Streamflow reduction induces early parental care in *Salaria fluviatilis* (Asso, 1801) males

Running head: Male of the freshwater blenny become parental at an early age

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Summary

This study investigated the effect of hydrological stress (streamflow reduction) on the reproductive and nesting behaviour of freshwater blenny (*Salaria fluviatilis*) males in a Mediterranean-type stream in NE Spain (a tributary of the Ebro River). The investigation included two study periods: (a) before stream flow reduction (sampling in 2004) and (b) afterwards (study year 2011). Nesting males in 2004 (N= 31) and 2011 (N= 11) were measured, weighed and photographed in the field. The size of the total egg cluster (male reproductive success) was measured and photographed for each nest found (N= 137 in 2004 and N= 28 in 2011). Nesting area was measured to determine nest density for each sampling period. The degree of secondary sexual traits (SSTs) development was measured later through the photographs. The age of males was assigned according to the length-intervals established by Vinyoles and Sostoa (2007) for this species in the same study area. After flow reduction in 2011, the flooded area of the river bed was reduced by more than 80%. A great proportion of small males (one year old) with developed SSTs (cephalic crest and anal glands) were found to defend a nest. This is the first time that parental care is found for the male of the freshwater blenny at a young age. Total cluster size (mean ± SE) is halved under the low flow conditions (from 45.4 ± 2.8 cm^2 to 22.9 ± 2.7 cm^2), but an increase in the number of partial clusters per nest was found. This study highlights the sexual plasticity of freshwater blenny males depending on environmental conditions and the vulnerability of this endangered species to the hydrological changes of anthropogenic origin in Spain.

Introduction

Over the past 40 years there has been a marked decrease of annual stream flows in many rivers of the Iberian Peninsula due to (a) an increase in climate variability, (b) changes in water management, (c) implementation of irrigated crops in rainfed areas, and (d) other water demands by human activities (Lorenzo-Lacruz et al., 2012). In the Ebro (NE Spain), for example, a significant decrease in river flows has been detected in over 90% of the water bodies (López-Moreno et al., 2011) at a 1% annual reduction rate (Lorenzo-Lacruz et al., 2012). In Spain, an exacerbated expansion of irrigated land (during the second half of the twentieth century) led to a current irrigation demand of 80% of the total human water consumption (Iglesias and Mínguez, 1997). Rivers in Mediterranean-climate regions, such as the Iberian ones, are characterized by a dynamic hydrology, resulting in droughts during summer and flash flooding during autumn and winter. Due to the hydrological changes described above, duration and intensity of droughts are increasing (Vicente-Serrano and Cuadrat-Prats, 2007). Furthermore, these areas are subjected to a greater water demand for human use (Lorenzo-Lacruz et al., 2010), and the excessive water extraction aggravates the effect of summer droughts (Moyle, 1995).

The most frequently demonstrated effects of drought on freshwater fish assemblages are declines in population abundance, changes in the age structure, altered movements within the watersheds and aggregation of fish in reduced microhabitats with suitable conditions (Matthews and Marsh-Matthews, 2003; Resh et al., 2012). Several studies describe how climate change may affect the current density and distribution of species of freshwater fish in the world (see, for example, Markovic et al., 2012). However, it is surprising that so little is known about how climate change and flow reductions (also increased due to human water extraction) may affect the biology, ecology and behaviour of particular fish species. Conservation of freshwater fish is at a critical point world-wide. In Europe, at least 41% of freshwater fish species are under threat (Freyhof and

Brooks, 2011). The situation is worse in the Iberian Peninsula, which contains a large number of endemic fish (70% of species; Doadrio 2001) and it is thus considered as a European area of high biodiversity. In water-stressed areas, knowledge about how freshwater fish respond to decreases in flow can be of great importance for their conservation. In this work we aimed to study the effect of a streamflow reduction on the reproduction of the freshwater blenny (classified in Spain as "Endangered" by Doadrio, 2001). Decline and current threats against this species in the Iberian Peninsula and elsewhere are habitat loss, water pollution, introduction of exotic fish predators, gravel extraction and an excessive water extraction (Doadrio, 2001).

The freshwater blenny (*Salaria fluviatilis)* exhibits parental care. During the breeding season from May to the end of July (Vinyoles and Sostoa, 2007), males excavate a nest under a stone, and several females lay clusters of eggs in a monolayer on the underside of the stone. After egg fertilization, only males provide care to the eggs. Among blennies, the presence of at least two alternative reproductive tactics is common: parental males (dominant, with developed secondary sexual traits; SSTs) and sneaker males (often with female appearance and with non-developed SSTs). In most blennies, male SSTs consist in the development of a cephalic crest and two anal glands covering the first spinous rays of the anal fin. Parental males show a typical male behaviour (defending a nest, courting females and caring for the eggs) while parasitic males (sneakers, without nest) attempt to steal fertilizations from the parental ones. In the freshwater blenny these two reproductive tactics (dominant and sneaker) were described by Neat et al. (2003). According to these authors, small (young) males are “obligatory sneakers”. However, the freshwater blenny mainly inhabits

Mediterranean-type streams where the reproductive populations can often be reduced to 1-year-old individuals (Vinyoles and Sostoa 2007). Changes in the fish age structure caused by flow reductions (such as summer droughts) are common in these environments (see, for example, Oliveira et al., 2002). According to Neat et al. (2003), these males should not adopt the parental tactic at such a young age. The aim of this study was to investigate whether young males of the freshwater blenny (which are the dominant age group in drought years; see Vinyoles and Sostoa 2007) were able to nest after a marked reduction in water flow in a Mediterranean-type stream (a tributary of the Ebro basin). If so, morphological traits (degree of development of the SSTs and body condition) and reproductive success of these males would be evaluated and compared to those of males that were nesting before the flow reduction. The possible implications of anthropogenic disturbances on the species' conservation will be discussed.

Materials and methods

Study area

The study was conducted at the River Matarranya, a 100 Km long tributary of the Ebro River (NE Spain). The freshwater blenny population is restricted to a 2 Km stretch close to the confluence with the main stem of the Ebro River (in Fayón). This work was done at a localized stretch of 100 m known to be a species nesting area since 1984. The first sampling (in 2004) was performed in this place. To make sure that males under low streamflow conditions (in the 2011 study period) did not move to adjacent areas in search of better spawning sites, we explored approximately 3 Km of river (upstream and downstream from the nesting site) before sampling. We found that the nesting site of the freshwater blenny was at the same specific location where it was found years before, despite the noticeable changes in the river conditions.

The study area has a typical Mediterranean lowland climate, where strong summer droughts and floods in spring and autumn are common. At the middle and lower reaches of the River Matarranya, the annual precipitation does not exceed 340 mm. Water extraction for irrigation activities has occurred in this river since 1986 (with the Spain's entry into the European Economic Community). Since then, fish abundance has been shown to be declining (Sostoa et al., 2004). In 2006 many peach-growing areas were implanted on the mid stretch of the Matarranya, in an area traditionally cultivated with dry farming crops. Water demand by farmers led to the building of irrigation pools with water pumped from the river. This took place especially during spring and summer months when most of the fish species spawns in the Matarranya. According to data provided by the Hydrographic Confederation of the Ebro Basin (available at: //www.chebro.es, last accessed on September 2015), from 2007 the river flow in the lower Matarranya (after implementation of the irrigated crop areas) was halved. During March to July 2011 the mean (± SD) river flow was one-sixth (0.6 ± 1.1 m^3/s) of what was during March to July 2004 (3.6 ± 3.4 m^3/s).

Data collection

Data were collected between the 16th and the18th June 2004 (before flow reduction) and the 16-18th June 2011 (after flow reduction). Nests of freshwater blennies were searched for by examining the underside of suitable stones (> 10 cm in diameter; Freeman et al., 1990) throughout the whole nesting area. A numbered flag was then placed near each nest stone to identify its location. The next day, we caugth as many males in their nests as we could. For this, a bucket measuring 30 cm in diameter, open at both ends, was placed over the nest stones (with care to prevent the escape of the guarding male) and the fish were caught through a small hand-net. Caugth males were anesthetized with MS-222 to avoid stress, and then their total body length was measured (TL, mm), they were weighed (W, g) and photographed. After recovery under oxygen supply, each male was released into its nest. The degree of development of male SSTs (cephalic crest height and diameter of the anal glands) was measured from these photographs using the program Sigma Scan Pro 5. The cephalic crest height (mm) was measured as the distance from the middle of the cranium to the top of the crest. The diameters of the first and second anal gland (mm) were calculated as an average of two measurements (width and length), thus avoiding a bias caused by the irregular or non-circular shape of these glands. The ratios of each of these variables with respect to the TL were obtained thus avoiding finding differences on SSTs development due to differences in the size of males. Male body condition was calculated using the Fulton index ($K = 10^5 \times (W / TL^3)$). We assigned the age of caugth individuals according to the length-intervals established by Vinyoles and Sostoa (2007) for the freshwater blenny in the River Matarranya.

The size of the total egg cluster was measured (long and short axis to the nearest mm) and the egg mass was photographed for each nest found. Nest stones were then measured (long and short axis length to the nearest cm), and restored to their original position. These measurements were then multiplied to obtain cluster size (cm^2) and stone area (cm^2) following Côté et al. (1999). Partial egg clusters on the underside of the nest stones were easily distinguished according to Côté et al. (1999). Water depth (cm) and water current velocity (m/s), taken in the middle of the water column with a MiniAir20 universal anemometer, were measured in front of each nest stone. Nesting area was measured to determine nest density for each sampling period.

All statistical analysis were made with Statistica V.8 (StatSoft). Non-parametric statistics, except for male length, were used due to the lack of homogeneity of variance or because variables were not normally distributed (Shapiro-Wilk's test, $P < 0.05$). When multiple tests were performed, significance levels were corrected using the sequential Bonferroni method (Rice, 1989). Real probability values are reported throughout. All statistical analyses were considered significant at 5% ($P < 0.05$).

Results

Nesting site characteristics

Before flow reduction (in 2004), when the river flow was six times higher during the nesting period of the freshwater blenny, 137 nests were found in a stretch of 47 m and a wet surface of 811.4 m^2 (0.14 nests per m^2). After flow reduction (in 2011), which took place after the implementation of irrigated crop areas in the medium stretch of the Matarranya, only 28 nests were found in a river stretch of 70 m and 214.5 m^2 (0.18 nests per m^2). Despite the considerable fall in the number of nests found in 2011, nest density was not significantly different between the two years ($\chi^2 = 0.00$, d. f. = 1, P > 0.05).

Mean water depth in front of nest stones was significantly lower in 2011 (mean ± S.D = 14.1 ± 5.9 cm, N= 27) than in 2004 (mean ± S.D = 26.4 ± 11 cm, N= 137; Mann-Whitney *U*-test: z = 5.97, P < 0.05). The water current velocity at nest sites was also significantly lower for the second study period (mean ± S.D = 0.14 ± 0.12 m/s, N= 28) than for the first one (mean ± S.D = 0.49 ± 0.25 m/s, N= 14; Mann-Whitney *U*-test: z = 3.9, P < 0.05). The river width (mean ± S.D = 17.7 ± 0.88 m) was also higher before flow reduction as compared to the reduced mean width found in 2011 (mean ± S.D = 3.1 ± 1.08 m). The observed changes between 2004 and 2011 represented a 70% reduction in the wet surface of the riverbed and an 80% reduction in the number of nests of the freshwater blenny in the River Matarranya.

Male traits

31 male blennies were caught in the nesting area of the River Matarranya in 2004 and 11 in 2011. Mean TL of nesting males in 2011 was significantly lower than that of nesting males in 2004 (t = 5.97, P < 0.05; Table 1). This decrease in length was due to a lower age of nesting males after water extraction and flow reduction found in the second study period (2011) (Fig. 1). Under the low flow conditions of 2011, the proportion of nesting males belonging to the age group I (young males of first reproduction) was 91% (N= 10), whereas in the previous study period

(2004) this value accounted for only 16% (N= 5) of young males, which were identified as those guarding and defending a nest (Fig. 1). In 2004, the dominant age group of nesting males was the age group II (54.8 % of individuals). Significant differences in male body condition were also found between the two study periods. In particular, males in 2011 were in worse condition than in 2004 (Mann-Whitney *U*-test: z = 2.02, P < 0.05; Table 1). Moreover, in 2011 the degree of development of the SSTs in male blennies (analyzed from the ratio between each of these variables to TL) was significantly lower than that found in 2004 (cephalic crest height: Mann Whitney *U*-test: z = 3.39, P < 0.05; 1st anal gland diameter: Mann Whitney *U*-test: z = 3.73, P < 0.05; 2^{ond} anal gland diameter: Mann Whitney *U*-test: z = 4.36, P < 0.05) (Table 1).

Male reproductive success

Nest stone size was similar between the two study years (Mann-Whitney *U*-test: z = 0.15, P > 0.05; Fig. 2a). However, a strong decrease in total cluster size was observed in 2011 (mean ± SE = 22.9 ± 2.7) as compared to that found in 2004 (mean ± SE = 45.4 ± 2.8; Mann-Whitney *U*-test: z = 4.15, P < 0.05). This decrease implied a 50 % reduction in cluster size as compared to 2004. Conversely, in 2011 (mean ± SE = 2.8 ± 0.2) an increase in the number of different partial clusters in the nests was found with respect to what was found before flow reduction in 2004 (mean ± SE = 2.2 ± 0.1; Mann-Whitney *U*-test: z = 3.44, P < 0.05; Fig. 2b). No relationship was found between male length and total size of the cluster in their nest in 2011 (Spearman's rank correlation: r_s= 0.22, P > 0.05; male TL ranged from 43 to 67 mm), whereas in 2004 there was such a relationship (Spearman's rank correlation: r_s= 0.56, $P < 0.05$; male TL ranged from 47 to 106 mm).

Discussion

In comparison with the 2004 observations, a 70% reduction in the wet surface of the river bed and an 80% reduction in the number of nests guarded by males were found in the second year (2011) of our study (under flow reduction). Moreover, a 50% reduction in male reproductive success was found. The nesting area of the freshwater blenny in the River Matarranya (at Fayón) was limited to the same place where it had previously been found, despite the occurrence of drastic hydrological changes.

Freshwater fish species inhabiting Mediterranean-type streams have developed life-history tactics such as fast growth, early sexual maturity and short life span to cope with the harsh conditions imposed by strong seasonal fluctuations in streamflow (Hermoso et al., 2009; Vinyoles et al., 2010). In these ecosystems, severe drought conditions are associated with decreased longevity (Lobón-Cerviá et al., 1991; Oliveira et al., 2002). The disappearance of parental cohorts after severe droughts is quite common among freshwater fish inhabiting such environments (Dolbeth et al., 2007). This has been also reported for the freshwater blenny in the River Matarranya where the extreme environmental conditions such as an abrupt drop in the trophic availability can cause the disappearance of most adult river blennies during the summer period (Vinyoles et al., 1999; Vinyoles and Sostoa, 2007). This explains why most males found in the nesting area of the River Matarranya in the second year of the study (in 2011) were young fish under conditions of hydrological stress [according to Vinyoles and Sostoa (2007) these fish had just turned one year old in May]. This is the first time that parental care is found in the male of the freshwater blenny at such a young age. These results are not consistent with those described by Neat et al. (2003), who found that small males do not develop SSTs and act as "obligatory sneakers". However, parental behaviour at an early age has also been found in another blenniid fish (*Aidablennius Sphinx*) by Neat and Locatello (2002).

In fish species with ARTs (alternative reproductive tactics), the plasticity in the adoption of one or the other tactic has been reported and often depends on environmental factors such as resource availability and social cues (Taborsky, 1994; Burmeister et al., 2005). In a recent study (conducted in captivity), Fabre et al. (2014) found that medium-sized males of the freshwater blenny (ranging from 53 to 66 mm in total length) may adopt the dominant tactic depending on the social context in which they are kept. According to these authors, the medium-sized males acted as dominant (i. e., exhibited SSTs and parental behaviour) when they were kept with males smaller than themselves, but the opposite was found when these males were kept with larger ones (in this case, the medium-sized males did not develop SSTs and did not defend a nest). The results of this study suggest that young males of river blennies adopted the dominant parental tactic when cohorts of adult males were absent in the nesting area. A careful examination of all the stones of the nesting area, and visual observations of the fish coming out of the shelters, ensures that there were actually no fishes of different sizes to those described in this study at the sampling site. The adoption of the parental tactic in such conditions may be an adaptive trait of the freshwater blenny typically inhabiting fluctuating environments. Our results highlight the plasticity of this species concerning the shift of reproductive tactics depending on environmental conditions.

It is well known that female fecundity in fish is directly related to their body length. Typically, a 44 mm TL female freshwater blenny (one year old) lays about 416 eggs during the reproduction period, whereas a 55 mm TL female (two years old) lays about 1206 eggs (Vinyoles and Sostoa, 2007), i.e. 65.5% more. This may explain why under hydrological stress a 50% decrease in cluster size was found. However, an increase in the number of clusters per nest was found. We hypothesize that females (fractional spawners), under low flow conditions could make more fractioned egg batches in two different ways: (1) by distributing them among a greater number of nests (i. e., increasing the number of matings with different males), or (2) by leaving smaller batches of eggs and laying them a higher number of times with the same male. Under certain conditions (related to the unpredictable nature of Mediterranean-type streams), the lack of female choice due to the need of breeding rapidly has already been suggested in this species by Côté et al. (1999). This is consistent with the lack of relationship found between male length and total cluster size in the second year of this study (under low flow). Moreover, young fathers in 2011 were in worse physical condition and they possessed less developed SSTs in relation to their body length than those found in the previous period (in 2004, when water flow was higher). Wong et al. (2007) suggested that anthropogenic disturbances can reduce the evolutionary potential of sexual selection by diminishing the efficacy of visual displays and weakening the signals of male quality in the three-spined sticklebacks. Female preferences varying over the years owing to environmental fluctuations (water temperature and food availability) have been found in the sand goby by Lehtonen et al. (2010).

In conclusion, the freshwater blenny presents biological and behavioural characteristics typical of a species adapted to fluctuating streams (as hydrological changes). However, harsh hydrological conditions in this species cause a significant reduction in the number of nests, a reduction in the number of eggs per nest, and an early onset of the parental care. These results highlight the vulnerability of this endangered species to the hydrological impacts of anthropogenic origin.

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