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Territorial and foraging behaviour of juvenile Mediterranean trout under changing conditions of food and competitors

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Abstract

Territoriality is probably the most important ecological mechanism regulating densities in stream-living salmonids. Body size is typically regarded as the best predictor of territory size, but food abundance and competitor density may be key driving factors. However, a global analysis of literature data showed no clear patterns on the relative causal role of those factors on determining territory size in juvenile salmonids. Thus, in a factorial experiment, we estimated to what extent simultaneous variations of fish size, competitor density and food abundance affected the size of foraging and defended areas of Mediterranean brown trout *Salmo trutta*. In contrast to former studies, we found that foraging areas were larger than defended territories. Foraging and territorial behaviour changed significantly under varying density and feeding regimes. Foraging areas decreased with increasing competitor density and food availability, and there was a strong interaction between these two factors. Defended territories decreased with increasing density, irrespective of food abundance. Although our findings showed a significant allometric relationship between fish length and territory size, the data contained much unexplained variability. Our findings suggest that defended areas are relatively fixed for a given trout length. However, at extremely high population densities, defended areas decreased. Thus, under extreme competition, such as during critical periods right after emergence, trout may subdivide available habitat and thereby moderate density declines.

Introduction

Juvenile stream-dwelling salmonids defend feeding territories under both experimental and field conditions (Elliott 1990; Keeley 2000; Imre et al. 2004). They begin defending feeding territories shortly after emergence; obtaining a territory appears to be essential for survival. At this early stage, mortality is usually density-dependent and population size is regulated primarily via territorial behaviour (Elliott 2001). Territoriality might be also responsible for the effects of density dependence on individual growth (Ward et al. 2007; Parra et al. 2011). Further, territoriality plays a relevant role in setting carrying capacity for salmonids in rivers (Grant and Kramer 1990), because maximum population numbers are not only determined by the abundance and distribution of limited resources but also by how individuals compete for their use. Determining the maximum number of individuals a system can support is a primary goal of fisheries management and important in assessing the effects of human actions on threatened populations (Ayllón et al. 2012a). Carrying capacity models for salmonids should account for both resource dynamics and territorial behaviour, though this second aspect is frequently neglected in population dynamics modeling. To fill this gap, Ayllón et al. (2012b) developed a model in which the maximum population abundance is limited by fluctuating habitat conditions and regulated through the behavioural adjustment of the size of feeding territories as a function of fish body size. Body size is the strongest predictor of territory size in salmonids, but any ecological factor affecting the body-territory size relationship could lead to changes in the maximum population abundance.

Classical models of optimal territory size (Hixon 1980; Schoener 1983) predict that feeding territories for energy maximizers animals, i.e. those whose potential reproductive success is

positively correlated with their net energetic intake vary inversely with food availability and competitor density. Competitor and resource abundances may change over time, so animals must be flexible in their territorial behaviour. Salmonid fish are known to defend feeding territories and adjust their aggressive behaviour to changing environmental conditions. Both field and laboratory work provides support for this prediction in territorial salmonids (Elliott 1990; Keeley 2000; Toobaie and Grant 2013). In addition to the energetic benefits or costs of defense, space requirements for young salmonids are also directly related to body size through metabolic demands (Grant and Kramer 1990; Keeley 2000). Previous observational studies that measured territory size in young salmonids found an allometric relationship with body size (Elliott 1990, *S. trutta*; Keeley and Grant 1995, *Salmo salar*; Keeley 2000, *Oncorhynchus mykiss*), which was also predicted by *in silico* simulation experiments (Ayllón et al. 2010, for the brown trout *S. trutta*). However, other studies did not find this relationship—for example, Steingrímsson and Grant (2008) for the Atlantic salmon *S. salar*.

A global analysis of previous observational and experimental studies on territorial juvenile salmonids shows no clear patterns, and even reveals somehow contradictory results, on the influence of food abundance or competitor density on either territory size or how individuals scaled territories with body size. To date, only Keeley (2000) has evaluated how juvenile salmonid territory size changes in response to simultaneous changes in food abundance and competitor density (but see Keeley and Grant 1995, and Keeley and McPhail 1998 in field studies), despite the fact that under natural conditions these factors act simultaneously and their interaction could have strong consequences on the adjustment of territory size. An experimental manipulation of the major determinants of territory size can determine the direction of causation

and the interactions between factors, and help incorporate them into population dynamics modeling.

Most of the information concerning the territorial behaviour of juvenile salmonids comes from northern latitudes (Sundström et al. 2003; Steingrímsson and Grant 2008; Toobaie and Grant 2013). The question of whether stream-dwelling salmonids show a similar territorial behaviour throughout their distribution range has been long neglected. In particular, factors controlling brown trout territory size in Mediterranean areas are unknown, despite the relevance that this may have for population management, especially when global warming is increasingly threatening their persistence (Almodóvar et al. 2012; Ayllón et al. 2013). The few existing experimental studies of brown trout territorial behaviour come from northern populations (Deverill et al. 1999; Johnsson et al. 2000; Sundström et al. 2003). Only Elliott (1990) developed a predictive model for territory size of 0+ Atlantic brown trout, which provides a baseline from which the maximum area of a territory can be calculated based on fish length.

In this study, we investigated the effects of simultaneous variations of fish size, competitor density, and food abundance on the territory size of Mediterranean brown trout. We predicted that territory size would be inversely proportional to food abundance and density of surrounding competitors and positively correlated with fish size. We also predicted that those factors would interact when trout are establishing territories. We tested these predictions in a factorial experiment using a distinct Mediterranean lineage of brown trout. The model developed by Elliott (1990) was additionally used to test whether brown trout territorial behaviour differs between Mediterranean and Atlantic lineages.

Materials and methods

Experimental set-up

In April 2010, about two weeks after emergence, 0+ brown trout were stocked into experimental channels at the Uña Hatchery (40°13'N, 1°58'W) near Cuenca, Spain. Fish were allowed to acclimate for six weeks before experiments started in June 2010. Fish were the offspring of wild breeders caught at the Mundo River (Segura River basin) as part of a supportive breeding program that stocks recreational fisheries. Brown trout from the Segura River basin belong to a Mediterranean lineage (Machordom et al. 2000).

Experiments were carried out between June and September 2010. To analyze the effects of food abundance and competitor density on the territorial behaviour of young brown trout, the experiment had a factorial design, consisting of two factors (food and density) and three levels per factor (low, intermediate, and high). We used six parallel artificial channels without a substrate in the indoor part of the hatchery (to avoid predation), each measuring 3.3×0.5 m (1.65 m^2). Each channel was divided into three 1.0×0.5 m (0.5 m^2) sections (experimental units) to allow for simultaneous replicates. Therefore, 18 experimental units were available for the experiments. Mesh screens were placed at both ends of each of the experimental units to prevent the escape of any fish. Water was supplied directly from the Rincón stream (Júcar River basin). The water entering the channels was filtered through fine-mesh nylon screening to remove any invertebrates that might have entered from the river. Light and temperature were ambient, with water temperature ranging from 10°C to 12°C across the experimental period. We assigned treatment levels to experimental units to eliminate any potential upstream-downstream effects.

We randomly assigned treatments such that each level of experimental factors appeared once in the upper, middle, and lower channel positions.

Fish were fed live zooplankton (water flea, *Daphnia pulex*) and chironomid and simuliid larvae. Prior to the experiments, we estimated an average satiation ration for the low-density treatment, i.e., the maximum quantity of food a trout consumed when under low competition. We randomly varied the feeding times for several days, then calculated an average satiation ration (number of items consumed by individual fish). The low food ration (*LFR*, 500 ind·m⁻²) was equal to the satiation ration, and the intermediate (*IFR*) and high (*HFR*) food rations were the double and the quadruple of the satiation ration, 1000 and 2000 ind·m⁻², respectively. Similar proportions of each prey type were used in each treatment. Food levels were within the upper range typically found in productive streams in Spain (Almodóvar et al. 2006; Nicola et al. 2010); therefore, it represented the largest amount of food encountered by juvenile salmonids in the wild. Similar food rations have been used by others (Keeley 2000; Imre et al. 2004; Toobaie et al. 2013).

To test our predictions, it was essential to elicit aggressive defensive behaviour among the experimental trout. Once this behaviour was guaranteed we could assess the size of territories under various conditions of food and competitors. Experimental competitor density was calculated using mean body size and percent habitat saturation (*PHS*) as described by Grant and Kramer (1990). *PHS* measures the total percentage of the streambed occupied by territories of all salmonids in a stream and has been used by some authors to assess the level of competition (Parra et al. 2011, 2012). We experimentally induced competition by increasing the density levels to double or quadruple the 100% habitat saturation, i.e., all the available space occupied

by brown trout territories. According to the logistic model of Grant and Kramer (1990), the probability of observing a density-dependent response in the *PHS* levels tested in our experiments (100, 200, and 400%) was between 0.90 and 1.00. The review by Grant and Kramer (1990) showed that 19 of 31 studies of various salmonid species had mean *PHS* values over 100% (range 105–775%). Hence, our experimental range (100–400%) could reflect juvenile densities observed elsewhere in natural salmonid populations shortly after emergence. Thus, the low (*LCD*), intermediate (*ICD*), and high (*HCD*) competitor densities were 100% saturation (46 ind·m⁻²), 200% saturation (92 ind·m⁻²), and 400% saturation (184 ind·m⁻²), respectively. Each experimental unit received 23, 46, or 92 trout, which corresponded to *LCD*, *ICD*, and *HCD* treatments respectively. There were two replicated experimental units for each density level and food ration level combination; altogether, we used 966 trout that averaged 32.82 ± 3.71 mm in length and 0.51 ± 0.16 g in weight. Trout occasionally died during the experiments (less than 5% of total used trout); the number of deaths was similar across treatments. We replaced dead trout with new trout of similar size to maintain the experimental densities. Replacement trout were reared in channels containing the same competitor densities as the treatment channels. Replacement trout might have found it harder than resident fish to establish new territories (see Johnsson and Forser 2002); however, they induce aggressive behaviour in the resident fish.

Behavioural measurements

Brown trout behaviour was recorded using a digital video camera on a tripod. Each experimental unit was recorded for 30 minutes once every week from June to September. The times of recording were randomized to ensure that each experimental unit was observed at different times of day (9:00-12:00, 12:00-15:00, or 15:00-18:00) during the experiment. In all, 288 recordings

were obtained (18 experimental units $\times$ 16 weeks. We decided to analyze data only from recordings performed every two weeks to include a higher variability in fish body length; therefore, 144 recordings were analyzed. At the start of each recording, food was distributed throughout the section to simulate invertebrate drift. The first 5 minutes of each recording were not used for data collection because fish were somewhat disturbed by the camera set-up and needed few minutes to return to their original feeding positions.

Recordings were played with VLC Media Player software (VideoLAN, France) for data collection. From each 30-min recording, the position of each trout within the experimental section was examined first. Only trout with a clear centrally located holding position, i.e. those fish that typically initiated aggressive acts and foraging attempts from a single central place maintained during the entire 30 min recording were considered for data collection. Multiple foraging stations were never observed. For each focal fish, all foraging movements and aggressive interactions with surrounding individuals were registered and measured. The foraging radius was defined as the distance travelled by a fish from its holding position to the point where it captured a food item (see Keeley 2000); each foraging attempt involved an attack on a single prey and return to the same central place. Similarly, the aggressive radius was the distance travelled by a fish from its holding position to the point where an intruder fish provoked an aggressive response; each aggressive act involved an aggressive response (mainly chases and nips) until the intruder was chased away then a return to the same central place.

To measure the distances, each experimental unit acted as an X/Y coordinate system, with the 0, 0 (X, Y) position in the lower left corner of the unit, as seen on screen. The software PMeter

(Pegtop, Germany) was used to place a ruler on the display screen to measure the distances and the length of each focal fish. Actual distances and fish lengths were calculated from a system of grid lines spaced 20 cm apart that were drawn on the bottom of each channel for scale. The foraging and defended areas of each focal trout were defined as the average foraging and aggressive radii respectively, assuming that the territory is a circle (Keeley and Grant 1995; Keeley 2000). To examine the allometry of territory size, we used the local competitor density instead of the treatment density. To calculate the local density, we ranked all measured aggressive radii for each recording and selected the longest one for each recording (maximum aggressive radius; *MAR*). For each aggressive and foraging movement within a treatment, we counted the number of trout within the circle defined by the treatment-specific *MAR*. The local density ($\text{ind}\cdot\text{m}^{-2}$) was then calculated as the number of trout within the circle divided by its area.

Statistical analyses

Prior to analysis, all continuous variables were subjected to Shapiro-Wilk tests for normality. When necessary, variables were log-transformed before analyses to meet the assumptions of parametric tests. General linear models (GLM) were used to assess the effects of food abundance and competitor density on the foraging and defended areas. First, an analysis of covariance (ANCOVA; density and food treatments as covariates) was used to test for differences in size between foraging and defended areas. Second, to test the prediction that territory size decreases with increasing food abundance and competitor density, two-way analyses of variance (ANOVA) and subsequent Tukey's tests were used to determine whether there was a main effect of competitor density, food abundance, or their interaction on foraging and defended areas among experimental fish. Third, we used least squares regressions to examine the effects of local

density and fish length on defended areas. Finally, the models given by Elliott (1990) were employed to estimate the expected defended area of each experimental trout. According to this model, the maximum area defended is linearly proportional to fish size on a log/log scale, but the relationship is significantly different for trout greater and less than 40 mm in length. The length of each experimental trout was used to calculate their expected defended areas using the power functions from Elliott (1990). The range of fish lengths used in Elliott's experiment (~ 22–60 mm) was comparable to the range used in the present study (21–67 mm). Observed (*ODA*, own data) and predicted (*PDA*, Elliott 1990) defended areas were then compared by ANCOVA tests (fish length as covariate). Before using ANOVA and ANCOVA, the assumption of homogeneity of variances was verified through Levene's test. All statistical analyses were performed using SPSS v.19 statistical software (SPSS Inc., Chicago, Illinois). The critical significance level α for all statistical tests was set at 0.05.

Results

Juvenile brown trout foraged and defended space around a primary holding station, but the relative use of space was different. In all treatment levels foraging areas (mean 394.6 cm²; min-max, 1.2–3676.4 cm², N= 544) were significantly larger than defended areas (mean 168.9 cm², min-max, 3.6–1 566.0 cm², N= 822) (ANCOVA, $F_{3, 1333} = 50.54$, $P < 0.001$). Foraging and aggressive behaviours of juvenile brown trout were directed mainly towards lateral and upstream directions from a central located foraging station; fish tended to move laterally most often.

Mean foraging areas were dependent on food abundance (two-way ANOVA, $F_{2, 506} = 4.40$, $P = 0.013$), and competitor density ($F_{2, 506} = 8.36$, $P < 0.001$). The interaction between food

abundance and competitor density was also significant ($F_{4, 506} = 6.36$, $P < 0.001$). Thus, foraging areas significantly decreased with increasing density of competitors and the amount of available food (Fig. 1). Regarding the effect of food abundance, post hoc tests showed that mean foraging areas of *LFR* and *IFR* treatments were similar, with averages of 401.5 and 423.5 cm² respectively; however, the mean foraging area in the *HFR* treatment was significantly smaller (285.1 cm²) than *LFR* and *IFR* treatments (Tukey tests, $P = 0.012$ and $P = 0.017$ respectively). It is worth noting that the average foraging area in the *HFR* treatment was similar in all density treatments (Fig. 1). Concerning the density effect, mean foraging areas were similar between *ICD* and *HCD* treatments, with averages of 327.1 and 310.8 cm² respectively; however, the mean foraging area in the *LCD* treatment was significantly larger (468.8 cm²) than in the *ICD* and *HCD* treatments (Tukey tests, $P = 0.010$ and $P = 0.004$, respectively). The foraging area decreased with increasing food availability within each density treatment (Fig. 1).

Conversely, mean defended areas were dependent on competitor density (two-way ANOVA, $F_{2, 826} = 21.06$, $P < 0.001$) but not on food abundance ($F_{2, 826} = 0.761$, $P = 0.468$). There was no significant interaction between food abundance and competitor density ($F_{4, 826} = 2.185$, $P = 0.069$). Thus, average defended areas were similar among low (*LFR*, 167.6 cm²), intermediate (*IFR*, 170.4 cm²), and high food rations (*HFR*, 166.3 cm²). However, defended areas gradually decreased with increasing competitor density (*LCD* mean 212.4 cm²; *ICD* mean 179.5 cm²; *HCD* mean 120.1 cm²; Tukey tests, *LCD/ICD*, $P = 0.034$; *LCD/HCD*, $P < 0.001$; *ICD/HCD*, $P < 0.001$). Further, within each density treatment, average defended areas were similar among food abundance levels, which evidenced the lack of interaction between factors

(Fig. 1). Consequently, we did not control for food abundance to estimate the allometry of defended areas.

Since neither food abundance nor its interaction with competitor density significantly influenced defended areas, we pooled the data to compare observed (*ODA*) against expected (*PDA*) defended areas by density treatment. There was a clear effect of competitor density on the size of the defended areas, so comparisons were carried out separately for the different density treatments. Observed and predicted size of defended areas were similar in *LCD* (mean *ODA* = 216.3 cm², mean *PDA* = 252.7 cm², ANCOVA, $F_{1,427} = 3.08$, $P = 0.080$) and *ICD* treatments (mean *ODA* = 176.7 cm², mean *PDA* = 185.3 cm, $F_{1,671} = 0.83$, $P = 0.362$). However, the mean observed defended area in the *HCD* treatment was significantly smaller than that predicted by Elliott's (1990) model (mean *ODA* = 122.3 cm², mean *PDA* = 385.1 cm², $F_{1,547} = 434.5$, $P < 0.001$; Fig. 2).

After controlling for the effect of local density of competitors on the size of defended areas ($\log_{10}$ defended area [cm²] = 2.688 – 0.326 $\log_{10}$ local density [ind·m⁻²], $F_{1,816} = 111.69$, $P < 0.001$, $r^2 = 0.35$), size of defended area (i.e., residuals of local density vs. defended area) increased significantly with fish size; however, the explanatory power of the model was not high (Residuals = -0.789 + 0.529 $\log_{10}$ length (cm), $F_{1,816} = 19.21$, $P < 0.001$, $r^2 = 0.15$). There was a significant relationship between the size of defended areas and fish length but high unexplained variance was also noted.

Discussion

Juvenile brown trout showed territorial behaviour according to the central-place territorial model, which assumes that sit-and-wait individuals forage and attack intruders from, and return to, a single foraging station (Elliott 1990; Grant and Kramer 1990). Our findings showed that trout actively defended a territory that decreased in size as competitor density increased irrespective of food abundance. From its central position, trout moved away from that territory to feed, to a distance according to food availability and density of competitors.

Our results support the hypothesis that increasing competitor density decreases the territory size (both foraging and defended areas) in brown trout. However, the size of the defended areas in our study did not change inversely with food abundance. Our findings do not agree exactly with the traditional optimality models (Hixon 1980; Schoener 1983), which assign the same importance to food abundance as to competitor density. Few studies exist on changes in territory size of juvenile salmonids in response to simultaneous changes in food abundance and competitor density. Actually, only Keeley (2000) tested this with rainbow trout *O. mykiss* and confirmed the predictions of the optimality models, but without a significant interaction between food abundance and competitor density. Table 1 summarizes 10 published studies (field and laboratory) on the effects of food abundance and density of competitors on the size of defended areas in juvenile salmonids. In all laboratory studies, there was a significant effect of competitor density on the size of the defended area, while only 60% of field studies showed this effect. Consistent with our study, the experiment of Imre et al. (2004) found that the size of the areas defended by juvenile *O. mykiss* decreased with increasing local population and did not change with variable food abundance. However, studies in the natural environment have contradictory results. Gunnarson and Steingrímsson (2011) found that invertebrate drift abundance had no

effect on the size of defended areas of the juvenile brown trout *S. trutta*, but areas unexpectedly increased with competitor density. The same authors reported the opposite results for the Arctic char *Salvelinus alpinus* in the same study. Keeley and Grant (1995) observed a significant effect of drift abundance on the size of defended areas of the juvenile Atlantic salmon *S. salar*, but no effect of intruders' pressure and competitor density; meaning intruder pressure the number of aggressive interactions between a focal fish and intruders per hour. Keeley and McPhail (1998) found that the size of the areas defended by juvenile *O. mykiss* was inversely related to the abundance of drifting invertebrates and local competitor density, but no effect of intruders' pressure. Finally, Dill et al. (1981) found that the size of defended areas of juvenile *O. mykiss* was inversely related to benthos abundance in the juvenile coho salmon *Oncorhynchus kisutch*, this effect being more clear when intruders' density increased. However, these authors found no effect of variable drift abundance on territory size.

On the other hand, in our experiments the amount of available food determined the size of the foraging areas. When competitor density increased, both defended and foraging areas were significantly reduced, but the latter decreased even more when food abundance increased. To our knowledge, there are no previous estimates of foraging areas in brown trout. The estimates for other salmonids only indicate that aggression distances are larger than foraging ones (Grant et al. 1989; Keeley and Grant 1995; Keeley 2000; Imre et al. 2002; Steingrímsson and Grant 2008), the opposite of our experiments. Keeley (2000), in a very similar experiment, found that maximum sizes of foraging and defended areas were the same. In agreement with our results, Keeley (2000) and Imre et al. (2004) found an inverse relationship between foraging areas of rainbow trout and both food abundance and stocking density.

339
340 While some previous studies on juvenile salmonids have indicated a negative relationship
341 between food abundance and defended areas (Slaney and Northcote 1974; Dill et al. 1981;
342 Keeley 2000), others only found a weak negative (Keeley and Grant 1995; Keeley and McPhail
343 1998; Wood et al. 2012; Toobaie and Grant 2013) or no relationship (Imre et al. 2004;
344 Gunnarson and Steingrímsson 2011) (Table 1). In general, previous studies suggest that the size
345 of the defended area changes very little with increasing food abundance and that only very large
346 increases in food abundance would decrease defended areas in natural streams. However, our
347 experimental brown trout did not seem to adjust defended areas even at very high levels of food
348 abundance. Our study is the first laboratory experiment where live invertebrates have been used
349 instead of pellets (Keeley 2000; Imre et al. 2004; Wood et al. 2012), simulating the available
350 food in wild conditions. Food abundance was manipulated to remain within the upper range
351 found in Spanish productive streams (Almodóvar et al. 2006; Nicola et al. 2010). Therefore, it
352 seems that food abundance needs to be even higher to be a limiting factor when defending a
353 territory. As Imre et al. (2004) pointed out, the intense competitive pressure exerted by neighbors
354 under conditions of extremely high density and impossibility of emigration (as in our study)
355 could prevent territory holders from expanding their territories when food abundance is low. In
356 this regard, our results agree with those found in previous experimental studies of salmonids and
357 other animals (reviewed by Adams 2001). Alternatively, defense of a given area may have other
358 implications for trout beyond feeding. They may simply maintain a minimum necessary physical
359 space for growth when energy requirements are already fulfilled; previous studies have shown
360 that juvenile trout grow larger in larger spaces (Greenberg and Giller 2001). Under this
361 hypothesis, trout should be flexible in changing their foraging behaviour (increasing foraging

area as food abundance decreases) to meet their metabolic requirements but defend only a minimum necessary physical space.

Irrespective of food abundance, brown trout showed an innate behaviour to defend an area against intruders. The size of the area was reduced when competitor density increased. To our knowledge, this is the first study showing experimentally how defended areas of juvenile brown trout change with variable competitor density. A similar study was carried out under wild conditions in Icelandic rivers, and the authors did not find a negative effect of competitor density on territory size (Gunnarsson and Steingrímsson 2011). However, fish densities in that study were much lower (mean $1.46 \text{ ind}\cdot\text{m}^{-2}$) than those used in the present study (46, 92, and $184 \text{ ind}\cdot\text{m}^{-2}$). The same pattern has been observed in experimental and field studies of other salmonids where competitor density was low (Keeley and Grant 1995, *S. salar*, mean $3.2 \text{ ind}\cdot\text{m}^{-2}$; Keeley and McPhail 1998, *O. mykiss*, mean $2.9 \text{ ind}\cdot\text{m}^{-2}$). In contrast, a negative significant relationship between competitor density and territory size has been reported at higher density conditions (Keeley 2000, *O. mykiss*, $74 \text{ ind}\cdot\text{m}^{-2}$; Imre et al. 2002, *O. mykiss*, $46 \text{ ind}\cdot\text{m}^{-2}$; Imre et al. 2004, *O. mykiss*, $25 \text{ ind}\cdot\text{m}^{-2}$; Wood et al. 2012, *O. mykiss*, $1.9\text{-}14.8 \text{ ind}\cdot\text{m}^{-2}$; Lindeman et al. 2015, *S. salar*, $0.25\text{-}8 \text{ ind}\cdot\text{m}^{-2}$). At low densities, territoriality may play a smaller role, as individuals have foraging areas large enough to survive and grow (note however that dominance plays a very important role since the most dominant individuals would occupy the positions providing the best conditions to grow; Newman 1993). As a result, density-dependent mortality is typically observed in salmonids only at high densities, while density-dependent effects on growth are stronger at relatively low densities (Imre et al. 2005, Einum et al. 2006).

Although our findings showed a significant trend where the size of defended territories increased with fish length, data still contained a high amount of unexplained variability. A similar outcome was obtained by Gunnarsson and Steingrímsson (2011) for Icelandic brown trout in wild conditions. In general, there are disparate results reported in the salmonid literature. Steingrímsson and Grant (2008) and Gunnarsson and Steingrímsson (2011) did not find a significant allometric relationship between territory and fish size in other salmonids (Table 2). This contrasts with Elliott's (1990) study on brown trout that found a close relationship between fish length and the area of the defended territory. Likewise, other authors have found a negative relationship in other salmonids (see review in Table 2). The scaling of territory size with body size was lower in our study than in other studies of salmonids (Table 2). During the experiments, we observed that fish were often distributed throughout the water column rather than confined to the bottom of the channel. Gunnarsson and Steingrímsson (2011) suggested that under high *PHS* values, juvenile salmonids might defend three-dimensional territories, and Ayllón et al. (2010) showed that the allometry of territory volume would be a better predictor of brown trout spatial requirements than territory area. Thus under keen competition, the spatial territory geometry may change. This could explain the low explanatory power of our allometric model and may be considered in future research. Alternatively, it is possible that the strongly competitive conditions imposed in our experiment prevented trout from acquiring the area required to fulfill their theoretical metabolic demands. The slope (b) of the defended area vs. fish length regression in juvenile salmonids reported in the present (Table 2) and previously published (fish length 21-85 mm) studies ranged from 2.24 to 4.75 (mean 3.12), which extends the range published by Grant and Kramer (1990). The rate at which salmonids scale territories to body size is highly species-specific.

408
409 The role that territorial behaviour plays in the regulation of population size depends on how
410 territory size responds to population density. Our findings show that, on the one hand, territory
411 size (both foraging and defended areas) decreases with increasing fish density. On the other
412 hand, the size of the defended area is relatively fixed for a given length of fish; our results were
413 comparable to those predicted by allometric regressions for similar sized trout in an Atlantic
414 population (Elliott 1990). Therefore, it can be concluded that at densities at which all available
415 habitat is saturated, the defended area would be set by fish length, being just qualified around
416 narrow ranges by local density. Only when competitor density was markedly extreme (four times
417 the 100% *PHS*) did territory size fall drastically below the expected values. This suggests that
418 under extreme competition, such as during the critical period right after emergence, trout
419 subdivide available habitat and therefore moderate the magnitude of density decline due to
420 density-dependent mortality. After some extreme point, trout may even cease to be territorial
421 because it is not profitable in terms of energy gain to defend a territory anymore (Elliott 1994).
422 Therefore, it can be concluded that trout maintain territories determined mainly by fish size
423 regardless of available food, and only under exceptionally high competition do they substantially
424 decrease territory size. Brown trout is flexible in its territorial behaviour under variable
425 competitor pressure, as are other vertebrate species (Maher and Lott 2000), but under natural
426 conditions an extremely high density of competitors is needed to significantly change their
427 territory size. The model developed by Elliott (1990) did not control for the density factor and
428 his experimental trout remained at lower densities than used in our experiments. However, it
429 seems to have a great predictive power except in conditions of extreme competition.
430

To summarize, territory size of juvenile brown trout is flexible under severe intraspecific competition, thus lending support to optimal territory size models. The scarce estimates of *PHS* in Iberian brown trout populations close to carrying capacity (Ayllón et al. 2010, Parra et al. 2011) indicate an average *PHS* around 52% (range 34–76%) in summer, which corresponds to a probability of a density-dependent response of less than 0.80, according to the logistic model of Grant and Kramer (1990). Our findings indicate that under this scenario, the defended areas would be set by fish length and would only slightly adjust depending on the local competitor density. Thus, territories will set a maximum density for a particular habitat, lending support to our previous work estimating the carrying capacity of brown trout in Mediterranean populations (Ayllón et al. 2012a,b, 2013). It should be noted that studies based on observations of territorial individuals provide information regarding the space and food requirements of the dominant individuals, but studies accounting for the diverse behavioural strategies displayed (dominants vs. subordinates, territorial vs. non-territorial) are still needed. This new approach could reveal the average spatial requirements of all fish in the population, which may be relevant for predictive modeling in populations or conditions with a high proportion of non-territorial fish. In addition, a review by Grant and Kramer (1990) showed that 19 of 31 studies of different salmonid species reported mean *PHS* values over 100% (range 105–775%). Therefore, there are natural conditions of severe competition where defended areas would be determined less by fish length and much more by competition, as we observed here.

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References

- Adams, E.S. 2001. Approaches to the study of territory size and shape. *Annu. Rev. Ecol. Syst.* **32**: 277-303.
- Almodóvar, A., Nicola, G.G., and Elvira, B. 2006. Spatial variation in brown trout production: the role of environmental factors. *T. Am. Fish. Soc.* **135**: 1348-1360.
- Almodóvar, A., Nicola, G.G., Ayllón, D., and Elvira, B., 2012. Global warming threatens the persistence of Mediterranean brown trout. *Glob. Change Biol.* **18**: 1549-1560.
- Ayllón, D., Almodóvar, A., Nicola, G.G., and Elvira, B. 2010. Modelling brown trout spatial requirements through physical habitat simulations. *River Res. Applic.* **26**: 1090-1102.
- Ayllón, D., Almodóvar, A., Nicola, G.G., Parra, I., and Elvira, B. 2012a. A new biological indicator to assess the ecological status of Mediterranean trout type streams. *Ecol. Indic.* **20**: 295-303.
- Ayllón, D., Almodóvar, A., Nicola, G.G., Parra, I., and Elvira, B. 2012b. Modelling carrying capacity dynamics for the conservation and management of territorial salmonids. *Fish. Res.* **134-136**: 95-103.

- 475 Ayllón, D., Nicola, G.G., Elvira, B., Parra, I., and Almodóvar, A. 2013. Thermal carrying
476 capacity for a thermally-sensitive species at the warmest edge of its range. PloS ONE **8**:
477 e81354.
- 478 Deverill, J.I., Adams, C.E., and Bean, C.W. 1999. Prior residence, aggression and territory
479 acquisition in hatchery-reared and wild brown trout. J. Fish Biol. **55**: 868-875.
- 480 Dill, L.M., Ydenberg, R.C., and Fraser, A.H.G. 1981. Food abundance and territory size in
481 juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. **59**: 1801-1809.
- 482 Einum, S., Sundt-Hansen, L., and Nislow, K.H. 2006. The partitioning of density-dependent
483 dispersal, growth and survival throughout ontogeny in a highly fecund organism. Oikos
484 **113**: 489-496.
- 485 Elliott, J.M. 1990. Mechanisms responsible for population regulation in young migratory trout,
486 *Salmo trutta*. III. The role of territorial behaviour. J. Anim. Ecol. **59**: 803-818.
- 487 Elliott, J.M. 1994. Quantitative Ecology and the Brown Trout. Oxford University Press.
- 488 Elliott, J.M. 2001. The relative role of density in the stock-recruitment relationship of salmonids.
489 In: Stock, recruitment and reference points: assessment and management of Atlantic
490 salmon (Eds E. Prévost & C. Chaput), pp. 25-66. INRA, Paris.
- 491 Grant, J.W.A., and Kramer, D.L. 1990. Territory size as a predictor of the upper limit to
492 population density of juvenile salmonids in streams. Can. J. Fish. Aquat. Sci. **47**: 1724-
493 1737.
- 494 Grant, J.W.A., Noakes, D.L.G., and Jonas, K.M. 1989. Spatial distribution of defence and
495 foraging in young-of-the-year brook charr, *Salvelinus fontinalis*. J. Anim. Ecol. **58**: 773-
496 784.

- 497 Greenberg, L.A., and Giller, P.S. 2001. Individual variation in habitat use and growth of male
498 and female brown trout. *Ecography* **24**: 212-224.
- 499 Gunnarsson, G.S., and Steingrímsson, S.O. 2011. Contrasting patterns of territoriality and
500 foraging mode in two stream-dwelling salmonids, Arctic char (*Salvelinus alpinus*) and
501 brown trout (*Salmo trutta*). *Can. J. Fish. Aquat. Sci.* **68**: 2090-2100.
- 502 Hixon, M.A. 1980. Food production and competitor density as the determinants of feeding
503 territory size. *Am Nat* **115**: 510-530.
- 504 Imre, I., Grant, J.W.A., and Keeley, E.R. 2002. The effect of visual isolation on territory size and
505 population density of juvenile rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat.*
506 *Sci.* **59**: 303-309.
- 507 Imre, I., Grant, J.W.A., and Keeley, E.R. 2004. The effect of food abundance on territory size
508 and population density of juvenile steelhead trout (*Oncorhynchus mykiss*). *Oecologia*
509 **138**: 371-378.
- 510 Imre, I., Grant, J.W.A., and Cunjak, R.A. 2005. Density-dependent growth of young-of-the-year
511 atlantic salmon *Salmo salar* in Catamaran Brook, New Brunswick. *J. Anim. Ecol.* **74**:
512 508-516.
- 513 Johnsson, J.I., Carlsson, M., and Sundström, L.F. 2000. Habitat preference increases territorial
514 defence in brown trout (*Salmo trutta*). *Behav. Ecol. Sociobiol.* **48**: 373-377.
- 515 Johnsson, J.I., and Forser, A. 2002. Residence duration influences the outcome of territorial
516 conflicts in brown trout (*Salmo trutta*). *Behav. Ecol. Sociobiol.* **51**: 282-286.
- 517 Keeley, E.R. 2000. An experimental analysis of territory size in juvenile steelhead trout. *Anim.*
518 *Behav.* **59**: 477-490.

- 519 Keeley, E.R., and Grant, J.W.A. 1995. Allometric and environmental correlates of territory size
520 in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **52**: 186-196.
- 521 Keeley, E.R., and McPhail, J.D. 1998. Food abundance, intruder pressure, and body size as
522 determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*).
523 Behaviour **135**: 65-82.
- 524 Lindeman, A.A., Grant, J.W., and Desjardins, C.M. 2015. Density-dependent territory size and
525 individual growth rate in juvenile Atlantic salmon (*Salmo salar*). Ecol. Freshw. Fish **24**:
526 15-22.
- 527 Machordom, A., Suárez, J., Almodóvar, A., and Bautista, J.M. 2000. Mitochondrial haplotype
528 variation and phylogeography of Iberian brown trout populations. Mol. Ecol. **9**: 1325-
529 1338.
- 530 Maher, C.R., and Lott, D.F. 2000. A review of ecological determinants of territoriality within
531 vertebrate species. Am. Midl. Nat. **143**: 1-29.
- 532 Newman, R.M. 1993. A conceptual model for examining density dependence in the growth of
533 stream trout. Ecol. Freshw. Fish **2**: 121-131.
- 534 Nicola, G.G., Almodóvar, A., and Elvira, B. 2010. Effects of environmental factors and
535 predation on benthic communities in headwater streams. Aquat. Sci. **72**: 419-429.
- 536 Parra, I., Almodóvar, A., Ayllón, D., Nicola, G.G., and Elvira, B. 2011. Ontogenetic variation in
537 density-dependent growth of brown trout through habitat competition. Freshw. Biol. **56**:
538 530-540.
- 539 Parra, I., Almodóvar, A., Ayllón, D., Nicola, G.G., and Elvira, B. 2012. Unravelling the effects
540 of water temperature and density dependence on the spatial variation of brown trout
541 (*Salmo trutta*) body size. Can. J. Fish. Aquat. Sci. **69**: 821-832.

- 542 Schoener, T.W. 1983. Simple models of optimal feeding-territory size: a reconciliation. Am. Nat.
543 **114**: 902-914.
- 544 Slaney, P.A., and Northcote, T.G. 1974. Effects of prey abundance on density and territorial
545 behavior of young rainbow trout (*Salmo gairdneri*) in a laboratory stream channel. J.
546 Fish. Res. Board Can. **31**: 1201-1209.
- 547 Steingrímsson, S.Ó., and Grant, J.W.A. 2008. Multiple central-place territories in wild young-of-
548 the-year Atlantic salmon *Salmo salar*. J. Anim. Ecol. **77**: 448-457.
- 549 Sundström, L.F., Lohmus, M., and Johnsson, J.I. 2003. Investment in territorial defence depends
550 on rearing environment in brown trout (*Salmo trutta*). Behav. Ecol. Sociobiol. **54**: 249-
551 255.
- 552 Toobaie, A., and Grant, J.W.A. 2013. Effect of food abundance on aggressiveness and territory
553 size of juvenile rainbow trout, *Oncorhynchus mykiss*. Anim. Behav. **85**: 241-246.
- 554 Ward, D.M., Nislow, K.H., Armstrong, J.D., Einum, S., and Folt, C.L. 2007. Is the shape of the
555 density-growth relationship for stream salmonids evidence for exploitative rather than
556 interference competition. J. Anim. Ecol. **76**: 135-138.
- 557 Wood, J.L.A., Grant, J.W.A., and Belanger, M.H. 2012. Population density and territory size in
558 juvenile rainbow trout, *Oncorhynchus mykiss*: implications for population regulation.
559 Can. J. Fish. Aquat. Sci. **69**: 1121-1128.

Table 1. Effects of food abundance and competitors on territory size of juvenile salmonids in present and published studies. Review only include studies where territory size, competitors' pressure (either density or intruder pressure) and food abundance were directly measured.

Table 2. Allometric relationships between fish length and territory size in juvenile salmonids in present and published studies. Regression equations were $\log_{10}T = a + b\log_{10}L$ (units of variables are indicated; ***= $P < 0.001$, **= $P < 0.01$, *= $P < 0.05$, n.s.= not significant), except in Keeley (2000) and this study where T were the residuals (res) from the linear regression between density and territory size.

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Fig. 1. Mean $\pm$ SE of foraging and defended areas (cm^2) of 0+ brown trout under nine experimental treatment combinations of competitor density (*LCD*: 100% *PHS*; *ICD*: 200% *PHS*, *HCD*: 400% *PHS*) and food abundance (*L*: low; *I*: intermediate; *H*: high). See Materials and methods for details.

Fig. 2. Frequency (%) distribution of observed (*ODA*) and predicted (*PDA*) defended areas (cm^2) of 0+ brown trout under low (*LCD*: 100% *PHS*), intermediate (*ICD*: 200% *PHS*) and high (*HCD*: 400% *PHS*) competitor density. *PDA* was calculated using the power functions from Elliott (1990) and length of each experimental trout. See Materials and methods for details.

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Table 1.

Reference	Species	Type [*]	Factors [†]	Competitors [†]	Food	Effects ^{†, ‡}
Keeley and McPhail (1998)	<i>O. mykiss</i>	F, O	D, IP, FA	D = 1–7 ind·m ⁻² IP = 4–27 ind·h ⁻¹	Drift, 18.0–993.1 mg dry w·m ⁻² ·h ⁻¹	D, sig. T↓ when ↑D IP, n.sig. FA, sig. T↓ when ↑FA
Keeley (2000)	<i>O. mykiss</i>	L, E	D, FA, D × FA	D = 32–127 ind·m ⁻²	Pellet, 1.44–5.76 g·day ⁻¹	D, sig. T↓ when ↑D FA, sig. T↓ when ↑FA D × FA, n.sig.
Imre et al. (2002)	<i>O. mykiss</i>	L, E	D	D = 30–65 ind·m ⁻²	Pellet, 1.44 g·day ⁻¹	Density, sig., T↓ when ↑D
Imre et al. (2004)	<i>O. mykiss</i>	L, E	FA	D = 24.8 ind·m ⁻²	Pellet, 0.09–2.88 g·day ⁻¹	Density, sig., T↓ when ↑D FA, n.sig.
Wood et al. (2012)	<i>O. mykiss</i>	L, E	D	D = 4–12 ind·m ⁻²	Pellet, 5% fish biomass	D, sig. T↓ when ↑D
Toobaie and Grant (2013)	<i>O. mykiss</i>	L, E	FA	D = 8 ind·m ⁻²	Pellet, 0.62–20% fish wet body weight	FA, sig. T↓ when ↑FA
Dill et al. (1981)	<i>O. kisutch</i>	F, O	D, FA	D = 5–125 ind·m ⁻² ·min ⁻¹	Benthos, 2–60 mg·100 cm ⁻² Drift, 0.5–15 mg·m ⁻² ·min ⁻¹	FA (drift), n.sig. FA (benthos), sig. T↓ when ↑FA
Keeley and Grant (1995)	<i>S. salar</i>	F, O	D, IP, FA	D = 1–6 ind·m ⁻² IP = 1–21 ind·h ⁻¹	Drift, 10.5–518.7 mg dry w·cm ⁻² ·h ⁻¹	D, n.sig. IP, n.sig. FA, sig. T↓ when ↑FA
Lindeman et al. (2015)	<i>S. salar</i>	F, E	D	D = 0.25–8 ind·m ⁻²	Natural food supply (not measured)	D, sig. T↓ when ↑D

Gunnarsson and Steingrímsson (2011)	<i>S. alpinus</i>	F, O	D, FA	D = 0.3–12 ind·m ⁻¹ radius	Drift, 0.1–443.6 no·min ⁻¹ ·net ⁻¹	D, n.sig.
Gunnarsson and Steingrímsson (2011)	<i>S. trutta</i>	F, O	D, FA	D = 0–6.7 ind·m ⁻¹ radius	Drift, 1.5–179.6 no·min ⁻¹ ·net ⁻¹	FA, sig. T↓ when ↑FA D, sig. T↑ when ↑D
This study	<i>S. trutta</i>	L, E	D, FA, D × FA	D = 46–184 ind·m ⁻²	Invertebrates, 500–2000 ind·m ⁻²	FA, n.sig. D, sig. T↓ when ↑D FA, n.sig.

*: Laboratory = L, Field = F, Experimental = E, Observational= O. †: D = density, IP = intruder pressure, FA = food abundance ‡: T = territory size, sig. = P < 0.05, n.sig. = P ≥ 0.05.

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Table 2.

Reference	Species	a	b	r^2	N	L (mm)	Units (L/T)
Grant and Kramer	Interspecific	-2.83	2.61	0.87***	23	25–150	cm/m ²
Keeley and McPhail	<i>O. mykiss</i>	-2.26	2.47	0.71***	50	30–50	cm/m ²
Keeley (2000)	<i>O. mykiss</i>	-1.77	3.05	0.55***	68	30–60	cm/res
Keeley and Grant (1995)	<i>S. salar</i> < 51	-3.23	3.91	0.88***	48	29–51	cm/m ²
Keeley and Grant (1995)	<i>S. salar</i> > 51	-0.63	1.12	0.86***	46	56–130	cm/m ²
Steingrímsson and Grant	<i>S. salar</i>	-0.37	0.53	0.01 n.s.	50	30–55	cm/m ²
Grant et al. (1989)	<i>S. fontinalis</i>	0.95	2.48	0.70***	23	25–85	cm/cm ²
Gunnarsson and	<i>S. alpinus</i>	-2.40	3.02	0.11 n.s.	31	34–54	cm/m ²
Elliott (1990)	<i>S. trutta</i> < 40	-2.84	3.45	0.97***	501	22–40	mm/cm ²
Elliott (1990)	<i>S. trutta</i> > 40	-0.90	2.24	0.96***	182	40–60	mm/cm ²
Gunnarsson and	<i>S. trutta</i>	-3.89	4.75	0.15*	30	33–46	cm/m ²
This study	<i>S. trutta</i>	-0.79	0.53	0.15***	816	21–67	cm/res

Note: intercept = a , slope = b , coefficient of determination = r^2 , sample size = N, fish length = L, and territory size = T.