

Radio-telemetry techniques in the study of displacement of freshwater anomurans

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Abstract

In an ecological context, information on the movement and activity is important for understanding the requirements of habitat, resource usage patterns and the potential of interspecific interactions. The aim of this study was to examine the displacement pattern on daily activity and occupancy of substrates by *Aegla manuinflata* using radio-telemetry technique on field on Southern Brazil. Four adult males were monitored during nine days. The aeglids showed a significantly greater displacement toward upstream than downstream. *Aegla manuinflata* individuals showed higher displacement activity during the night period. Although activity was not constant: the animals spent one or more days without displacement. All aeglids showed locomotion activity concentrated between 11:43 p.m. and 02:25 a.m., although it was not restrict to sunset period and night. Individuals showed specific occupation of different types of substrate, but a pattern in the occupation of substrates as a function of the photoperiod was not observed. In this study, using a new technique for tracking aeglids, it was concluded that *A. manuinflata* is capable of actively dislocate throughout the stream, either toward upstream and downstream, passing through obstacles which may represent ability of re-colonization. The animals are more active during the night period, probably a strategy to avoid predators that are active during the day; the selection of substrate is not associated to sediment texture.

Key words: Aeglidae, Circadian rhythm, Substrate preference.

Introduction

Information on the movements and activity of animals is important for understanding their requirements of habitat,

resource usage patterns and the potential of interspecific interactions (Sutherland, 1996). The movements of animals are connected both to the acquisition of primary resources (for instance, food, shelter and partners for mating) and also to avoidance of stress sources

(predators, thermal extremes and dehydration) (Barbaresi *et al.*, 1997). The movement patterns of individuals and populations may reflect ecological and growth responses to environmental heterogeneity (Southwood, 1977; Levine *et al.*, 1984; Cohen and Levin, 1991).

Movement is a fundamental, yet relatively poorly understood population process. Although population ecology has traditionally concentrated on understanding temporal fluctuations in abundance (Turchin, 1998), more recently the focus has shifted to spatially explicit approaches, leading to a greater appreciation of the importance of movement (Steinberg and Kareiva, 1997). Moreover, it has been acknowledged that some important population phenomena depend not only on spatial changes in average population density, but also on individual movement behaviour (Morales and Ellner, 2002; Kokko and López-Sepulcre, 2006). Interactions between life history, physiology, behaviour and habitat make individual movement an exceptionally complex phenomenon (Patterson *et al.*, 2008).

A number of studies have been conducted in crustaceans using different telemetry techniques, in order to provide information on home range, migration patterns and activity of these animals (Palinura: Smith *et al.*, 1998; Watson III *et al.*, 1999; Astacidea: Bubb *et al.*, 2002; Anomura: González-Gurriarán *et al.*, 2002; Jørgensen *et al.*, 2007; Brachyura: Clark *et al.*, 1999; Turner *et al.*, 2003; Guerra-Castro *et al.*, 2007; Lynch and Rochette, 2007). Stand out the studies using radio-telemetry techniques, such as those conducted by Robinson *et al.* (2000), who examined the movement patterns and the living area of *Austropotamobius pallipes* (Lereboullet, 1858), by Bubb *et al.* (2002a), who evaluated the movements and activities of *Pacifastacus leniusculus* (Dana, 1852) and by Bubb *et al.* (2004), who studied the movement and dispersion of *P. leniusculus*.

Crustaceans show some advantages as potential targets of telemetry, such as the existence of an exoskeleton, which represents a solid and relatively inert surface to which

transmitters and a series of sensors may be fixed. In aquatic environments, where most crustaceans live, the capacity of direct observation in nature is limited by water clarity, substratum structural complexity and diving propensity (Wolcott, 1995). Another advantage of telemetry is that a number of individuals can be monitored at the same time by using an individual marker for each organism. This differs from traditional tagging and recapturing techniques, in which individuals are tagged only at specific places and, generally, not very frequently during their lives, such that long-range movements are difficult to detect for many animals (Hedin and Ranius, 2002).

Many decapod crustaceans synchronize certain behaviors with daily light/dark cycles. Both laboratory and field data suggest that these rhythms are controlled by circadian clocks that are strongly influenced by environmental factors, particularly by light (Aréchiga and Atkinson, 1975; Karnofsky *et al.*, 1989; Aréchiga *et al.*, 1993; Palmer, 1995; Aréchiga and Rodríguez-Sosa, 1997; Skajaa *et al.*, 1998; Jury *et al.*, 2005). However, although laboratory studies are useful to identify trends in a species with respect to being active in a determined time of the day or night, or for determining whether this pattern is triggered by an endogenous clock, the real activity pattern expressed by an animal in its natural habitat is often influenced by additional factors (Golet *et al.*, 2006). It is well known that exposure to light may affect a number of physiological processes in crustaceans, such as locomotion, food, mating, changes in color and metabolism (Meyer-Rochow, 1994).

The micro- and medium-scale movements of aeglids, the only continental water anomuran, are not very well known (Lopez, 1965). The Aeglidae family is restricted to the South American Neotropical region, occurring in streams, flooding rivers and lakes. These animals play a fundamental role in the food chain of limnetic systems (Arenas, 1976; Santos *et al.*, 2008; Cassini *et al.*, 2009). With the aid of radio-telemetry, it is possible to accurately follow the movements of these

animals in time and space, thus providing unprecedented information on aeglids biology and behavior in their natural environment.

Sixty nine species are known for the Aeglididae (McLaughlin *et al.*, 2010), some of which are highly threatened by environmental degradation (Pérez-Losada *et al.*, 2002; Bond-Buckup *et al.*, 2003), and in Brazil, twelve of these species are considered threatened according to IUCN criteria (Critically Endangered, Endangered and Vulnerable) (Magris *et al.*, 2010). Information about movement patterns of aeglids can be relevant to analyze the impacts on these animals and also to understand the possible strategies of species that inhabit threatened environments.

Radio-telemetry was used in the present study to answer the following questions about *Aegla manuinflata* Bond-Bukup and Santos, 2009 in their natural environment in southern Brazil: 1) what are the displacement patterns of this species; 2) what are their activity rhythms in their natural environment; and 3) how does their occupation of different substrates occur?

Material and Methods

Study area: Our experiment was conducted in the Passo da Taquara Stream (29°36'01"S - 54°10'37"W, 158 m altitude) (Fig. 1), state of Rio Grande do Sul, Brazil. This stream is characterized by a temperature range of 16.8 to 24.1°C and a flow range of 0.01 to 0.07 m³/s throughout the year.

The bottom of Passo da Taquara Stream is composed of five kinds of substrate according to sedimentary scale of Wentworth: boulders ($\phi = 256 - \infty$ mm), cobble ($\phi = 64 - 256$ mm), pebble ($\phi = 4 - 64$ mm), granule ($\phi = 2 - 4$ mm) and sand ($\phi < 2.0$), with a predominance of boulders and their subdivisions. The bottom of the river was previously mapped for kind substrate through the use of sieves. The stream banks exhibit variations in the ground cover by vegetation from absent to scattered or dense; in the last case, the streambed is covered with its branches. This vegetation supplies the

watercourse with organic matter, that is mainly present in the deeper stretches of the stream because of the slower speed of floodwater in these sites.

Capturing and tagging: Traps, baited with bovine liver, were used for capturing aeglids. The transmitters were attached with nontoxic glue (adhesive cyanoacrylate) to four adult males in the intermolt stage (Tab. 1). The low number of tagged animals is justified by the small size of the stream, sectioned into three branches, each of which is approximately 80 m. Besides, a similar number of animals has been used satisfactorily in other studies with crustaceans in large areas, such as estuaries, the open sea and even in large rivers (Clark *et al.*, 1999, with *Callinectes sapidus* Rathbun, 1896 – n = 5; Gherardi *et al.*, 2000, with *Procambarus clarkii* (Girard, 1852) – n = 5, Carr *et al.*, 2004, with *C. sapidus* – n = 8; Jørgensen *et al.*, 2007, with *Paralithodes camtschaticus* (Tilesius, 1815) – n = 7; Lynch and Rochette, 2007, with *Carcinus maenas* (Linnaeus, 1758) – n = 6).

All captured aeglids were measured and weighed but only four individual of the appropriate size and weight to bear the transmitters were selected (Tab. 1). In a previous analysis conducted in a laboratory and in the natural habitat, in *ad libitum* observations it was verified that the experimental individuals behaved in the same way as non-tagged individuals. In addition, they were able to hide under rocks in the same manner as non-tagged animals. Therefore it was concluded that the transmitters did not significantly affect the behavior of tagged individuals. It is largely known that transmitters (tags) weighing up to 10% of the of specimens body mass do not interfere in its activity, behavior and/or survival (Clark *et al.*, 1999; Robinson *et al.*, 2000; Bubb *et al.*, 2002a and 2004; Guerra-Castro *et al.*, 2007). In this study, transmitters weighing between 0.3 and 0.4 g were used, which are less than 10% of the body weight of studied aeglids.

Monitoring: The surface of aeglids was dried, and the transmitters were glued to

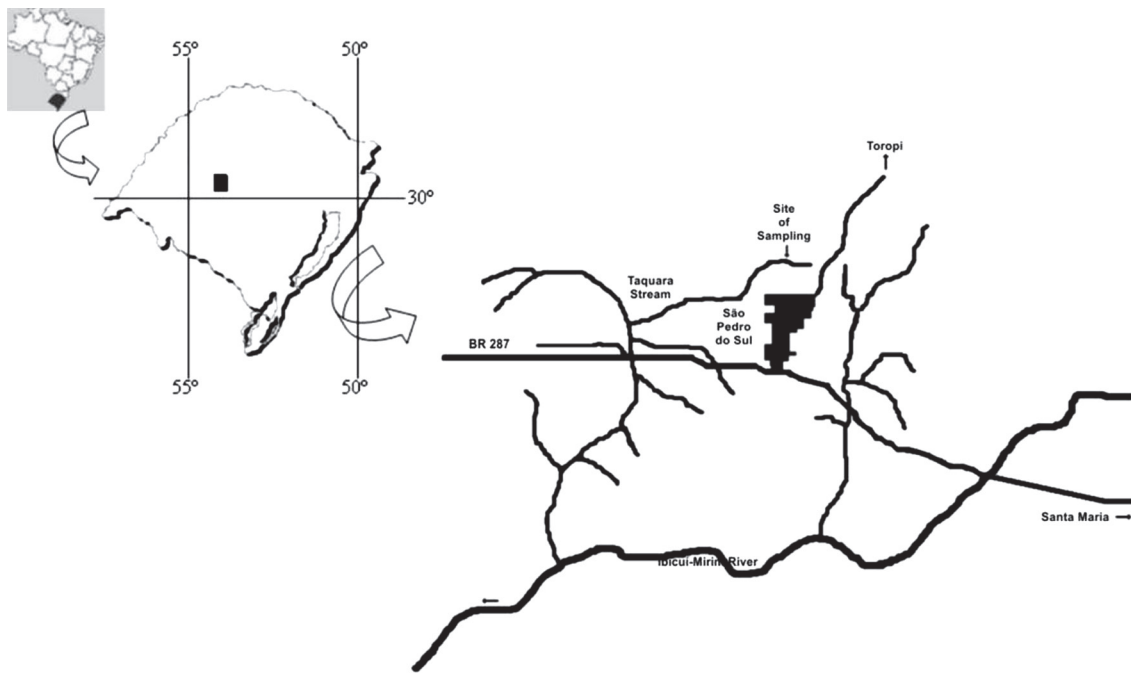


Figure 1. Schematic representation of localization of Passo da Taquara Stream on Southern Brazil.

the cephalothorax (Fig. 2). The transmitters used (model BD-2N) have a lifespan of approximately 12 days within a range of 50 to 100 meters in the frequencies shown in Tab. 1. The transmitters were acquired from Holohil Systems Ltd. The signals were received by a receptor (model TRX-16S) equipped with a Yagi three element antenna from Wildlife Material International, Inc.

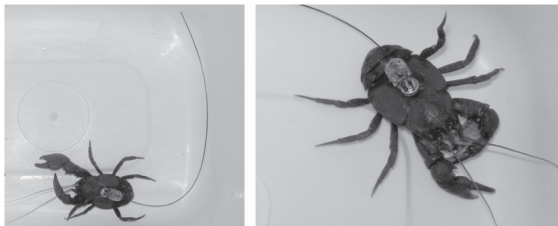


Figure 2. *Aegla manuinflata*. Aeglid with transmitter attached on its cephalothorax.

Tagged animals were monitored for a period of approximately nine days (the lifespan of tags in the field) with radio sightings conducted every three hours to obtain an estimate of day and night movements. After their release, the aeglids were observed to determine their movements downstream or upstream from the point of release, in addition to the record of their displacement area during that period of time.

Data analysis

Displacement: For each specimen, the day and night displacement was calculated, i.e., the displacement was quantified in meters between each monitoring time (3, 6, 9 and 12 a.m., and 3, 6, 9 and 12 p.m.). Before the displacement analysis, the data normality was checked by the Shapiro-Wilk test, as they were non-parametric ($p < 0.0005$), the Kruskal-Wallis test (H test) was used to compare the displacement rate among the four individuals, and the G test was used to compare the displacement rate between day and night. The statistical tests were conducted using Past 1.95 software (Hammer *et al.*, 2001).

Circadian activity: To determine whether the aeglids exhibited different activity patterns during the circadian cycle, a circular statistic was employed, which analyzes the distribution of displacement (in m) during the day. To determine their activity, the sum of the displacement of the animals at each sighting times was used (3, 6, 9 and 12 a.m., and 3, 6, 9 and 12 p.m.) over seven experimental days (from the 2nd to the 8th day; the 1st and 9th days were excluded from this analysis because on

the 1st day, monitoring was performed after 12 p.m., and on the 9th day, a complete set of data was not available for all animals due to the loss of the signal from the transmitters). The activity levels were tested for a non-random distribution using a Rayleigh Z-Test, which was conducted using Oriana software (Kovach, 1994). To compare the activity patterns among the four individuals, a χ^2 test was applied.

Space occupation: The relationship between the kinds of substrate occupied by the four individuals of *A. manuinflata* and their distribution was determined by the Mantel Test (Manly, 2000). For this purpose, the data were standardized by the arcsin function (because data were presented as %), and the Euclidian distance was used to evaluate similarity.

The Mantel test is a non-parametric procedure based on randomization that generates statistics at significant levels for a correlation between matrix distances without supposing specific probability distributions (Cesaroni *et al.*, 1997). This test utilizes permutation, and the association between these two matrices can be tested by the Z distribution, such that it can establish the statistical significance of the matrix correlation (Sokal, 1979; Smouse *et al.*, 1986; Manly, 1990; Cesaroni *et al.*, 1997). Normalization was conducted to transform Z into a coefficient (r), which varies from -1 to +1. The significance of Z was determined using a Monte Carlo permutation test (Smouse *et al.*, 1986) with 5,000 permutations. The Mantel test was conducted in the statistical program NTSYS 2.1 (Rohlf, 2000).

For the Mantel test, two matrices were built based on two hypotheses to be compared to the similarity matrix of the substrate composition (sand, cobble, granule, slab, boulder and gravel):

1. Similarity matrix 1 (SM1): considering the structuring related to the four individuals, i.e., the evaluation of whether the usage of different substrates reflects individual preferences.

2. Similarity matrix 2 (SM2): considering whether there is structuring by

photoperiod (day/night) depending on the occupied substrates.

In addition to the hypothesis tests, the kind of substrate preferentially occupied by the four individuals was evaluated.

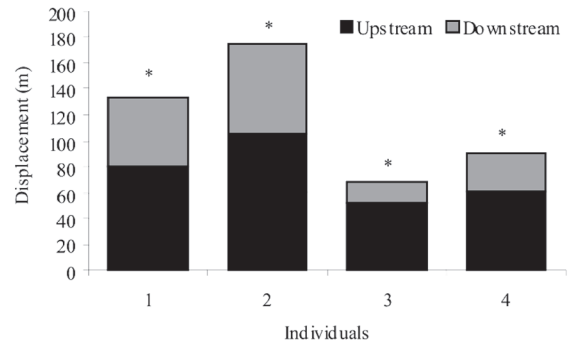


Figure 3. *Aegla manuinflata*, animals monitored by radio-telemetry at a stream on Southern Brazil on February/2009: displacement of four individuals (1, 2, 3 and 4) downstream and upstream during eight days of observation. (*) Represents significant difference using χ^2 test (p<0.0001).

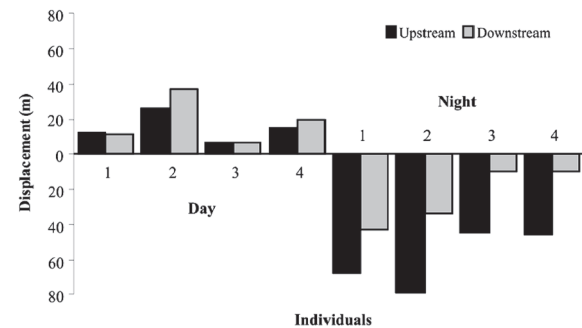


Figure 4. *Aegla manuinflata*. Animals monitored by radio-telemetry at a stream on Southern Brazil on February/2009: displacement of four individuals (1, 2, 3 and 4) downstream and upstream during eight days of observation considering displacement on day time (9 a.m., 12 a.m., 3 p.m. and 6 p.m.) and night time (12 p.m., 3 a.m., 6 a.m. and 9 p.m.).

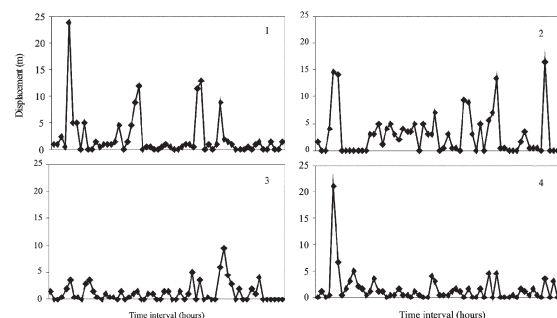


Figure 5. *Aegla manuinflata*. Daily locomotion activity (at each three hours) of four individuals. Animals monitored by radio-telemetry at a stream on Southern Brazil on February/2009.

Table 1. *Aegla manuinflata*. Data of animals used on radio-telemetry experiment on Southern Brazil on February/2009. CL = Cephalothorax Length; RPL = Right Propod Length; LPL = Left Propod Length; PH = Propod Height; W = Weight of animal and TF = Transmitter Frequency.

| Individual | CL (mm) | RPL (mm) | LPL (mm) | PH (mm) | W(g) | TF (MHz) |
|------------|---------|----------|----------|---------|------|----------|
| 1 | 18.64 | 15.02 | 17.71 | 11.06 | 4.72 | 150.019 |
| 2 | 19.02 | 16.94 | 13.18 | 11.17 | 4.72 | 150.037 |
| 3 | 19.23 | 14.21 | 16.48 | 11.10 | 4.77 | 150.057 |
| 4 | 18.64 | 14.31 | 16.51 | 10.52 | 4.64 | 150.097 |

Table 2. *Aegla manuinflata*. Displacement of four individuals (1, 2, 3 and 4) monitored by radio-telemetry at a stream on Southern Brazil on February/2009.

| | Displacement (m) | | | | | |
|------|------------------|--------------|---------------|---------------|--------------------|----------------|
| | Minimum (3h) | Maximum (3h) | Minimum (24h) | Maximum (24h) | Mean ± sd (8 days) | Total (8 days) |
| 1 | 0 | 24 | 4.5 | 40.5 | 2.48±4.39 | 134 |
| 2 | 0 | 16.5 | 5.5 | 39 | 3.24±4.08 | 175 |
| 3 | 0 | 9.5 | 2.0 | 21 | 1.33±1.86 | 69 |
| 4 | 0 | 21 | 1.5 | 41 | 1.67±3.09 | 90.5 |
| Mean | 0 | 17.75 | 3.37 | 35.37 | - | 117.87 |

Table 3. *Aegla manuinflata* Animals monitored by radio-telemetry at a stream on Southern Brazil on February/2009: total sum in meters of displacements of four individuals, and in parenthesis the percentage (%) downstream and upstream during eight days of observation.

| Displacement (m) | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Total |
|--------------------|------------|-------------|-----------|-------------|-------------|-------------|-------------|------------|--------------|
| Total downstream | 6.5 (46.4) | 14.5 (11.9) | 20 (42.6) | 45 (73.2) | 11.5 (31.5) | 23 (23.6) | 20.5 (38.7) | 31 (82.7) | 172 (36.7) |
| Total upstream | 7.5 (53.6) | 107 (88.1) | 27 (57.4) | 16.5 (26.8) | 25 (68.5) | 74.5 (76.4) | 32.5 (61.3) | 6.5 (17.3) | 296.5 (63.3) |
| Total displacement | 14 | 121.5 | 47 | 61.5 | 36.5 | 97.5 | 53.05 | 37.5 | 468.5 |

Table 4. *Aegla manuinflata*. Circular statistical results indicating periods of higher circadian activity of four individuals. Animals monitored by radio-telemetry at a stream on Southern Brazil on February/2009. (*) Represents significant difference using Rayleigh's Test.

| | Indiv. 1 | Indiv. 2 | Indiv. 3 | Indiv. 4 |
|-------------------------------|------------|------------|------------|------------|
| Mean Vector (μ) | 02:25 a.m. | 00:55 a.m. | 00:35 a.m. | 11:43 p.m. |
| Length of Mean Vector (r) | 0.366 | 0.202 | 0.238 | 0.221 |
| Rayleigh Test (Z) | 21.116 | 8.076 | 5.838 | 5.776 |
| Rayleigh Test (p) | 6.75E-10* | 3.11E-4* | 0.003* | 0.003* |

Results

Displacement

Among the monitored animals, the highest displacement was performed by individual n° 2 that displaced 175 m during the eight days of monitoring (Tab. 2). Only individuals 2 and 3 showed significant differences related to the displacement rate ($H = 5.813$; $p = 0.02$).

Regarding downstream and upstream movement (Tab. 3), the animals exhibited a significantly displacement upstream ($\chi^2 =$

768.3; $p < 0.0001$), which was also observed when the four individuals were analyzed separately (Fig. 3). The night period was characterized by larger displacement than that daytime period (downstream and upstream) (G test = 153.79; $p < 0.0001$ for the four analyzed individuals together) (Fig. 4). It became evident that aeglids were capable to overcome natural barriers because at least one individual (individual n° 2) traversed three small waterfalls (60, 70 and 80 cm high) that exhibited high-speed flows.

The displacement of aeglids was not constant since each animal spent one or more

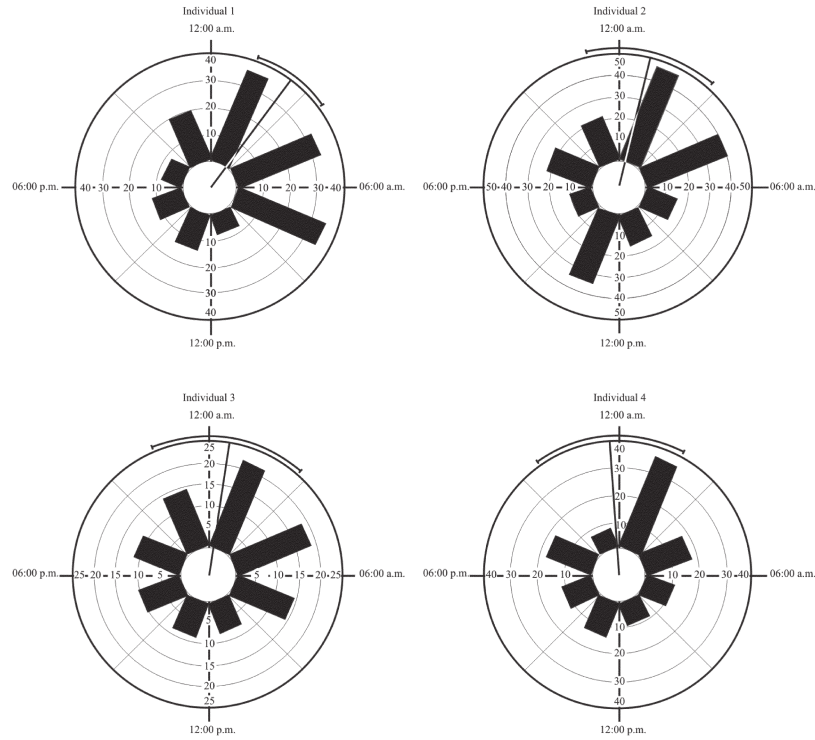


Figure 6. *Aegla manuinflata*. Circadian activity of four individuals during seven days of observation (at each three hours). Bars represent the total sum of displacement (in meters) and vectors indicate the average and confidence intervals (95%). Animals monitored by radio-telemetry at a stream on Southern Brazil on February/2009.

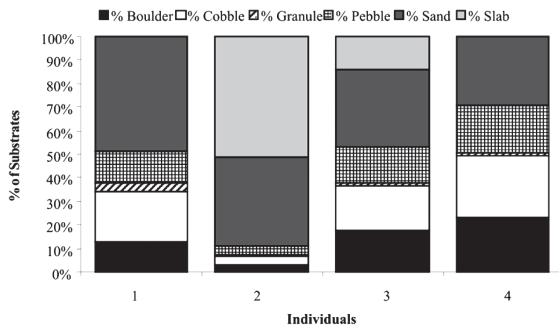


Figure 7. *Aegla manuinflata*. Occupation percentage of different types of substrate (sand, cobble, granule, slab, boulder and gravel) by four individuals (1, 2, 3 and 4) at natural environment. Animals monitored by radio-telemetry at a stream on Southern Brazil on February/2009.

days without any movement, or in almost total inactivity (Fig. 5), followed by re-establishment of locomotion.

Circadian activity

The four aeglids individuals exhibited higher displacement activity during the dark photophase. Although their activity was not restricted to the sunset period and night (Fig. 6), it was concentrated between 11:43 p.m. and 2:25 a.m. (Rayleigh Test) (Tab. 4, Fig. 6). There was a significant difference in the activity patterns between individuals 1 and 2

($\chi^2 = 24.632$; $p = 8.81E-4$) and 1 and 4 ($\chi^2 = 22.122$; $p = 0.002$).

Space occupation

The individual specificity regarding to the occupation of different kinds substrate was proved by Mantel test (SM1: $r = 0.20$; $p = 0.0002$), but a pattern in the substrate occupation as a function of the photoperiod (day/night) was not observed (SM2: $r = -0.005$; $p = 0.06$).

Among the four analyzed individuals, n° 2 exhibited a differentiated mode of occupation, using mainly slate as a substrate, whereas the other three used more often the sand substrate. It should also be noted that n°4 used boulder substrates more often than the other individuals (Fig. 7).

Discussion

The present study is the first research employing the radio-telemetry technique that proved to be an efficient method for improving the biological knowledge and behavior of aeglids. These animals usually spend most

of time under rocks and other substrates in rivers and streams making almost impossible to observe and follow these individuals. The results obtained in this study supply information about displacement circadian activity and substrate occupation patterns of *A. manuinflata* in their natural habitat. In spite of the use of only few individuals, radio-telemetry data were sufficient to reveal variability among them, as well as for the different activities of the same individual in the space and in the time, confirming the success of this technique in previous studies with other crustaceans (Clark *et al.*, 1999; Gherardi *et al.*, 2000; Lynch and Rochette, 2007).

Aegla manuinflata exhibited significant displacement rates. The knowledge of displacement and migration patterns of Aeglidae species is scanty. Lopez (1965) using tagging and recapturing methods between the months of July and December, reported that *A. paulensis* Schmitt, 1942 displaced approximately 300 m along a stream. This value is about three times of the average displacement of the present study (117.87 m), but *A. manuinflata* could displace much more if the observation lasted six months as conducted by Lopez (1965). On the other hand, after one year of observations using the same technique and 32 records, Gherardi *et al.* (2000), verified that *Procambarus clarkii* crayfish showed an average movement of 44 m in Italy, which could be considered a minor displacement compared to the average displacement exhibited by *A. manuinflata* during the eight days of monitoring we conducted in southern Brazil. Barbaresi *et al.* (1997) recorded a maximum displacement of 22.5 m per day, when studying the fresh water crab *Potamon fluviatile* (Herbst, 1785) using radio-telemetry techniques. Finally, Robinson *et al.* (2000) calculated an average daily movement pattern of less than 5 meters when studying the crayfish *Austropotamobius pallipes*; in addition, the authors also reported movements of more than 300 m in 10 days. These previous results related to daily movement are inferior to those obtained for *A. manuinflata* in the present study, which

may be related to natural conditions such as the high mobility of aeglids or even to a higher motivation to move in search of different resources. The displacement degree was highly variable among individuals, both for daily displacement and for total (considering all days and periods of observation) displacement. The dispersion rate in the habitat could be related to a combination of different factors, including the occupation of shelters, which serve as an ephemeral home range (Gherardi *et al.*, 1998, Robinson *et al.*, 2000; Aquiloni *et al.*, 2005), where some individuals remaining there for some time, whereas others choose to disperse.

Streams are dynamic systems and the distribution pattern of invertebrates that inhabit this environment may vary as a response to changes in stream flow velocity and temperature and biotic interactions. Small-scale movements in response to changes in flow velocity have been demonstrated for a number of macroinvertebrates that inhabit lotic environments (Lancaster, 1999). For many invertebrates living in streams, drift is an important means of transportation (Allan and Feifarek, 1989; Lancaster *et al.*, 1996), but large vagile invertebrates are able to perform active movements, including traveling substantial distances against the flow (Robinson *et al.*, 2000).

We found that displacement of *A. manuinflata* took place both downstream and upstream, being the latter were considerably greater. In other crustaceans that live in similar environments to aeglids', such as crayfishes, it has been shown that understanding the spatial and temporal patterns of their movements and activities is relevant to predicting their expansion and colonization capacity. Numerous studies with crayfish species have shown that radio-telemetry is a very effective technique to analyze the movement patterns of adults in river systems (Schütze *et al.*, 1999; Bohl, 1999; Gherardi and Barbaresi, 2000; Robinson *et al.*, 2000).

In the present study, specimens moved actively upstream, i.e., they were able to move against the stream flow. Upstream movement

in rivers and sometimes on land has previously been reported for some crayfish species, such as *Orconectes nais* (Faxon, 1885) (Momot, 1966), *P. leniusculus* (Light, 2003) and *P. clarkii* (Kerby *et al.*, 2005), which usually moved downstream. According to these authors, both stream flow velocity and height of obstacles can decrease or prevent the movement of crayfishes upstream. The aeglids are clearly able to move upstream which would allow them to invade or reoccupy areas upstream from which, in turn, they eventually could have been removed due to flooding (Light, 2003). Apparently, these animals prefer to move upstream where they find more oxygenated water, an important feature for aeglids.

Previous studies indicated that obstacles (natural and artificial barriers) such as waterfalls limit the movement of aquatic species in rivers negatively affecting their dispersion mechanisms (Utzinger *et al.*, 1998; Benstead *et al.*, 1999; Luttrell *et al.*, 1999; Joy and Death, 2001; Kerby *et al.*, 2005). However, *A. manuinflata* proved to be able to overcome barriers (waterfalls) of almost 1 m high, as previously reported for *A. paulensis* (Lopez, 1965). This information reinforces the concept of the capability of a habitat recolonization by aeglids, which have already been suggested by the fact that these animals tend to move upstream.

By means of radio monitoring one could verify that some individuals may become totally inactive and occupy the same area for a long period that can be few hours or more than a day, until they start moving actively again. These changes in movement activity did not follow a periodic pattern, and showed some variation for the same individual. A similar pattern has been reported for various crustacean species: *A. pallipes* (Gherardi *et al.*, 1998), *C. sapidus* (Carr *et al.*, 2004), *Cancer pagurus* Linnaeus, 1758 (Skajaa *et al.*, 1998), *P. leniusculus*, (Bubb *et al.*, 2004), *P. fluviatile* (Barbaresi *et al.*, 1997), *P. clarkii* (Gherardi and Barbaresi, 2000; Gherardi *et al.*, 2000; Aquiloni *et al.*, 2005; Barbaresi *et al.*, 2004; Barbaresi and Gherardi, 2006).

According to Skajaa *et al.* (1998) after

being fed to satiation or in periods of low motivation to eat, *C. pagurus* remains inactive, probably to minimize the energetic cost of moving. This could be an explanation for the fluctuation between activity and inactivity periods in *A. manuinflata*. In addition, the preference to a specific area might indicate that such site serves as a “shelter”, being associated with food supply and prevention of predation. Therefore, aeglids are only expected to move when there is an additional cost (competition, predation, scarce food) to remaining in the same area. In the present study, individuals that remained inactive for a long period of time were those who occupied areas with submerged vegetation. It is possible that these areas provided them an ideal supply of food and shelter, without any necessity in expending energy for displacement.

The movements of specimens of *A. manuinflata* were guided by nyctohemeral rhythms, and these animals were observed to have nocturnal habits, although they did not move exclusively during the night. In laboratory studies, Sokolowicz *et al.* (2007) reported nocturnal habits for *A. longirostri* Bond-Buckup and Buckup, 1994, confirming this tendency to be more active in periods of low luminosity, both in their natural environment and in the laboratory.

Nocturnal habits have been broadly reported for decapod crustaceans (Atkinson and Naylor, 1976; Barbaresi *et al.*, 1997; Gherardi *et al.*, 2000; Barbaresi and Gherardi, 2001; Carr *et al.*, 2004; Aquiloni *et al.*, 2005; Jury *et al.*, 2005; Golet *et al.*, 2006; Sokolowicz *et al.*, 2007). More activities during the night might be an adaptive response because it may allow these animals to escape from temperature extremes and to avoid possible predators that are visually orientated and therefore most active during the day (Barbaresi *et al.*, 1997; Skajaa *et al.*, 1998; Sokolowicz *et al.*, 2007).

According to Atkinson and Naylor (1976), changes in light intensity during sunset and sunrise could have direct effects on activity of Norway lobster, *Nephops norvegicus* (Linnaeus, 1758). Moreover, light might also have an indirect effect in controlling the

availability of food and predators. Concerning aeglids, the possibility of avoiding predators is probably one of the main reasons for their nocturnal habits, since they feed mostly on allochthonous plant matter (Bueno and Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004; Santos *et al.*, 2008).

Aeglids exhibited specificity in the occupied substrate. However, this specificity did not occur due to circadian rhythms, such variation is a function of individuals (n° 1, 2, 3 and 4) independent of periods. Benvenuto *et al.* (2008) observed that the crayfish *A. pallipes* of different size classes occurred mainly in substrate of mud, gravel and pebbles, but adults preferred boulders. In addition, the occupation of a certain substrate by crayfish is independent from its availability but is related to the size of the crayfish with larger animals associated with larger substrates (grossly divided) (Benvenuto *et al.* 2008). Adequate substrates may offer shelter against predators (Naura and Robinson, 1998; Ilhéu *et al.*, 2003, Barbaresi *et al.*, 2007) and prevent drifting. Surprisingly, *A. manuinflata* occupied a finely divided substrate (sand). Actually, sandy substrates were associated with marginal and submerged vegetation, which could also serve as shelter and food source. This assumption is supported by the observation that most aeglids spent long time in sandy substrates, but further studies are desirable in order to find a pattern of occupation according to the substrate composition.

The result obtained in this study emphasizes the importance of preserving natural environments, aiming the conservation of aeglid species. According to Kerby *et al.* (2005), both velocity and high barriers reduce or even eliminate, the movement of the crayfish *P. clarkii* upstream the watershed. It is known that, Brazilian southern river systems are more fragmented by dams than those in other South American regions (Nilsson *et al.*, 2005). The construction of big barriers, such as hydroelectric power plants, surely, may block the displacement of aeglids upstream and consequently the genetical exchange between populations will be permanently prevented.

In this study, the new technique allowed to answer three questions that had previously been raised: firstly, *A. manuinflata* was able to move actively along the stream, both downstream and upstream, including overcoming of obstacles; secondly, the studied aeglids are active mainly during the night, supporting previous observations made in laboratory, similar to many know decapod crustaceans; thirdly, in the studied area, considering the microhabitat diversity supplied by the sedimentary scales and the presence of vegetation over banks, the selectivity of the substrate was not generally associated with the sediment texture, but it was a clear individual response of each studied organism. The observations and results showed in the present study were obtained from aeglids monitored in their natural habitat resulting in a high degree of reliability of the biological and behavioral data collected from these crustaceans.

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