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Conservation Challenges Under Taxonomic Uncertainty: Introgression Patterns and Environmental Correlates in Mediterranean Brown Trout

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ABSTRACT

The Mediterranean brown trout (*Salmo trutta* species complex) comprises genetically and ecologically distinct lineages primarily distributed across the Western Mediterranean region. Conservation of the Mediterranean brown trout is complicated by unresolved taxonomy. Although some populations have recently been recognised as distinct species, most—including those in the Iberian Peninsula—remain classified under the nominal *S. trutta*, which is currently listed as Least Concern by the IUCN. This taxonomic ambiguity masks severe conservation threats, particularly the widespread genetic introgression from stocked Atlantic-origin trout. In this study, we assessed introgression patterns, environmental drivers and conservation implications for Mediterranean brown trout populations in Catalonia (northeastern Iberian Peninsula). Genetic data from 134 sites were analysed using microsatellite markers and LDH-C1* allele frequencies, and the influence of historical stocking intensity and climatic variables on introgression was evaluated using hierarchical partitioning and regression models. Our results revealed extensive introgression, with only 10.4% of the populations retaining genetic purity. Stocking intensity was the primary predictor of introgression, followed by temperature and precipitation seasonality. Although the overall distribution of brown trout in the region remains stable, genetically pure Mediterranean populations have experienced severe range contraction, with a 61.5% reduction in extent of occurrence (EOO) and an 89.6% reduction in area of occupancy (AOO). This decline might meet IUCN criterion B for Endangered, although a formal assessment is currently impeded by taxonomic uncertainty and data limitations. The recognition of Mediterranean brown trout as a distinct species, alongside urgent measures to halt ongoing introgression and preserve remaining pure populations, is essential for its long-term conservation.

1 | Introduction

The brown trout (*Salmo trutta*) is a cold-adapted freshwater fish widely distributed across Europe, western Asia and North Africa (MacCrimmon et al. 1970). It is probably the most important freshwater sport fish, having been introduced worldwide and extensively studied (Lobón-Cerviá 2017). Its taxonomic classification as a single species, however, overlooks significant genetic and ecological diversity. Molecular phylogenetic analyses based on mitochondrial DNA (mtDNA) have identified five major evolutionary lineages among European brown trout populations: Atlantic (AT), Mediterranean (ME), Adriatic (AD), Danubian and Duero (Bernatchez 2001; Vera et al. 2010; Sanz 2017). The marmoratus (MA) lineage, once considered part of *S. trutta*, is now recognised as representing a distinct species, *Salmo marmoratus*, the marble trout, which is endemic to Alpine river systems draining into the northern Adriatic Sea and is currently classified as Vulnerable (VU) by the IUCN (Ford 2024a). However, MA haplotypes are not exclusive to *S. marmoratus* and have also been detected in other Mediterranean trout populations around the Adriatic region, either due to natural historical distributions or ancient translocations (Tougard 2022; Talarico et al. 2023). Although many authors consider *S. trutta* to be a species complex, it is still a matter of discussion whether it should be treated as a single polytypic species or as a complex of incipient species that may include vulnerable taxa (Sanz 2017; Segherloo et al. 2021). Such taxonomic uncertainty is particularly relevant for species conservation, because policies and protection measures often rely on well-defined taxonomic classifications (Mace 2004; Allendorf 2012). The difficulty in delineating species boundaries can lead to misclassification on global conservation platforms, such as the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN Standards and Petitions Committee 2024). Because species complexes are often treated as a single taxonomic unit, their broad distributions result in their classification as Least Concern, which can mask significant threats to individual, undescribed species (Scherz et al. 2019; Liu et al. 2022). This situation applies to *S. trutta*, which is listed as Least Concern on the IUCN Red List, although this designation is currently restricted to the AT lineage (Freyhof 2024). Nevertheless, the binomial name is still used in several countries bordering the Mediterranean to refer to native brown trout populations.

In the Mediterranean region, characterised by its unique climatic and hydrological conditions, the *S. trutta* complex is predominantly represented by the ME and AD lineages (Bernatchez 2001; Sanz 2017), which are distributed across the Iberian and Italian peninsulas, major Mediterranean islands (Sicily, Sardinia and Corsica), Balkan rivers and southern Anatolia (Turkey). The ME lineage likely diverged from the AD lineage within the Pyrenean region, with their current distributions reflecting admixture across most of the Mediterranean catchments (Cortey et al. 2004). Both lineages (ME and AD) constitute a long-diverged branch within the *S. trutta* complex (Sanz 2017) and are commonly referred to as Mediterranean brown trout (hereafter MED). MED exhibit discernible morphological traits—particularly in pigmentation (Aparicio et al. 2005; Lorenzoni et al. 2019; Vera et al. 2023)—as well as ecological differences from other lineages, such as an extended reproductive period (Larios-López et al. 2015). Despite well-established genetic, morphological and ecological differentiation, the taxonomic status of MED populations remains unresolved, and they may even constitute a complex of distinct species. Several species have been proposed within MED, including *Salmo rhodanensis* (Rhône and Roia drainages), *Salmo ghigii* (Corsica, Sardinia and mainland Italy), *Salmo cetti* (Sicily) and *Salmo farioides* (eastern Adriatic basins) (Polgar et al. 2022; Ford 2024b, 2025; Ford and Duchi 2024). However, their formal taxonomic status and precise distribution ranges remain unresolved. Notably, although *S. cetti* is geographically a Mediterranean island endemic, molecular evidence shows that its mtDNA belongs to the southern Atlantic (AT) lineage rather than to the MED lineages (Tougard et al. 2018; Splendiani et al. 2019). The situation is even more uncertain in the Iberian Peninsula, where despite the pronounced mitochondrial and nuclear genetic differentiation observed among trout populations (revised in García-Marín et al. 2017), no formal taxonomic separation of MED from *S. trutta* has been proposed. Two of the four putative Mediterranean trout species—regardless of their genetic lineages—are currently categorised as threatened by the IUCN, with *S. cetti* (AT lineage) listed as Critically Endangered and *S. ghigii* (MED lineages) as Endangered (Ford and Duchi 2024; Ford 2025).

The conservation significance of MED is exceptionally high, as they represent a substantial component of the genetic, ecological and life-history diversity within the *S. trutta* complex (Larios-López et al. 2015; Sanz 2017; Aparicio et al. 2023). MED populations are exclusively freshwater, exhibit limited movement and lack anadromous forms (Aparicio et al. 2018). These populations are subject to multiple anthropogenic threats throughout their range. Habitat alteration due to damming and water diversion, coupled with overexploitation through recreational fishing, has significantly diminished their abundance and distribution (Benejam et al. 2016; Carosi et al. 2022). Furthermore, stocking programmes for recreational fisheries have historically introduced hatchery-reared trout of the Atlantic lineage (hereafter ATL) originally native to North Atlantic basins (Berrebi et al. 2021). The lack of reproductive isolation between MED and ATL stocks has led to extensive introgressive hybridisation, disrupting locally adapted gene pools and compromising the genetic integrity of MED populations (Garcia-Marin et al. 1991; Berrebi et al. 2000). In addition to genetic threats, global warming poses a major risk to MED conservation. Brown trout is a stenothermal species, adapted to cold-water environments (Elliott and Elliott 2010). The Mediterranean Basin is recognised as a climate change hotspot, making MED populations particularly vulnerable to rising temperatures (Almodóvar et al. 2012; Ayllón et al. 2013). Projections indicate that 35%–80% of Mediterranean waterbodies suitable for brown trout may be lost under projected climate scenarios (Estrela-Segrelles et al. 2023).

Numerous studies have evaluated the genetic status of MED populations and have shown the extent of introgression with ATL stocks, consistently reporting high levels of genetic mixing across their range. In Italy, approximately 60%–70% of MED populations exhibit severe introgression from ATL strains, with fewer than 5% remaining genetically pure, as initially reported using microsatellite and mtDNA analyses (Splendiani et al. 2016) and supported by recent genomic studies employing SNP-based markers (Splendiani et al. 2016, 2024; Talarico et al. 2023). In France, molecular surveys similarly revealed widespread introgression, with Atlantic alleles detected across the Pyrenean and southern Alpine catchments, as well as in Corsica, and only 10%–20% of the analysed populations were identified as pure MED (Poteaux and Berrebi 1997; Berrebi et al. 2018). In Mediterranean rivers of the Iberian Peninsula, an estimated 75%–85% of brown trout populations exhibit introgression from non-native Atlantic hatchery strains or are entirely of hatchery origin. This pattern was first revealed by allozyme and mtDNA analyses (Almodóvar et al. 2006) and more recently confirmed by genomic studies (Casanova et al. 2022). In Catalonia (northeastern Iberian Peninsula), genetic surveys using SNP panels and microsatellites indicate widespread hatchery influence, with 70%–95% of populations displaying substantial Atlantic introgression as a result of decades of stocking with homogenised central European strains (Araguas et al. 2017; Vera et al. 2023).

A consistently reported pattern across studies is the high variability in introgression among catchments. This variation appears to be driven primarily by differences in stocking intensity (Almodóvar et al. 2006). However, environmental factors such as hydrological and temperature regimes also seem to contribute (Splendiani et al. 2013; Folio et al. 2021; Vera et al. 2023), although they have not been well investigated. Moreover, despite numerous genetic studies on MED, significant gaps remain concerning its current distribution, population size and extinction risk—factors that are essential for accurately assessing its conservation status (IUCN Standards and Petitions Committee 2024). This study aims to assess introgression patterns and the conservation status of MED in the Iberian Peninsula, treating MED as a distinct taxonomic unit from *S. trutta*, and to discuss the challenges and limitations of conservation management. The specific objectives of our study are: (1) to evaluate the degree of introgression of MED in Catalonia and its environmental correlates and (2) to identify the key conservation risks threatening the persistence of MED.

2 | Methods

2.1 | Study Area

The study was conducted across the historical distribution range of MED in Catalonia, defined based on historical records and contemporary field observations. This distribution includes major river basins originating near or above 2000 m a.s.l. and flowing southward in the Eastern Pyrenees. In these basins, MED predominantly inhabit headwater streams and middle river sections characterised by cool summer temperatures and coarse substrates suitable for spawning. A total of 134 sampling sites along 86 watercourses were surveyed, spanning the Fluvià, Ter, Llobregat, Cardener, Segre, Noguera Pallaresa and Noguera Ribagorçana (hereafter N. Pallaresa and N. Ribagorçana, respectively) basins (Figure 1). ATL stocks were extensively introduced into MED habitats in the study region until 2013, when legal restrictions prohibited further releases of non-native brown trout (Araguas et al. 2017; Vera et al. 2023). Other basins in the region (e.g., Muga, Tordera, Besòs, Francolí) currently support brown trout populations derived exclusively from released ATL fish, as no native MED populations are known to have inhabited them. Consequently, these river basins were excluded from the study. Non-native trout species, including rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*), were also introduced in the study basins (Miró and Ventura 2013). However, only a few naturalised populations persist in localised stretches, posing minimal threats from competition or predation to MED populations.

2.2 | Sampling Sites and Trout Abundance

Fish sampling was conducted at 134 sites across the seven major river basins indicated above (Figure 1, Table S1). Sampling sites were selected based on stream reach characteristics, with boundaries defined by major changes in physical characteristics (e.g., slope, flow, habitat connectivity). Between 2014 and 2019, each site was surveyed twice, separated by a minimum of 2 years, under stable flow conditions (late June–October). Fish were sampled using two-pass depletion electrofishing (Zippin 1956) with a backpack electrofisher (Hans Grassl GmbH ELT60II; pulsed DC, 1.3 kW, 300/500 V). Sampling sites ranged from 100 to 150 m in length

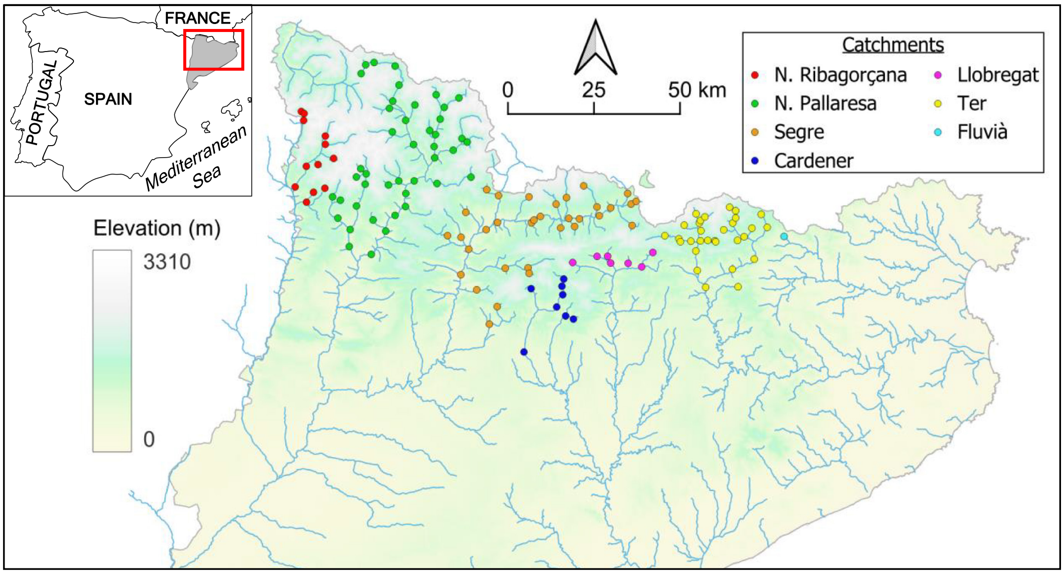

FIGURE 1 | Geographical location of the study area within Catalonia, northwestern Mediterranean Basin. Colour points correspond to sampling sites (134), distributed across seven major catchments.

(mean ± SE = 117.9 ± 1.7 m) and were naturally bounded to minimise fish escape. Captured fish were identified, measured (fork length, FL, in mm), weighed (in g) and released. During the first sampling survey at each site, tissue samples (ventral fin clips) from a subsample of individuals (mean sample size $N \pm SE = 29.05 \pm 0.61$ fish per site) were preserved in 99% ethanol for subsequent genetic analyses.

Fish density was estimated using POP/PRO software (Kwak 1992) and standardised per 100 m stream length. The average of the two surveys was used as the estimated density at each site. Total trout abundance per river reach was extrapolated under the assumption of spatially uniform density. While this approach can underestimate the absolute number of fish present, both because two-pass electrofishing removal methods tend to yield conservative estimates (Riley and Fausch 1992) and because spatial extrapolation may not capture local variations in fish distribution, it provides a sufficiently cautious estimate when the goal is conservation assessment rather than determining precise population sizes (Cowx et al. 2009).

2.3 | Genetic Analyses

DNA was extracted from ventral fin clips of 3935 specimens using the Chelex protocol (Walsh et al. 2013). As described by Araguas et al. (2017) and Vera et al. (2023), whose data are included in this study, fish were genotyped at the lactate dehydrogenase C1 locus (LDH-C1*) and five microsatellite loci (SsHaeIII14.20, Str591INRA, Str73, Ssa85 and SSoSL438) to determine the genetic impact of ATL stocks at both the population and individual levels.

The LDH-C1 genotypes were obtained using a PCR Restriction Fragment Length Polymorphism (PCR-RFLP) protocol (McMeel et al. 2001). The frequency of the LDH-C1*90 allele was used as the primary estimate of population introgression, as it is fixed in ATL stocks and absent from native MED populations (Garcia-Marin et al. 1991; Berrebi et al. 2021). This frequency provided a direct estimate of ATL introgression at each location and was used in subsequent analyses to test its relationship with environmental and anthropogenic variables.

The five microsatellite loci used in this study revealed significant differences between MED and ATL stocks (Fernández-Cebrián et al. 2014; Araguas et al. 2017; Vera et al. 2023). These loci were amplified and genotyped following the methodology described in Araguas et al. (2017). For each individual, estimates of the introgression rate (q) were obtained using the STRUCTURE software following Sanz et al. (2009), when only a baseline for the hatchery ATL stock is available because the native genetic composition before releases of ATL fish is unknown, and assuming an admixture model with two populations (hatchery and native), in which hatchery individuals were constrained to be non-admixed. As in Araguas et al. (2017) and Vera et al. (2023), we used a sample of the stock at the regional hatchery in Bagà collected in 2003, before the banning of hatchery releases. It is important to note that this approach consistently attributes a small fraction (2%–4%) of the genome to ATL even in pure MED individuals (Sanz et al. 2009; Araguas et al. 2017). Microsatellites provide a more efficient tool than the LDH-C1 locus for classifying individuals as native ($q \leq 0.04$), hybrid ($0.04 < q \leq 0.80$) or stocked ($q > 0.80$).

2.4 | Environmental Variables and Stocking Pressure

To examine the relationship between environmental and human variables and the introgression of ATL genes into MED populations, a set of site-specific hydrological and climatic variables was compiled. Catchment characteristics, including altitude (m a.s.l.), drainage area (km^2) and reach slope (m km^{-1}), were extracted from publicly accessible geographic databases. Climatic variables (maximum summer air temperature, minimum winter temperature, precipitation seasonality) were obtained from the BioClim database (Karger et al. 2017).

As stocking has been recognised as the primary driver of introgression in MED populations (Splendiani et al. 2016; Berrebi et al. 2018), the impact of historical stocking practices on introgression rates was evaluated. Historical records of ATL strain releases, spanning from 1984 to 2013, were retrieved from the archives of the Government of Catalonia (Departament d'Acció Climàtica, Alimentació i Agenda Rural, Generalitat de Catalunya; https://agricultura.gencat.cat). Because exact release locations were frequently unspecified, stocking data were aggregated as follows: Fluvià, Ter, Llobregat, Cardener, Segre-mainstem, Segre-tributaries, N. Pallaresa-mainstem, N. Pallaresa-tributaries, N. Ribagorçana-mainstem and N. Ribagorçana-tributaries. An index of stocking intensity was developed to quantify the relative impact of stocked fish on wild populations. The stocking intensity index was calculated as the ratio of the total number of fish stocked over all years to the total estimated trout abundance within a given basin. Because historical trout abundance data at the time of stocking were unavailable, stocking intensity was weighted using current fish abundance estimates, assuming relative long-term stability of trout abundance, as it is largely determined by the natural productivity of each river (Almodóvar et al. 2006).

2.5 | Data Analyses

We delineated the current distribution of MED in Catalonia using reach-specific genetic data. Populations with no evidence of hybridisation with the ATL stocks were categorised as 'Pure MED' or 'Unaltered' (0% introgression). Introgressed populations were categorised as 'Low introgression' (< 5%), 'Moderate introgression' (5%–25%) or 'High introgression' (> 25%). To assess the current status and distribution of MED, we estimated the total length of stream reaches by genetic status. Stream reach lengths (km) were measured using a 1:50,000-scale digital hydrologic layer in QGIS software (http://QGIS.org 2022, http://www.qgis.org). MED distribution data were also used to calculate two key IUCN Red List metrics: area of occupancy (AOO) and extent of occurrence (EOO) (IUCN Standards & Petitions Committee 2024). For both historical and current pure populations, AOO and EOO were estimated using the Geospatial Conservation Assessment Tool (GeoCAT), with a 2 km^2 cell size (Bachman et al. 2011).

Following Arranz et al. (2025), we applied three complementary statistical techniques to disentangle the factors influencing introgression rate and stocking intensity: univariate correlations, hierarchical partitioning (HP) and multiple linear regression (MLR). Altitude, catchment area, slope, maximum and minimum monthly air temperatures, precipitation seasonality, latitude, longitude and (for introgression) stocking intensity were used as predictors. The univariate correlations provide information on the association between predictors. HP provides a better measure of variable importance than MLR by estimating both the unique and shared (common) effects of predictors; thus, it is less affected by collinearity and model selection (Arranz et al. 2025). The full MLR model was obtained to test for unique effects and to provide the sign and magnitude of the relationships. Added-variable plots, also called partial-regression plots, were used to display the relationship between a predictor and the response variable while controlling for the effects of other predictors. We also provide the results of beta regression, which should be preferable for response variables bounded between 0 and 1, but we focus on MLR because it provided the same qualitative results whereas added-variable plots do not seem available for the former technique. All statistical analyses were conducted in R Version 4.5.0 (R Core Team 2025). We used the `PerformanceAnalytics` package (Version 2.0.8; Peterson and Carl 2024) for matrix scatterplots, `rdacca.hp` (Version 1.1.1; Lai et al. 2022) for HP, `car` (Version 3.1.3; Fox and Weisberg 2018) for the added-variable plots, `betareg` (Cribari-Neto and Zeileis 2010) for beta regression and `viridis` (Version 0.6.5; Garnier et al. 2024) for colourblind-friendly graphs.

2.6 | Risk Assessment

Threats to unaltered and low-introgressed MED populations were categorised into four risk classes: (1) demographic/inbreeding risks, (2) introgression from ATL, (3) habitat quality and (4) climate change. Overharvesting risk was excluded due to its reduced impact following the implementation of catch-and-release regulations in most of the rivers within the study area. Demographic risk was assessed using the census size (N_c) of mature adults (> 140 mm FL), assuming a 1:1 sex ratio (Aparicio et al. 2023). This value was used as a proxy for effective population size (N_e), based on the assumption that $N_e \approx N_c$ when sex ratios are balanced and all mature individuals contribute equally to reproduction (Waples 2024). While this provides a conservative estimate—because N_e is typically lower than N_c due to variance in reproductive success—it represents a practical and appropriate approximation for the aims of this assessment. Risk levels were classified as follows: low ($N_e > 2500$), moderate (250–2500) and high (< 250) (Jorde and Ryman 1996; Hansen et al. 2002). Genetic risk increased with proximity to hybridised populations lacking dispersal barriers. Habitat risk was based on channel morphology and flow alterations. Climate risk is linked to altitude, with lower reaches facing greater warming. Thermal habitat projections classified risk by expected habitat loss under mean air temperature increases of 0.5°C (high), 1°C (moderate) and 1.5°C (low) (Almodóvar et al. 2012). Each reach received risk scores (1 = low, 2 = moderate, 3 = high) for each risk class, and the average score determined extinction vulnerability: low (< 1.5), moderate (1.5–1.75) or high risk (≥ 2).

3 | Results

3.1 | Hatchery Introgression

The non-native LDH-C1*90 allele was detected in the majority of sampled localities. Introgression estimates derived from microsatellite loci showed a strong correlation with LDH-C1*90 allele frequency (Pearson's $r = 0.82$; $p < 0.001$). However, the relationship was significantly nonlinear (quadratic linear model, $p < 0.05$) because microsatellite loci tended to provide slightly lower values, particularly when introgression was low (Figure S1). In the majority of sites, ATL stocks have hybridised with MED populations, and only 14 out of 134 (10.4%) analysed populations were genetically unaltered (Table S1).

Between 1984 and 2013, more than 13 million individuals of ATL brown trout were released across the study basins. The Llobregat River received the highest number of introduced individuals (2.85 million), followed by the Segre and N. Pallaresa mainstems. The stocking intensity index was also highest within the Llobregat River, with variation among other basins reflecting the size of the recipient populations (Figure 2).

Stocking intensity was associated with several environmental variables (Figure S2), but HP suggested that it was mostly related to climatic variables (temperature) and a longitudinal gradient (all with 2%–7% of explained individual variation and 0%–7% of unique effects), with the other predictors explaining little variation and no unique effects (Figure S3, Table S2). Stocking intensity slightly increased towards eastern basins and in warmer streams (Figures S2 and 2). Introgression degree was related to many variables (Figure S2), but HP suggested that stocking intensity was by far the most important variable, with 26% of individual contribution and 20% of unique contribution (Figure 3, Table S3). Other significant predictors showed smaller individual contributions: climatic variables (temperature and precipitation seasonality) were most important (3%–5% of explained individual variation each, mostly unique), whereas geographical and physical predictors were least, mostly sharing explained variation with other predictors (Figure 3, Table S3). An MLR confirmed the results of HP (Table S4) and that introgression degree increased with stocking intensity, minimum temperature and decreased with maximum temperature. A beta regression model provided very similar results but suggested that other predictors that were only marginally significant with MLR, such as geographical longitude and precipitation seasonality, also influenced (Table S5). Therefore, higher introgression levels were observed in southern and eastern streams (Figure S2), but this was due to increased stocking intensity and climatic variation (Table S4, Figures 3 and 4). In contrast to univariate correlations (Figure S2), MLR suggested that introgression decreased with increasing maximum temperature after accounting for other predictors (Figures 4 and S4).

3.2 | Range Extension and Abundance of Mediterranean Brown Trout

The historical distribution range of MED in Catalonia was conservatively estimated to be 927.6 km of stream length. Most of this range was located within the N. Pallaresa (32.3%), Segre

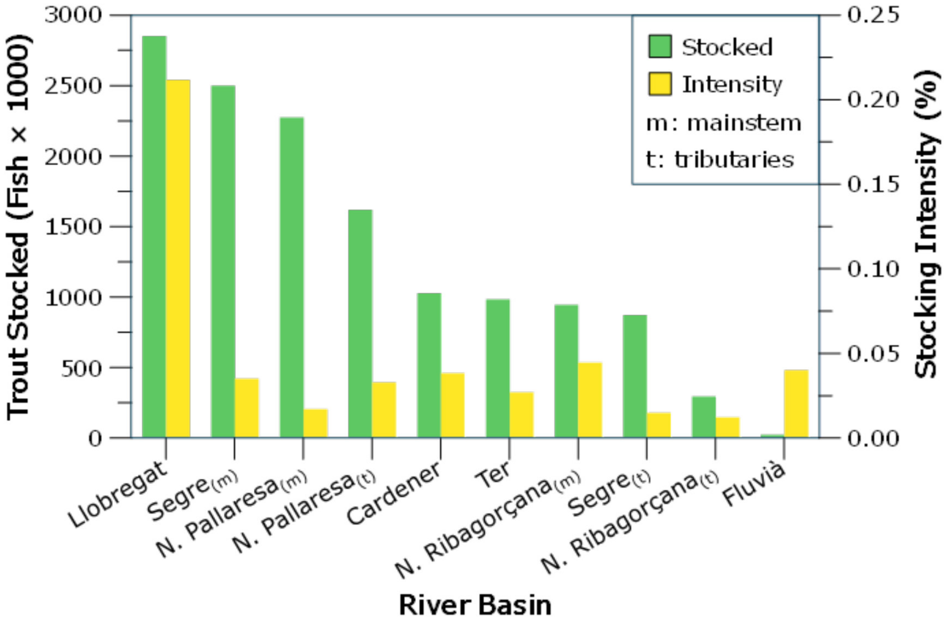

FIGURE 2 | Number of stocked brown trout individuals from 1984 to 2013 and stocking intensity index across major basins.

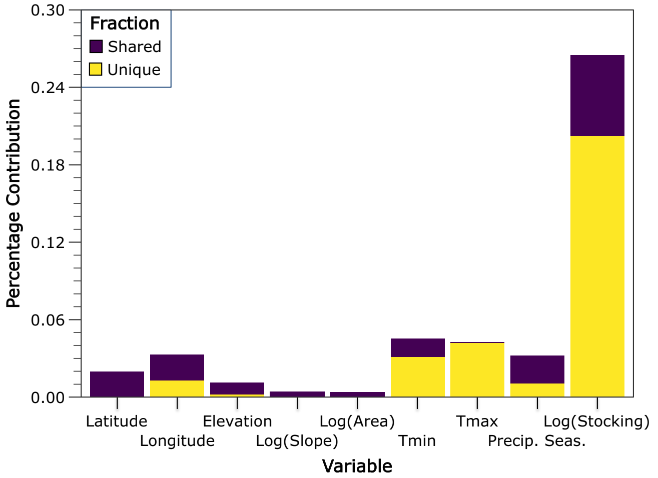

FIGURE 3 | Hierarchical partitioning of the introgression degree of Mediterranean brown trout as a function of the nine predictors. The unique (yellow) and shared (purple) proportional contributions are shown. Slightly negative unique contributions were equated to zero. See Table S3 for permutation tests and further details.

(29.5%) and Ter (18.1%) basins, followed by the N. Ribagorçana (9.2%), Cardener (5.7%), Llobregat (4.8%) and Fluvià (0.4%). The current distribution of brown trout populations (encompassing both genetically pure MED and introgressed) has remained largely consistent in terms of presence, with extirpation occurring in only a limited number of streams ($<1\%$ of the historical range), primarily due to reduced flows or desiccation associated with human activities. Brown trout was the only fish species present in most sampling sites (75.4%).

Genetically unaltered MED populations currently occupy 99.8 km of streams, representing 10.76% of their historical distribution. The remaining 89.24% of the historical range is now inhabited by introgressed populations, with low ($<5\%$), moderate (5%–25%) and high ($>25\%$) introgression detected over 216.2, 365.3 and 246.3 km of stream length, respectively (Figure 5). Approximately half of the genetically unaltered MED populations are found in isolated headwater tributaries. Notably, several mainstem reaches within the N. Pallaresa basin continue to support pure MED populations.

In terms of abundance, linear fish density in genetically unaltered MED populations was categorised as low (<500 fish km^{-1}) in 16.8% of occupied streams, intermediate (500–1000 fish km^{-1}) in 53.2% of occupied streams and high (>1000 fish km^{-1}) in 30.0% of occupied streams. The highest density of MED individuals was recorded in the N. Pallaresa basin, followed by the Ter and N. Ribagorçana basins (Figure 6). MED individuals were not detected in both the Llobregat and Fluvià basins.

The historical EOO and AOO for MED in Catalonia were estimated to be 7464 and 536 km^2, respectively. Currently, these values have declined to 2873 km^2 (EOO) and 56 km^2 (AOO), representing a reduction of 61.5% and 89.6%, respectively.

3.3 | Risk Assessment

A simple risk assessment identified several threats to the persistence of pure or low-introgressed MED populations (Figure S5), which are distributed across a total stream length of 316.0 km. Climate change poses a high risk to 72.2% of this stream length because these reaches are situated in relatively low-elevation areas. Habitat alteration, primarily attributable to hydropower infrastructures, poses a high risk to 24.0% of the area occupied by pure or low-introgressed MED populations. Genetic and demographic risks were generally lower, as many MED populations are isolated from introgressed populations by dams or natural barriers (e.g., waterfalls) and exhibit high densities that maintain sufficiently large effective population sizes. The extinction vulnerability of these critical populations was estimated by averaging the scores of all risk scores. A total of 36.4% of the stream length occupied by pure or low-introgressed

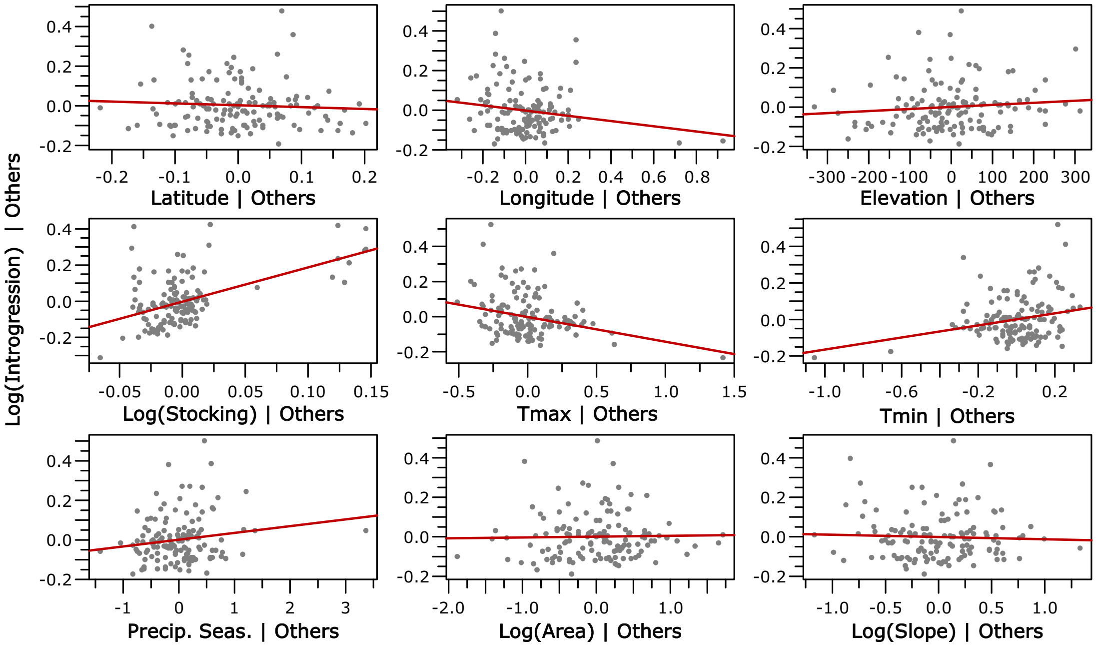

FIGURE 4 | Added-variable plots of the linear model of introgression degree of Mediterranean brown trout as a function of the nine predictors. See Table S4 for further details.

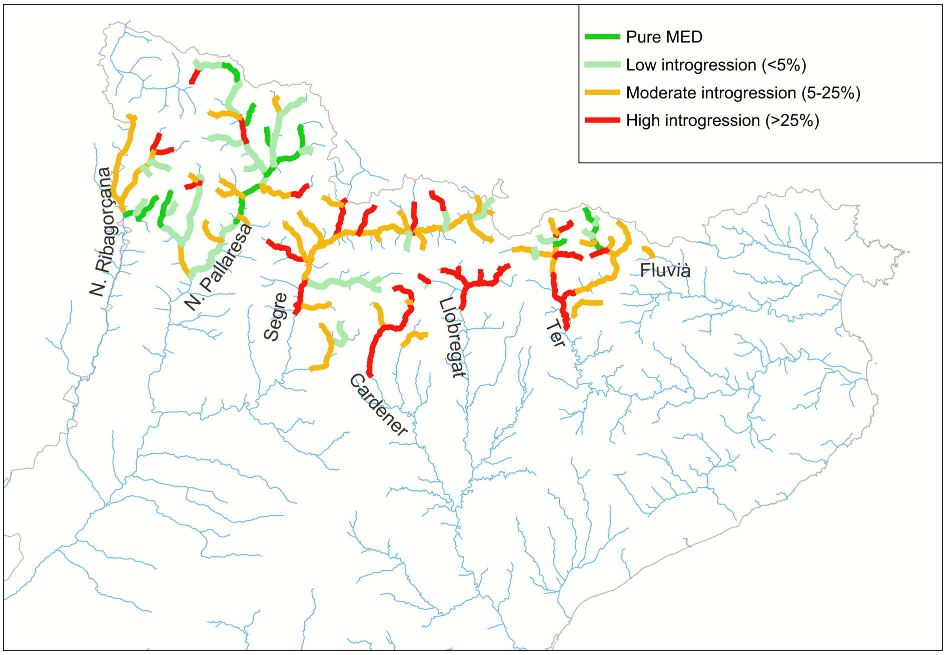

FIGURE 5 | Genetic status of the Mediterranean brown trout in the study area.

MED populations was classified as high risk (mean score ≥ 2), and only 11.6% was categorised as low risk (mean score < 1.5).

4 | Discussion

The findings of this study demonstrate that hybridisation and genetic introgression with ATL brown trout are the principal drivers of the decline of MED populations across north-eastern Iberian rivers. Genetic analyses revealed widespread replacement of native MED genomes by the AT lineage, with stocking intensity emerging as the primary driver of genetic admixture. However, introgression outcomes were also shaped by environmental variables, particularly thermal extremes and precipitation seasonality, which influenced the vulnerability and resilience of MED under Mediterranean climatic regimes. The severe reduction of the MED historical range has confined remnant pure populations to fragmented headwater refugia, with profound implications for their long-term persistence and conservation status.

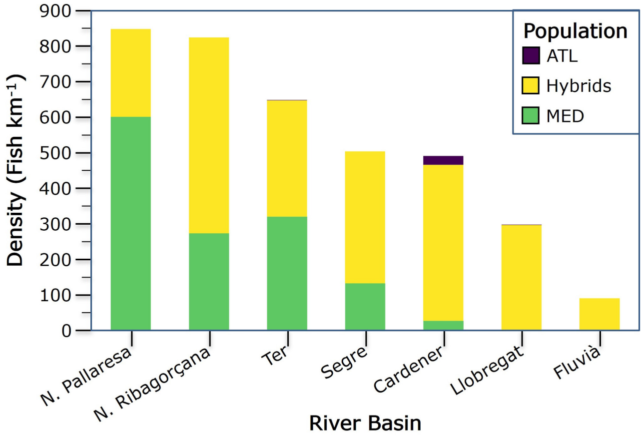

FIGURE 6 | Average density per river basin of Mediterranean brown trout (MED), domesticated Atlantic lineage brown trout (ATL) and their hybrids.

4.1 | The Degree and Correlates of Genetic Introgression

The current distribution of pure MED populations in Catalonia has contracted substantially, declining by almost 90% compared to their historical range. Extant populations are largely confined to headwater streams, where better quality habitats persist and anthropogenic disturbances are less pronounced. However, because the overall distribution of brown trout in Catalonia (encompassing both pure MED and introgressed populations) has not significantly diminished compared to historical data, environmental degradation alone does not fully account for the MED decline. This contrasts with other Iberian fish species, whose distribution ranges have contracted primarily due to ecosystem perturbations (Aparicio et al. 2000; Alcaraz et al. 2015). Instead, this study corroborates that hybridisation and genetic introgression with released ATL stocks are the main drivers of MED population loss. In three of the study basins (Segre, Llobregat and Cardener), the surveys conducted in this study did not detect any native MED populations. The observed decline may accelerate in the short term because nearly half of the extant pure populations persist in small, shallow streams with limited carrying capacity. Although trout densities can be locally high in some of these streams, the overall number of individuals remains low due to spatial constraints. The long-term consequences of ATL releases are reflected in the widespread occurrence of multi-generational hybrids across most populations. However, populations consisting solely of ATL individuals or first-generation (F1) hybrids, indicative of recent hybridisation, are now rare and restricted to isolated localities. The cessation of stocking within the MED distribution range, implemented in 2013 in accordance with European and regional regulations, has mitigated the immediate risk of further genetic introgression. However, previously hybridised populations may contribute to the spread of ATL gene pools into neighbouring streams, as reported by Vera et al. (2023) in the Segre River. Furthermore, ATL stocks are still present in hatcheries operating in Catalonia to supply fish for put-and-take fisheries located in tailwaters and the middle reaches of main rivers beyond the MED distribution area. Consequently, the risk of future hybridisation depends on the continuation and enforcement of current management policies. To circumvent regulatory restrictions on the stocking of non-native trout, some angling associations advocate for breeding programmes using native Mediterranean trout. However, this approach introduces additional risks to the conservation of native diversity as it could lead to the undetected loss of local or regional genetic singularities (Fernández-Cebrián et al. 2014; Leitwein et al. 2018). In Italy, the introduction of domesticated stocks of Mediterranean brown trout has already disrupted the genetic integrity of local populations, thereby complicating future conservation efforts (Talarico et al. 2023; Righi et al. 2024). In addition, prolonged captive breeding can lead to deleterious effects such as domestication, inbreeding and reduced genetic variation, which may negatively affect wild populations when stocked—even if the breeding programme originated from local wild fish (Araki et al. 2008).

The impact of introgression varies across river systems, with some maintaining higher levels of MED resilience while others have experienced near-complete replacement by hybridised populations. Stocking intensity emerged as the principal factor explaining variation in ATL introgression among the river basins studied. Heavily stocked rivers exhibited the highest levels of introgression, consistent with patterns reported in most of the Iberian basins (Almodóvar et al. 2006). Although stocked trout often exhibit reduced performance in natural environments compared to locally adapted native genotypes (Hansen et al. 2002; Rusco et al. 2023), sustained stocking pressure can counteract the rate at which stocked trout are removed by natural selection, leading to increased hybridisation over time (Hansen et al. 2002; Bourret et al. 2022). Moreover, adaptive introgression may facilitate the persistence of exotic alleles within native gene pools. Talarico et al. (2021) suggested that rare allele advantage (RAA) at the major histocompatibility complex (MHC) could promote introgression in Italian MED trout, as alleles introduced at low frequency through hybridisation with hatchery fish may gain a selective advantage under negative frequency-dependent selection. Such alleles can be maintained and even spread within populations, particularly at adaptive loci like the MHC, or at linked genes through hitchhiking. However, large-scale hatchery trout releases do not always lead to high introgression rates, as shown in Italy, where the resulting degree of ATL introgression varied among streams and was not always proportional to stocking intensity (Splendiani et al. 2016). Similarly, in Iberian rivers, MED populations are more prone to hybridisation with ATL stocks than wild Atlantic populations, even under similar stocking intensities (Almodóvar et al. 2006). Therefore, factors other than stocking intensity influence introgression outcomes, as evidenced by previous studies identifying hydrological dynamics, population demography and habitat connectivity as significant contributors to the modulation of introgression patterns (Splendiani et al. 2016; Vera et al. 2023).

In this study, temperature significantly influenced introgression dynamics. Higher average maximum temperatures and lower average minimum temperatures were both associated with reduced levels of introgression. Thermal tolerance is known to vary among species and lineages as a consequence of adaptive responses to local environmental histories, and populations inhabiting highly variable environments often evolve greater thermal plasticity, enabling acclimation to a broader temperature range under strong selection pressures (Seebacher et al. 2012; Meek et al. 2025). Consistent with this, previous studies have shown that AT lineage offspring exhibit lower survival rates at low temperatures (Ojanguren and Braña 2003; Régnier et al. 2013), while MED juveniles present higher survival rates in cold environments (Folio et al. 2021). Moreover, the observed negative relationship between high maximum temperatures and introgression rate may therefore reflect an adaptive trait of MED, which is better suited to high summer temperatures typical of the Mediterranean region (Splendiani et al. 2024).

Introgression rate is also influenced by hydrological variability. Trout populations inhabiting Mediterranean catchments characterised by unstable flow regimes are more vulnerable to genetic displacement (Splendiani et al. 2013; Vera et al. 2023). Consistent with these findings, our analyses showed a significant association between precipitation seasonality, which was used as a proxy for hydrological variability, and elevated hybridisation rates. One possible explanation for this pattern is that hydrological instability at lower latitudes may have impeded the establishment of high-density native brown trout populations prior to the onset of stocking programmes (Nicola et al. 2009). This, in turn, could have facilitated introgression following large-scale releases of hatchery trout (Splendiani et al. 2013).

4.2 | Conservation Status and Management Implications

Our risk assessment identified climate change and habitat alterations as the most important threats to the remaining MED populations. These populations occupy the southernmost extent of salmonid distribution in Europe—a region particularly susceptible to reduced water availability (Lionello and Scarascia 2018). As a result, local river systems are exposed to both rising temperatures and more frequent, prolonged droughts, further exacerbating unfavourable conditions for trout survival (Jonsson 2024). This warming trend is driving an upstream shift in the thermal range suitable for MED populations (Almodóvar et al. 2012), accompanied by a decline in adult trout densities (Tissot et al. 2025). Within the study area, up to 74% of the stream length currently occupied by MED populations is situated in relatively low-elevation areas, thereby increasing their vulnerability to climate change. Habitat degradation, primarily due to hydrological alterations induced by hydropower operations, further threatens MED populations. Nearly half of the stream length occupied by MED has been classified as being at medium to high risk due to these disturbances. Small hydropower plants divert water, resulting in lowered water levels, reduced fish cover and degraded mesohabitat quality, ultimately leading to declines in trout abundance and condition (Benejam et al. 2016).

The assessment of the conservation status of MED is currently complicated by taxonomic uncertainty and data limitations. In the study area, MED has experienced a 61.5% reduction in EOO, from 7464 to 2873 km^2, and an 89.6% reduction in AOO, from 536 to 56 km^2. Although these declines mostly coincided with the period of intensive stocking practices between 1960–1970 and 2012 (i.e., over a longer timeframe than the last 10 years required for IUCN Criterion A), they clearly meet the thresholds for classification as Endangered under Criterion B (EOO $<$ 5000 km^2; AOO $<$ 500 km^2, with continuing decline). A definitive conservation assessment is constrained by the lack of detailed data from other Mediterranean river systems, but evidence from Catalonia provides strong support for an Endangered status. Moreover, elevated introgression rates reported in other Mediterranean basins of Spain (Almodóvar et al. 2006; Sanz et al. 2006; Vera et al. 2013), France (Berrebi et al. 2000) and Italy (Splendiani et al. 2016) suggest that similar declines are occurring across the broader range of MED. Although some putative species have already been granted elevated threat status—such as *S. ghigii* (Endangered) and *S. cetti* (Critically Endangered) in the 2025 IUCN assessments (Ford and Duchi 2024; Ford 2025)—Mediterranean populations in the Iberian Peninsula remain taxonomically subsumed within *S. trutta*, which is currently listed as Least Concern.

This misclassification poses a substantial challenge to conservation efforts, as legislative and regulatory frameworks typically rely on formal taxonomic status (Cook et al. 2023). Without a standardised classification, some MED populations lack the legal protections necessary for targeted conservation measures. Under integrative taxonomy criteria for species delineation (de Queiroz 2007; Pante et al. 2015), MED could be classified as a distinct species due to its well-established genetic differentiation and large genomic divergence from other lineages (Leitwein et al. 2016; Casanova et al. 2022). Divergence estimates suggest that MED split from other brown trout lineages between 500,000 and 1.2 million years ago (Bernatchez 2001; Sanz 2017). Additional supporting evidence includes morphological differentiation (Aparicio et al. 2005) and geographic isolation (Sanz 2017). Although some researchers advocate for the recognition of Iberian MED as an Evolutionarily Significant Unit (ESU) (Almodóvar et al. 2006), this classification lacks legal recognition within the majority of European legislation. For example, Directive 92/43/EEC of the European Council on habitat and species conservation, EU LIFE Programme and the IUCN Red List acknowledge only formally described species, not ESUs. This limitation restricts access to conservation funding and legal protections for MED populations. Therefore, expediting a formal taxonomic description is essential for securing long-term conservation resources and ensuring effective management strategies.

Alongside taxonomic clarification, immediate conservation actions are urgently needed. The highest priority should be the identification and protection of the remaining genetically pure MED populations, the designation of genetic refuge areas where these populations are located and the prevention of further introgression by maintaining or enforcing policies that prohibit the release of non-native stocks. The recovery of pure MED populations should not rely solely on preventing further introductions of non-native trout because natural selection alone appears insufficient to eliminate non-native gene pools within the study region (Araguas et al. 2017). Therefore, active management interventions, such as electrofishing campaigns to remove non-native and hybrid trout, followed by the translocation of genetically pure MED individuals from the geographically and genetically closest source population, should be considered (Caudron et al. 2011; Carosi et al. 2020).

Effective implementation of such measures may be supported by adapting models from established conservation programmes in other Mediterranean regions. For example, two EU-funded LIFE projects in Italy—LIFE TROTA and LIFE STREAMS—implemented coordinated strategies to restore Mediterranean trout populations by rehabilitating habitats, removing non-native genotypes and reinforcing populations with genetically pure stocks (Carosi et al. 2025). These projects utilised the listing of *Salmo macrostigma* in Annex II of the EU Habitats Directive, despite the name being taxonomically outdated. However, because Iberian MED populations are officially recognised as *S. trutta*, a species not covered by the Directive, this prevents access to comparable EU conservation instruments.

Mitigating the impacts of rising water temperatures due to climate change is complex and challenging. The most feasible strategy appears to be the genetic restoration of MED populations in higher-altitude stream sections through the selective removal of non-native trout and the re-establishment of MED lineages, as previously discussed. Effective implementation of this approach requires the regulation of water extraction in target streams to ensure adequate flow regimes, which are critical for thermal buffering and habitat stability (Fuso et al. 2023).

In summary, our results demonstrate that MED is threatened by genetic introgression, climate change and other environmental pressures. Its effective conservation requires formal recognition of its evolutionary distinctiveness, which transcends current taxonomic classifications.

Author Contributions

Conceptualisation: E.A., E.G-B. and J-L.G-M. Developing methods: E.A., J-L.G-M., M.V., S.H. and R.R. Conducting the research: E.A., R.R., J-L.G-M., M.V. and S.H. Data analysis and data interpretation: E.A., E.G-B., J-L.G-M., M.V. and C.A. Preparation of figures and tables: E.A., E.G-B., R.R. and C.A. Writing: E.A., J-L.G-M., E.G-B., C.A., M.V., S.H. and R.R.

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Ethics Statement

All procedures complied with applicable Spanish and EU regulations for animal research and welfare.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.30449975.v1.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Summary of sampling site characteristics, including geographic coordinates, date of genetic sampling, number of genotyped individuals (N), estimates of genetic introgression (%) based on the frequency of the *LDH-C1**90 allele (LDH), classification of individuals as pure Mediterranean (MED), hybrids, or Atlantic (ATL) based on microsatellite markers (Ms), and effective population size (N_e) estimates used for demographic risk assessment in pure and low-introgressed populations. **Table S2:** Hierarchical partitioning of stocking intensity with the eight predictors. The unique and average shared proportional contributions for each predictor and the total are given. Individual importance is the sum of the unique and shared contributions. The *p* value corresponds to the permutation tests of individual contributions with 999 permutations; significant *p* values ($p < 0.05$) are bolded. **Table S3:** Hierarchical partitioning of the degree of introgression with the nine predictors. The unique and average shared proportional contributions for each predictor and the total are given. Individual importance is the sum of the unique and shared contributions. The *p* value corresponds to the permutation tests of individual contributions with 999 permutations; significant *p* values ($p < 0.05$) are bolded. **Table S4:** Multiple linear regression model of introgression degree with the nine predictors. $R^2_{adj} = 0.440$, $n = 136$. **Table S5:** Beta regression model (logit function) of introgression degree with the nine predictors. NA = not available. **Figure S1:** Relationship between the introgression degree of Mediterranean brown trout measured with LDH-C1*90 allele frequency (LDH) and microsatellite markers (Ms). On top, the Spearman correlation and a smother is given (histogram in the diagonal). On bottom, linear regression and Pearsons correlation (***, < 0.001). **Figure S2:** Pairwise relationships between the degree of introgression in Mediterranean brown trout and the nine predictors. Below the diagonal, the bivariate scatterplots with the linear regression function are shown. Above the diagonal, Pearson correlation coefficients are displayed, with font size proportional to the absolute value of the coefficient. Significance levels are indicated as follows: (▪, $p < 0.10$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). **Figure S3:** Hierarchical partitioning of stocking intensity of Mediterranean brown trout as a function of eight predictors. The unique (yellow) and shared (purple) proportional contributions are shown. Slightly negative unique contributions were equated to zero. See Table S3 for permutation tests and further details. **Figure S4:** Relationship of the degree of introgression with stocking intensity and maximum temperature. The linear model is shown. Note the logarithmic scales for both axes. **Figure S5:** Extent of stream length occupied by unaltered and low-introgressed populations of Mediterranean brown trout affected by low, medium, and high risk levels for the evaluated risk factors.