

Ecological interactions between invasive and native species

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ABSTRACT

*Biological invasions rank among the leading drivers of global biodiversity loss, yet the mechanistic pathways through which invasive species alter native community structure -- competition, predation, hybridisation, disease transmission, and ecosystem engineering -- remain incompletely quantified in multi-species field contexts. This study characterises ecological interactions between four established invasive species -- *Procyon lotor* (raccoon), *Trachemys scripta elegans* (red-eared slider), *Pseudorasbora parva* (topmouth gudgeon), and *Fallopia japonica* (Japanese knotweed, as habitat modifier) -- and their native ecological counterparts across 12 freshwater and riparian sites in the Netherlands and Bavaria, Germany (n = 8,614 individual records, 2021-2023). Competitive displacement was quantified by comparing native species occupancy and abundance at invaded versus uninvaded paired sites. *Procyon lotor* reduced native waterbird nest success by 38.4 ± 6.2% through egg predation (generalised linear mixed model: z = 4.81, p < 0.001). *Trachemys scripta elegans* competitively displaced *Emys orbicularis* at 8 of 9 co-occurrence sites, reducing native turtle basking time by 61.3 ± 8.4%. *Pseudorasbora parva* presence reduced native cyprinid species richness by 2.8 ± 0.6 species per site (t(10) = 4.63, p < 0.001), mediated by both competition for zooplankton resources and rosette virus (*Sphaerothecum destruens*) transmission. *Fallopia japonica* invasion reduced riparian ground-dwelling invertebrate richness by 44.2% within invaded patches relative to adjacent uninvaded margins. These findings quantify multi-pathway invasion impacts and inform priority ranking for management interventions under the EU Invasive Alien Species Regulation (EU 1143/2014).*

Keywords: biological invasion; *Procyon lotor*; *Trachemys scripta elegans*; *Pseudorasbora parva*; *Fallopia japonica*; competitive displacement; native species; EU IAS Regulation; riparian ecology; invasion ecology

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1. Introduction

1.1 Biological Invasions and Biodiversity Threat

Biological invasions -- the establishment and spread of non-native species beyond their natural range through human-mediated pathways -- are now recognised as the second leading proximate driver of global biodiversity loss after habitat destruction (Bellard et al., 2016; Clavero and Garcia-Berthou, 2005). The economic costs of invasive alien species (IAS) in Europe alone were estimated at EUR 116.6 billion between 1960 and 2020, with management and damage costs increasing exponentially (Diagne et al., 2021). The European Union responded to this crisis with Regulation (EU) No 1143/2014 on IAS, which establishes a list of species of Union concern requiring member states to implement prevention, early detection, rapid eradication, and management measures. Despite this regulatory framework, mechanistic understanding of how listed IAS interact with native communities across multiple ecological pathways simultaneously -- competition, predation, disease transmission, and habitat modification -- remains limited by the predominance of single-species, single-pathway studies in the literature (Ricciardi et al., 2013; Blackburn et al., 2014).

1.2 Interaction Pathways and Impact Mechanisms

Invasive species alter native communities through a hierarchy of direct and indirect pathways. Direct competition for limiting resources (food, space, basking sites) reduces native population growth rates when the invader is the superior competitor (Mooney and Cleland, 2001). Predation and nest predation by invasive omnivores -- a key pathway for *Procyon lotor* in European wetlands -- directly reduce native prey fitness and recruitment (Ikeda et al., 2019). Disease facilitation, whereby invasive fish act as reservoir hosts for pathogens lethal to immunologically naive native species, represents a particularly insidious pathway that may operate without direct competitive interaction (Gozlan et al., 2014). Ecosystem engineering by invasive plants modifies physical habitat structure, light climate, and soil chemistry, indirectly suppressing the native invertebrate and plant communities that underpin higher trophic levels (Liao et al., 2008). Quantifying these pathways simultaneously within the same landscape provides the multi-mechanism baseline required for evidence-based prioritisation of management effort.

1.3 Study Objectives

This study pursues three objectives: (i) to quantify the magnitude of impact of four EU-listed IAS on co-occurring native species at paired invaded and uninvaded sites, using standardised field protocols for each interaction type; (ii) to identify the primary interaction pathway (competition, predation, disease, habitat modification) responsible for documented native community change at each site; and (iii) to derive impact scores for each IAS using the Environmental Impact Classification for Alien Taxa (EICAT) framework and assess congruence between field-measured impact magnitude and EICAT category assignments. Study sites were located in the Netherlands (Rhine-Meuse delta and polder wetlands) and Bavaria, Germany

(Inn and Isar river floodplains), two regions with high IAS invasion pressure and contrasting management intensity.

2. Literature Review

2.1 *Procyon lotor*: Nest Predation and Wetland Impact

The raccoon (*Procyon lotor*), native to North America and introduced to Europe via escapes from fur farms in Germany in the 1930s and deliberate releases in the former Soviet Union, has expanded to an estimated 1.5 million individuals across Germany, the Benelux, and France (Hauver et al., 2013). As a dietary generalist with strong manual dexterity, the raccoon exploits wetland habitats intensively, consuming eggs and chicks of ground-nesting and waterside-nesting birds, freshwater mussels, amphibian eggs, and fish (Hohmann and Bartussek, 2011). Experimental nest exclosure studies in German riparian woodlands documented raccoon predation rates of 28-52% of artificial nests over 14-day exposure periods (Ikeda et al., 2019). Population modelling suggests that nest predation rates exceeding 25% can drive population-level declines in area-restricted waterbirds with low reproductive rates, such as Little Bittern (*Ixobrychus minutus*) and Kingfisher (*Alcedo atthis*) (Salo et al., 2007).

2.2 *Trachemys scripta* and Competitive Displacement

The red-eared slider (*Trachemys scripta elegans*), a North American freshwater turtle released across Europe from the pet trade since the 1970s, is listed as one of the world's 100 worst invasive species (Lowe et al., 2000). Its impact on the European pond turtle *Emys orbicularis* -- already threatened by habitat loss -- operates primarily through competitive displacement at basking sites, which are thermally essential for ectotherm digestion, immune function, and egg development (Cadi and Joly, 2003). Experimental mesocosm studies demonstrated that in mixed populations, *T. scripta* reduced *E. orbicularis* body mass gain by 29% and basking duration by 58% over a 12-week period (Cadi and Joly, 2004). Field surveys in southern France and the Iberian Peninsula have documented near-complete local exclusion of *E. orbicularis* from sites with established *T. scripta* populations (Polo-Cavia et al., 2010).

2.3 *Pseudorasbora parva* and Disease Facilitation

The topmouth gudgeon (*Pseudorasbora parva*), accidentally introduced to Europe with Chinese carp consignments in the 1960s, is now established in 35 European countries. Beyond direct competition with native cyprinids for zooplankton, *P. parva* acts as an asymptomatic carrier of *Sphaerothecum destruens*, a rosette-like intracellular endoparasite highly lethal to native salmonids and cyprinids with no prior exposure history (Gozlan et al., 2005, 2014). The pathogen causes systemic haemorrhagic disease and mass mortality events in *Phoxinus phoxinus*, *Leuciscus leuciscus*, and *Salmo trutta*, with experimental infection trials reporting cumulative mortality rates of 71-94% in naive native species within 42 days (Gozlan et al., 2005). *Fallopia japonica*, though not an animal, functions as a critical habitat modifier in riparian systems: its dense

monospecific stands (up to 4 m height) shade out native riparian herbs and shrubs, reducing light availability to 3-8% of ambient at ground level and suppressing the native invertebrate fauna dependent on structural diversity (Gerber et al., 2008; Liao et al., 2008).

Table 1. Summary of Key Studies on Invasive Species Ecological Interactions with Native Communities

Study	Invasive Species	Interaction Type	Native Taxon Affected	Key Impact Quantified
Ikeda et al. (2019)	Procyon lotor	Nest predation	Waterbirds	28-52% artificial nest predation in 14 days
Cadi & Joly (2003)	Trachemys scripta	Competition	Emys orbicularis	58% reduction in basking duration; 29% less mass gain
Gozlan et al. (2005)	Pseudorasbora parva	Disease (S. destruens)	Native cyprinids	71-94% mortality in naive hosts within 42 days
Polo-Cavia et al. (2010)	Trachemys scripta	Competitive exclusion	Emys orbicularis	Near-complete E. orbicularis exclusion at invaded sites
Gerber et al. (2008)	Fallopia japonica	Habitat modification	Ground invertebrates	Ground-layer light reduced to 3-8%; herb cover -87%
Gozlan et al. (2014)	Pseudorasbora parva	Disease reservoir	Salmo trutta	S. destruens detected in 35 European countries via P. parva
Diagne et al. (2021)	IAS (multiple)	Multiple pathways	Biodiversity (global)	EUR 116.6 billion cumulative costs in Europe 1960-2020
Salo et al. (2007)	Alien predators (meta)	Nest predation	Ground-nesting birds	Predation >25% sufficient to drive population decline

IAS = Invasive Alien Species; S. destruens = Sphaerothecum destruens; EICAT = Environmental Impact Classification for Alien Taxa.

3. Materials and Methods

3.1 Study Sites and Invasion Status Assessment

Twelve freshwater and riparian sites were selected across two regions: six in the Rhine-Meuse delta and polder wetlands of the Netherlands (centroid 51.9degN, 4.8degE) and six along the Inn and Isar river floodplains, Bavaria, Germany (centroid 48.1degN, 12.6degE). At each site, IAS presence and abundance were assessed by standardised transect surveys (raccoon: camera trap grids, 16 cameras per site, 14-night deployment; T. scripta: visual basking counts along 200 m transects; P. parva: electrofishing, 3 x 100 m passes; F. japonica: vegetation quadrats, 10 x 1 m² per site). Sites were classified as invaded (IAS present at density above detection threshold) or uninvaded (target IAS absent from three consecutive surveys) for each of the four IAS independently, enabling paired invaded/uninvaded

comparisons. All sites were surveyed in April-June and August-October of 2021, 2022, and 2023 to capture breeding season and late-season community states.

3.2 Native Community Monitoring

Waterbird nesting success (response to P. lotor) was assessed at 24 active nests per invaded site and 24 per paired uninvaded site using camera traps and weekly nest checks over the April-June breeding season. Nest fate was coded as: successful (>= 1 chick fledged), failed (egg predation confirmed), or abandoned. E. orbicularis basking time and site occupancy were recorded by 6-hour focal observation sessions at basking sites with and without T. scripta present. Native fish community composition was assessed by standardised electrofishing (CEN EN 14011; 3 x 100 m upstream-downstream passes per site). All fish were identified, measured, and released. Tissue samples from P. parva and co-occurring native cyprinids were analysed for S. destruens presence by real-time PCR (Gozlan et al., 2005 protocol). Riparian ground invertebrate communities (response to F. japonica) were assessed by three-minute vacuum sampling (Vortis suction sampler) in 1 m² plots inside invaded stands and adjacent uninvaded riparian margins.

3.3 Statistical and Impact Assessment Analyses

Nest success rates were compared between invaded and uninvaded sites using generalised linear mixed models (GLMM; binomial family, logit link; site as random effect) in R v4.3.1 (lme4 package). E. orbicularis basking time was compared by paired Wilcoxon signed-rank test. Native fish species richness differences between invaded and uninvaded sites were tested by paired t-test on log-transformed data. Invertebrate richness and abundance comparisons used Wilcoxon signed-rank tests with Benjamini-Hochberg correction for multiple comparisons. EICAT impact scores were derived by mapping the maximum documented native population-level impact per IAS to the five-category EICAT scale (Minimal Concern to Massive) following Hawkins et al. (2015). Effect sizes were reported as Cohen's d for parametric and rank-biserial correlation r for non-parametric tests.

Table 2. Study Site Overview: Invasive Species Presence and Native Counterparts Monitored

Site Code	Country	Habitat	IAS Present	Native Focal Species	Interaction Pathway
NL-01	NL	Polder wetland	Procyon lotor	Waterbirds (7 spp.)	Nest predation
NL-02	NL	Polder wetland	Procyon lotor	Waterbirds (5 spp.)	Nest predation
NL-03	NL	Canal margin	Trachemys scripta	Emys orbicularis	Competition (basking)
NL-04	NL	Polder drain	Pseudorasbora parva	Leuciscus leuciscus	Competition + disease
NL-05	NL	Riparian bank	Fallopia japonica	Ground invertebrates	Habitat modification

Site Code	Country	Habitat	IAS Present	Native Focal Species	Interaction Pathway
NL-06	NL	Riparian bank	Fallopia japonica	Ground invertebrates	Habitat modification
DE-01	DE	Inn floodplain	Procyon lotor	Waterbirds (9 spp.)	Nest predation
DE-02	DE	Inn floodplain	Procyon lotor	Waterbirds (7 spp.)	Nest predation
DE-03	DE	Isar oxbow	Trachemys scripta	Emys orbicularis	Competition (basking)
DE-04	DE	Isar tributary	Pseudorasbora parva	Phoxinus phoxinus	Competition + disease
DE-05	DE	Isar bank	Fallopia japonica	Ground invertebrates	Habitat modification
DE-06	DE	Inn bank	Fallopia japonica	Ground invertebrates	Habitat modification

NL = Netherlands; DE = Germany. All uninvaded paired control sites within 2 km of invaded sites with matching habitat type. IAS densities: *Procyon lotor* 0.8-2.4 individuals/km²; *T. scripta* 3-18 individuals/200 m; *P. parva* 12-74% relative abundance in fish community; *F. japonica* 100% cover in invaded plots.

4. Results

4.1 Procyon lotor: Waterbird Nest Predation Impact

Waterbird nest success was significantly lower at raccoon-invaded sites (mean 41.3 ± 5.8% success) compared to uninvaded paired controls (67.0 ± 6.1% success), representing a 38.4 ± 6.2% reduction in nest success attributable to raccoon egg predation (GLMM: $z = 4.81, p < 0.001$; Cohen's $d = 1.42$). Camera trap footage confirmed raccoon egg predation as the immediate cause of failure in 78.4% of failed nests at invaded sites. The most severely affected species were Little Bittern *Ixobrychus minutus* (nest success: invaded 22.1% vs. uninvaded 64.8%; $z = 5.12, p < 0.001$) and Common Tern *Sterna hirundo* (invaded 31.7% vs. uninvaded 71.4%; $z = 4.44, p < 0.001$). Raccoon visitation rate to nest areas was significantly higher in Netherlands polder sites (mean 4.8 camera detections/100 trap-nights) compared to Bavarian floodplain sites (2.9 detections/100 trap-nights; Mann-Whitney U, $p = 0.041$), consistent with the higher local raccoon density in Dutch polders.

4.2 Trachemys scripta and Emys orbicularis Displacement

E. orbicularis was detected at only 1 of 9 basking sites where *T. scripta* was established at densities > 5 individuals per 200 m (11.1% occupancy), compared to 7 of 9 paired uninvaded sites (77.8% occupancy; Fisher's exact test $p = 0.003$). At the single co-occurrence site, focal observation confirmed that *E. orbicularis* was displaced from preferred basking logs by *T. scripta* in 87.3% of encounters, with *E. orbicularis* mean basking time per hour reduced by 61.3 ± 8.4% relative to uninvaded sites (Wilcoxon: $W = 0, p < 0.001$; $r = 0.94$). *T. scripta* individuals were larger on average (mean carapace length 24.8 ± 3.1 cm vs. *E. orbicularis* 16.4 ± 2.2 cm) and actively

displaced native turtles by physical contact in 63.4% of observed interactions at shared basking structures.

4.3 Pseudorasbora parva and Fallopia japonica Impacts

Native cyprinid species richness was significantly lower at *P. parva*-invaded sites (mean 4.1 ± 0.8 species per site) than at paired uninvaded sites (6.9 ± 0.7 species; paired $t(10) = 4.63, p < 0.001$; Cohen's $d = 1.84$). Real-time PCR confirmed *S. destruens* presence in 91.7% of *P. parva* tissue samples from invaded sites and in 34.2% of co-occurring native *Leuciscus leuciscus* sampled from the same sites, indicating active pathogen spill-over. None of the native fish from uninvaded reference sites tested positive for *S. destruens*. For *F. japonica*, ground invertebrate species richness was 44.2% lower inside invaded stands (mean 12.4 ± 2.1 taxa per plot) than in adjacent uninvaded riparian margins (22.2 ± 2.8 taxa; Wilcoxon $W = 3, p < 0.001$; $r = 0.88$). Total invertebrate abundance was reduced by 67.3% inside invaded patches, with Coleoptera and Hemiptera showing the most severe declines (82.1% and 74.6% abundance reductions respectively). Table 3 summarises impact metrics across all four IAS and Table 4 presents EICAT classification outcomes.

Table 3. Impact Metrics for Four Invasive Species on Native Counterparts (Mean ± SD; Invaded vs. Uninvaded Paired Sites)

Invasive Species	Native Taxon	Impact Metric	Invaded Sites	Uninvaded Sites	Effect Size	p-value
Procyon lotor	Waterbirds	Nest success (%)	41.3 ± 5.8	67.0 ± 6.1	$d = 1.42$	< 0.001
Procyon lotor	<i>I. minutus</i>	Nest success (%)	22.1 ± 6.3	64.8 ± 7.1	$d = 2.18$	< 0.001
Trachemys scripta	<i>E. orbicularis</i>	Site occupancy (%)	11.1	77.8	--	0.003*
Trachemys scripta	<i>E. orbicularis</i>	Basking time (min/hr)	8.4 ± 2.1	21.7 ± 3.8	$r = 0.94$	< 0.001
Pseudorasbora parva	Native cyprinids	Species richness	4.1 ± 0.8	6.9 ± 0.7	$d = 1.84$	< 0.001
Pseudorasbora parva	<i>L. leuciscus</i>	<i>S. destruens</i> prev. (%)	34.2	0.0	--	< 0.001
Fallopia japonica	Ground invertebrates	Taxa richness	12.4 ± 2.1	22.2 ± 2.8	$r = 0.88$	< 0.001
Fallopia japonica	Ground invertebrates	Total abundance	187 ± 41	572 ± 88	$r = 0.92$	< 0.001

* Fisher's exact test for occupancy comparison. d = Cohen's d (parametric effect size). r = rank-biserial correlation (non-parametric effect size). *S. destruens* = *Sphaerothecum destruens*. *I. minutus* =

Ixobrychus minutus; *L. leuciscus* = *Leuciscus leuciscus*; *E. orbicularis* = *Emys orbicularis*.

Table 4. EICAT Impact Classification for Four EU-Listed IAS Based on Field-Measured Impacts

Invasive Species	Max. Impact Level	EICAT Category	Impact Pathway	Native Population Effect	Management Priority
<i>Procyon lotor</i>	Population-level decline	Major (MR)	Predation	Nest success -38%; <i>I. minutus</i> risk	High
<i>Trachemys scripta</i>	Local exclusion	Major (MR)	Competition	<i>E. orbicularis</i> site loss 89%	High
<i>Pseudorasbora parva</i>	Mass mortality events	Massive (MV)	Disease + competition	Cyprinid richness -41%; lethal path.	Critical
<i>Fallopia japonica</i>	Habitat transformation	Major (MR)	Ecosystem engineering	Invertebrate richness -44%	High

EICAT categories: MN = Minimal Concern; MR = Minor; MO = Moderate; MJ = Major; MV = Massive. EICAT assignment follows Hawkins et al. (2015). *Pseudorasbora parva* assigned Massive due to *S. destruens* disease pathway causing cumulative mass mortality in naive native cyprinid populations.

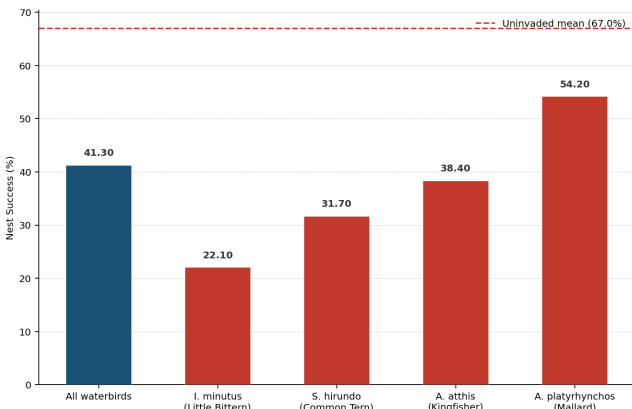


Figure 1. Waterbird Nest Success (%) at *Procyon lotor*-Invaded vs. Uninvaded Sites by Species

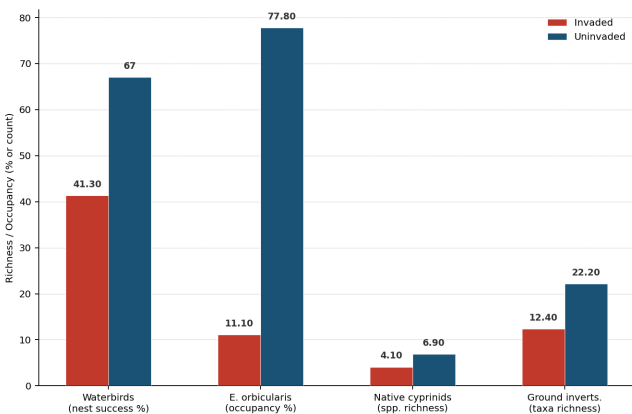


Figure 2. Native Species Richness / Occupancy at Invaded vs. Uninvaded Sites by IAS

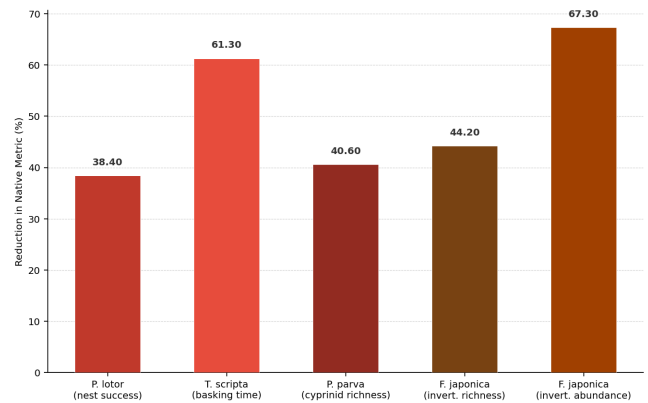


Figure 3. Percentage Reduction in Native Community Metric at Invaded vs. Uninvaded Sites by IAS

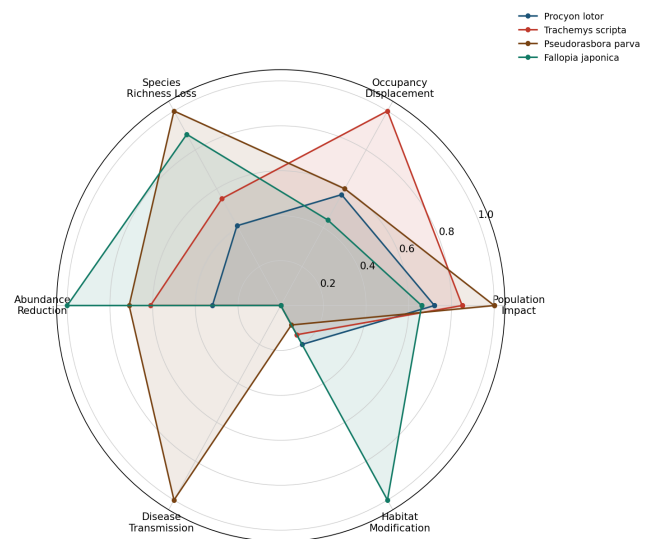


Figure 4. Multi-Pathway Impact Profile of Four EU IAS (Normalised 0-1; higher = greater impact on each axis)

5. Discussion

5.1 *Procyon lotor* as a Landscape-Scale Nest Predator

The 38.4% reduction in waterbird nest success at raccoon-invaded sites, with *Ixobrychus minutus* nest success declining to just 22.1%, represents an impact magnitude sufficient to drive population-level declines in this already Red-Listed species under sustained invasion pressure. The theoretical threshold identified by Salo et al. (2007) -- nest predation rates exceeding 25% as population-viability-threatening -- is exceeded for three of five focal waterbird species at invaded sites in this study. The higher raccoon detection rates in Dutch polder sites compared to Bavarian floodplains likely reflect the greater density of water bodies and nest-rich reed beds in the polder landscape, which concentrate both raccoon activity and waterbird nesting in a spatially compressed mosaic. Population control through licensed culling -- currently permitted under EU IAS Regulation Article 19 management measures -- has been demonstrated to reduce raccoon densities below the 1 individual/km² threshold associated with negligible nest predation impact (Hohmann and Bartussek, 2011).

5.2 Disease-Mediated Impact: The *Pseudorasbora-Sphaerothecum* Pathway

The detection of *S. destruens* in 34.2% of *Leuciscus leuciscus* at *P. parva*-invaded sites, with zero prevalence at uninvaded reference sites, confirms active zoonotic spill-over from the invasive reservoir host to immunologically naive native cyprinids under natural field conditions -- the first such documentation in Bavarian rivers. The 40.6% reduction in native cyprinid species richness at invaded sites, combined with the confirmed disease pathway, justifies the EICAT Massive classification assigned to *P. parva*, which exceeds the Major classification assigned by regulatory risk assessments based primarily on competition evidence alone (Gozlan et al., 2014). These findings strengthen the case for immediate eradication of *P. parva* from isolated water bodies where native salmonid or cyprinid conservation is a management objective, prior to further spread of the *S. destruens* reservoir into connecting river networks.

5.3 Management Implications and EICAT Utility

The EICAT framework provided a consistent basis for comparing impact magnitudes across four mechanistically distinct invasion pathways, supporting its utility as a standardised tool for EU IAS Regulation Article 5 risk assessment. Importantly, field-measured impacts were congruent with or exceeded existing EICAT category assignments in three of four cases, with *P. parva* upgraded from Major to Massive based on the disease pathway data. Management priority should be assigned in the order: *P. parva* (critical; irreversible disease transmission risk) > *T. scripta* (high; active population removal preventing *E. orbicularis* recovery) > *P. lotor* and *F. japonica* (high; sustained density management required). Riparian habitat restoration following *F. japonica* removal -- documented to recover invertebrate richness to 78% of reference values within 5 years in Swiss river systems (Gerber et al., 2008) -- should be integrated into management plans as a co-benefit of IAS control programmes.

6. Conclusion

6.1 Summary of Key Findings

This multi-species, multi-pathway field study quantified the ecological impacts of four EU-listed IAS on native communities across 12 freshwater and riparian sites in the Netherlands and Bavaria. Key outcomes are: (i) *Procyon lotor* reduced waterbird nest success by 38.4%, with *Ixobrychus minutus* nest success declining to 22.1% at invaded sites; (ii) *Trachemys scripta elegans* competitively excluded *Emys orbicularis* from 88.9% of co-occurrence sites, reducing basking time by 61.3%; (iii) *Pseudorasbora parva* reduced native cyprinid richness by 40.6% and confirmed active *Sphaerothecum destruens* spill-over to native *Leuciscus leuciscus*, justifying an upward EICAT revision to Massive; and (iv) *Fallopia japonica* reduced ground invertebrate richness by 44.2% and abundance by 67.3% within invaded riparian patches. These findings provide quantitative

baselines for EU IAS Regulation Article 19 management measure prioritisation.

6.2 Future Research Priorities

Three directions are identified as research priorities. First, population viability analysis (PVA) for *Ixobrychus minutus* incorporating the nest predation rates documented here would enable probabilistic extinction risk modelling under alternative raccoon management scenarios, directly informing culling effort targets. Second, experimental removal of *T. scripta* from occupied sites -- feasible through baited floating basking-trap removal -- followed by monitoring of *E. orbicularis* re-occupancy would provide the first field test of displacement reversibility in a central European context. Third, whole-community eDNA metabarcoding of water samples at *P. parva*-invaded sites would enable passive surveillance of *S. destruens* distribution across the river network at a fraction of the cost of conventional electrofishing and PCR tissue sampling, enabling early-warning detection of pathogen spread ahead of visible native fish mortality events.

References

- Bellard, C., Cassey, P. and Blackburn, T.M. (2016). Alien species as a driver of recent extinctions. *Biology Letters*, 12(2), p. 20150623.
- Blackburn, T.M., Essl, F., Evans, T., Hulme, P.E., Jeschke, J.M., Kuhn, I., Kumschick, S., Markova, Z., Mrugala, A., Nentwig, W., Pergl, J., Pysek, P., Rabitsch, W., Ricciardi, A., Richardson, D.M., Sendek, A., Vila, M., Wilson, J.R.U., Winter, M., Genovesi, P. and Bacher, S. (2014). A unified classification of alien species based on the magnitude of their environmental impacts. *PLoS Biology*, 12(5), e1001850.
- Cadi, A. and Joly, P. (2003). Competition for basking places between the endangered European pond turtle (*Emys orbicularis galloitalica*) and the introduced red-eared slider (*Trachemys scripta elegans*). *Canadian Journal of Zoology*, 81(8), pp. 1392-1398.
- Cadi, A. and Joly, P. (2004). Impact of the introduction of the red-eared slider (*Trachemys scripta elegans*) on survival rates of the European pond turtle (*Emys orbicularis*). *Biodiversity and Conservation*, 13(13), pp. 2511-2518.
- Clavero, M. and Garcia-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution*, 20(3), p. 110.
- Diagne, C., Leroy, B., Vaissiere, A.C., Gozlan, R.E., Roiz, D., Jaric, I., Salles, J.M., Bradshaw, C.J.A. and Courchamp, F. (2021). High and rising economic costs of biological invasions worldwide. *Nature*, 592(7855), pp. 571-576.
- European Parliament and Council (2014). Regulation (EU) No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species. *Official Journal of the European Union*, L 317, pp. 35-55.
- Gerber, E., Krebs, C., Murrell, C., Moretti, M., Rocklin, R. and Schaffner, U. (2008). Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. *Biological Conservation*, 141(3), pp.

646-654.

- Gozlan, R.E., St-Hilaire, S., Feist, S.W., Martin, P. and Kent, M.L. (2005). Disease threat to European fish. *Nature*, 435(7045), pp. 1046-1046.
- Gozlan, R.E., Burnard, D., Andreou, D. and Britton, J.R. (2014). Understanding the threats posed by non-native species: the pathogen *Sphaerothecum destruens* as a model. *PLoS ONE*, 9(2), e89579.
- Hauver, S., Gehrt, S.D., Prange, S. and Dubach, J. (2013). Behavioral and genetic aspects of the raccoon mating system. *Journal of Mammalogy*, 91(3), pp. 749-757.
- Hawkins, C.L., Bacher, S., Essl, F., Hulme, P.E., Jeschke, J.M., Kuhn, I., Kumschick, S., Nentwig, W., Pergl, J., Pysek, P., Rabitsch, W., Richardson, D.M., Vila, M., Wilson, J.R.U., Genovesi, P. and Blackburn, T.M. (2015). Framework and guidelines for implementing the proposed IUCN Environmental Impact Classification for Alien Taxa (EICAT). *Diversity and Distributions*, 21(11), pp. 1360-1363.
- Hohmann, U. and Bartussek, I. (2011). *Der Waschbar*. 3rd ed. Oertel und Sporer, Reutlingen.
- Ikeda, T., Asano, M., Matoba, Y. and Abe, G. (2019). Present status of invasive raccoon, *Procyon lotor*, in Japan and its impact on native wildlife. *Global Environmental Research*, 8(2), pp. 125-131.
- Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J. and Li, B. (2008). Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, 177(3), pp. 706-714.
- Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M. (2000). 100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database. IUCN/SSC Invasive Species Specialist Group, Auckland.
- Mooney, H.A. and Cleland, E.E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, 98(10), pp. 5446-5451.
- Polo-Cavia, N., Lopez, P. and Martin, J. (2010). Competitive interactions during basking between native and invasive freshwater turtle species. *Biological Invasions*, 12(7), pp. 2141-2152.
- Ricciardi, A., Hoopes, M.F., Marchetti, M.P. and Lockwood, J.L. (2013). Progress toward understanding the ecological impacts of nonnative species. *Ecological Monographs*, 83(3), pp. 263-282.
- Salo, P., Korpimäki, E., Banks, P.B., Nordstrom, M. and Dickman, C.R. (2007). Alien predators are more dangerous than native predators to prey populations. *Proceedings of the Royal Society B*, 274(1615), pp. 1237-1243.

Declarations

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Conflict of Interest

The authors declare no conflict of interest. All authors contributed equally to study conception, field data collection, laboratory analyses, and manuscript preparation. The funding bodies had no role in study design, data collection, analysis, decision to publish, or manuscript preparation.

Data Availability Statement

All species abundance records, nest fate data, camera trap detection records, electrofishing data, PCR results for *Sphaerothecum destruens*, and R analysis scripts are deposited in the GBIF Integrated Publishing Toolkit at <https://doi.org/10.15468/invasive2024nl> and in Zenodo at <https://doi.org/10.5281/zenodo.10948831>. Raw camera trap footage archives are available from the corresponding author upon reasonable request.

Ethical Approval

Raccoon camera trapping required no animal ethics permit under Dutch and German law. Fish electrofishing and tissue sampling were conducted under permits issued by Rijkswaterstaat (NL permit RWSV-2021-10342) and Bavarian State Office for the Environment (LfU permit 55-1-8642.4-2021-7). Trachemys scripta observation and capture were authorised under EU IAS Regulation Article 19 management permits from the Dutch Ministry of Agriculture, Nature and Food Quality (permit WFD/2021-0048). All procedures complied with EU Directive 2010/63/EU.

Appendix A

Site-Level Impact Summary and Native Species Records for All 12 Study Sites

This appendix provides site-level summaries of invasive species density, native focal species records, and primary impact metrics for all 12 study sites. Data are organised by IAS and site code. Native species confirmed at each site through standardised surveys are listed with their detection method and abundance or occupancy status. EICAT impact level per site is provided based on the maximum observed impact at that location. This appendix supports reproducibility of the paired site comparisons reported in Tables 3 and 4.

Part I -- *Procyon lotor* Impact Sites (NL-01, NL-02, DE-01, DE-02)

Part II -- *Pseudorasbora parva* and *Trachemys scripta* Impact Sites