animals with which it has congregated.

The aggregation of individuals of *A. fulica* occurs in both wild animals (Fischer & Colley, 2004, 2005; Simião & Fischer 2004) and in the laboratory (Chase *et al.*, 1980). Although environmental factors (e.g.: luminosity, the time of day and moisture) and individual factors (e.g.: age, relationships, individual dispositions and population size) (Chase *et al.*, 1980) affect the way that aggregation occurs in the laboratory, there has not been any evaluation of the factors that cause aggregation in nature.

It is extremely important to have an understanding of how *A. fulica* makes use of its own odours, those of other individuals and plants, or the quality of its micro-habitat and the way that this can act as a stimulus or reference-point for aggregation. This understanding allows measures of control to be taken, enables methods to be devised for selective capture, and allows for management of the environment in a way that can prevent these animals from becoming established. On the basis of the premise that *A. fulica* possesses a long-lasting memory of the odours of plants that are used for food (Croll & Chase, 1977), a hypothesis can be postulated that *A. fulica* selects the substrates that can provide protection and conditions for reproduction by using a variety of cues to return to shelter after searching for food. As a way of addressing these issues, our objective has been to characterize and quantify the ecological mediators that give rise to aggregation in *A. fulica*.

MATERIALS AND METHODS

The study was carried out in four localities on the coastline of Parana State in the south of Brazil during the period January 2003 – March 2010. Most of the land (2,315,733 sq.m) that comprises the municipality of Guaraqueçaba (25º18′24″S, 48º19′44″W) constitutes a Federal Environmental Protection Zone, the center of which has less than 1000 dwellings and was built on marshlands that are often subject to flooding. Ilha Rasa (25º20′3″S, 48º25′54″W), which covers 10.5 sq.m., was included in the Protection Zone of Guaraqueçaba and its 430 inhabitants were spread out along a narrow coastal strip where they maintained the naturally-preserved vegetation. There are few anthropic substrates; however, much of the terrain is bordered with exotic plants. The municipality of Guaratuba (25º52′48″S, 48º34′29″W) has an area of 1,328,480 sq.m. and a population of 33,058 inhabitants and is mainly used for summer holidaymakers which means a lot of the houses are closed after the holiday season. The municipality of Morretes (25º28′37″S, 48º50′04″W) is located in Serra do Mar. Its altitude is 8.5 m. above sea-level and it has an area of 687,541 sq.m. It is characterized by the presence of small rural holdings surrounded by natural reserves.

The study zone is part of the Atlantic Forest biome characterized by the dominance of dense rain forest vegetation (Veloso, Filho & Lima, 1991). The climate is defined as Cfa (subtropical climate) according to the Koppen classification based on vegetation, temperature and rainfall. The average annual temperature is 21º to 22ºC, while the coldest quarter varies between 16º and 17ºC and warmer quarter between 26º and 27ºC. The annual rainfall is of 2000–2500 mm with a coefficient of variation between 15–20% and the wettest quarter between December and February (800–900 mm) and the driest quarter from June to August (250–350 mm). The annual relative humidity is 80–85% and evapotranspiration of 700–800 mm (IAPAR, 2012).

Aggregation in wild snails The analysis of gregarious behaviour in wild animals was conducted by sampling specimens in the urban zones of Guaraqueçaba and Ilha Rasa as well as in the native forest of Morretes, in compliance with the protocols previously established for the region (Fischer & Colley, 2004, 2005, Fischer *et al.*, 2006). We consider aggregation as the mutual occurrence of organisms in response to an environmental stimulus, due to changes in undirected movement or increasing circling in areas with large densities of other individuals. The snails were considered to be aggregating when two or more animals were resting together in the same substrata, at least 10 cm distant from other groups or solitary animals. This distance was standardized based on the animals' natural environment and relative to shell length of the largest specimens included in the sample of the region. Daily inspections were conducted during the dry, cold season (autumn and winter) and in the hot, wet season (spring and summer). Because each site has peculiar environmental characteristics, the sampling needed to be adjusted accordingly. In

Guaraqueçaba, the most urbanized region, sampling was carried out in gardens and backyards, over the course of tree days and covering 59 different plots. On the island Ilha Rasa, the snails were present only in anthropic environments, but the plots were less limited. We conducted sampling at 16 locations totaling 8 h of active search. The interior of the forest in Morretes consisted of a small, continuous and heterogeneous area, and involved three hours of sampling with three groups and three catchers. In all three areas the same sampling protocol (Fischer & Colley, 2004, 2005, Fischer *et al.,* 2006) was applied: (1) determining the identity of an individual by observing presence of deformities on the body and on the shell as part of a general characterization, (2) recording the density, distribution (horizontal and vertical) and distance from the nearest individual, (3) monitoring the activity and behaviour of the animals, and (4) determining the presence of dead shells, eggs and newly hatched individuals. The following environmental factors were considered: size of the area; type of soil; plant species; substrate type; presence of garbage; temperature; humidity; and rainfall. Due to differences in sampling effort in different areas of the study, all comparisons involved estimating the proportions of isolated animals to aggregated animals.

In addition to the locality and the season, the other variables taken into account were the aggregation substrate, vegetation type, soil and anthropic substrates (such as wood, bricks, walls, fences and debris), and the reproductive stage of the molluscs. The molluscs were categorized as: juvenile (2–4 cm); juvenile adult (4–6 cm); and old adult (6–10 cm). This was determined by measuring the length of the shell (Tomiyama, 1992, 2002). The following aggregation parameters were employed: frequency of occurrence of isolated compared to aggregated individuals; size of aggregations; distribution of each reproductive phase in the aggregations.

Homing among wild animals The evaluation of homing was carried out on waste ground in an area 8.5×63.7 m within Guaratuba. This area was selected due to the availability of multiple rest and feeding sites, low illumination, lack of human disturbance and the ability to monitor the snails overnight. At the outset, five pre-existing rest sites were mapped out, with snail presence mapped and site characters such as depth of soil noted. Snails were marked using a pen and sealed with a thin coat of enamel. This followed the protocol of Fischer & Colley (2004, 2005). Animals were monitored overnight for six consecutive nights in the spring and six consecutive nights in the summer of 2009 and the exact position and behaviour of animals recorded every hour until they came to rest on vegetation in the morning. The animals' movements were estimated without touching them. At the end of the night rest sites were examined and the exact disposition of each snail noted.

Laboratory experiments Experiments were conducted in the laboratory Núcleo de Estudos do Comportamento Animal (NEC-PUCPR) between December 2002 and January 2003. Two hundred and seventy snails were kept in 100 litre containers filled with organic soil, dry feed (coarse-grained maize), fresh vegetables and water at a temperature of 25 ± 3°C, with relative air humidity of 77 ± 5% and environment luminosity using a clear/dark cycle of 12 h/12 h. The molluscs were divided into three classes of maturity: juvenile, 2–4 cm; subadult, 4–6 cm; mature adult, 6–10 cm (Tomiyama, 1992).

Two hypotheses were tested: a) that aggregation pattern varied in response to the reproductive state of individuals; b) that snails tended to select substrates that favoured aggregation independent of reproductive state. Thirty replicate trials were carried out.

Influence of the co-specific on aggregation Thirty snails of each class of maturity were marked on the upper region of the shell and divided into two groups. Each group (15 juveniles, 15 subadults, 15 mature adults) was enclosed in a container (33×60×17 cm) with 2/3 of its area filled with a layer of organic soil to a depth of 5 cm. The remaining area was free of soil and used for food and water. Every morning, over a period of 28 days, the positions of each animal were measured and recorded. Dividing the snails into two groups allowed for two replicates.

Influence of substrate on aggregation The final experiment was used to evaluate the influence of the substrate on aggregation. Examination of the degree of aggregation was recorded as above, only noting the position of individuals in relation to the soil and cleared bottoms of the containers.

The occurrence of individuals on vertical surfaces above the soil or above the cleared areas of the containers was noted. Each of the soil/clear areas was divided into two smaller quadrants to allow measurement of the number of times the snails were recorded in the same quadrant over the period of 28 consecutive days.

Statistical procedures To analyze the frequency of aggregation and the relationship between the stage of maturity and the location, season, and substrate, the absolute values were compared by means of a G test. For this purpose, when a comparison of values was performed using one of the variables, we tested the null hypothesis that the frequencies had a similar distribution between compared variables. In comparing the frequencies obtained between variables, comparison was made between two of the variables, on which we tested the observed proportions of one of the frequencies, considering as expected the proportion of the variable to which it was compared. The same procedure was adopted to compare the combinations of each stage of maturity in the groups. The G test was also used to evaluate the frequency of homing, travel route, and behaviour, and to test the preference for substrates between stages. The data for mean (proportion of each stage in the groups, size of the groups, distance of travel, occurrence in groups, and records of resting with the same individual or on the same substrate) were compared by the Kruskal-Wallis and Mann-Whitney nonparametric tests, since the data did not show a normal distribution. To analyze the spatial distribution, we used the index of dispersion (ID = Variance^2/mean) and the Morisita Index of dispersion, applying the test of departure from randomness (Krebs, 1999). In both tests, values obtained close to 1 represent a random distribution; values less than 1, a uniform distribution; and greater than 1, a clumped distribution.

Legal procedures The study was undertaken in compliance with the legislation of Brazil regarding the capture and keeping of animals. This was issued by IBAMA [Brazilian Institute of the Environment (Register No. 10846–2) and authorized by the Committee of Ethics on the Use of Animals CEUA-PUCPR (Register No. 227).

Results

Aggregation in wild animals During field sampling, 3623 snails were recorded, most of which were found as individuals. Frequency of aggregation was greater among the Ilha Rasa and Guaraqueçaba populations than that of Morretes (Table 1) and more common in urban areas (32%) than in natural areas (Gtest$_{(1)}$ = 727.8, $p < 0.01$).

The season and locality sampling showed that aggregation in wild animals was more common in the dry season (Table 2), although there were variations in each locality and the greatest aggregation frequencies were recorded during the dry season in Guaraqueçaba and in the wet season in Ilha Rasa (Table 2).

The frequency of aggregated animals was the same for those on open wild ground compared to anthropic substrates, and significantly greater than that under vegetation (Gtest$_{(1)}$ = 29.4, $p < 0.01$) (Tab. III). However, on the basis of evaluations carried out independently at each locality, it was found that, with the exception of Ilha Rasa, the aggregated snails were more frequent in anthropic environments (Table 3). The aggregation in each of these substrates, when the two seasons were compared, provided evidence that in the wet season there was a greater frequency in the anthropic substrates, while in the dry

Table 1 The total number and relative frequency of the sample of isolated and aggregated of the *A. fulica*.

	N	*Gtest$_{(1)}$*	*Isolated*		*Agregate*		*Gtest$_{(1)}$*	*Id*	*IM*
Morretes	536	A	440 (82%)	a	96 (18%)	b	G(1)=239; P<0.01	0.66	0.86
Guaraqueçaba	2010	B	1409 (71%)	a	601 (29%)	b	G(1)=384; P<0.01	2.85*	3.23*
Ilha Rasa	1077	C	639 (60%)	a	438 (40%)	b	G(1)=301; P<0.01	2.45*	3.18*
Total	3623		2488 (69%)	a	1135 (31%)	b	G(1)=517; P<0.01	2.8*	2.8*

The absolute values of those isolated and aggregated in each locality and between the different communities were compared by means of the G-test and the significantly different values ($p < 0.05$) marked by different letters (small letters for values at the localities and capital letters for values between the localities). Id = dispersion index and IM = Morisita dispersion index, - the significant values ($p < 0.05$) were marked with an asterisk.

Table 2 The total number and relative frequency of the sample of isolated and aggregated of the *A. fulica* in the hot, wet season and the cold, dry season.

		Wet and hot					*Dry and cold*				
	Gtest	Isolated	Agregate		*Id*	*IM*	Isolated	Agregate		*Id*	*IM*
Morretes	A	207 (78%)	60 (22%)	a	0.74	0.62	233(87%)	36(13%)	a	0.56	0.79
Guaraqueçaba	B	1134(84%)	229(16%)	a	0.83	0.85	275(43%)	372(57%)	b	6.7*	7.9*
Ilha Rasa	C	150(34%)	293(66%)	a	4.2*	2.1*	489(77%)	145(23%)	b	0.6	0.72
Total		1491(61%)	582(39%)	a	1.7*	1.5*	997(45%)	553(55%)	b	3.4*	2.5*

The absolute values of those isolated and aggregated in each locality and between the localities were compared by means of the G-test and the significantly different values ($p < 0.05$) marked by different letters (small letters at the localities and capital letters between the localities). Id = dispersion index and IM = Morisita dispersion index, - the significant values ($p < 0.05$) were marked with an asterisk.

Table 3 Relative frequency of the sample of aggregated *A. fulica* in the substrates – at ground level, in the vegetation and anthropic substrate.

		Morretes				*Guaraqueçaba*				*Ilha Rasa*				*Total*			
		%	G	ID	IM	%	G	ID	IM	%	G	ID	IM	%	G	ID	IM
Total	Ground	17	a	0.6	0.68	31	a	4.3	3.1	48	a	2.6*	1.8*	32	a	3.1*	2.3*
	Vegetation	3	b	0.8	0.82	11	b	0.7	2*	46	a	2.9*	2.1*	24	b	1.6*	1.5*
	Anthropic	31	c	0.4	0.6	39	c	2.6*	0.75	15	b	0.8	0.8	32	a	2.2*	1.8*
Dry	Ground	19	a	0.54	0.67	21	a	1.6*	1.5*	71	a	3.3*	1.7*	38	a	2*	1.7*
	Vegetation	3	b	0.62	0.67	10	b	0.5	0.5	86	b	5.9*	2.9*	24	b	2*	1.8*
	Anthropic	31	a	0.43	0.61	18	a	0.53	0.6	2.9	c	2.4*	1.6*	20	c	0.7	0.8
Wet	Ground	16	a	0.71	0.79	50	a	6.4*	3.7*	24	a	0.71	0.77	32	a	4.4	3.1*
	Vegetation	6	B	0.92	0.96	23	b	2.4*	1.9*	30	b	0.78	0.88	24	b	0.96	0.97
	Anthropic	–	–	–	–	68	c	5.7*	2.9*	4.7	c	0.15	0.23	51	c	4.1	2.7*

The absolute values of the isolated and aggregated animals in each locality (determined separately for the total in the wet and dry season) were compared by means of the G-test and the significantly different values ($p < 0.05$) marked with different letters. Id – dispersion index and IM = Morisita dispersion index – the significant values ($p < 0.05$) were marked with an asterisk.

season, the aggregation was less in the anthropic substrates (Table 3).

In total, and particularly in Guaraqueçaba, aggregation was more frequent among juveniles, while in Ilha Rasa aggregation was also frequent among adults (Table 4). When seasonal factors are taken into account, in the dry season the frequency of juveniles and subadults found aggregated was higher than that observed for mature adults. In the wet season, the frequency of subadults was higher (30%) than juveniles (24%) or mature adults (28%). However, a seasonal comparison showed that there was no variation in the case of the Morretes population, while in Guaraqueçaba, juvenile adults are aggregated more in the dry season and mature adults in the wet season. In Ilha Rasa, juveniles were aggregated more in the dry season and subadults and juveniles in the wet season (Table 4).

Although groups of up to 34 wild snails were observed, the average rate for aggregation was 4.6 ± 4 (Table 5). In total, there was no variation in the size of the groups in different seasons; however, apart from the groups that were greater in Guaraqueçaba than in the other localities ($H_{(2;314)} = 14.4$, $p < 0.01$), there was greater aggregation in the dry season, while in Ilha Rasa, it was greater in the wet season (Table 5). The groups recorded in the field largely consisted of mature adult snails (mature adult 63%; subadult 23%; juvenile 14%), which formed the groups that were most frequently found in the wet season (wet 70%; dry 50%; $U=9102$, $p < 0.01$), while the juveniles were more common in the dry season (wet 7%; dry 24%; $U=9$, $p < 0.01$).

The most frequent combination found in all the sampling places was that between mature adults and between mature adults and juveniles (Tab.

Table 4 Relative frequency of the sample of old adult, juvenile adult and juvenile of the aggregated *A. fulica* in the total period and for each season.

	Total			*Dry and cold*			*Wet and warm*		
	Old Adult	Juvenile Adult	Juvenile	Old Adult	Juvenile Adult	Juvenile	Old Adult	Juvenile Adult	Juvenile
Morretes	16% a	21% a	21% a	16%a	11%a	19%a	21%a	23%a	25%a
Guaraqueçaba	21% a	37% a	43% b	47%a	68%b	56%c	17%a	21%a	3%b
Ilha Rasa	31% a	21% b	29% a	24%ac	17%bc	30%c	63%a	76%b	74%b
Total	25% a	29% b	35% c	47%a	68%b	57%c	16%a	21%b	3%c

The absolute values of the isolated and aggregated creatures were compared by means of the G-test and the significantly different letters ($p < 0.05$) of the total period and for each season were marked with different letters.

Table 5 The average number of the *A. fulica* in the aggregations for the total period and for each season.

		Total	*Dry*		*Wet*		*Mann Whitney*
Morretes	A	3.3±2; 2 (42; 2–10)	3.6±2.4; 3 (23; 2–10)	a	2.8±1.5; 3 (19; 2–8)	a	NS
Guaraqueçaba	B	5.2±4.9; 6 (142;2–34)	7.8±6.5; 9 (54;2–34)	a	3.6±2.6; 3 (88; 2–22)	b	U=965; p<0.01
Ilha Rasa	Ab	4.3±3.4; 6 (128; 2–19)	2.9±1.6; 2 (68;2–10)	a	5.9±4.1; 5 (60; 2–19)	b	U=1117; p<0.001
Total		4.6±4; 5 (312; 2–34)	4.9±4.9; 6 (136; 2–34)	a	4,1±3; 5 (176; 2–22)	a	NS

The averages of the values between the localities were compared by means of the Kruskall Wallis non-parametric test and the significantly different averages ($p < 0.05$) were marked with different letters (capitals). The averages shown for each locality were compared in the dry and wet seasons by means of the Mann Whitney non-parametric test and the significantly different averages ($p < 0.05$) marked in different letters (small letters). The values represented are: average ± SD; median (sample; range).

VI) and a seasonally-based comparison showed that this combination was more common in the wet season, while the others were more common on the dry season ($\text{Gtest}_{(1)} = 91.5$, $p < 0.01$).

Homing in wild animals The five rest sites in which the homing of the *A. fulica* were monitored contained groups with an average of 5.1 ± 2.3 (N = 60; 2–9) animals. When account is taken of the six consecutive days of monitoring, the average time spent in the same site was 4.5 ± 1.9 days (N = 57; range: 1–6) which is the equivalent of 78.6% of the observation period. Thus, it was estimated that the average amount of time spent by the snail in the same place was 0.78 ± 0.28 (N = 60; range = 0.16–1).

In the initial evaluation, a greater frequency of subadults was recorded, although in consecutive inspections, it was found that there was no difference in the classifications by size between the proportion of molluscs that settled for six days in the same place, migrants, emigrants and snails that were recorded in the same place on non-consecutive days (Fig. 1). Of the 57 animals that were monitored, 51% remained for six days in the same site since more snails left than entered. With regard to the migrants, a much smaller proportion returned (between non consecutive days) to the rest site (Fig. 1). About 84% of the snails rested for a longer period with the same individuals, and when the representative proportion of the observation time is taken into account, on average the animals settled 0.73 ± 0.3 (N = 158; 0.17–1) with the same individual and with an average distance between them of 4.1 ± 1.9 cm (N = 429;1–6).

Snails were more active at night, moving on average 98.7 ± 53.8 cm (N = 253; range 10–327 cm) and moving away from the rest site to a distance of approximate 42.9 ± 31.6 cm (N = 253; range 8–210 cm), both these distances were significantly greater in the case of juvenile adults. The nocturnal displacement was characterized by linear (29%), non-linear (28.5%) and intermediary (43.5%) routes, the intermediary paths being most common among the juvenile adults (35%, $G_{test}=28.6$, $p<0.01$) and old adults (51% $G_{test}=13.9$, $p<0.01$) and the non-linear among the juveniles (49%, $G_{test}=13.1$, $p<0.01$).

Influence of individuals on aggregation In the laboratory experiments, the average number

Table 6 The proportion of participation of old adult, juvenile adult and juvenile of the *A. fulica* in the aggregation for the total period and for each season.

		Total	*Dry*		*Wet*		*Mann Whitney*
Old Adult	A	0.6±0.3; 0.66 (325;0–1)	0.5±0.4; 0.5 (138;0–1)	a	0.7±0.3; 0.8 (187;0–1)	b	U=9102; p<0.01
Juvenile Adult	B	0.23±0.3; 0.09 (330; 0–1)	0.26±0.3; 0.15 (138; 0–1)	a	0.21±0.2; 0 (192; 0–1)	a	NS
Juvenile	C	0.14±0.3; 0 (320; 0–1)	0.24±0.3; 0 (138; 0–1)	a	0.07±0.2; 0 (182; 0–1)	b	U=9103; p<0.001

The proportions were compared between the seasons and between the localities through the G-test and the significantly different values (p < 0.05) were marked in different letters (small letters for seasons; capital letters for localities). The values represented are: average ± SD; median (sample; range).

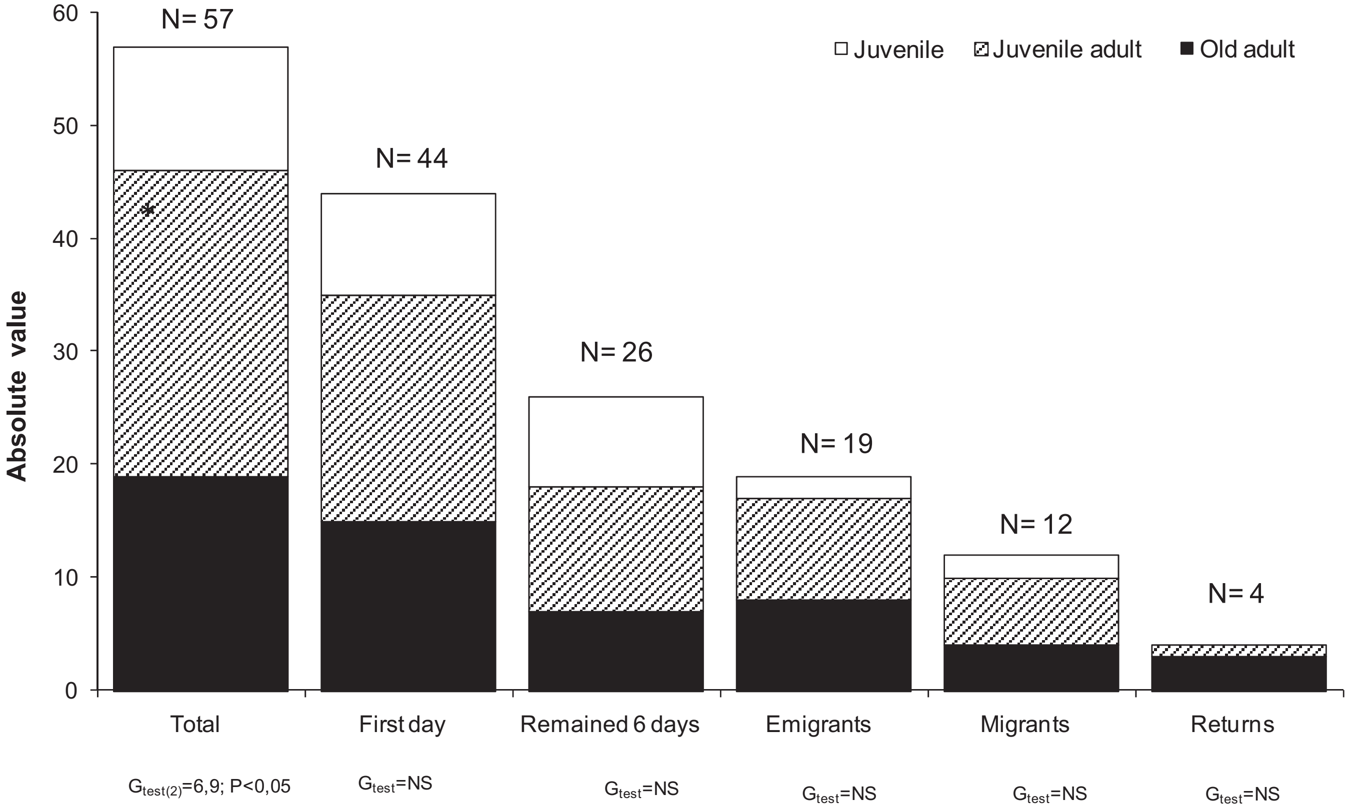

Figure 1 Evaluation and comparative studies of the *A. fulica* that remained in the same site for six days, and those which migrated, emigrated or returned to the site. The absolute values were compared by means of the G-test and the differences that were significantly different (p< 0.05) are highlighted with an asterisk.

of animals that were found in an isolated state was 4 ± 1.9 (N = 90; range 0–8), in groups with less than five snails 3.9 ± 1.7 (N = 90; range 0–8) and in groups with more than five snails 2.1 ± 1.4 (N = 90; range 0–6), the last being the most frequent ($H_{(2;\ 270)}$ = 64.2, p < 0.01). Solitary individual specimens were more often found among mature adults ($H_{(2;\ 90)}$ = 33.6, p < 0.01), groups of up to five examples among the subadults ($H_{(2;\ 90)}$ = 16.6, p < 0.01) and more than five snails among the juveniles.

During the 28 days of monitoring, the record of the exact position of the snails revealed that the snails aggregated with the same individual on consecutive days at an average rate of 6.5 ± 2.7 times (N = 632, range 1–15) and on non-consecutive days 5.1 ± 1.9 times (N = 632, range 1–12) (U = 136473, p < 0.001). These values when viewed with regard to the total time period of the study represent an average proportion of 0.23 ± 0.1 (N = 632, range 0–0.6). However, it was found that the frequency at which the snails aggregated with the same neighbouring individual varied in accordance with their level of maturity, since the average number of times that two individual specimens were recorded in the same group did

Table 7 The average number of times that old adults, juvenile adults and juveniles of the *A. fulica* were recorded in the same rest site, on vertical surfaces, on horizontal areas with soil and horizontal areas without soil during a monitoring period of 28 days.

	Vertical surface		*Horizontal surface with soil*		*Horizontal surface without soil*		*Kruskal wallis*
Juvenile	2.1±1.3; 2 (168; 1–8)	aA	9.7±3.4; 9 (59; 2–18)	bA	1.9±0.9; 2 (50; 1–5)	aA	H(2;273)= 138; p<0.01
Juvenile adult	1.7±0.9; 1 (134; 1–7)	aB	8±2.6; 8 (60; 2–12)	bB	2.1±1.2; 2 (48; 1–5)	aA	H(2;242)=138.6; p<0.01
Old adult	1.5±0,8; 1 (139; 1–5)	aAB	6.6±2.3; 7 (61; 2–12)	bC	2.8±1,5; 3 (60; 1–8)	cB	H(2;260)=153.9; p<0.01
Kruskal wallis	H(2; 437)= 14.3; p<0.01		H(2; 180)= 26.2; p<0.01		H(2; 158)= 9.6; p<0.01		

The averages were compared by means of the Kruskall-Wallis test with a subsequent analysis conducted with the Mann Whitney test and the significantly different averages ($p < 0.05$) were represented by different letters (small letters for each stage of maturity; capital letters for each surface). The values represented are: average ± SD; median (sample; range).

not differ on consecutive and non-consecutive days in the case of mature adults, although in the case of juveniles ($U=10763$, $p < 0.01$) and subadults, it was greater on consecutive days.

Influence of the substrate on the aggregation In 28 days of observation, the average number of days that the same animal rested at the same site was 1.9 ± 1.2 times (N = 719, range 0–8) for vertical surfaces, 8.1 ± 3.1 times (N = 180, range 2–18) for horizontal surfaces with soil and 2.1 ± 1.4 times (N = 180, range 0–8) for horizontal surfaces without soil ($H_{(2;1079)} = 498$, $p < 0.001$). The substrates that were made available were employed in a distinctive way in the three stages of maturity and where there were vertical and horizontal surfaces with soil, there was a greater degree of permanence among the juveniles (Table 7).

DISCUSSION

The data obtained from this study have been drawn on to characterize gregarious behaviour and homing in *A. fulica* and shows complex mechanisms mediated by a wide range of factors. The fact that along the coastline of Parana State, *A. fulica* specimens tend to be isolated rather than aggregated, and that aggregation varies, depending on the site, season and degree of maturity, suggests that aggregation cannot be used as a behavioural pattern for the species. Rather, it is adopted as a strategy that can be employed in several contexts such as a protection against biotic and abiotic factors, as well as a result of over-population and used also as a reproductive strategy.

The use of aggregation as a defense strategy was found to be more useful among juveniles and during the dry season. According to Chase *et al.* (1980) and Tomiyama (1992), closer proximity is a means of avoiding dehydration and predation, which are both factors that are very important for smaller animals. Chase *et al.* (1980) believed that the formation of dense groups prevents the invasion of predators and, thus, it is probable that there is competition to reach the most central position in the group, which has the effect of making the group more compact. This has been corroborated by the results of laboratory experiments where the juveniles have formed the most numerous groups. However, although groups of up to 34 snails can be found, there seems to be a pattern regarding the number of their components since a similar average size of the groups was found in this study, under different conditions. In other terrestrial pulmonata, the aggregations also show a limited number of components (Dan & Bailey, 1982) and the juveniles form larger groups (Cameron & Carter, 1979; Dan & Bailey, 1982; Lind, 1989). It may be possible to relate the tolerance limit of the co-specific to territoriality and discover that the mechanisms employed in individual recognition may be used in the formation of the groups. According to Fischer (2009), although aggregation is a form of protection against desiccation, *A. fulica* can adopt other strategies such as aestivation, burial, vertical displacement and the distinct use of substrates, to overcome stressful situations like having to undergo either a lack or excess of moisture and extremes of temperature.

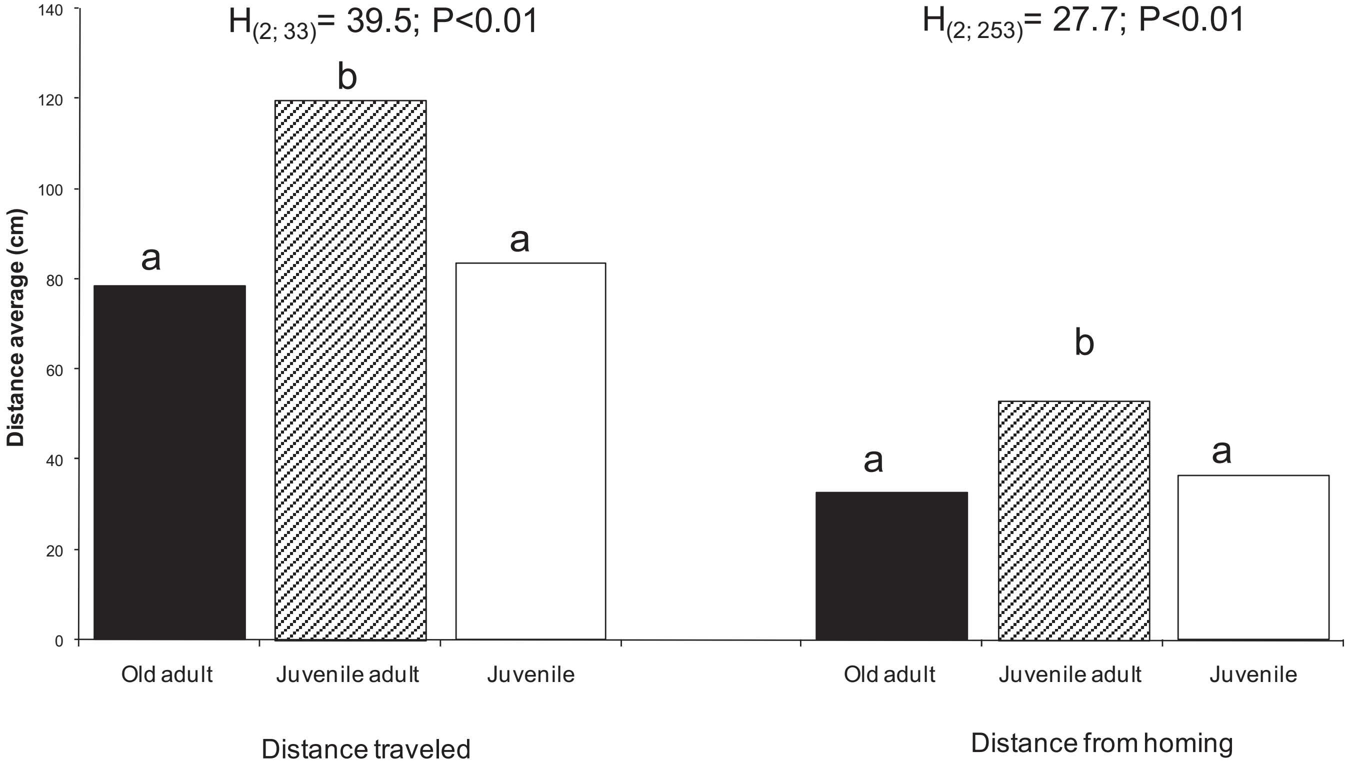

Figure 2 The average distances (cm) travelled by the old adult, juvenile adult and juvenile of the *A. fulica* during their periods of activity and the distances from the rest site. The averages of each group were compared after each inspection by means of the Kruskall Wallis Test and the values that are significantly different (p < 0.01) are marked in different letters.

The distinct use of substrates by *A. fulica* throughout its ontogenetic development was confirmed, as well as the fact that aggregation varies in accordance with the locality, season and substrates. These factors characterize its relationship to chosen sites. Hence, in the event of the suitable shelter being limited or else dispersed in an irregular way, it can be expected that this will cause a degree of aggregation (Raut & Barker, 2002; Tomiyama, 1992). In fact, wild animals are found at focal points of infestation (Simião & Fischer, 2004; Fischer & Colley, 2004, 2005) where a large amount of resources are available. Tomiyama (1992) is among those who do not consider the aggregation of *A. fulica* to be an intentional phenomenon, because in Japan it has been found that the individual snails only aggregate in vegetation. According to Chase *et al.* (1980), external stimuli such as the concentration of food resources, moisture and luminosity can attract snails so that they arrive at the aggregation site in an independent way, as has also been found in the case of *Helix pomatia* (Lind, 1989). The heterogeneous nature of the environment and irregular supplies of food act as mediators of aggregation, as was evident when the different localities under study were compared here. While the dispersion was random in the more stable and uniform environments, like the interior of forests in Morretes, the same was not the case in the urbanized environments like Guaraqueçaba. In this locality, the soil is subject to flooding which stimulates a vertical distribution and aggregation during the dry season when they are more exposed to the problem of desiccation. In Ilha Rasa, the greater existence of natural substrates (Fischer & Colley, 2004) makes aestivation feasible during the period of burial in the dry season (Fischer, 2009), while it leads to greater aggregation in the wet season as a result of the flooding of the ground and the increase of activity among the snails.

Overpopulation can also act as a mediator of aggregation because the aggregation varies in accordance with site and substrate. In the anthropic environment, which by mitigating climatic conditions can be conducive to the reproduction and attraction of other snails, it is expected that there will be a greater population density. In general, low density is regarded as a rate of

7–32 snails per sq. m. and high density anything above 201 snails per sq. m. (Raut & Barker, 2002; Southwick & Southwick 1969). However, the overpopulation may only be temporary because, according to Civeyrel & Simberloff (1996), the initial phase of colonization by *A. fulica* involves large and resistant animals whose descendants are drawn on to increase the population density. Nonetheless, in successive generations, increasing competition and homozygous adults result in precocious maturity, a reduction of nutrients, cannibalism, susceptibility to genetic deformities and a depression of the immune system, all of which reduce the population levels. Thus, both vertical distribution and aggregation are used as parameters to estimate the stage of colonization, both of which are greatest among the oldest populations (Fischer & Colley, 2004, 2005; Fischer *et al.*, 2006).

The fact that the groups are predominantly formed by adults and mainly in the wet season, suggests that reproduction acts as a mediator of aggregation. Fischer & Colley (2005) and Fischer *et al.* (2006) both believe that one of the factors that brings about an increase in population in urban areas is the restricted space between the snails which allows simultaneous sexual coupling to occur. It should be stressed, however, that the mating takes place during the nocturnal wanderings and at ground level (Tomiyama, 1992), a fact that fails to explain the occurrence of aggregation after mating. Whereas authors like Croll (1983) do not regard it as evident that sexual aggregation occurs in gastropods, Fearnley (1996) described the temporary aggregation of *Helix aspersa* in a sexual context although he stressed that the partner is not chosen at random, since there is a preference for members of the same population group. In the case of sedentary gastropods, like *Subulina octona*, aggregation is seen by D'Avila *et al.* (2006) as a strategy to enable reproduction.

The individual specimens of *A. fulica* search for food outside their rest site and as a result, the extent of their territory can be inferred from the fact that the pattern of activity and distance from the rest site is smaller in old adults than in young adults. Our data support the observations of Tomiyama (1992, 2000) and Tomiyama & Nakani (1993) about *A. fulica* in Japan. These researchers state that territoriality is more important for mature adult snails since they have to find a site that is suitable for protection, oviposition and recruitment of their progeny. However, for competition to occur, there must first be scarcity or a difference in value resources. Thus, after a microhabitat has been selected, the snail signals that its unfamiliar odours should repel other snails that are seeking their own shelter. The lack of physical structures that can cause a direct confrontation is replaced by chemical communication which involves releasing the repellant substances referred to by Chase *et al.* (1978). Nonetheless, in this study, homing was evident at every stage of maturity, possibly due to the restriction to smaller areas. It is also possible remaining settled in the site for a longer period of time serves to strengthen the mollusc's ability to find it again.

Reflection on the question of territoriality must take into account what it is that stimulates the animals to move about. There are both intrinsic factors (hunger and reproduction) and extrinsic factors (moistness and clarity) that stimulate displacement (Takeda & Ozaki, 1986; Tomiyama, 1992). Although *A. fulica* displays a circadian cycle, its predisposition for locomotion mainly stems from an increase in moisture and the alteration of the concentration of haemolymph (Takeda & Ozaki, 1986). Mead (1961) refers to an "escape reaction" in *A. fulica* leading to "mass migration" which could be partly triggered by overpopulation. If this phenomenon could be proved, it would greatly contribute to a better understanding of the mechanisms behind the dispersal of *A. fulica*.

The verification of the frequency and the factors that benefit aggregation and homing raises questions about the underlying mechanisms that cause this. When account is taken of the fact that the epidermis of *A. fulica* produces different secretions that serve different functions (Chase & Boulanger, 1978; Chase, 1982), it can be concluded that the snails might, in the course of evolution, have begun to use these signals for navigation and orientation in their environment. It should also be noted that the odours emitted by plants might be used as points of reference. Thus, it is hoped that both the aggregation and the homing might be mediated, either by means of chemical communication resulting from the odours released by the animal itself through trails of mucus or faeces, or by other individuals or by the molecules present in the environment around the rest site (Gelperin, 1974). The presence of the same individual snail in the shelter on consecutive days makes it likely that it uses odoriferous paths to refind the place where it had settled on the previous day (Chase *et al*., 1980). The chemical molecules released by the mucus, together with its moistness and viscosity have probably come to be used as pathways for navigation (Chase *et al*., 1978). Chase & Boulanger (1978) have confirmed that *A. fulica* reacts more to mucus when it is emitted by a foot rather than by a mouth, which suggest that pheromones can be found around sites of aggregation. In this study, it was evident that a trail was used at the exit of the shelter and when the snails wandered in a file, they followed the route of the animal in front, as recorded by Mead (1961). The information obtained from the trail is probably more effective for juveniles since they reveal a more irregular route that remains closer to the shelter, while the adults are able to make use of other information. It should be noted that apart from the molecules associated with the aqueous matrix of the track, more volatile molecules are also released that give signs of their place of origin without any need for the wandering molluscs to touch the trail. In view of the fact that plants used as a shelter, the faeces and other settled individuals had also combined to release volatile molecules, there may be a synergy that helps bring about convergence in the rest site (Chelazzi, Levoci & Parpagnoli, 1988).

Aggregation is a multi-adaptive system in molluscs which can be used as a determinant of survival, a suitable means of reproduction or a way of responding to environmental factors such as seasonal variations, microclimates, heterogeneity and the uneven distribution of food resources. Aggregations can be permanent or temporary, the former arising from a restricted area and the latter being linked to sites that are used for reproduction, feeding, rest and protection against dehydration. In general, gregarious molluscs possess smaller living areas and because they are mediated by chemical communication, tend to remain close to them. Chemoreception studies can take place through the release of volatile substances or through contact where the mucus is used to indicate the identity and physiological state of the animal. By concentrating on the habitats, life stories and evolutionary factors involved, it can be confirmed that each species adopted the best strategy to allow it to adapt to a particular niche (Baur, 1993; Croll, 1993; Chelazzi, 1990; D'Avila *et al*., 2006). However, the data of this study indicate that *A. fulica* can use aggregation for any one of these contexts and displays great flexibility when faced with different situations which can make aggregation become one of the main factors that allow this species to be potentially highly invasive. A knowledge of the variables of gregarious behaviour and of the mechanisms that act as mediators of environmental orientation, constitute a valuable aid in drawing up management plans, as well the knowledge of the attractive compounds used by *A. fulica* can also be used to develop species-specific baits. Further studies of attractive features involving mucus and organic matrices are the next step to be carried out by our group to help in the understanding of the complex mechanism of *A. fulica* orientation in the environment.

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