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**Stage-structured prediction of establishment, spread and integration of non-native freshwater fishes: within-country translocations pose greater risk**

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20 **Abstract**

21 Building on trait-based invasion theory, we developed a stage-structured profiling framework  
22 to evaluate both foreign introductions and extralimital species (within-country translocations  
23 beyond historical ranges) of non-native freshwater fishes in Greece, predicting establishment,  
24 spread and integration. We compiled a dataset of 63 species (36 foreign, 27 extralimital), and  
25 characterized each species with ecological, biogeographic, and anthropogenic attributes.  
26 Predictors were evaluated with cross-validated logistic/multiple regression and CART models  
27 including a taxonomy-based covariate to reduce bias from shared ancestry. Extralimital  
28 translocations were frequent and all 27 species established, emphasizing their disproportionate  
29 role in reshaping regional faunas. Establishment increased with physiological tolerance and  
30 proximity to the nearest native source, but declined with maximum body size, and propagule  
31 pressure showed only weak additional support. Spread across drainage basins was driven  
32 mainly by introduction effort and physiological tolerance. Integration increased with  
33 introduction effort, while CART identified distance from nearest native source as the primary  
34 discriminator of widespread and abundant outcomes, with trophic level further structuring  
35 outcomes among extralimital taxa. These results indicate that management frameworks  
36 focused solely on foreign non-native fish species may underrepresent risk from within-country  
37 translocations. Incorporating both pathways into screening and surveillance can strengthen  
38 prevention and early detection, addressing a European policy gap where translocations remain  
39 more weakly regulated than foreign introductions. Our study provides a practical, trait- and  
40 pathway-informed screening tool that integrates organismal traits with invasion-history and  
41 biogeographic proxies to guide prevention, pathway management and targeted monitoring in  
42 Mediterranean river networks.

43

44 **Keywords:** establishment, extralimital, foreign, integration, quantitative risk assessment,  
45 spread

46

## 47 **Introduction**

48 Freshwater ecosystems are essential to human well-being and global biodiversity, yet  
49 they remain highly vulnerable to degradation (Haubrock et al. 2025). Freshwater areas with  
50 high species richness, or high endemism (Vavalidis et al. 2025), often coincide with intensive  
51 human use, creating extinction-risk hotspots (Reid et al. 2019). Habitat alteration, pollution,  
52 over-exploitation, invasive species and climate change jointly continue to drive the decline of  
53 freshwater biota (Reid et al. 2019; Su et al. 2021; Sayer et al. 2025). Among the most widely  
54 introduced vertebrates, freshwater fishes have established self-sustaining populations across  
55 nearly all biogeographic realms (except Antarctica), reshaping regional ichthyofaunas and  
56 leaving a pronounced Anthropocene signature (Gozlan et al. 2010; Bernery et al. 2022;  
57 Leroy et al. 2023). Once established, non-native fish species (hereafter NNFS) may impose  
58 ecological impacts that depend on interactions among invader traits (e.g., trophic position, body  
59 size, tolerance and life history), introduction conditions (e.g., propagule pressure) and recipient  
60 ecosystem properties (e.g., habitat structure and species richness - biotic resistance). Where  
61 these factors align, NNFS may negatively alter native assemblages through predation, resource  
62 competition, hybridization and ecosystem disruption, thereby driving genetic, community and  
63 ecosystem-level processes (Cucherousset and Olden 2011).

64 The invasion process of NNFS in a new region can be divided into a series of stages:  
65 transport, introduction, establishment, spread, followed ultimately by ecological and/or socio-  
66 economic impacts in the recipient area (Blackburn et al. 2011; Lawson and Hill 2021). Each  
67 stage is separated by physical, biological, or environmental barriers that species must overcome  
68 (Blackburn et al. 2011). Because organisms face different challenges at each stage, traits that  
69 promote success in one stage might be irrelevant or even detrimental at the next (Kolar and  
70 Lodge 2002; Cassey et al. 2004; Blackburn et al. 2011; Richardson and Pyšek 2012). Invasion  
71 process models therefore treat progression as a series of conditional probabilities, requiring  
72 species to complete one stage before advancing to the next (Hill 2008; Leung et al. 2012).  
73 Human-mediated introductions may intentionally or accidentally favour species with tolerant  
74 or desirable characteristics, thereby increasing the likelihood of overcoming early barriers  
75 (Blackburn and Duncan 2001; Jeschke and Strayer 2006). While the establishment phase has  
76 been the most extensively studied (García-Berthou 2007; Hayes and Barry 2008), species must  
77 first survive transport and introduction before establishment can occur (Kolar and Lodge 2002;  
78 Hill 2008).

79 Previous studies have shown that the factors predicting success at each invasion stage  
80 can differ, with much research focusing on traits linked to establishment (García-Berthou 2007;

81 Bernery et al. 2022). Although predictors vary, several traits are consistently associated with  
 82 higher establishment probability. For example, climatic similarity between a species' native  
 83 range and the introduced region functions as an environmental filter and a prerequisite for  
 84 survival and reproduction at the recipient ecosystem (Bomford et al. 2010; Howeth et al. 2016).  
 85 Likewise, a prior history of invasion success elsewhere is positively correlated with  
 86 establishment probability, the more ecosystems in which a species has established, the stronger  
 87 its record of invasion success, rather than a standalone causal driver (Kolar and Lodge 2002;  
 88 Marchetti et al. 2004a,b; Ribeiro et al. 2008). Successful invaders often share life-history and  
 89 ecological traits such as larger body size (Marchetti et al. 2004a,b; Ribeiro et al. 2008; Lawson  
 90 and Hill 2021; Lawson et al. 2025), broad physiological tolerance to environmental conditions  
 91 (Kolar and Lodge 2002; Marchetti et al. 2004a,b; Lawson et al. 2025), flexible or low spawning  
 92 requirements (Mandrak 1989; Olden et al. 2006), and generalist or high-level trophic status  
 93 (Marchetti et al. 2004a,b; Liu et al. 2017). In contrast, traits associated with ecological impacts  
 94 are more variable (García-Berthou 2007; Bernery et al. 2022; Britton 2023), suggesting that  
 95 impact severity is highly context-dependent. Moreover, the predictive values of traits can vary  
 96 geographically, with factors linked to establishment in one region proving less relevant  
 97 elsewhere (Bernery et al. 2022; Britton 2023). This regional variability emphasizes the need  
 98 for additional research and more contextualised models to improve predictions across invasion  
 99 stages under diverse ecological and socio-economic settings.

100 Greek freshwater ecosystems represent a critical hotspot in the Mediterranean region  
 101 for trait-based risk screening of NNFS. Under a biogeographic criterion of nativeness (Soto et  
 102 al. 2024; Vilizzi et al. 2025), translocation of a narrowly endemic taxon to another ecosystem,  
 103 even within the same biome or ecoregion, renders it non-native if the recipient site lies outside  
 104 its natural native range. Over the past century, at least 63 NNFS have been recorded in Greece  
 105 across numerous drainages (Economidis et al. 2000; Economou et al. 2007; Perdikaris et al.  
 106 2016; Koutsikos et al. 2019a; Koutsikos et al. 2021; Vardakas et al. 2022; Koutsikos et al.  
 107 2025), comprising 36 foreign NNFS (introduced across international borders; Richardson &  
 108 Pyšek 2008), and 27 extralimital NNFS (within-country translocations across biogeographic  
 109 barriers; native in part of the country, non-native elsewhere; Robinson et al. 2016). Dominant  
 110 invaders such as the Pumpkinseed sunfish *Lepomis gibbosus*, the Topmouth gudgeon  
 111 *Pseudorasbora parva* and the Eastern mosquitofish *Gambusia holbrooki* have accelerated  
 112 faunal homogenisation and threatened endemic species such as the two Greek killifish *Valencia*  
 113 *letourneuxi* and *Valencia robertae* (Kalogianni et al. 2019), ultimately degrading freshwater  
 114 ecosystem services (Koutsikos et al. 2021; 2025). The main vectors include escapes from

115 aquacultures, angling/fish bait, intentional stocking and the ornamental aquarium trade  
 116 (Koutsikos et al. 2019a; 2021; 2025), aligning with the principal pathways recognised for  
 117 freshwater fish invasions in Europe (Nunes et al. 2015; Piria et al. 2018). Ongoing propagule  
 118 pressure through these pathways highlights the need for predictive, region specific risk  
 119 assessment tools to prevent further introductions into Greece’s biodiverse yet vulnerable  
 120 freshwaters.

121 Here, we develop a stage-structured profiling framework that evaluates introductions  
 122 and within-country translocations of NNFS in Greece and predicts three sequential outcomes:  
 123 establishment, spread and integration. Using a comprehensive species list (Perdikaris et al.  
 124 2010; Koutsikos et al. 2019a, 2021, 2025; Vardakas et al. 2022), we jointly analyse successful  
 125 and failed introductions of both foreign and extralimital NNFS, which are categories that were  
 126 rarely evaluated together (Ruesink 2005; Lawson and Hill 2021; Su et al. 2023), to enhance  
 127 the robustness and predictive power of trait-based invasion models. This approach addresses  
 128 well-recognised gaps in invasion ecology, where failures are often overlooked and  
 129 translocations underexplored, and generates broader insights into the traits driving invasion.  
 130 We examine invasion as three discrete stages, establishment, spread, and integration, and  
 131 model each stage independently using classification and regression trees, and logistic or  
 132 multiple linear regressions (Kolar and Lodge 2002; Marchetti et al. 2004a,b; Ruesink 2005;  
 133 Ribeiro et al. 2008; Allen et al. 2013; Howeth et al. 2016, 2025; Lawson & Hill 2021). After  
 134 identifying key life-history traits of NNFS in Greek freshwaters, we address four questions: (i)  
 135 how do life-history traits differ between successful and failed introductions? (ii) which  
 136 introduction-related factors and traits best predict the spread of established NNFS? (iii) what  
 137 characteristics facilitate the integration of NNFS into Greek fish communities? and (iv) which  
 138 modelling approach offers the highest predictive accuracy at each invasion stage? The resulting  
 139 trait-screening assessment is intended to guide management strategies and policy aimed at  
 140 preventing invasion pathways and protecting the ecosystem services of freshwater systems.

141

## 142 **Materials and methods**

### 143 *Study area*

144 Greece’s hydrologic network is highly fragmented (Fig. 1), with numerous drainage  
 145 basins descending from a predominantly mountainous landscape and draining into an  
 146 extensive, convoluted coastline (Economou et al. 2007; Skoulikidis et al. 2009).  
 147 Biogeographically, the country is divided into eight freshwater ecoregions, namely Thrace,  
 148 Macedonia-Thessaly, Southeast Adriatic, Ionian, Western Aegean, Crete, Eastern Aegean and

149 South Anatolia (Zogaris and Economou 2017). Each supports a distinctive freshwater fish  
 150 fauna, with the exception of the islet territory located in the South Anatolian ecoregion, which  
 151 lacks perennial water bodies and thus fishes. Further, species richness in Greece exhibits strong  
 152 geographic structure, with higher species richness toward central and northeastern Greece  
 153 while endemism is greatest toward the west, northwest and south (Zogaris and Economou  
 154 2017).

155

156 *Data acquisition*

157 We compiled a list of NNFS species recorded in Greek inland waters, incorporating  
 158 both successful and failed introductions (Economou et al. 2007; Barbieri et al. 2015; Koutsikos  
 159 et al. 2019a; Vardakas et al. 2022). A species was classified as successful when reproduction  
 160 had been documented in the wild and as failed when establishment was explicitly reported as  
 161 unsuccessful or when no evidence of reproduction was available. Variable selection and  
 162 definitions follow descriptions widely used in trait-based invasion studies (Marchetti et al.  
 163 2004a,b; Ribeiro et al. 2008; Lawson & Hill 2021; Marcolin et al. 2025; Table 1) and were  
 164 categorised as follows. We extracted eleven variables and grouped them into three categories:  
 165 (1–6) ecological and biological traits: maximum adult size, maximum lifespan, maximum  
 166 fecundity, parental care, physiological tolerance, and trophic level; (7–8) biogeographical  
 167 parameters: size of native range and distance from nearest native source; and (9–11)  
 168 anthropogenic factors: prior invasion success, propagule pressure, and introduction effort. For  
 169 each species, we searched Google Scholar and Web of Science using scientific and common  
 170 names, with “life history” as an additional keyword. We extracted trait data for Greek NNFS  
 171 primarily from the literature (Economidis et al. 2000; Economou et al. 2007; Zenetos et al.  
 172 2009; Perdikaris et al. 2010; Barbieri et al. 2015; Koutsikos et al. 2019a; Vardakas et al. 2022)  
 173 but also from databases such as FishBase (Froese and Pauly 2021), IUCN (IUCN 2025) and  
 174 grey literature to fill remaining gaps. With the exception of prior invasion success and trophic  
 175 level, all variables were coded as categorical or ordinal due to limited availability of reliable  
 176 continuous data.

177

178 Maximum adult size (MAS)

179 We recorded the maximum total or standard length (L, cm) attained in the wild, assigning  
 180 individuals to the following categories to reduce measurement bias: 1:  $L < 10$ ; 2:  $10 \leq L < 20$ ;  
 181 3:  $20 \leq L < 40$ ; 4:  $40 \leq L < 80$ ; 5:  $80 \leq L < 160$ ; 6:  $L \geq 160$  cm.

182 Maximum lifespan (ML)

183 We defined lifespan as the maximum age observed for wild individuals under natural  
184 conditions, categorised as: 1:  $\leq 2$  yr; 2: 3–4 yr; 3: 5–8 yr; 4: 9–16 yr; 5:  $> 16$  yr.

185

186 Maximum fecundity (MF)

187 We quantified MF as the highest number of eggs per female recorded under natural conditions  
188 on a five-point scale: 1:  $MF < 100$ ; 2:  $100 \leq MF < 1,000$ ; 3:  $1,000 \leq MF < 10,000$ ; 4:  $10,000 \leq$   
189  $MF < 100,000$ ; 5:  $MF \geq 100,000$  eggs.

190

191 Parental care (PC)

192 We categorised PC as: 1: open-substrate spawners with no parental care; 2: brood hiders that  
193 hide eggs but provide no further care; 3: guarders that guard embryos and/or larvae; and 4:  
194 bearers that carry their embryos.

195

196 Physiological tolerance (PT)

197 We coded tolerance to sub-optimal water quality (temperature, dissolved oxygen, turbidity,  
198 salinity) as follows: 1: intolerant, fishes with low tolerance to change or extremes in water  
199 quality (e.g. salmonids); 2: moderately tolerant fishes capable of living in water with moderate  
200 variability in water quality (e.g. sturgeons); 3: tolerant fishes capable of living in waters in  
201 which water quality often reaches their limits of physiological tolerance for short periods (e.g.  
202 cyprinids); 4: extremely tolerant fishes capable of living in systems with poor water quality  
203 (e.g. centrarchids, poecilids).

204

205 Trophic level (TL)

206 We treated TL as a continuous variable using FishBase. TL was extracted from DietTroph  
207 when based on quantitative stomach-content data, or FoodTroph when inferred from qualitative  
208 food-item lists. Values typically span from 2.0 for primary consumers (herbivores/detritivores)  
209 to  $\geq 4.5$  for large piscivores, with higher TL indicating greater reliance on animal prey.

210

211 Size of native range (SNR)

212 We quantified native range size ( $\text{km}^2$ ) for all NNFS using distribution polygons from the IUCN  
213 Red List of Threatened Species, acknowledging that precise distributional ranges are  
214 unavailable for many species. Areas were calculated in an equal-area projection. We then  
215 classified SNR using a four-level categorization: 1, range occupies  $< 5\%$  of a single  
216 zoogeographic sub-region (e.g., Yliki roach *Rutilus ylikiensis*); 2, 5–50% of one sub-region

217 (e.g., Thracian chub *Squalius orpheus*); 3, >50% of one sub-region (e.g., Bighead carp  
 218 *Hypophthalmichthys nobilis*); and 4, range extending across >1 sub-region (e.g., European  
 219 perch *Perca fluviatilis*), following Abell et al. (2008). Species occurring in more than one sub-  
 220 region but with a very restricted distribution (<5% in each) were scored as “3” to reflect their  
 221 limited overall occupancy (e.g. Greek barbel *Luciobarbus graecus*). For eight species lacking  
 222 IUCN spatial data, we drew on comparable trait-based invasion studies and expert knowledge  
 223 to assign SNR classes (Marchetti et al. 2004a,b; Ribeiro et al. 2008; Lawson and Hill 2021;  
 224 Marcolin et al. 2025).

225

226 Distance from nearest native source (DNNS)

227 We coded DNNS as an ordinal variable, because exact distances were not known: 1: within  
 228 Hellenic Peninsula; 2: Central and Eastern Europe east of the Danube eastwards; 3: Western  
 229 Europe to the Danube River (excluded); and 4 = intercontinental.

230

231 Prior invasion success (PIS)

232 We quantified PIS as the number of countries in which each species has established self-  
 233 sustaining populations, using records from the FAO Introduced Species Fact Sheets database  
 234 (<https://www.fao.org/fishery/en/introsp/search>). To improve normality and homoscedasticity,  
 235 we log-transformed this count as  $\ln(x + 1)$  (LnPIS) before analysis.

236

237 Propagule pressure (PP)

238 We quantified PP as the number of individuals released in unsuccessful introductions and the  
 239 number of individuals used to establish the first self-sustaining population in successful  
 240 introductions. Four ordinal classes were assigned: 1: < 100 inds, typically small releases by  
 241 anglers or aquarists; 2: 100–1,000 inds, generally escapees from aquaculture facilities; 3 =  
 242 1,000–10,000 inds, commonly associated with water transfers or forage-fish stockings; and 4:  
 243 > 10,000 inds, usually the result of large-scale recreational-fishery stocking programmes.  
 244 These categories reflected the coarse resolution of historical records and expert reconstructions  
 245 of likely release sizes. We assumed that unrecorded, illegal introductions by anglers (e.g.  
 246 Aristotle's catfish *Silurus aristotelis*) or aquarists (e.g. the Sailfin molly *Poecilia latipinna*)  
 247 involved < 100 individuals. For some of the native species translocated via water-transfer  
 248 schemes, we assigned a propagule size of 100–1,000 individuals. For certain translocated  
 249 species that established populations outside of their native range through water transfers, we  
 250 estimated propagule size to be 100–1,000 individuals.

251

252 Introduction effort (IE)

253 We quantified IE as the number of introduction release events in Greek freshwater: 1 = a single  
 254 introduction (e.g., the Western Greece goby *Economidichthys pygmaeus*); 2 = several  
 255 introductions by aquaculture escapees, aquarists, or anglers (e.g. the European catfish *Silurus*  
 256 *glanis*); and 3 = repeated introductions conducted by government run stocking programs (e.g.,  
 257 the Common carp *Cyprinus carpio*).

258

259 To account for potential effects of shared ancestry, we included a phylogenetic  
 260 covariate following the approach of Howeth et al. (2016, 2025). Species were assigned an  
 261 ordinal rank based on family membership, with families ordered from basal to more derived  
 262 lineages according to Nelson (2006). Post-2006 cypriniform splits (e.g. Leuciscidae,  
 263 Xenocyprididae) were treated at the Cyprinidae level to maintain consistency with the Nelson  
 264 framework. This ordinal scale was incorporated as a family-rank phylogenetic correction  
 265 (Tax\_rank) to reduce bias from phylogenetic non-independence (Howeth et al. 2025).

266 A binary response variable (1 = successful, 0 = unsuccessful) was defined to evaluate  
 267 establishment among NNFS. Because reliable counts of successful and unsuccessful  
 268 translocations were available (Koutsikos et al. 2019a; Vardakas et al. 2022), translocated NNFS  
 269 were incorporated into the dataset.

270 We quantified spread as the number of distinct drainage basins in Greece in which each  
 271 species was recorded, restricting counts to occurrences most plausibly resulting from human-  
 272 mediated introductions rather than natural dispersal from adjacent basins. We considered 140  
 273 basins, delimited based on native ichthyofaunal composition, with endemic species indicating  
 274 isolation. A detailed map of the drainage-basin framework, together with further  
 275 methodological information on sampling and basin delineation, is provided in Koutsikos et al.  
 276 (2019a). Drainage basins served as the reporting unit because they encompass entire  
 277 watersheds (including lentic and/or lotic habitats), thereby capturing spatial extent even when  
 278 the precise limits of spread are uncertain, given that many species continue to expand their  
 279 ranges. To avoid duplication, multiple records of the same species within a basin (e.g., from  
 280 different sites or from both lentic and lotic environments) were consolidated into a single basin-  
 281 level presence. In addition, isolated lentic ecosystems, notably natural lakes with distinctive  
 282 fish assemblages and no surface connectivity to surrounding drainage networks, were treated  
 283 separately to reflect their unique faunal composition. For modelling, counts were transformed  
 284 as  $\ln(x + 1)$  (Marchetti et al. 2004a,b; Ribeiro et al. 2008).

285 For the integration stage, we quantified the average value of species distribution and  
286 abundance per drainage basin for Greece as a response variable, categorized in the following  
287 way: 1: species very rare, probably a single record; 2: isolated population in small, restricted  
288 region/s; 3: species widespread and abundant everywhere. This classification followed  
289 Macdonald et al. (2003), as adapted by Koutsikos et al. (2019a; 2021) and was validated by the  
290 authors' personal knowledge of Greek watersheds.

291

### 292 *Data analysis*

293 We examined three stages of the invasion process for NNFS in Greece, establishment,  
294 spread and integration, following Marchetti et al. (2004a; 2004b), Ribeiro et al. (2008) and  
295 Lawson et al. (2021). For each stage, we began with a full model and applied a consistent  
296 predictor-screening workflow to limit multicollinearity. Specifically, we used pairwise  
297 Kendall's tau-b correlations (Siegel and Castellan 1988) to identify strongly associated  
298 predictors  $|\tau| \geq 0.60$  and retained a single surrogate from each correlated set. Surrogates were  
299 selected using the lower univariate AIC for the stage-specific response, with occasional  
300 overrides based on ecological justification. We then calculated variance inflation factors (VIF;  
301 Zuur et al. 2010) iteratively, removing the term with the highest VIF until all values were  $< 5$ .

302 For establishment, we fitted a logistic-regression model (11 candidate predictors) and,  
303 after correlation screening, discarded ML, IE, PC, and PIS. We then applied manual forward  
304 selection with a probability of entry of  $< 0.15$  and removal of  $> 0.20$  of each variable (Hosmer  
305 and Lemeshow 2000; Tabachnick and Fidell 2001). Model fit was evaluated using  
306 classification tables (Tabachnick and Fidell 2001). As a second method, we applied  
307 classification-tree analysis to model binary invasion outcomes. This technique recursively  
308 partitions predictor variables to predict a categorical response (Breiman et al. 1984). Because  
309 the response variable was binary, classification rather than regression trees was used. Given  
310 the small sample size, we used ten-fold cross-validation to select tree size and retained the  
311 smallest tree within one- standard-error (1-SE) of the minimum classification rate (Howeth et  
312 al. 2016; 2025).

313 Spread and integration stages were analysed using the same subset of successfully  
314 established NNFS, so correlation screening produced the same set of excluded predictors in  
315 both stages. Accordingly, we removed MF, ML, PC, PP and PIS from spread and for  
316 integration analysed, and then applied the same VIF filtering and manual forward-backward  
317 selection criteria (entry  $p < 0.15$ , removal  $p > 0.20$ ; Ribeiro et al. 2008). We analysed spread  
318 using multiple regression models and regression trees. Spread was quantified as the number of

319 invaded drainage basins and modelled on the log-transformed scale for regression trees.  
320 Regression-tree analysis was fitted using regression trees, which recursively partitions  
321 predictor space to minimise within-node residual sum of squares, with terminal nodes returning  
322 node means (Breiman et al. 1984). We assessed predictive performance using 10-fold cross-  
323 validation and selected the simplest subtree satisfying the 1-SE rule (excluding the trivial root),  
324 balancing parsimony and fit. For the integration stage, we modelled average abundance of  
325 successful NNFS using multiple regression, applying the same correlation and VIF screening  
326 and the same selection criteria as above. We also analysed abundance with a classification tree,  
327 retaining the minimal tree that met the one-standard-error rule.

328 All data processing and analyses were performed using R version 4.5.1 (R Development  
329 Core Team 2025). Logistic and multiple regression analyses were conducted with the car  
330 package (Fox and Weisberg 2019) for multicollinearity diagnostics (VIF) and Type II  
331 ANOVA, pscl (Jackman 2024) for pseudo- $R^2$  estimation, and lmtest (Zeileis and Hothorn,  
332 2002) for goodness-of-fit and heteroskedasticity testing. Regression and classification trees  
333 were developed using rpart (Therneau and Atkinson, 2025) and visualized with rpart.plot  
334 (Milborrow 2025) packages.

335

## 336 **Results**

337 Our survey analysed 63 NNFS in Greece, including 36 foreign and 27 extralimital  
338 species. Among the foreign NNFS, 11 established self-sustaining populations while 25 failed,  
339 whereas all 27 translocations of extralimital NNFS were successful (Table 1), based on  
340 reproduction records or persistent multi-year detections. MAS, ML, and PIS were closely  
341 linked to establishment (Fig. 2, Table 1).

342 Logistic regression indicated that Distance from Nearest Native Source, Physiological  
343 Tolerance, Maximum Adult Size, and Propagule Pressure were significant predictors of  
344 establishment, achieving an overall classification accuracy of 84.1% (Table 2). Coefficient  
345 signs showed that establishment probability decreased with greater Distance from Nearest  
346 Native Source and larger body size but increased with higher Physiological Tolerance.  
347 Propagule Pressure had a positive but limited effect, indicating weak support for higher  
348 establishment with increasing release magnitude (Table 2). Cross-validation indicated that the  
349 classification tree model classified established NNFS more accurately by 60% than failed  
350 species (Fig. 3a, AUROC = 0.84; 65% upon 10-fold cross-validation). The classification tree  
351 for the establishment stage showed that NNFS introduced from nearby origin resulted in 100%  
352 establishment. In contrast, for introductions originating farther from the native range,

353 establishment depended strongly on tolerance, with extremely tolerant species showing high  
 354 establishment success (71%), whereas species with lower Physiological Tolerance were  
 355 predominantly unsuccessful, with establishment dropping to 29%.

356 The spread model explained 56% of the variance and retained Introduction Effort and  
 357 Physiological Tolerance (Table 3). Both predictors were positively associated with spread,  
 358 indicating that NNFS introduced on more occasions, and those able to tolerate a wider range  
 359 of conditions, tended to occupy more drainages. The regression tree for the spread stage was  
 360 driven primarily by Introduction Effort, which formed the root split and separated the dataset  
 361 into a large group corresponding to single introduction events with limited spread and a smaller  
 362 group with multiple or repeated introduction events with more invaded drainages (Fig. 3b; 60%  
 363 upon 10-fold cross-validation). Species introduced only once made up 66% of observations  
 364 and showed limited spread, whereas species with multiple or repeated introductions comprised  
 365 34% and spread more widely on average. Within this higher effort group, Physiological  
 366 Tolerance further structured spread. Intolerant to tolerant species comprised 13% of  
 367 observations and showed intermediate spread, whereas extremely tolerant species comprised  
 368 21% and showed the greatest spread across basins.

369 The integration model was significant, accounting for 20% of the variance (Table 4).  
 370 Integration increased with Introduction Effort, indicating that species released more frequently  
 371 tended to reach higher average abundance across drainages. Maximum Adult Size showed a  
 372 weak negative association and was not significant, indicating little evidence that larger-bodied  
 373 species attained lower mean abundance once established.

374 The classification tree for the integration stage retained a simple hierarchical structure,  
 375 with Distance from Nearest Native Source emerging as the root splitter (Fig. 3c; 65% upon 10-  
 376 fold cross-validation). Species with greater Distance from the Nearest Native Source region  
 377 comprised 29% of observations and were most often assigned to the high-abundance class.  
 378 Species with lower distance comprised 71% and were further split by trophic level, indicating  
 379 that introductions from nearby source regions did not converge on a single outcome. Within  
 380 this group, lower trophic-level species ( $< 3.5$ ; 50%) were more frequently classified into an  
 381 intermediate abundance class, whereas higher trophic-level species ( $\geq 3.5$ ; 21%) were most  
 382 often assigned to the highest abundance class, suggesting that high abundance can occur both  
 383 among introductions from distant source regions and among introductions from nearby source  
 384 regions, especially at higher trophic levels.

385

386 **Discussion**

387 We developed a cross-stage framework that combined regression models and  
388 classification/regression trees (CART) to evaluate predictors of NNFS establishment, spread,  
389 and integration in Greek watersheds. Overall, predictors from both approaches converged on a  
390 coherent pattern in which Distance from the Nearest Native Source and Physiological  
391 Tolerance were essential for NNFS success across stages, while Maximum Adult Size tended  
392 to constrain establishment and more weakly integration. Human-interest proxies were stage  
393 specific, with Propagule Pressure retained during establishment and introduction effort shaping  
394 both spread and also integration, where higher mean abundance was associated with more  
395 frequent releases and higher Trophic Level further structured outcomes, indicating context-  
396 dependent effects. Taken together, our cross-stage analysis shows that key constraints persist  
397 across stages, but their relative importance shifts from establishment to spread and integration  
398 in Greece, a biogeographic crossroads between the broader Balkan and Danubian region and  
399 Aegean systems toward Anatolia that also supports high freshwater endemism (Vardakas et al.  
400 2022).

401

#### 402 **What distinguishes successful from failed introductions?**

403 Establishment of NNFS in Greek drainage basins was primarily filtered by  
404 biogeographic proximity to the nearest native source (lower DNNS) and high physiological  
405 tolerance, with maximum adult size reducing establishment probability and propagule pressure  
406 showing only weak additional support. The classification tree corroborated this hierarchy by  
407 indicating high establishment probability for short-distance introductions, whereas successful  
408 long-distance introductions were largely restricted to extremely tolerant taxa and were rare for  
409 low-tolerance species introduced intercontinentally. Similar patterns have been observed in  
410 climate-matching and environmental-filtering studies, which highlight the influence of  
411 tolerance thresholds and local habitat constraints in shaping establishment success across  
412 Mediterranean climate systems (Koutsikos et al. 2018). Mechanistically, distance from nearest  
413 native source plausibly captures declining environmental matching (and, often, lower transfer  
414 frequency) with increasing translocation distance, while physiological tolerance reflects  
415 resilience to hydrologic variability and water-quality stress, two filters repeatedly emphasized  
416 in stage-based fish screening and climate-/tolerance-based risk assessments (García-Berthou  
417 2007; Howeth et al. 2016; 2025; Marchetti et al. 2004a). Notably, establishment in Greece was  
418 dominated by extralimital translocations, consistent with frequent, short-range, human-assisted  
419 movements (e.g., ad hoc stocking and angling-linked transfers) that readily bypass basin  
420 isolation in Greece's fragmented hydrographic setting and contribute to ongoing faunal

421 reshuffling and homogenization (Koutsikos et al. 2019a; Vardakas et al. 2022; Koutsikos et al.  
422 2025).

423 By contrast, only a small subset of foreign NNFS have progressed beyond the strong  
424 establishment barrier and become truly widespread in Greece, most notably *G. holbrooki*, *C.*  
425 *gibelio*, *P. parva*, and *L. gibbosus* (Koutsikos et al. 2025). These taxa closely match the trait–  
426 pathway profile implied by our establishment results: they are generally small-to-moderate  
427 bodied, highly tolerant and ecologically flexible, and have typically experienced sustained or  
428 repeated releases through human activities (Koutsikos et al. 2021; Koutsikos et al. 2025). Their  
429 ability to dominate degraded and modified habitats and build large populations helps explain  
430 why they disproportionately restructure both lentic and lotic assemblages and accelerate faunal  
431 homogenization across Greece (Koutsikos et al. 2025). Their expansion is further facilitated by  
432 anthropogenically altered lowland environments common in Mediterranean basins, slow-  
433 flowing, vegetated reaches, fine substrates, wetlands, reservoirs, and regulated channels, where  
434 warm-water, highly tolerant fishes can build high densities and drive homogenization (Vargas  
435 and De Sostoa 1996; Clavero et al. 2004; Ferreira et al. 2007; Koutsikos et al. 2021; Koutsikos  
436 et al. 2025). More broadly, our results align with cross-region evidence that biogeographic  
437 proximity and physiological tolerance operate as recurrent establishment filters, with human-  
438 mediated introduction dynamics contributing additional (often stage- and region-dependent)  
439 explanatory power (Howeth et al. 2025; Marchetti et al. 2004b). In another Mediterranean  
440 peninsula, successful establishment was similarly associated with shorter distance from nearest  
441 native ranges and smaller adult size and was strongly supported by prior invasion success  
442 (Ribeiro et al. 2008). Extending this Mediterranean signal, a comparative analysis across five  
443 Mediterranean-climate regions found that the breadth of establishment (number of regions  
444 where a species established) was best predicted by previous introduction success, with mean  
445 latitude adding secondary explanatory power (Marr et al. 2010). By comparison, California  
446 models also emphasized tolerance and distance-related constraints but highlighted additional  
447 life-history components (e.g., Parental Care and Maximum Adult Size), reinforcing that “core”  
448 filters can be shared while the surrounding trait set is context contingent (Marchetti et al.  
449 2004b). Subtropical Florida provided a clearer contrast, where establishment success was more  
450 strongly differentiated by reproductive investment (notably parental care and egg traits) and  
451 tended to involve larger-bodied species (Lawson and Hill 2021). At broader spatial scales,  
452 establishment had likewise been linked to climatic/thermal tolerance and climate-zone  
453 matching (Marcolin et al. 2025), while in tropical systems climate match and Prior Invasion  
454 Success can dominate risk signals (Chan et al. 2021), highlighting that some invasion filters

455 are broadly consistent, but their relative strength is reshaped by regional environments and  
 456 introduction pathways.

457

458 **Which introduction-related factors and traits best predict spread?**

459 Spread across Greek drainage basins was driven primarily by Introduction Effort and  
 460 Physiological Tolerance. The regression tree supported this pattern by selecting introduction  
 461 effort as the primary split, separating species recorded from a single release event, which  
 462 showed limited basin occupancy, from species with multiple or repeated release events, which  
 463 occupied more basins on average. Within the repeated-introduction branch, Physiological  
 464 Tolerance further structured spread, with intolerant to tolerant species showing intermediate  
 465 basin occupancy and extremely tolerant species achieving the widest distributions. This is  
 466 consistent with stage-based evidence that post-establishment expansion is often sustained by  
 467 repeated releases and redistribution, and that broad tolerance to suboptimal conditions can  
 468 facilitate spread across heterogeneous freshwater environments (Marchetti et al. 2004a,b;  
 469 Howeth et al. 2025).

470 Among foreign NNFS that have expanded widely, the omnivorous *G. holbrooki*  
 471 combines extreme tolerance, extensive prior invasion history, and sustained human-assisted  
 472 dispersal, conferring a strong advantage in Greek basins. Its spread is further reinforced by life-  
 473 history traits, well suited to Mediterranean freshwater systems, including high reproductive  
 474 output, rapid juvenile development, and fast population turnover (Gkenas et al. 2012). These  
 475 traits enable the species to thrive in shallow or slow-flowing waters and in disturbed or  
 476 modified habitats where native fishes are often scarce, as well as to disperse between  
 477 watersheds through irrigation and drainage canals that connect adjacent basins (Pen et al. 1993;  
 478 Vargas and De Sostoa 1996; Pyke 2008). *Carassius gibelio* likewise combines broad tolerance  
 479 with a long invasion history, with its wide occupancy linked to accidental introductions during  
 480 fish-stocking (Koutsikos et al. 2019a). The expansion of *P. parva* follows a similar pathway:  
 481 the species is now widespread in transboundary rivers and lakes of northern Greece, reflecting  
 482 initial accidental introduction during fish-stocking operations in the Albanian section of Lake  
 483 Prespa in the 1970s (Rosecchi et al. 1993) and subsequent dispersal through shared river  
 484 systems with Albania, Bulgaria and Turkey (Gozlan et al. 2010; Piria et al. 2017).

485 Among extralimital fishes, *C. carpio* illustrates how repeated human-mediated releases  
 486 and translocations can sustain broad spread despite large body size, through angling- and  
 487 stocking-driven movements (Koutsikos et al. 2019a; 2025). In Greece, the Common Carp is  
 488 the most widely distributed extralimital taxon (Economidis et al. 2000; Perdikaris et al. 2010),

489 often involving domesticated or non-native strains linked to stocking programmes (Barbieri et  
490 al. 2015). The species is particularly abundant in ecoregions identified as major recipient zones  
491 for carp introductions and characterized by relatively low native fish diversity but high  
492 conservation importance (Economou et al. 2016; Koutsikos et al. 2019a). Collectively, these  
493 examples support the inference from our models that spread in Greece is primarily a function  
494 of repeated human-assisted releases and redistribution (Introduction Effort), amplified by  
495 tolerance (Physiological Tolerance).

496 At a continental European scale, spread is promoted by strong introduction pathways,  
497 including aquaculture escapes, stocking, and releases linked to recreational fisheries and the  
498 ornamental trade, together with repeated human mediated movements among basins, with  
499 spread driven largely by invasion history variables that capture the type and number of  
500 introduction causes (Marcolin et al. 2025). Reproductive pace can also contribute, since species  
501 that spawn more frequently may colonise a wider range after establishment (Marcolin et al.  
502 2025). This aligns with our Greek pattern, where Introduction Effort dominated spread and  
503 Physiological Tolerance further differentiated the most widely distributed taxa. By contrast, in  
504 the Iberian Peninsula, spread was linked to larger body size and detritivory, increased with  
505 prior invasion success, and showed an inverse association with size of native range, suggesting  
506 that species angling popularity can reshape which predictors best explain expansion even  
507 within Mediterranean contexts (Ribeiro et al. 2008; Banha et al. 2024). Habitat change can also  
508 amplify spread opportunities, as shown in the Colorado River Basin where altered flow regimes  
509 and widespread reservoir conditions created niche opportunities that favoured many non-native  
510 warm water generalists (Olden et al. 2006). Finally, large-scale trait-based analyses indicate  
511 that invasiveness reflects both human-mediated pressure and a life-history axis (e.g., fecundity  
512 and size–longevity), while susceptibility to invasion increases where native assemblages leave  
513 functional space available for invaders (Su et al. 2023). Together, these studies support the  
514 same general inference as our results: spread is often accelerated by repeated releases and  
515 redistribution, but the extent of expansion is ultimately filtered by trait–environment fit and the  
516 opportunity structure created by altered recipient systems.

517

### 518 **What facilitates integration into recipient communities?**

519 Introduction Effort was the main positive predictor of integration across drainages,  
520 whereas Maximum Adult Size showed only weak support for a negative association. In  
521 contrast, the classification tree identified distance from the nearest native source region as the  
522 first split, with species introduced from more distant source regions most often assigned to the

523 highest integration class. This is consistent with broad-scale evidence that a subset of taxa  
 524 repeatedly succeed across regions. For example, Ruesink (2005) highlighted common carp and  
 525 mosquitofish among species with particularly high establishment success across many  
 526 countries, and these globally repeat-success taxa also tend to progress further along the  
 527 invasion continuum, often reaching high integration and, in some cases, high impact stages in  
 528 recipient systems. For species introduced from closer sources, integration outcomes were  
 529 further filtered by trophic level. Within this subset, lower-trophic level species were more  
 530 frequently assigned to the lower integration class, whereas higher-trophic level species were  
 531 more frequently assigned to the highest class. Overall, the models indicate that repeated release  
 532 history can elevate average abundance, while biogeographic origin and trophic strategy further  
 533 sort which established populations reach the strongest integration, matching stage-based  
 534 studies where invasion history and human support dominate late-stage performance and traits  
 535 act as secondary filters (Marchetti et al. 2004a, b). The trophic level also aligns with trait-based  
 536 work showing that piscivory and higher trophic positions are often linked to stronger  
 537 establishment and impacts (Howeth et al. 2016; Chan et al. 2021). This suggests that in Greek  
 538 drainages, similar to the Colorado River Basin, extralimital predators such as *S. glanis*, *S.*  
 539 *lucioperca* and *P. fluviatilis* may be exploiting "vacant niches" or using novel resources in  
 540 hydrologically altered environments (Olden et al. 2006). Furthermore, the negative influence  
 541 of body size in our linear models contrasts with continental-scale findings favouring large-  
 542 bodied invaders (Su et al. 2023) but mirrors results from California, where integration was  
 543 associated with smaller to medium-sized fishes better suited to variable regimes (Marchetti et  
 544 al. 2004b). Finally, the prominent role of history and human support in the Greek integration  
 545 stage aligns with the broader European pattern in which pathway intensity and introduction  
 546 history dominate post-establishment outcomes (Marcolin et al. 2025), as illustrated by the  
 547 widespread translocation and continued fisheries-driven support of Common carp (*C. carpio*)  
 548 and by rainbow trout (*Oncorhynchus mykiss*), which is often maintained through regular  
 549 stocking (Vardakas et al. 2022; Koutsikos et al. 2025).

550

### 551 **Model complementarity and data structure**

552 Our profiling framework used ordinal scoring for several predictors because  
 553 information was compiled from heterogeneous sources and continuous trait or introduction  
 554 data were incomplete. This screening approach is common in stage-based assessments, but it  
 555 can retain residual measurement error and reduce sensitivity to ecological gradients even when  
 556 models are cross-validated (Marchetti et al. 2004b; Ribeiro et al. 2008). Likewise, we used

557 prior invasion success as a pragmatic invasion-history proxy, consistent with stage-structured  
558 fish invasion studies that quantify “success elsewhere” from global occurrence/establishment  
559 records to capture broad differences among species (Marchetti et al. 2004b; Ribeiro et al. 2008;  
560 Marcolin et al. 2025). Although FAO Introduced Species Fact Sheets can vary in completeness  
561 among countries, the database provides a consistent, repeatable basis for comparative scoring,  
562 and the log-transformation further reduces sensitivity to extreme values while preserving rank  
563 differences among taxa.

564 To address potential phylogenetic non-independence, we incorporated a taxonomy-  
565 based surrogate for relatedness (Nelson family rank) in the CART analyses, which preserves  
566 interpretable splits while capturing clade-level signal in mixed, partly categorical datasets, as  
567 in other regional risk-model applications (Howeth et al. 2016; Howeth et al. 2025).  
568 Methodologically, we applied both parametric regression and tree-based CART models at each  
569 invasion stage, and the two approaches were complementary. Regression models provided  
570 effect sizes and directionality, whereas CART highlighted non-linear thresholds and  
571 interactions and yielded transparent, manager-relevant decision rules. This matches evidence  
572 that tree-based models perform well with mixed ecological attributes and early-stage screening,  
573 even when parametric models can explain more variance overall (Howeth et al. 2016; Lawson  
574 and Hill 2021). Moreover, the Greek invasion context is strongly shaped by pathway-driven  
575 taxonomic bias, which likely explains why human-mediated predictors recur across stages. In  
576 Greece, major pathways for foreign NNFS include aquaculture- and angling-related releases,  
577 while extralimital translocations are dominated by angling/fish-bait movements alongside a  
578 substantial fraction of unknown pathways (Koutsikos et al. 2019a). These pathways  
579 disproportionately involve lineages closely linked to fisheries, stocking, or trade, and they  
580 include taxa repeatedly linked to faunal homogenization such as *G. holbrooki*, *C. gibelio*, and  
581 extralimital *C. carpio* (Vardakas et al. 2022; Koutsikos et al. 2025). Future work should  
582 prioritise (i) improved introduction-history metrics that directly quantify introduction effort,  
583 including the number of independent release events, their timing and duration, and where  
584 possible the magnitude of releases, (ii) hydrographic and infrastructure connectivity measures  
585 that capture opportunities for secondary translocation among basins, including inter-basin  
586 water transfers, canals, and reservoir networks, and (iii) explicit tests of model transferability  
587 across Mediterranean and temperate regions to ensure screening performance remains robust  
588 under environmental change (Howeth et al. 2016; Howeth et al. 2025).

589

590 **Extralimital translocations: a disproportionate and under-regulated risk**

591 A central management insight of this study is the disproportionate success of  
 592 extralimital NNFS which were all established and many now occupy a wide range of basins  
 593 (Koutsikos et al. 2019a,b; Vardakas et al. 2022). Short distances, strong environmental  
 594 matching, and dense pathways (angling, stocking, water transfers) likely contribute to this  
 595 pattern. Yet EU Regulation 1143/2014 targets foreign NNFS and largely omits within-country  
 596 translocations, creating a regulatory blind spot for extralimital NNFS. Moreover, extralimital  
 597 taxa have often been omitted from, or inconsistently treated in, biodiversity surveys (Zenetos  
 598 et al. 2009) and in invasive species reporting and prioritisation (Arianoutsou et al. 2023).  
 599 Importantly, extralimital taxa can be highly detrimental in endemic-rich Mediterranean  
 600 regions, leading to irreversible impacts such as hybridisation (e.g. *S. aristotelis*, *S. glanis*)  
 601 (Paschos et al. 2004).

602 While national instruments (e.g., Presidential Decrees, biodiversity strategies, Water  
 603 Framework Directive plans) could address the issue of extralimital species, no consistent  
 604 framework currently exists. Therefore, we urge for the creation of National binding legislation  
 605 in order to regulate the introduction and spread of the extralimital NNFS. Previous work in  
 606 Greece has repeatedly signalled the urgency of addressing this policy gap, forming a  
 607 continuous call for coordinated action before the ecological and biodiversity impacts of  
 608 extralimital species become irreversible (Economidis et al. 2000; Perdikaris et al. 2016, 2025;  
 609 Koutsikos et al. 2019a,b, 2025; Vardakas et al. 2022; Gkenas et al. 2024; Zogaris et al. 2024).  
 610 Management should therefore prioritise pathway controls that regulate within-country  
 611 translocations as firmly as on foreign NNFS introductions, including stricter measures on  
 612 stocking and live-bait use, containment of aquaculture escapes, and systematic early detection,  
 613 including eDNA screening where appropriate (Koutsikos et al. 2019a; Kalogianni et al. 2024).

614

## 615 **Conclusions and future directions**

616 Our stage-structured assessment shows that invasion risk in Greek freshwaters is not  
 617 uniform along the introduction pathway but instead different traits and context filters dominate  
 618 at each step. Establishment was most strongly associated with shorter biogeographic distance  
 619 from nearest native source and higher physiological Tolerance, while maximum adult size  
 620 tended to constrain establishment and propagule pressure contributed only weakly. Once  
 621 established, spread across drainage basins was driven primarily by introduction effort and  
 622 physiological tolerance. Integration increased most clearly with introduction effort, while the  
 623 classification tree highlighted distance from nearest native source as the primary partition of  
 624 widespread and abundant outcomes and body size and trophic level further structured outcomes

625 among taxa. A striking and management-critical result is that all extralimital translocations in  
626 our dataset established successfully, emphasising the outsized role of within-country  
627 movements in reshaping Greek ichthyofaunas.

628 These patterns translate into clear guidance. First, prevention and early detection should  
629 adopt a pathway-inclusive, stage-structured screening approach that evaluates foreign and  
630 extralimital taxa consistently and prioritizes a compact set of predictors reflecting proximity,  
631 tolerance, and introduction history. Second, surveillance should focus on (i) basins and sectors  
632 with recurring release pathways, including stocking, angling and live-bait use, aquaculture  
633 escape risk, and water-transfer activity, which increase introduction effort and secondary  
634 redistribution, and on (ii) taxa with broad physiological tolerance, higher trophic level, or  
635 strong invasion histories that are most likely to spread and integrate once introduced. Third,  
636 regulation should be aligned across pathways by explicitly addressing within-country  
637 translocations (stocking, live-bait use, aquaculture escape risk, and water-transfer biota) to the  
638 same standard applied to foreign NNFS.

639 Finally, while our models captured robust, convergent signals across methods, they also  
640 indicate where future work will sharpen prediction and management: integrating direct  
641 measures of propagule supply and hydrologic connectivity, leveraging genomics to resolve  
642 sources and cryptic spread, and testing transferability of stage-specific models across  
643 Mediterranean and temperate regions. Implementing the recommended screening and  
644 surveillance framework now, particularly for extralimital movements, offers a practical route  
645 to slow biotic homogenization, safeguard endemic-rich basins, and better align policy with the  
646 ecological dynamics of Mediterranean river networks.

647

#### 648 **Additional information**

#### 649 **Conflict of interest**

650 The authors have declared that no competing interests exist.

651

#### 652 **Ethical statement**

653 No ethical statement was reported.

654

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#### 664 **Author contributions**

665 CG and FR contributed to the study conception and design. Material preparation and data  
666 collection were performed by CG, NK, KL and LV. Data analysis and visualisation were  
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670

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677

#### 678 **Data availability**

679 All of the data that support the findings of this study are available in the main text

680

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**Table captions**

Table 1: List of non-native freshwater fish species (NNFS) in Greece, classified by origin as foreign introductions (introduced across international borders) or extralimital translocations (within-country movements across biogeographic barriers; native in part of Greece but non-native elsewhere), and by invasion outcome as successful (established) or failed. For each species, we report values for the 11 predictor variables used in the analyses and the number of Greek drainage basins in which the species is established (see material and methods for detailed explanation of the variables and basins).

Species	Family	Success	Maximum adult size	Maximum fecundity	Maximum lifespan	Parental care	Physiological tolerance	Adult trophic level	Size of native range	Distance to nearest native source	Prior invasion success	Propagule pressure	Introduction effort	Number of drainage basins
<b>Foreign NNFS</b>														
<i>Acipenser baerii</i>	Acipenseridae	0	6	5	5	1	2	3.3	4	4	13	3	2	2
<i>Acipenser gueldenstaedtii</i>	Acipenseridae	0	6	5	5	1	2	3.3	3	2	11	3	2	4
<i>Acipenser ruthenus</i>	Acipenseridae	0	5	5	5	1	2	3.6	3	2	11	1	2	1
<i>Lepomis gibbosus</i>	Centrarchidae	1	4	2	4	3	2	3.3	3	4	33	1	2	42
<i>Micropterus nigricans</i>	Centrarchidae	0	5	5	4	3	3	3.8	3	4	76	1	1	1
<i>Oreochromis niloticus</i>	Cichlidae	0	4	3	4	4	3	2.0	4	4	133	2	2	5
<i>Clarias ganepinus</i>	Clariidae	0	6	5	4	1	4	3.8	4	4	37	1	1	1
<i>Misgurnus fossilis</i>	Cobitidae	0	3	3	5	1	4	3.4	4	2	3	1	1	3
<i>Coregonus lavaretus</i>	Coregonidae	1	4	4	4	1	2	3.1	1	4	22	4	3	3
<i>Coregonus cf. albus</i>	Coregonidae	0	4	4	4	1	2	3.1	3	4	10	1	1	1
<i>Coregonus cf. peled</i>	Coregonidae	0	4	4	4	1	3	4.1	3	4	19	1	1	1
<i>Carassius auratus</i>	Cyprinidae	1	4	5	5	1	4	2.0	4	4	70	2	2	29
<i>Carassius gibelio</i>	Cyprinidae	1	4	5	4	1	4	2.5	4	4	9	2	2	85
<i>Carassius langsdorffii</i>	Cyprinidae	1	3	4	4	1	4	2.8	2	4	5	1	1	1
<i>Pseudorasbora parva</i>	Cyprinidae	1	2	2	3	3	3	3.1	4	4	33	2	1	26
<i>Babka gymnotrachelus</i>	Gobiidae	1	2	3	3	3	2	3.3	3	2	2	1	2	1
<i>Neogobius fluviatilis</i>	Gobiidae	0	3	3	3	3	3	3.4	3	2	4	1	1	1
<i>Ameiurus nebulosus</i>	Ictaluridae	1	4	4	4	3	4	3.7	3	4	30	1	2	2
<i>Ictalurus punctatus</i>	Ictaluridae	0	5	4	5	3	3	4.2	3	4	37	1	1	1
<i>Pterygoplichthys joselimaianus</i>	Loricariidae	0	3	3	3	3	4	2.0	2	4	0	1	1	1
<i>Gymnocephalus cernua</i>	Percidae	1	3	5	4	1	2	3.3	4	4	6	1	1	1
<i>Gambusia holbrooki</i>	Poeciliidae	1	1	2	1	4	4	3.1	2	4	26	4	3	137
<i>Poecilia latipinna</i>	Poeciliidae	1	2	1	1	4	4	2.0	3	4	14	1	1	1
<i>Polyodon spathula</i>	Polyodontidae	0	6	5	5	1	2	3.1	3	4	7	1	1	1
<i>Oncorhynchus kisutch</i>	Salmonidae	0	5	3	3	2	1	4.2	3	4	20	2	2	5
<i>Oncorhynchus mykiss</i>	Salmonidae	0	5	3	4	2	2	4.1	3	4	110	4	3	64
<i>Salmo letnica</i>	Salmonidae	0	4	2	3	2	1	4.0	1	2	1	3	2	1
<i>Salmo salar</i>	Salmonidae	0	5	3	4	2	1	4.5	4	4	24	1	1	2
<i>Salmo cf. trutta</i>	Salmonidae	0	5	4	5	2	2	3.4	4	3	37	3	2	2
<i>Salvelinus fontinalis</i>	Salmonidae	0	5	3	5	2	1	3.3	3	4	53	3	3	3
<i>Pygocentrus nattereri</i>	Serrasalminae	0	4	4	3	3	4	3.7	4	4	8	1	1	2
<i>Ctenopharyngodon idella</i>	Xenocypridae	0	5	5	5	1	2	2.0	3	4	120	4	3	19
<i>Hypophthalmichthys molitrix</i>	Xenocypridae	0	5	5	5	1	3	2.1	3	4	92	4	3	19
<i>Hypophthalmichthys nobilis</i>	Xenocypridae	0	5	5	5	1	3	2.8	3	4	64	4	3	4
<i>Mylopharyngodon piceus</i>	Xenocypridae	0	6	6	5	1	4	3.2	3	4	33	1	1	1
<i>Parabramis pekinensis</i>	Xenocypridae	0	4	3	5	1	4	2.0	3	4	6	1	1	1
<b>Extralimital NNFS</b>														
<i>Rhodeus meridionalis</i>	Acheilognathidae	1	2	2	3	1	1	2.5	3	1	0	1	1	3

<i>Acipenser naccarii</i>	Acipenseridae	1	6	5	4	1	2	3.4	3	2	0	3	1	1
<i>Cobitis hellenica</i>	Cobitidae	1	2	3	3	1	3	3.2	1	1	0	1	1	1
<i>Barbus sperchiensis</i>	Cyprinidae	1	3	4	2	1	2	3.1	1	1	0	1	1	1
<i>Cyprinus carpio</i>	Cyprinidae	1	5	5	5	1	4	3.1	4	4	154	4	3	116
<i>Luciobarbus graecus</i>	Cyprinidae	1	5	4	3	1	3	2.7	3	1	0	1	1	5
<i>Tinca tinca</i>	Cyprinidae	1	4	5	5	1	4	3.7	4	2	27	2	2	17
<i>Esox lucius</i>	Esocidae	1	5	5	5	1	2	4.1	4	3	16	2	2	10
<i>Economicichthys pygmaeus</i>	Gobiidae	1	1	2	1	3	1	3.5	1	1	0	1	1	8
<i>Kripowitschia caucasica</i>	Gobiidae	1	1	2	1	3	2	3.3	4	2	2	1	1	2
<i>Abramis brama</i>	Leuciscidae	1	5	5	5	1	3	3.1	4	1	8	1	1	1
<i>Pachychilon macedonicum</i>	Leuciscidae	1	2	4	4	1	4	2.9	3	1	0	1	1	2
<i>Pelagus marathonicus</i>	Leuciscidae	1	1	3	1	1	3	3.1	1	1	0	1	1	1
<i>Pelagus stymphalicus</i>	Leuciscidae	1	1	3	2	1	3	2.8	1	1	0	1	1	3
<i>Rutilus panosi</i>	Leuciscidae	1	3	4	4	1	3	2.9	1	1	0	1	1	2
<i>Rutilus ylikiensis</i>	Leuciscidae	1	3	4	4	1	3	2.9	1	1	0	1	1	2
<i>Scardinius acarnanicus</i>	Leuciscidae	1	3	4	4	1	3	2.0	1	1	0	1	1	1
<i>Scardinius graecus</i>	Leuciscidae	1	4	4	4	1	3	2.0	1	1	0	1	1	4
<i>Squalius orpheus</i>	Leuciscidae	1	4	3	3	1	3	3.3	2	1	0	1	1	2
<i>Squalius peloponensis</i>	Leuciscidae	1	3	3	3	1	3	3.4	2	1	0	1	1	2
<i>Squalius vardarensis</i>	Leuciscidae	1	4	3	3	1	3	3.3	3	1	0	1	1	3
<i>Oxynoemacheilus bureschi</i>	Nemacheilidae	1	2	3	3	1	2	2.8	3	1	1	1	1	2
<i>Perca fluviatilis</i>	Percidae	1	4	5	5	1	2	4.4	4	2	10	1	2	14
<i>Sander lucioperca</i>	Percidae	1	5	5	5	3	2	4.0	4	3	22	1	1	1
<i>Salmo cf. farionides</i>	Salmonidae	1	4	2	3	2	1	3.5	3	2	0	4	3	3
<i>Silurus aristotelis</i>	Siluridae	1	4	4	4	3	3	3.9	1	1	0	1	1	6
<i>Silurus glanis</i>	Siluridae	1	6	5	5	3	4	4.4	4	2	17	2	2	15

Table 2: Summary of the logistic-regression model (AIC = 57.37;  $R^2 = 0.44$ ) identifying predictors of establishment success for non-native fish species (NNFS) in Greece. The table lists estimated coefficients (B), their standard errors (SE), Wald test and p-values.

Model	Variables	B	SE	Wald	p-value
G = 38.96, p <0.001	Distance from nearest native source	-1.34	0.40	10.99	<0.01
	Physiological tolerance	1.18	0.49	5.80	0.01
	Maximum adult size	-0.75	0.36	4.44	0.03
	Propagule pressure	0.62	0.40	2.50	0.11
	Constant	2.99	2.19	1.85	0.17

Table 3: Summary of multiple regression model (AIC = 99.52;  $R^2 = 0.56$ ) identifying predictors of spread stage (number of catchments a species has invaded) for non-native fish species (NNFS) in Greece. The table lists estimated coefficients (B), their standard errors (SE), and p-values.

Model	Variables	B	SE	p-value
F = 22.07, p <0.001	Introduction effort	1.05	0.20	<0.00
	Physiological tolerance	0.60	0.17	<0.01
	Constant	-1.59	0.57	<0.01

Table 4: Summary of multiple-regression model (AIC = 69.37;  $R^2 = 0.20$ ) identifying predictors of integration stage (average value of species distribution and abundance per drainage basins) for non-native fish species (NNFS) in Greece. The table lists estimated coefficients (B), their standard errors (SE), and p-values.

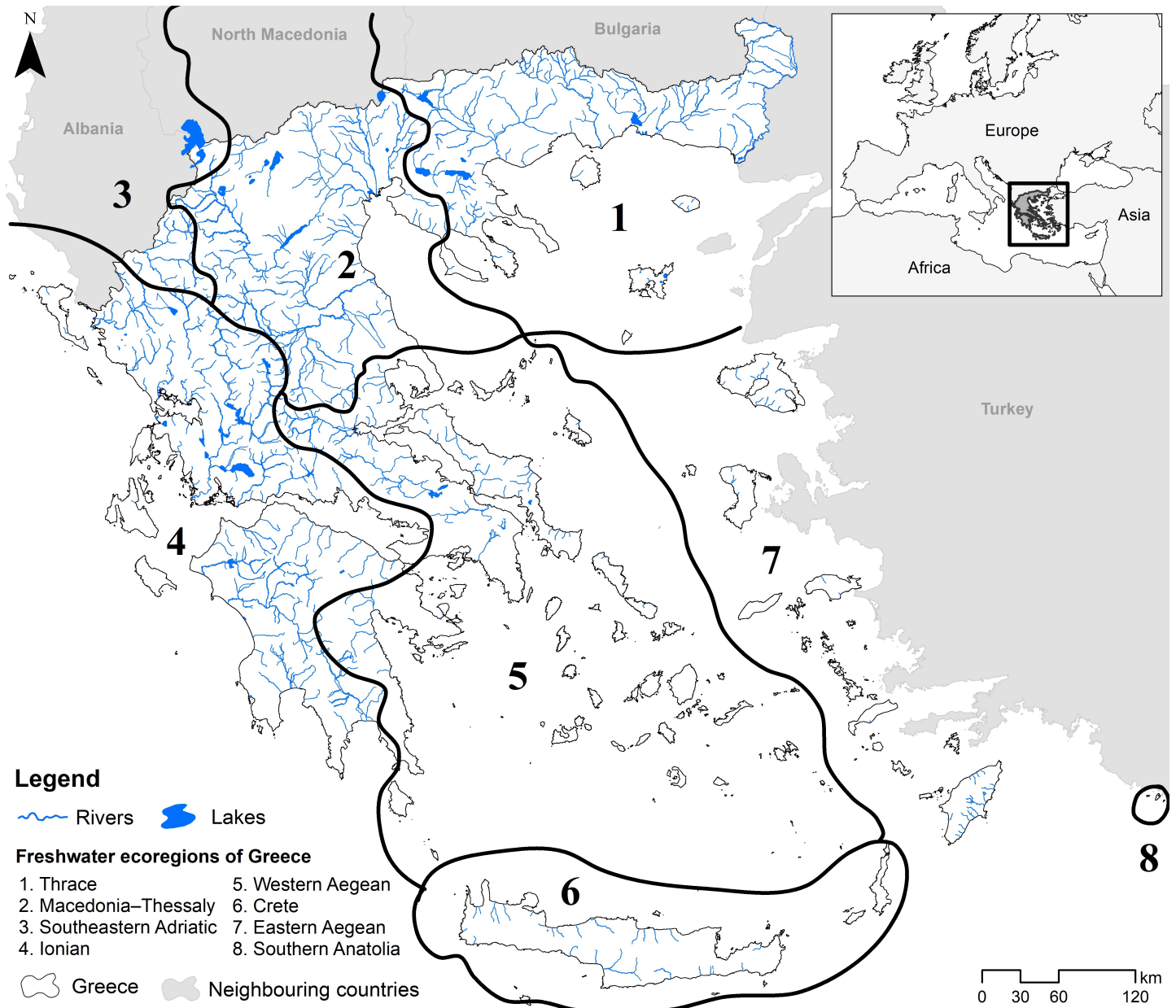
Model	Variables	B	SE	p-value
F = 3.58, p = 0.03	Introduction effort	0.34	0.14	0.02
	Maximum adult size	-0.11	0.07	0.11
	Constant	2.35	0.28	<0.01

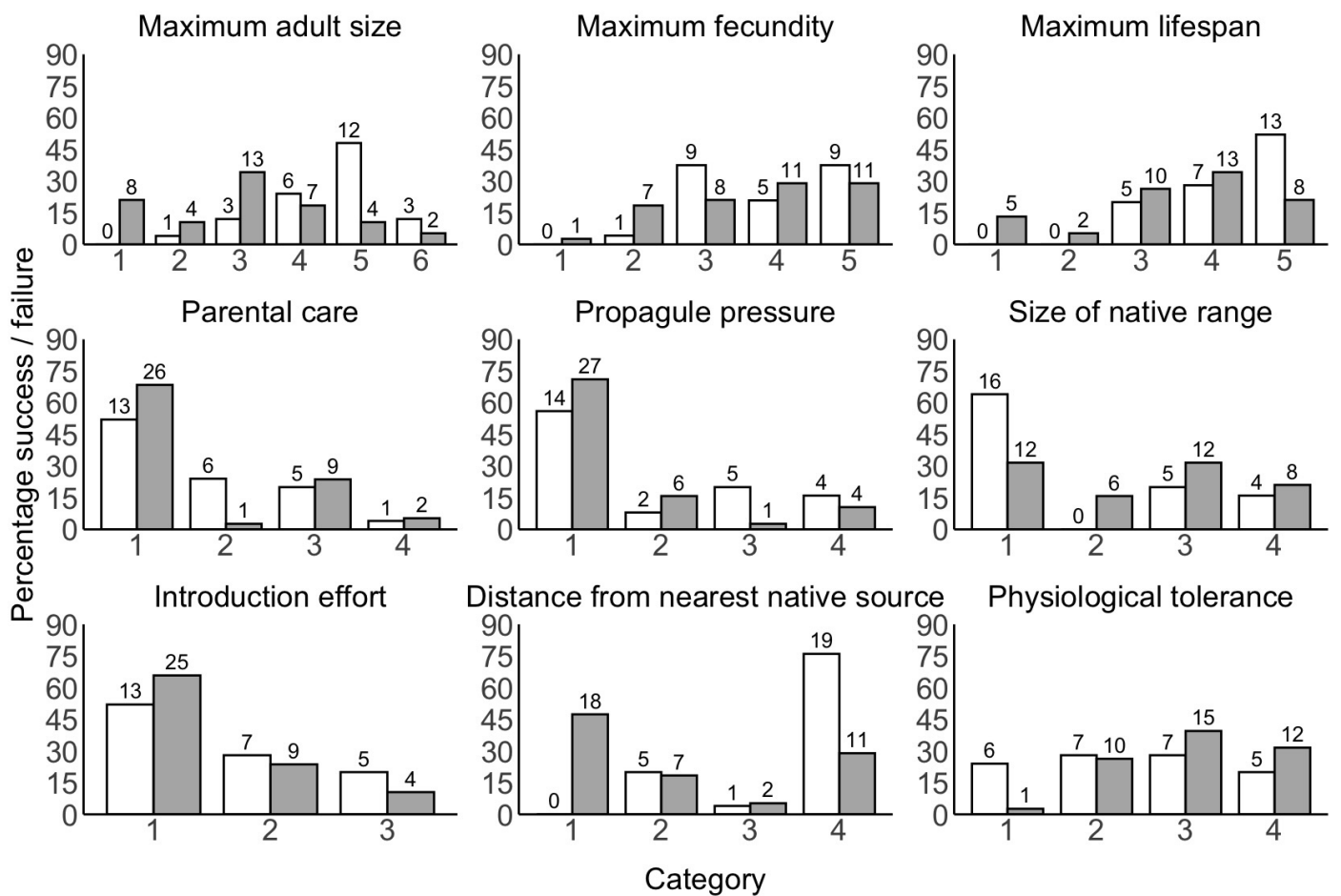
### Figure captions

Fig. 1: Freshwater ecoregions of Greece (numbered 1–8) following Zogaris and Economou (2017). Thick black lines delineate ecoregion boundaries; rivers (lines) and lakes (polygons) are shown in blue. The inset indicates the location of Greece within the broader Mediterranean region.

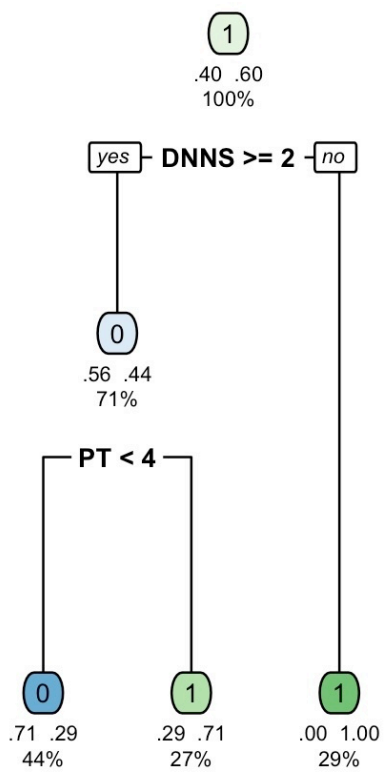
Fig. 2: Percentage of successful (grey bars) versus failed (white bars) of non-native fish species (NNFS) introductions across each variable subcategory. Numbers above the bars indicate the counts of successful and failed species, respectively. See text for detailed subcategory definitions

Fig. 3: Classification and regression trees (CART) summarizing predictors of three invasion stages of non-native fish species (NNFS) in Greece. (a) Establishment: classification tree predicting failure (0) versus establishment (1) from distance from the nearest native source (DNNS), and physiological tolerance (PT). (b) Spread: regression tree with splits on introduction effort (IE) and physiological tolerance (PT). (c) Integration: classification tree predicting abundance status (1 = very rare; low abundance and highly localized occurrences, 2 = isolated/restricted; persistent but geographically limited populations, 3 = widespread and abundant; high abundance across multiple basins), with splits on distance from the nearest native source (DNNS), and trophic level (Troph\_lev). Trees were selected by 10-fold cross-validation and pruned using the one-standard-error rule. Terminal nodes report the predicted outcome (class or mean), class probabilities (classification trees), sample size (n), and the percentage of species per node.

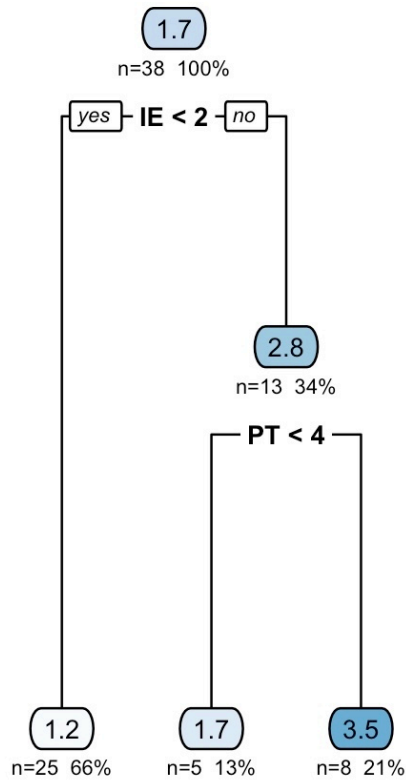




**(a) Establishment**



**(b) Spread**



**(c) Integration**

