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Combining Radio and PIT-Telemetry to Study the Large and Fine-Scale Movements of Stocked and Wild Brown Trout (*Salmo trutta* L.) in a Northeastern Stream, Portugal

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1. Introduction

Stream-resident salmonid movements have been the subject of numerous studies and their behaviour is relatively well-known (Harcup et al., 1984; Heggenes, 1988). For example, brown trout (*Salmo trutta*) is described as a sedentary species based on the behaviour displayed, often associated to the strong site attachment to a territory or home range (Bridcut & Giller, 1993; Armstrong & Herbert, 1997). Other salmonids like brook (*Salvelinus fontinalis*) (Roghair & Dolloff, 2005) and cutthroat trout (*Oncorhynchus clarki*) (Hegennes et al., 1991) showed similar behaviour. However, there are studies reporting a wide range of movements for brown (Meyers et al., 1992; Young, 1994), cutthroat (Hilderbrand & Kershner, 2000) and brook (Gowan & Fausch, 1996) trout populations. Trout behaviour can be modified by natural (*e.g.* fish density, food availability) and especially by man induced factors (*e.g.* environmental degradation, harvest and stocking) responsible for major threats of wild populations (Laikre et al., 2000). Indeed, stocking of hatchery-reared brown trout is a management tool commonly used to improve the recreational fishing (Cowx, 1999). This activity is responsible for a sudden artificial increase of fish density in a particular area. Negative impacts on wild populations, such as genetic contamination, competition, predator attraction and disease transmission were often referred (White et al., 1995; Einum & Fleming, 2001; Weber & Fausch, 2003) and are potentially amplified with the dispersal failure, since many hatchery-reared trout tend to remain near of the stocking site (Cresswell, 1981; Aarestrup et al., 2005). There are also contradictory results, as reported by Bettinger & Bettoli (2002) where stocked trout dispersal reached over 12 km in the downstream direction, just 24 hours after their release. Cortes et al. (1996) found for Portuguese salmonid streams that, during three successive years (2000 to 2003), less than 20% of stocked brown trout remained in the stream segment, one month after the release. However, in this study a mark-recapture method was used that did not allow to assess the main causes of the fish depletion and was not appropriate for the observation of fish behaviour. In fact, a wide variety of techniques, grouped as capture dependent (*e.g.* mark-recapture, telemetry) and independent (*e.g.* visual observation) methods, were used for the investigation of the spatiotemporal behaviour of freshwater fish (Lucas & Baras, 2000), although the comparisons and the validity of some results have been questioned (Gowan & Fausch, 1996). Recent technology and the development of a set of techniques (e.g. passive integrated- PIT, acoustic, radio and electromyogram- EMG transmitters), broadly referred as biotelemetry, enabled new information for researchers in basic and applied ecology, namely related with a better understanding of the physiology, behaviour and energetic status of free-living animals (Cooke et al., 2004). Radiotelemetry has been widely used, providing a high-resolution, in temporal and spatial scale, of information at individual level. Despite of the high costs of individual radio-tags and the detection equipment that restrict the number of tagged fishes, different studies were made to evaluate the home range of target species, like diel (Belanger & Rodriguez, 2001) and seasonal movements (Burrell et al., 2000), the influence of environmental factors (Ovidio et al., 1998) and the efficacy of fishways (Scruton et al., 2002). On the other hand, passive integrated transponder (PIT) technology has been developed for monitoring the individual movements of free-ranging fish for tracking (Prentice et al., 1990a; Armstrong et al., 1996; Greenberg & Giller, 2000), even small aquatic animals in shallow waters, involving low equipment costs and the possibility of addressing numerous questions in fields of animal behaviour, habitat use and population dynamics not covered by radiotelemetry (Roussel et al., 2000, Quintella et al., 2005). The indefinite life span and high tag retention with no apparent effects on growth and survival of tagged animals are other advantages mentioned to the PIT telemetry (Ombredane et al., 1998; Bubb et al., 2002). Several improvements occurred in the PIT technology throughout the last decades. Initially, stationary systems were used to evaluate the migration and survival of fish passing through fishway orifices (Prentice et al., 1990b; Castro-Santos et al., 1996) or streamwide antennae (Barbin-Zydlewski et al., 2001). In recent years, different types of portable equipments, like the flat-bed antenna design (Armstrong et al., 1996), the multipoint decoders connected to several flat-bed antennae (Riley et al., 2003) and the portable antenna (Roussel et al., 2000; Coucherousset et al., 2010), were developed and adapted to assess the behaviour of local populations in shallow streams. However, there is a lack of studies combining both radio and PIT telemetry technologies to study the behaviour of trout populations and this possibility is important to enhance the data quality.

The objective of the present study was to evaluate the spatial and temporal behaviour of wild and hatchery-reared brown trout populations in a stream of northeastern Portugal after stocking. Radio and PIT telemetry technologies were combined in order to study the movements of these sympatric populations. Radiotelemetry was used for large-scale continuous monitoring of individual fish and detailed information on movements was obtained at two distinct temporal scales: day-by-day and hourly diel cycles. Complementarily, PIT telemetry allowed a fine-scale approach considering the microhabitat use and activity pattern of each tagged fish in a confined area. This information was relevant to analyse the efficiency of stocking, the evolution of stocked fish condition and the potential impacts on the wild populations in order to define the most appropriate management measures for the Portuguese salmonid streams.

2. Material and methods

The study was carried out in summer and autumn of 2002 and 2005 in a salmonid stream, the Baceiro River, tributary of the Douro River, located in the Montesinho Natural Park, northeastern Portugal (Figure 1).

2.1 Study area

The Baceiro River is a third-order stream, approximately 25 km long, mean annual discharge of 1.93 m^3.s^{-1} and mean gradient of 4%, subjected to a reduced human pressure and a land use cover dominated by oak (*Quercus pyrenaica* Willd.) forests and also some meadows and planted chestnut (*Castanea sativa* Mill.) and *Pinus* spp., which contributes to the low impact on water composition (conductivity < 70 µS.cm^{-1}, dissolved oxygen > 9 mg.l^{-1}, alkalinity < 25 mg HCO_3^-.l^{-1}, hardness < 15 mg $CaCO_3$.l^{-1}, $NO_3^- < 0.5$ mg.l^{-1}, $PO_4^{3-} < 0.1$ mg.l^{-1}). This stream is characterized by a constrained channel, gravel-pebble over sand streambed and riparian vegetation is well developed and dominated by alder (*Alnus glutinosa* (L.) Gaertn.), although willow (*Salix salvifolia* Brot. and *S. atrocinerea* Brot.), poplar (*Populus nigra L.*) and ash (*Fraxinus angustifolia* Vahl) trees are also present. The stream width ranged between 5 m in the riffle to 12 m in the pool habitats, with maximum depth of 3 m. During summer (late) and autumn (early), the water temperature ranged from 5.0 to 19.0 ºC and discharge from 0.05 to 2.1 m^3.s^{-1} (the last after a storm event). It is important to mention that, during 2005, an extremely dry period was observed in the region and the stream became intermittent during a part of the summer. In the stream segment, the fish community consisted almost exclusively of wild brown trout populations and few numbers of nase (*Pseudochondrostoma duriense* Coelho) and Iberian chub (*Squalius carolitertii* Doadrio). Otter (*Lutra lutra* L.), water snakes (*Natrix maura* L. and *Natrix natrix* L.) and heron (*Ardea cinerea* L.) were the natural predators found in this stream.

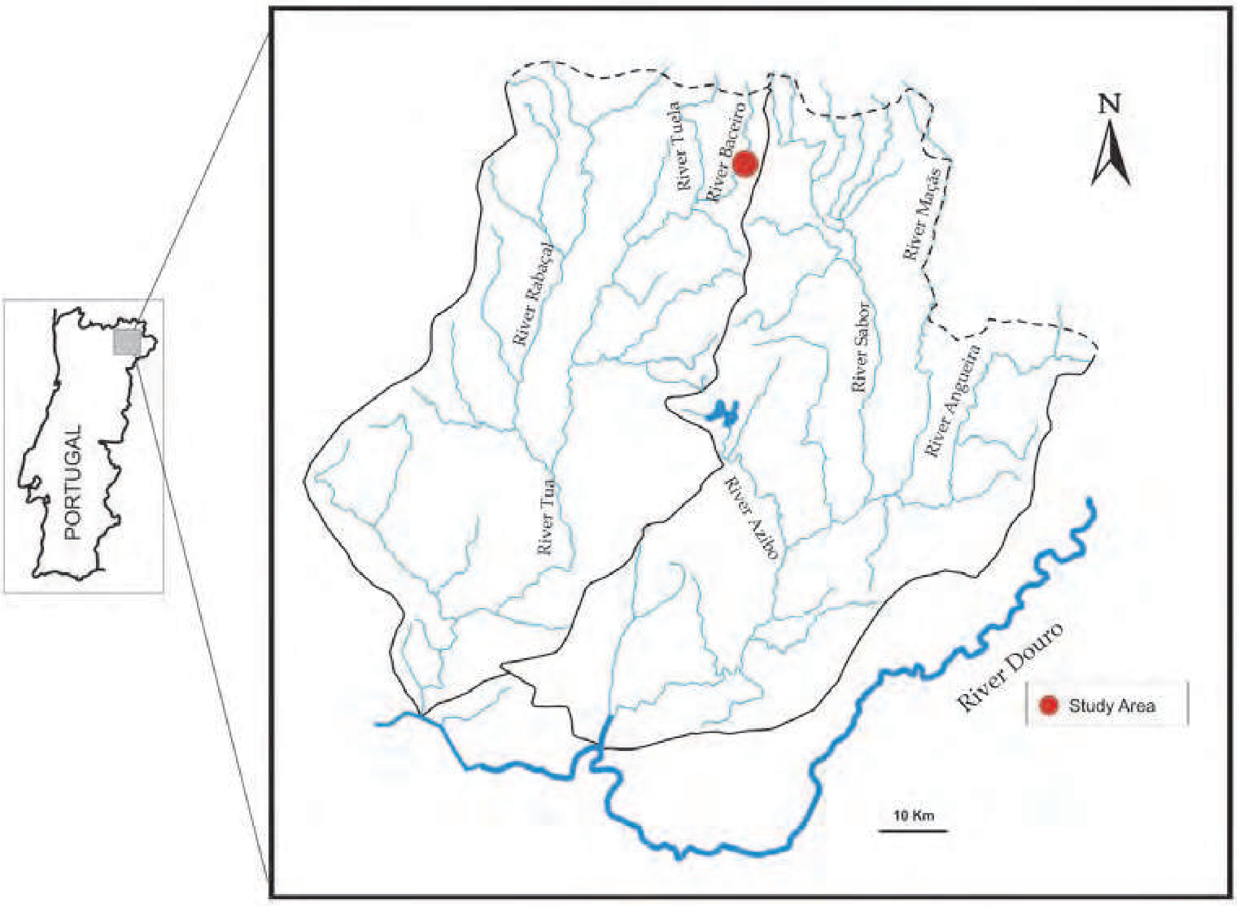

Fig. 1. Map of study area in the Baceiro River, a salmonid stream located in the Douro basin.

2.2 Field survey: Radio-tagging and tracking procedures

Fish activity and movements were monitored using a sequential scanning receiver- Lotek Eng. Inc. SRX_400 and a hand-held directional Yagi antenna (flexible elements) (Figure 2). Two different microprocessor coded radio transmitters (Lotek Engineering Ltd.) were used for the experiments carried out during 2002 and 2005. In the first and exploratory experiment, from 15 to 28 October 2002, one non-resident (from Sabor stream belonging to the contiguous watershed) native (330 mm total length, L_T) and one stocked brown trout (270 mm L_T) were tagged using a MCFT-3KM model (18 mm long x 7.3 mm diameter, 1.4 g in water) with 14 warranty life days and 5.00 sec. of signal burst rate. The transmitters operated with two codes (10 and 11) at the same frequency (149.420 MHz) and were attached (adding 0.5g, in air), alongside the base of the dorsal fin (Figure 3). The fish were previously anesthetized with 2-phenoxy-ethanol solution (0.25 $mg.l^{-1}$) and the radio-tags externally attached with nylon cords, which passed through the body muscles (inside of a hypodermic needle) to plastic plates cushioned with foam on the two sides of the fish to minimise scale damage.

Fig. 2. Radiotelemetry monitoring session in the Baceiro river (summer 2005).

This previous study allowed to set the methodology for the 2nd experiment, which was conducted from 16 September to 18 November 2005 and a MCFT-3D model used with the following characteristics: 61 warranty life days, 5.00 sec. of signal burst rate, 29 mm long x 10.3 mm diameter and weight of 2.1g in water. They operated with six different codes (001 to 006) at the same frequency (149.460 MHz) and were externally attached on six hatched-reared brown trout (size range 255-277 mm in total length, L_T, mean 265 ± 0.745 S.D. mm). Stocked trout were tagged according to the methodology defined, and maintained during one day in the hatchery to recover from the surgical procedures (Figure 3). After this period, fish were conditioned and transported in aerated tanks and, subsequently, released in the stream.

Fig. 3. Trout radio-tagging procedures and recover period of stocked trout in fishfarms, located near of Baceiro River.

The habitat unit selected for the release of stocked trout was 210 m long by 9.0 m mean width by 2.5 m of maximum depth, comprising all representative microhabitats of stream segment. Temperature (thermometer, accuracy of 0.1 ºC) and water column velocity (Valeport flowmeter, accuracy of 0.01 $m.s^{-1}$) were daily measured (Figure 4) and stream discharge determined near the stocking site. Velocity at 0.6 of total depth was considered as the mean water column velocity when the depth was less than 0.75 m. At deeper points the readings were averaged at 0.2 and 0.8 of total depth.

Fig. 4. Measuring temperature (ºC) and water column velocity ($m.s^{-1}$) in the Baceiro River (summer 2005).

The fish were monitored and located at least once a day until the end of their transmitter's battery life during the whole study period (14 days in 2002 and 64 days in 2005). Net daily journeys were registered, which were defined as the distance between locations at two consecutive days. During 2005, the fish were also monitored hourly for a partial diel cycle (from 06.00 a.m. to 24.00 p.m.) for eight days (week periodicity). Such registrations took place on 23 and 30 September, on 7, 14, 21 and 28 October and on 4 and 12 November. All tracks were conducted along the stream banks and the potential disturbance of fish activity minimized. To measure the trout movements, yellow fluorescent marks were sprayed on the stream bank (alder branches or rocks were selected) at regular intervals of 25 meters. The identification of a fish position was registered after the detection of the maximum signal strength for at least 1 min. The positions of each fish were used to determine the dispersal (defined as the distance travelled by individual fish from the stocking site), the daily home range (D.H.R., the difference between the most upstream and most downstream positions), and the total distance moved (T.D.M., the sum of all displacements detected). Non-parametric Mann-Whitney *U*-tests were performed to detect statistical differences between native and stocked fish dispersal in 2002 and between stocked trout for dispersal, D.H.R. and T.D.M. throughout 2005. Spearman rank order correlations (r_S) were made to assess the significant relationship between the dispersal of stocked fish and two relevant environmental variables: water temperature and discharge. All statistical analyses were performed using STATISTICA 7.0 package (Statsoft, 2004).

2.3 Field survey: PIT-tagging and monitoring design

The Passive Integrated Transponder (PIT) technology is composed of PIT tags, which are internally implanted in the fish, and one or several antennae connected to a transceiver. The PIT tag is detected and their individual code recorded when a tagged fish passed within the read range of the antenna. The fish detection is recorded when the transceiver energizes the tag by sending an electric current through the antenna, which emits an electromagnetic signal captured by the circuit board of the PIT tag that sends their individual code back to the transceiver (Riley et al., 2003; Gibbons & Andrews, 2004). The PIT technology used was based on a multi-point decoder (MPD) unit (UKID Systems Ltd, Preston, U.K.). This unit consists of DC integrated MPD/antenna multiplexer (8-channel) powered by a 24 V (18 Ah) rechargeable lead-acid battery pack, which provided more than 24 hours of continuous use, and eight black circular panel antennae connected to the PIT–tag reader by cable lengths of 10 m. Each panel antenna (22 mm deep and 300 mm in diameter) operates at a frequency of 134 kHz. Two distinct PIT tags (UKID Systems) were used in this study: 1) 12.0 mm long x 2.1 mm in diameter (122IJ) (defined as Type I) and 2) 34 mm (L) x 4 mm (D) (Type II) (344GL), with detection ranges of approximately 90 mm and 300 mm, respectively. This system enables logging up to 1000 time-stamped events from an onboard Real Time Clock and the Battery Backed-up Memory. In order to reduce the number of repetitive events, resulting from a fish that remained over the same antenna, a data repeated filter precluded the repeat reading of the same tag code within each 25 seconds period. The identification data (ID) output was further downloaded from MPD (via RS232) to a personal computer. The battery pack and the MPD was safeguard by a special enclosure (Peli-Plastic case) (Figure 5). A Casper Handheld reader was used when fish were captured and a unique identification required.

A stream segment (30 m long by a width ranging from 3 to 10 m), with riffle and pool habitats, was selected in the Baceiro stream. Before PIT telemetry experiment, the aquatic habitat was assessed based on transects (starting point randomly chosen), made perpendicular to the stream, with intervals of 5 m throughout each stream segment. Point measurements were done at 0.5 m intervals across each transect for the variables of total depth, surface velocity (measured 10 cm below the surface), bottom velocity (10 cm above the streambed) and mean water column velocity (0.6 of total depth), substrate composition and cover. Substrate composition was classified according to a modified Wentworth scale, adopting the following categories: 1) organic detritus; 2) silt and sand (< 2 mm); 3) gravel (2-16 mm); 4) pebble (17- 64 mm); 5) cobble (65- 256 mm); 6) boulder (> 256 mm) and 7) bedrock. Cover types were divided into five categories: 1) objects > 15 cm (substrate emerging from the streambed); 2) overhanging vegetation; 3) roots, undercut banks and submerged woody debris; 4) surface turbulence and 5) no cover. Total depth was directly measured with a stick meter and the velocities were measured with a Valeport electronic flowmeter. The following characteristics were determined for the available habitat: mean total depth of 40 cm (maximum depth= 90 cm); maximum water column velocity detected near the riffle zone of 0.90 m.s^{-1}; substrate composition dominated by sand, cobbles and boulders; main cover for fish provided by undercut banks and boulders. Water temperature ranged from 12 to 19 ºC. Between 12 August and 30 September 2005, the entire stream reach section selected was closed with stop nets. Previously to the beginning of the experiments, the study area was depleted of fish through several electrofishing sweeps (Hans Grassl ELT60 DC, 1.5W, 300/600 volts) and biometric data of local trout population recorded. Twenty-five resident native trout, distributed into three size classes (Table 1), were marked with 12 mm PIT tags and the adipose fin clipped.

Fig. 5. PIT equipment (battery-pack and multi-point decoder- MPD) unit and PIT tagging procedures.

After a recovery period of two hours, the wild trout population was released into the blocked stream reach. At the same time, a sympatric condition was promoted in the confined area adding a total of fifty PIT tagged stocked trout using transponders Type I and II (Table 1). Before tagging, individual fish were anesthetized with a solution of 2-phenoxy-ethanol (0.25 ml.l^{-1}) and the abdominal region disinfected (Betadine®). A sterilised needle linked to a special tagging gun was used for surgical implantation of the Type I tag in the fish peritoneal cavity (Figure 5). The Type II tag was manually implanted through an incision of approximately 4 mm made in the midventral line, without suturing the incision.

The MPD unit, the antennae installation and the data acquisition were made following a similar design described in Riley et al. (2003) and Teixeira & Cortes (2007), using a random distribution of antennae, changing their position every two days (Figure 6). During the study period the dry weather conditions verified and the values of microhabitat measurements were assumed constant for every two days. Biometric data of both sympatric populations were obtained five weeks after stocking through an electrofishing survey and unique identification codes obtained for all tagged fish.

Trout Group	Fish Number	L_T (cm)	M (g)	K*	Tag ratio ** (%)
1) Stocked					
Type I PIT tag	25	22.3 ± 1.6	126.7 ± 28.2	1.12 ± 0.08	0.08 ± 0.02
Type II PIT tag	25	23.2 ± 1.3	146.2 ± 24.9	1.16 ± 0.07	0.85 ± 0.17
2) Native					
A) < 15.0	8	13.3 ± 1.2	23.5 ± 6.7	1.00 ± 0.07	0.10 ± 0.03
B) 15.0-20.0	8	17.0 ± 1.2	48.6 ± 9.8	0.98 ± 0.08	0.22 ± 0.04
C) > 20.0	9	22.0 ± 1.9	108.9 ± 30.3	0.97 ± 0.04	0.46 ± 0.15

* $K = 100(M.\ L_T^{-b})$, *where* M- trout mass (g); L_T- total lenght (cm); b- alometric coefficient
** 100.(tag mass).(trout mass)$^{-1}$

Table 1. Mean ± standard deviation (S.D.) of total length (L_T), mass (M), Fulton's condition factor (K) and tag ratio of the pit-tagged brown trout in the Baceiro stream, during summer 2005.

Fig. 6. PIT-tagged trout passing over an antenna, during field experiment in the Baceiro River (summer 2005).

The analyses of movement and activity patterns of stocked and native trout populations were based on the non-repeated data (the continuous repeated records of each fish in the same antenna were not considered) recorded by the MPD unit during the five weeks of the sampling period. Non-metric multidimensional scaling (NMDS) analysis, an ordination method based on a rank order of Bray-Curtis similarities, was applied to non-repeated frequency to detect the behaviour differences between native and stocked trout. The NMDS was computed using the log transformed [log (x+1)] data. A multivariate analysis of similarities- one-way ANOSIM test, as a nonparametric randomization approach, was then applied to the Bray-Curtis similarity matrix to test the statistical differences between the two considered groups. These analyses were performed through the package PRIMER 5 (Clarke & Gorley, 2001). The relationship between the microhabitat variables and stocked and wild trout were assessed through a canonical correspondence analysis (CCA), a method of direct gradient analysis, where the ordination of objects (stocked and native fish) is based on species data (fish positions) and on environmental information associated (Jongman et al., 1987). Two CCA's were performed for two distinct periods: 1) first week- the adaptation period of stocked fish to wild environment; 2) from the 2nd to the 5th week, considering the post-adaptation period. This analysis was performed using the CANOCO software package (ter Braak & Smilauer, 1998). Data were standardized for microhabitat variables and log transformed [log (x+1)] for the non-repeated frequency data of the detected fish in all antennae positions. Only those variables with a variation inflation factor (VIF) of less than 20 were included to avoid multicollinearity (ter Braak, 1986). In addition, a Monte Carlo permutation test (199 permutations) was performed to test the significance of the axes. The fish activity was analysed based on the antennae non-repeated frequency data for the tagged trout populations, considering the following classes for *1)* native trout: A< 15.0; B- 15.0 to 20.0; C> 20.0 cm using Type I PIT tags and *2)* stocked brown trout: Type I and Type II PIT tags. Polynomial regressions were fitted to the data. Trout activity pattern was analyzed over 24-hours cycles, but discriminated for dawn, day, dusk and night periods. The influence of distinct detection range of the two types of PIT tags (only for stocked fish) and the ontogenetic variation of native trout was assessed. The differences between the trout classes for the defined periods were assessed using non-parametric Mann-Whitney *U*-tests (data did not fit to the assumptions of normality- Bartlet test). These *U*-tests were also performed for the comparisons between size (total length, L_T), mass (M) and the Fulton's condition factor (K) of stocked and native trout. A significant level of $P < 0.05$ was accepted.

3. Results

3.1 Radio-telemetry analysis

A distinct movement pattern was detected comparing stocked and native brown trout (Mann-Withney *U*-test, $P < 0.001$), from the radiotelemetry survey carried out in autumn 2002 (Figure 7). An initial stationary behaviour of stocked trout (for five days remaining in the stocking site) was replaced by their migration in a downstream direction, and at the end of 14 days (transmitter battery life) the fish were located in a small pool, 1,500 m from the stocking site. The magnitude of the displacement was correlated with the increase of stream discharge (Spearman correlation $r_S > 0.85$, $P < 0.01$). Conversely, wild trout remained near the stocking site hiding under a fallen tree, in spite of the non-residency status. It was only detected a downstream movement of 200 m coinciding with a sudden rainstorm, which raised the water level by 1 m. Nevertheless, after this period, the wild trout followed the upstream migration and travelled to feeding zones (90 m from stocking site) near a riffle/run habitat.

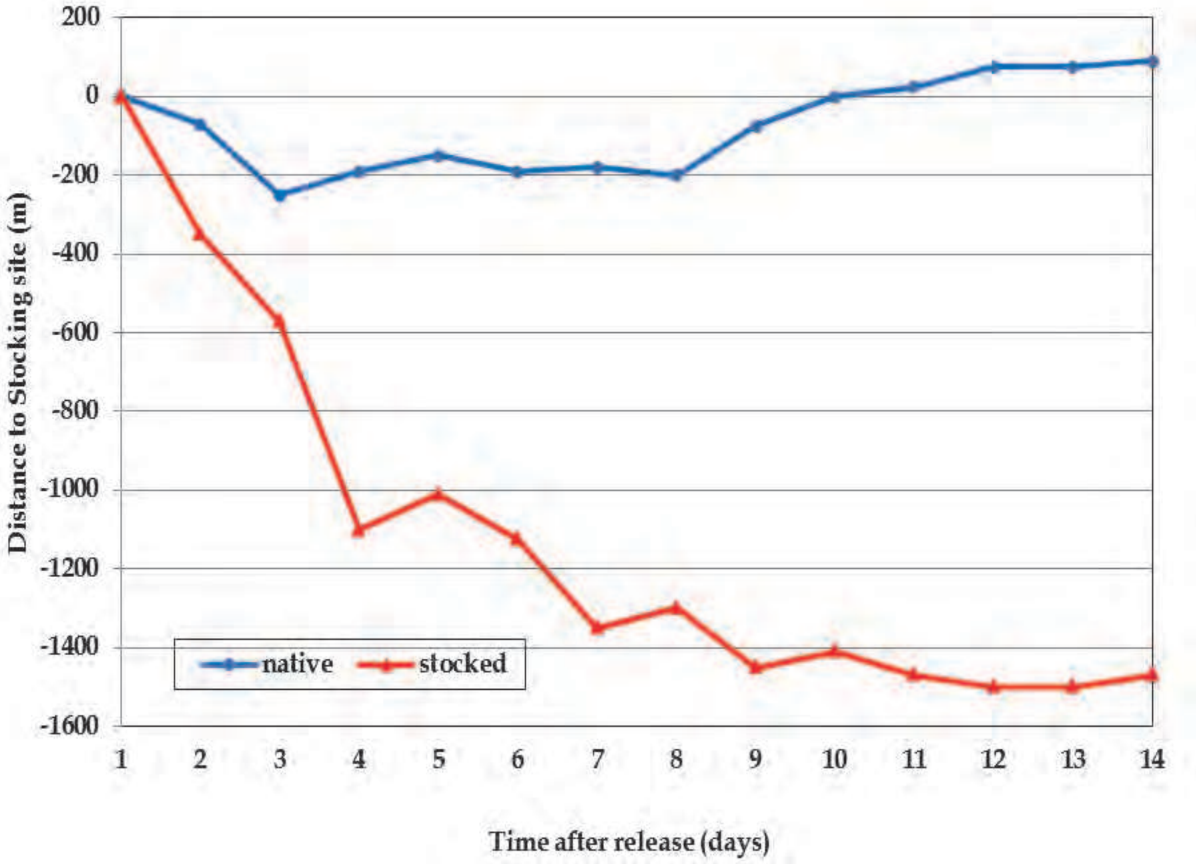

Fig. 7. Dispersal of one stocked and one native brown trout after being released in the Baceiro stream, on 15 October 2002. Symbols are daily positions of radio-tagged trout for 14 days (transmitter battery life).

Of the experiment conducted during summer/autumn of 2005 with six radio-tagged stocked trout, four individuals (T1, T3, T5 and T6) were tracked during the entire study period (64 days) and trout T2 and T4 signals were missed early, respectively on the 27th of October (after 42 days) and on the 31st of October (after 46 days) (Figure 8). Individual movements of stocked radio-tagged trout released in the Baceiro stream exhibited different patterns: a wide-range of displacements was recorded, and at the end of the registration period, the displacement of fish from the stocking site (dispersal) varied from 0 to 4,500 meters (Table 2).

Trout code	Total Length L_T (cm)	Mass (g)	Days tracked	Total Dispersal (m)
T1	26.8	223.5	64	0
T2	26.5	193.5	42	-350
T3	26.5	228.5	64	-4500
T4	25.5	178.4	46	-200
T5	27.7	209.6	64	-1025
T6	26.0	171.3	64	-1125

Table 2. Characteristics of stocked radio-tagged trout in the Baceiro stream (September to November 2005).

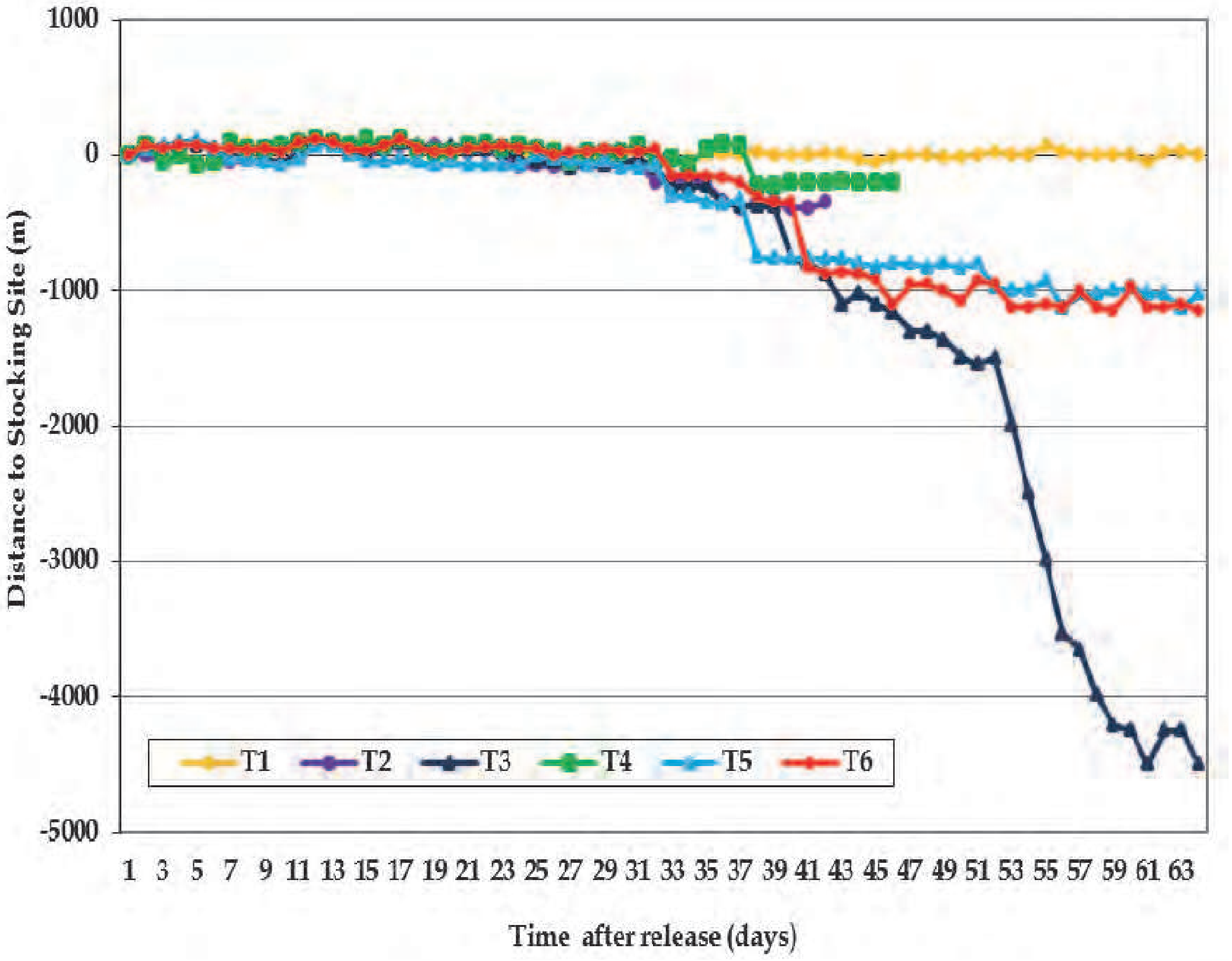

Fig. 8. Dispersal of six stocked brown trout (T1 to T6) after being released in the Baceiro stream, on 16 September 2005. Symbols are daily positions of radio-tagged trout for 64 days (transmitter battery life).

A common feature observed was the progressive downstream migration of the fish that displayed movement. The longest movement was reported for T3, which travelled over 4,500 m within 64 days, and for T5 and T6 located over 1,100 m from the stocking site, corresponding, respectively, to mean downstream progression velocities of 66.2 m.d^{-1} and 16.5 m.d^{-1}. This downstream movement was not detected continuously over every diel cycle and the interruptions in the migratory behaviour were more prolonged in the artificial pools. Indeed, during the study, 95% of fish locations were recorded in pool instead of run/riffle habitats. In opposition, T1 remained in the same pool habitat. Consequently, significant differences (*U*-tests, P< 0.001) were found for dispersal between fish showing stationary (T1) and a higher mobility behaviour (T3, T5, T6) and for the T.D.M. (*U*-test, P< 0.05) between T3 and the remaining fish, based on the sixty-eight diel tracks (trout were located once per day). The environmental conditions influenced the stocked trout behaviour. In fact, fish showed distinct movement patterns depending on two distinct periods identified during this study: 1) from 16 September to 9 October, characterized by dry and hot conditions (mean water temperature 12 °C and discharge < 0.05 m^3.s^{-1}) stocked fish exhibited restricted movements, confined to the stocking site; 2) from 10 October to 18

November, coinciding with successive precipitation events (discharge > 0.40 $m^3.s^{-1}$) and the lowering of water temperature (mean= 8.4 ºC), the stocked trout displayed an obvious dispersal. Most of stocked trout (83%) began the displacement, towards downstream almost immediately after the flow increase. These movements were significant and positively correlated with stream discharge (r_S> 0.54, P< 0.05), except for T1 (r_S= 0.22, P> 0.05) and T4 (r_S= 0.26, P> 0.05), and negatively correlated with water temperature (r_S> 0.54, P< 0.05). D.H.R. was calculated for tagged fish, based on the hourly monitoring movements (partial diel cycle from 06.00 a.m. to 24.00 p.m.), and ranged from 0 to 475 m (mean= 82 m). Significant differences were only detected for D.H.R. between T1 vs. T3 and T3 vs. T4 (*U*-tests, P< 0.01; Table 3).

Trout code	Daily Home Range (D.H.R., m)	Total Distance Moved (T.D.M., m)	Exploitation (T.D.M./D.H.R.)
T1	54 ± 20	95 ± 73	1.77 ± 0.96
T2	88 ± 35	194 ± 52	2.45 ± 1.01
T3	118 ± 64	255 ± 77	2.54 ± 1.14
T4	40 ± 21	87 ± 73	2.08 ± 1.20
T5	74 ± 26	272 ± 277	3.29 ± 1.04
T6	117 ± 148	266 ± 188	2.98 ± 1.16

Table 3. Variations of daily home range, mobility and exploitation (mean ± standard deviation, S.D.) of habitat by stocked trout in the Baceiro stream (based on eight partial diel cycles).

T.D.M. for the same period, showed values varying from 0 to 950 m and trout that displayed a superior migratory behaviour, like T3, T5 and T6, exhibited also greater daily movements and differed significantly from T1, T2 and T4, suggesting that fish showing higher mobility exploited more intensively their D.H.R. (*U*-tests, P< 0.01; Table 3). During the eight partial diel tracks, stocked fish was more active during day and twilight periods, and their mobility decrease at night (*U*-tests, day vs. night and twilight vs. night, P< 0.05). However, these results are limited to a short night period (21.00 to 24.00 hours).

3.2 PIT-telemetry analysis

A total of 44,934 fish records (identified tag codes) were successfully registered by the MPD unit for both populations, during five consecutive weeks after stocking release, with 80.3 % corresponding to stocked fish and 19.7% to native trout.

The NMDS ordination showed, in a two-dimensional space, the separation between native and stocked trout (Figure 9). The ANOSIM one-way analysis demonstrated significant differences between stocked and native trout (P< 0.001).

With regard to non-repeated data, a similar proportion was obtained for both populations (83.2% for stocked and 16.8% for native trout) considering the total of 14,369 registrations. However, stocked tagged trout density was double that of the native trout. During the study period, a small number of tag codes were not identified (0.02 %). Three stocked and two native trout (both from B class) were not identified by any antenna during the study period, suggesting that mortality (one native fish was captured dead) or/and tag expulsion can occur after the surgical implantation of the transmitters. Nevertheless, all tagged fish that survived and were captured by electrofishing, five weeks after stocking, showed the incisions to be healed up and only two stocked trout had signs of tag expulsion. At the same time, only 28% (Type I tags) and 32% (Type II) of stocked fish survived, reaching a minimum of 4% at the end of the study (7th week). On the other hand, 92% of native tagged fish remained alive in the study area. Furthermore, the variables *K* and *M* (only for Type II- PIT tagged fish) displayed a significant decrease (Mann-Whitney *U*-tests, $P < 0.05$) for the stocked fish and smaller size classes (A and B) of native trout. The CCA's ordinations (Figure 10 and 11) showed a similar relationship between the microhabitat variables and stocked and native trout for the two defined periods (eigenvalues of 0.472 and 0.177 for the 1st period and 0.228 and 0.124 for the 2nd one), and the first two axes explained, respectively, 63.8% and 65.0% of the variation relating to trout populations and the environmental variables. The Monte Carlo randomization test detected for both CCA's showed significant results for the sum of all eigenvalues (199 permutations, $P < 0.05$). For both CCA's a set of variables (*e.g.* total depth, dominant and subdominant substrate, aquatic cover, overhanging vegetation, distance to riffle and the distance to the nearest streambank) presented a similar importance that contributed to the distribution of stocked and native trout along the sympatric period and no substantial differences were detected between the initial adaptation period (1st week) and the remaining weeks.

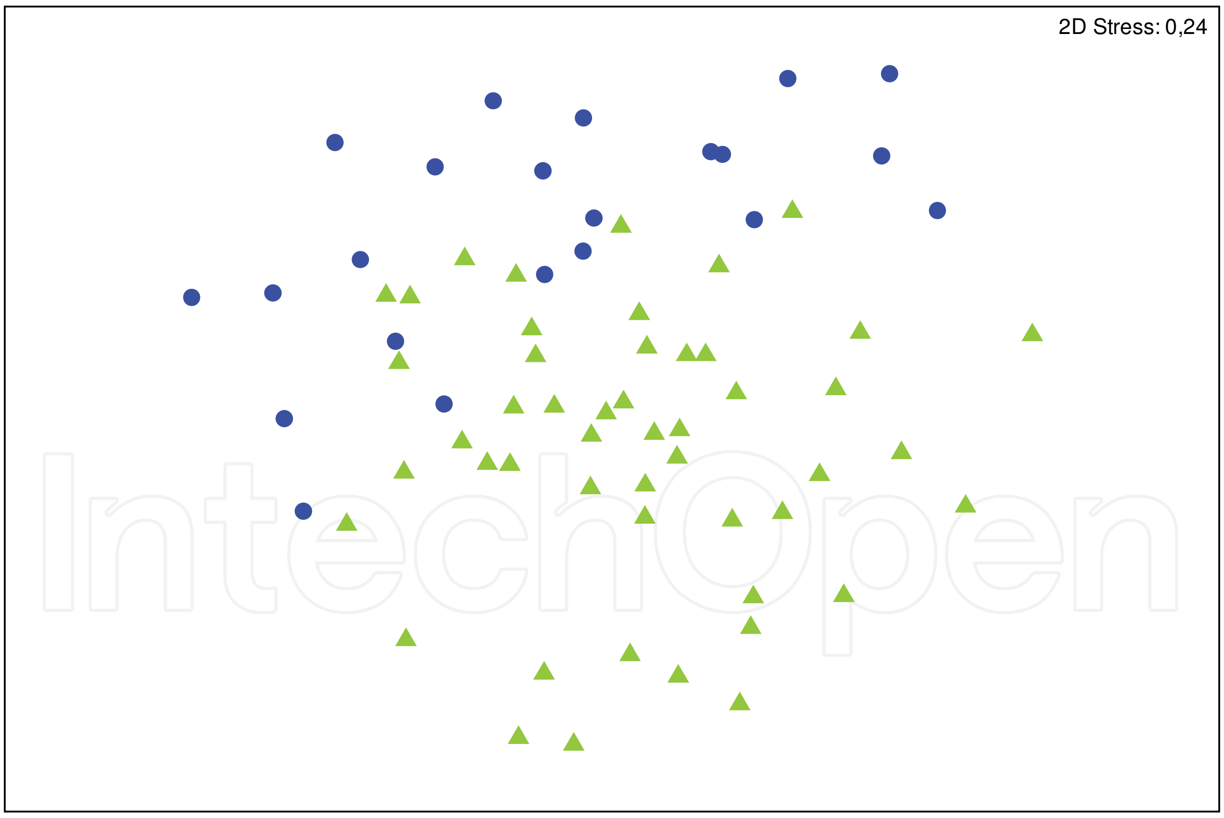

Fig. 9. NMDS ordination of stocked and native brown trout in the Baceiro stream (summer 2005). Ordination was based on a matrix of pair-wise Bray-Curtis similarities coefficients constructed from non-repeated records, log transformed (Log[x+1]). Symbols: ● = native trout; △ = stocked trout.

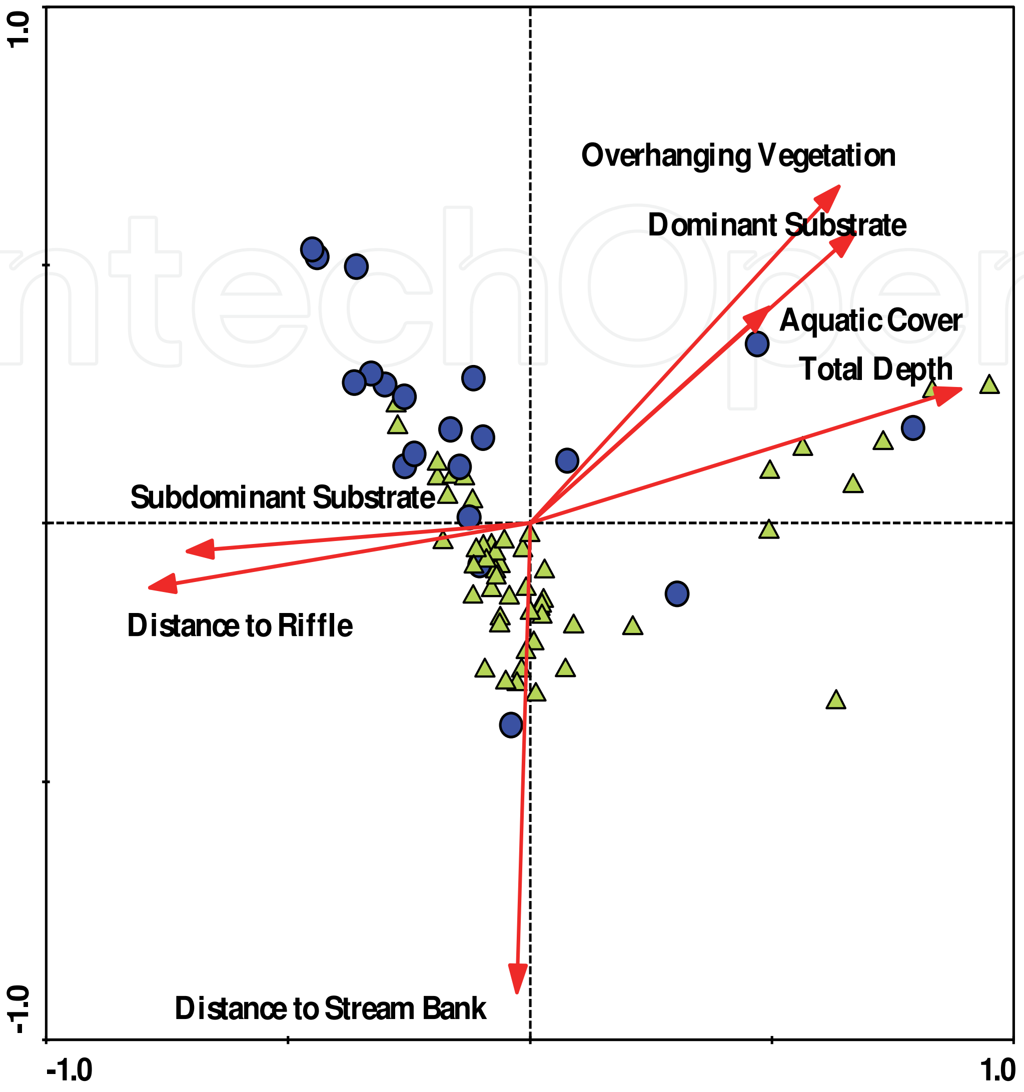

Fig. 10. CCA ordination diagrams of the 1st week for the Baceiro stream: distribution of native and stocked trout according to the selected microhabitat variables for the two first axes. The arrows represent the microhabitat variables and the symbols are the trout identification: *A)* arrows- total depth ; aquatic cover; overhanging vegetation; distance to riffle; distance to the stream bank; dominant and subdominant substrate; *B)* Symbols:● = native trout; ▲ = stocked trout. The length of the arrow is a measure of the importance of the environmental variable and the arrowhead points at the direction of increasing influence.

Comparatively, a greater proportion of the overall movements recorded for the dominant native trout occurred during the day period but no obvious activity pattern was detected among fish of same class. The diel activity pattern of trout varied substantially between both populations and with the type of PIT tags used. A significantly higher number of movements (62% of total non-repeated records) was detected by the MPD unit between Type II PIT tagged stocked trout and the remained groups for every diel period (dawn, day, dusk and night) defined (*U*-tests, $P < 0.05$). Despite of the distinct detection range of the PIT tags, the Type I- PIT tagged stocked trout movements were also significantly different from all native trout classes, except for adult native fish during the dawn period (*U*-tests, P< 0.05). The activity rhythm pattern of both stocked fish groups took place mainly at dawn and day and to a lesser extent at night periods. This activity pattern was roughly adopted by adult native trout, which differed significantly compared with the smaller size classes, precisely for dusk and night periods (*U*-tests, P< 0.05). Complementary analyses, based on the polynomial regressions (Figure 12), confirmed the distinct behaviour displayed by dominant trout (C Class > 20.0 cm), more active during the daylight period, related to the smaller native classes (A and B classes ≤ 20.0 cm), showing higher mobility during dusk and night periods. In fact, a temporal segregation was observed and probably dependent on the high density (three times more) promoted in the confined area as a result of the stocking experiment established.

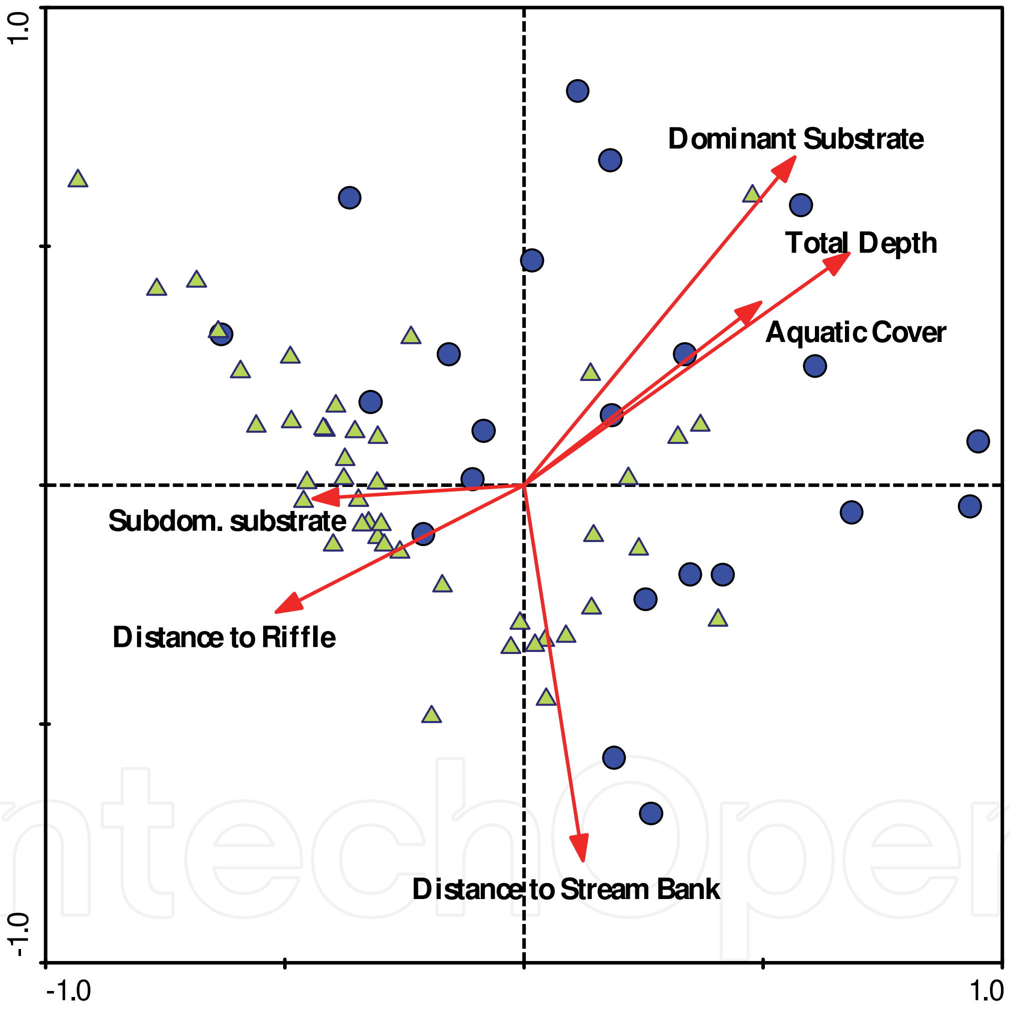

Fig. 11. CCA ordination diagrams of the 2nd to 5th week for the Baceiro stream: distribution of native and stocked trout according to the selected microhabitat variables for the two first axes. The arrows represent the microhabitat variables and the symbols are the trout identification: *A)* arrows- total depth ; aquatic cover; overhanging vegetation; distance to riffle; distance to the stream bank; dominant and subdominant substrate; *B)* Symbols: ● = native trout; △ = stocked trout. The length of the arrow is a measure of the importance of the environmental variable and the arrowhead points at the direction of increasing influence.

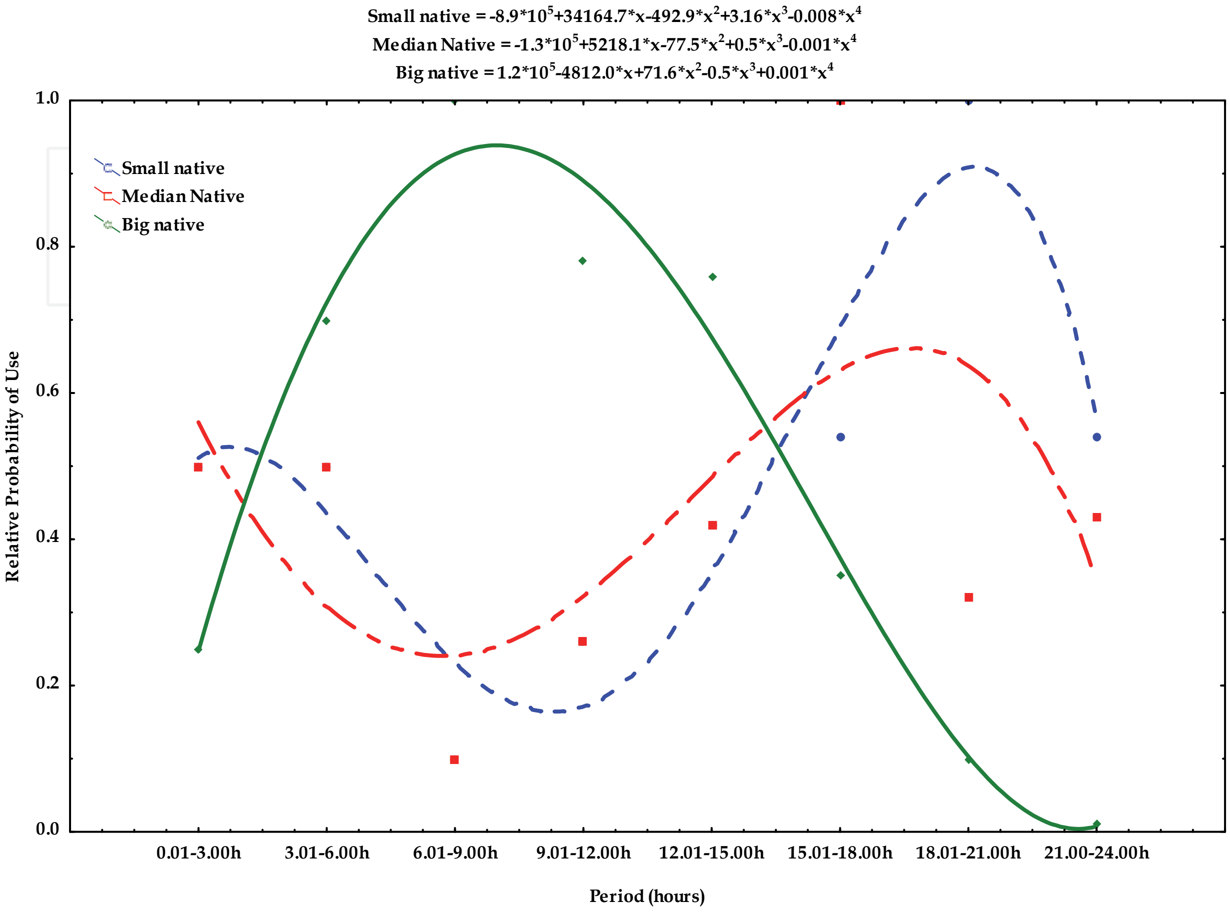

Fig. 12. Activity pattern based on polynomial regressions, performed for native trout, considering the different size classes: A) small < 15.0 cm; B) median 15.0-20.0 cm; C) big > 20.0 cm) using PIT Telemetry technology relative to eight diel periods and three hours classes in the Baceiro stream, during summer 2005. The dependent variable represents the relative probability of use (standardized to a 0-1 scale).

The comparisons between polynomial regressions calculated for the native size classes and stocked brown trout (Figures 12 and 13), showed similar behaviour only for the bigger individuals (dominant native and stocked trout) in spite of the increased probability of spatial competition and agonistic events. However, the morphological (fin deformities, hyperbuoyancy), physiological (stress response) and behavioural (lack of social hierarchy, weak territorial behaviour) characteristics presented by many stocked trout could explain their potentially disadvantageous performance in relation to dominant wild fish. The higher density referred for the PIT experiment established, did not affect the body condition of dominant native trout and contribute to explain the superior capacity to explore the available resources, namely in terms of feeding and resting activities. This pattern was not observed for smaller native trout and for stocked trout populations that showed a significant decrease in the body condition during the five weeks experiment.

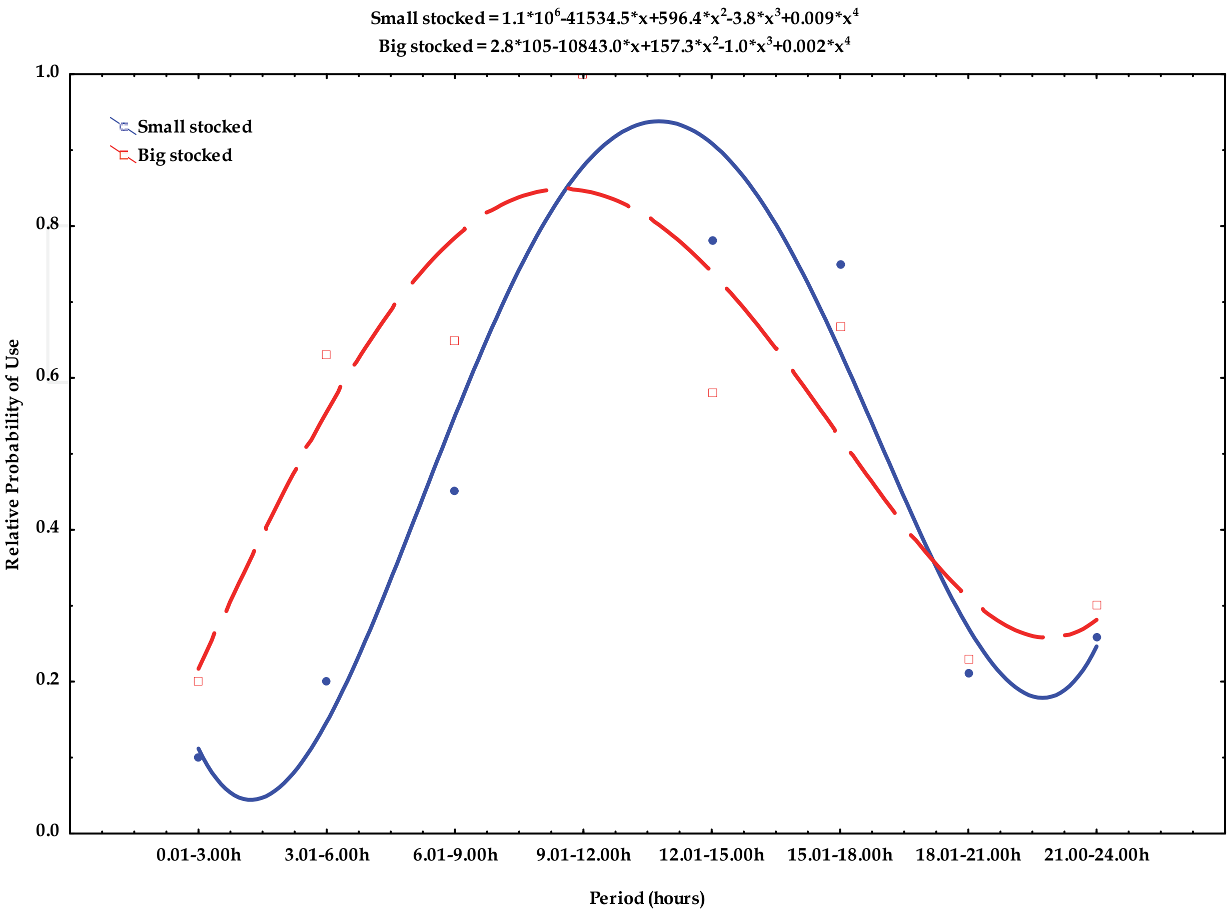

Fig. 13. Activity pattern based on polynomial regressions, performed for stocked trout: small- Type I PIT-tags; big- Type II PIT tags) using PIT Telemetry technology relative to eight diel periods and three hours classes in the Baceiro stream, during summer 2005. The dependent variable represents the relative probability of use (standardized to a 0-1 scale).

4. Discussion

Native brown trout showed a significantly less dispersal behaviour than stocked trout. However, caution should therefore be taken in the interpretation of this result since only two fish were considered in the exploratory experiment. Nevertheless, the limited movement exhibited by native fish was also identified in several studies (Knouft & Jutila, 2002; Maia, 2003), although, as referred by Bunnel et al. (1998), it may be function of the size of the habitat that provides adequate feeding and resting zones. These authors mentioned that brown trout movement varied among individuals of a same population, but most fish moved within a single continuous riffle/run-pool sequence in a diel cycle. In opposition to the resident trout movement, strictly related with the energetic cost/benefit ratio, Bachman (1984) found an erratic behaviour for the recently stocked trout. According to this study, confirmed by personal observations, hatchery-reared brown trout displayed a typical behaviour acquired in raceway tanks and moved constantly, leading to an excessive expenditure of energy in the swimming activity and agonistic encounters, which contributed to poor growth and survival rates in the wild environment. Stocked radio-tagged trout in the Baceiro stream showed a clear tendency to dispersal in downstream direction, although they exhibited distinct distance ranges. The dispersion of stocked brown trout is relatively well documented in the literature and, similarly to the present study, distinct individual movements were also found including stationary (maintaining the position near the stocking site) and mobile (ranging from upstream to downstream migrations) behaviour in the same stocked trout population (Cresswell, 1981). For example, Aarestrup et al. (2005) found that four brown trout left the study area whereas the remaining fish (*n*= 46) were stationary. However, in our study it was observed a decline in the water flow that conditioned the potential displacement of fish during the radio-telemetry study (32 days). Different factors can be associated with the downstream dispersal and often the harsh environmental conditions like higher levels of discharge originate the referred fish mobility (Ovidio et al., 2000), which was confirmed now. However, the river regulation (e.g. rapid fluctuations in flow resulted from hydroelectric peaking operations), water temperature regimes, different variables of physical habitat (e.g. channel slope, presence of coarse substrate particles, woody debris and roots functioning like potential cover refuges) and fish condition contribute to distinct migration behaviour of stocked fish in the natural environment. On the other hand, the influence of tagging implantation on fish movement was minimized, since external attachment of radio-transmitters and a low body/transmitter weight ratio was used, according to Brown et al. (1999). In the present study, the total distance moved (mean= 203 m) by stocked trout over the partial diel cycle considered (from 06.00 to 24.00 h) averaged about two times the length of home ranges (mean 82 m). However, total distance moved and home ranges are probably underestimated, since it was not possible to conduct registrations during a part of the night (from 00.00 to 06.00 h) and comparisons with other studies must be analysed taking into account this situation. The daily home ranges (minimum of 40 m for T4 and maximum of 118 m for T3) observed for stocked brown trout in the Baceiro stream are within the range of values observed for the same species by Young (1999) in a south-eastern Wyoming river (mean of 41 m), Ovidio et al.(2002) in a Belgian stream (8-480 m; mean 48 m), Knouft & Spotila (2002) in a Pennsylvania stream (20-2000 m) and Maia (2003) in a north-western Portuguese river (Vade River, 0-300m), excluding the migratory behaviour caused by spawning activity linked to reproduction. However, D.H.R. mentioned in the literature corresponded to resident trout and, because these fishes have a consistent fidelity to their home range or territory (Bunnel et al., 1998), it would be expected that non-resident fish, like stocked trout used in this study, had a superior mobility justifying greater dispersal, distance moved and home range, either in seasonal and diel scale analyses. Probably the dry climate during 2005 and the low discharge observed linked to high water temperatures recorded during the initial post-stocking period have restricted the movement of stocked fish. Stocked trout were shown to be more active during the day periods than during the night period in the Baceiro stream and can be related to the feeding habits acquired in the fishfarms. Brown trout is a visual feeder and the foraging efficiency decreases as light intensity declines (Fraser & Metcalfe, 1997; Klemetsen, 2003), but nocturnal (Clapp et al., 1990) and crepuscular (Bunnel et al., 1998) feeding patterns were also reported. However, the tendency of stocked trout in the Baceiro stream must be confirmed in complete (24 h) diel cycles.

Complementary analyses based on PIT-telemetry showed that only 30% of stocked fish survived five weeks after their release in the stream reach, while 92% of native trout were recaptured alive for the same period, considering the confined area of the experiment. Poor stocked brown trout survival rates were also reported in several studies (Pedersen et al., 2003; Aarestrup et al., 2005). The significant decrease in the condition of stocked brown trout suggested a lower ability to explore the available resources. In fact, the inefficient behaviours displayed by hatchery-reared fish contributed to a lesser adaptation to wild environment when compared with native trout. For example, higher aggression levels (Deverill et al., 1999), lack of social dominance structures (Jenkins, 1971), lower efficiency at feeding on wild prey (Olla et al., 1994), higher metabolic rates (Ersbak & Haase, 1983) and reduced swimming ability are reported for stocked salmonids. On the other hand, the hatchery-reared fish are more vulnerable to angling and natural predation ((Ludwig et al., 2002; Jacobsen, 2005) and higher mortality rates are associated near large stocked fish releases (Marnell, 1985). Observations from the stream bank revealed, during the experimental periods, a reduced fright response of stocked trout to human presence, confirming their potential weakness to avoid natural predation. In fact, most of the Portuguese northeastern salmonid streams supported growing populations of otter (*Lutra lutra* L.) and the higher number of spraints (4 PIT tags were detected) observed on stream banks suggested the higher mortality detected, mainly on stocked fish.

Stocked trout movements, for larger and even for smaller PIT tagged fish, were greater than for every native trout class defined. These fishes displayed an activity pattern more intense during day-light hours, namely during the dawn period. Normally, hatchery-reared trout are more active than wild fish (McLaren, 1979) and the higher mobility pattern showed during day-light hours in this study was, probably, related to the rearing environment (feeding habits in the hatchery) and the increasing ability of fish to detect food as light intensity increases (Fraser & Metcalfe, 1997). Furthermore, these results are according to the exploratory experiments made over two weeks in the previous year and confirmed the lack of a capacity to define a territory and a non-cost-effective behaviour, also detected by other authors (Bachman, 1984). In fact, the importance of habitat variables for both populations was similar during the 5 weeks suggesting that the stocked fish did not change their strategy in terms of habitat use. Hatchery-reared fish occupied habitats away from the stream banks, mainly, without aquatic cover (*e.g.* boulders, roots) and overhanging vegetation (shading). Other studies confirmed the distinct behaviour between wild and stocked salmonids based on the referred variables (Magoulick & Wilzback, 1997). Obviously, the different habitat use and the less concealment behaviour (Bachman, 1984) of stocked trout relative to wild fish increase their visibility and the vulnerability to avian and aquatic predators. A low influence of PIT tag surgical implantation in the fish peritoneal cavity was reported on different studies, with regard to survival, growth, swimming performance and general behaviour (Riley et al. 2003). During this study, trout were recaptured five weeks after their release in the stream, and healing was completed, without signs of inflammation or necrosis in the tissues, suggesting that the behaviour of both sympatric populations was not affected by the tagging procedures. Furthermore, only two stocked fish presented signs of tag expulsion, in spite of the incisions not having been closed with sutures for both 12 and 34 mm PIT tags used. However, low tag loss rates for PIT tags were also recorded for brown trout (4%, after seven months) and other salmonid species (0-2 %, 3 to 4 months) (Ombredane et al., 1998). Differences between the detection range of both PIT tags used (9 cm for 12 mm long vs. 30 cm for 34 mm PIT tags) produced a distinct amount of information (approximately three times more for 34 mm PIT tags) on fish behaviour confirmed by the number of repeated and non-repeated records, but higher mortality rates were found when the larger PIT tags are implanted in the smaller (< 15.0 cm) fish, in previous experiments conducted in the hatchery. However, caution should be taken in the interpretation of data, since a low proportion of area (eight panel antennae) was sampled for every diel cycle. This limitation was reported in several studies using PIT telemetry technology and further improvements are needed to increase the detection range of PIT reading units. With regard to the experimental design of this study, protocols combining a superior number of stationary flat-bed antennas and MPD units covering, at the same time period, the entire stream reach selected, and the use of portable antenna technology will improve the quality of data acquisition related to the small-scale movements by sympatric stocked and native trout populations.

5. Conclusion

The combination of the different methodologies used, *i.e.* radio and PIT-telemetry, allowed a better understanding of the movement patterns and spatial distribution of stocked and native trout through intensive tracking of a small number of radio-tagged fish over a short time scale and continuous monitoring of movements and microhabitat use by PIT-telemetry. As pointed out by Ovidio et al. (2009) gaps in the fish behaviour can be closed using complementary methodologies. This study also confirmed previous observations following distinct methodologies (*e.g.* snorkelling, electrofishing), which detected, just one month after, a low proportion of stocked brown trout in the stream segment where they had been released (Cortes et al., 1996; Teixeira et al., 2006). The potential negative impacts of stocking on wild population seemed to be limited in time and space and were demonstrated by the monitoring of fish movement (*e.g.* radiotelemetry), since a high dispersion was registered, mainly in downstream direction, of the majority of stocked fish. The rapid decrease of stocked fish condition, the variation of hydrological parameters and the vulnerability to predation were factors that contributed to the low efficiency of the stocking programs. For these reasons, stocking of brown trout as a management tool for supplementing the recreational fisheries in rivers must be questioned based on the reduced adaptation of stocked trout to wild environment. However, in specific conditions it could be a cost-effective option, namely if catchable-size trout were used and applied to selected areas where angling pressure is intense. It is possible that a greater proportion of stocked trout never adopt the adequate behaviour that normally is displayed by native trout. Probably the minor adaptation of hatchery-reared fish to the wild environment is more visible when stocking is made recurring to fish of superior size/age (> 1^+). Although Pedersen et al. (2003) had found a higher survival and adaptation of smaller brown trout (0^+) over a period of 11 months, a longer time is needed to fish reach the legal catch size for anglers and, as referred by Aarestrup et al. (2005), stocking with trout over the legal size limit could be the correct management tool for supplementing the recreational fisheries. Finally, it is important to consider alternative management techniques with low ecological risks like the improvement of the fish habitat and protective measures (*e.g.* catch-and-release, better management and angling regulations), to promote a superior biogenic capacity of the aquatic system and assure the conservation and/or exploitation of self-sustainability of wild trout populations.

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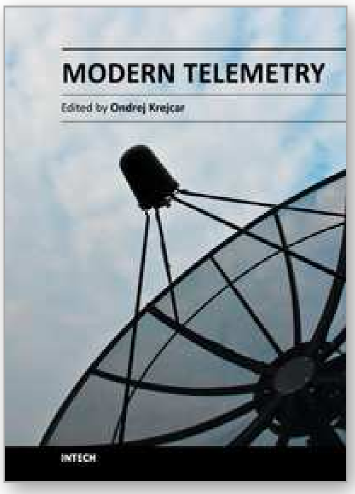

Modern Telemetry
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Telemetry is based on knowledge of various disciplines like Electronics, Measurement, Control and Communication along with their combination. This fact leads to a need of studying and understanding of these principles before the usage of Telemetry on selected problem solving. Spending time is however many times returned in form of obtained data or knowledge which telemetry system can provide. Usage of telemetry can be found in many areas from military through biomedical to real medical applications. Modern way to create a wireless sensors remotely connected to central system with artificial intelligence provide many new, sometimes unusual ways to get a knowledge about remote objects behaviour. This book is intended to present some new up to date accesses to telemetry problems solving by use of new sensors conceptions, new wireless transfer or communication techniques, data collection or processing techniques as well as several real use case scenarios describing model examples. Most of book chapters deals with many real cases of telemetry issues which can be used as a cookbooks for your own telemetry related problems.

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