HELLENIC CENTRE FOR MARINE RESEARCH

INSTITUTE OF MARINE BIOLOGICAL RESOURCES AND INLAND WATERS

REINTRO-HELLAS

A FRESHWATER FISH REINTRODUCTION MANUAL

Feasibility Assessment, Fish Translocation Guidelines

and Post-Release Monitoring Protocol

Version 1.0

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The Institute of Marine Biological Resources and Inland Waters (IMBRIW)

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To fulfil this mission, IMBRIW carries out multidisciplinary field, laboratory and experimental research, spanning both basic and applied science. It monitors and assesses the status and trends of fish and shellfish stocks in Greek waters, evaluates the ecological quality of inland waters, and advises on the sustainable exploitation of fisheries resources in Greek and Mediterranean seas. The Institute also undertakes targeted pilot studies, develops innovative tools and methodologies, and prepares management plans on specific issues at national, Mediterranean and EU scales. Research results are disseminated through scientific publications, technical reports, training activities and a wide range of organised events, in close collaboration with the other two Institutes of HCMR.

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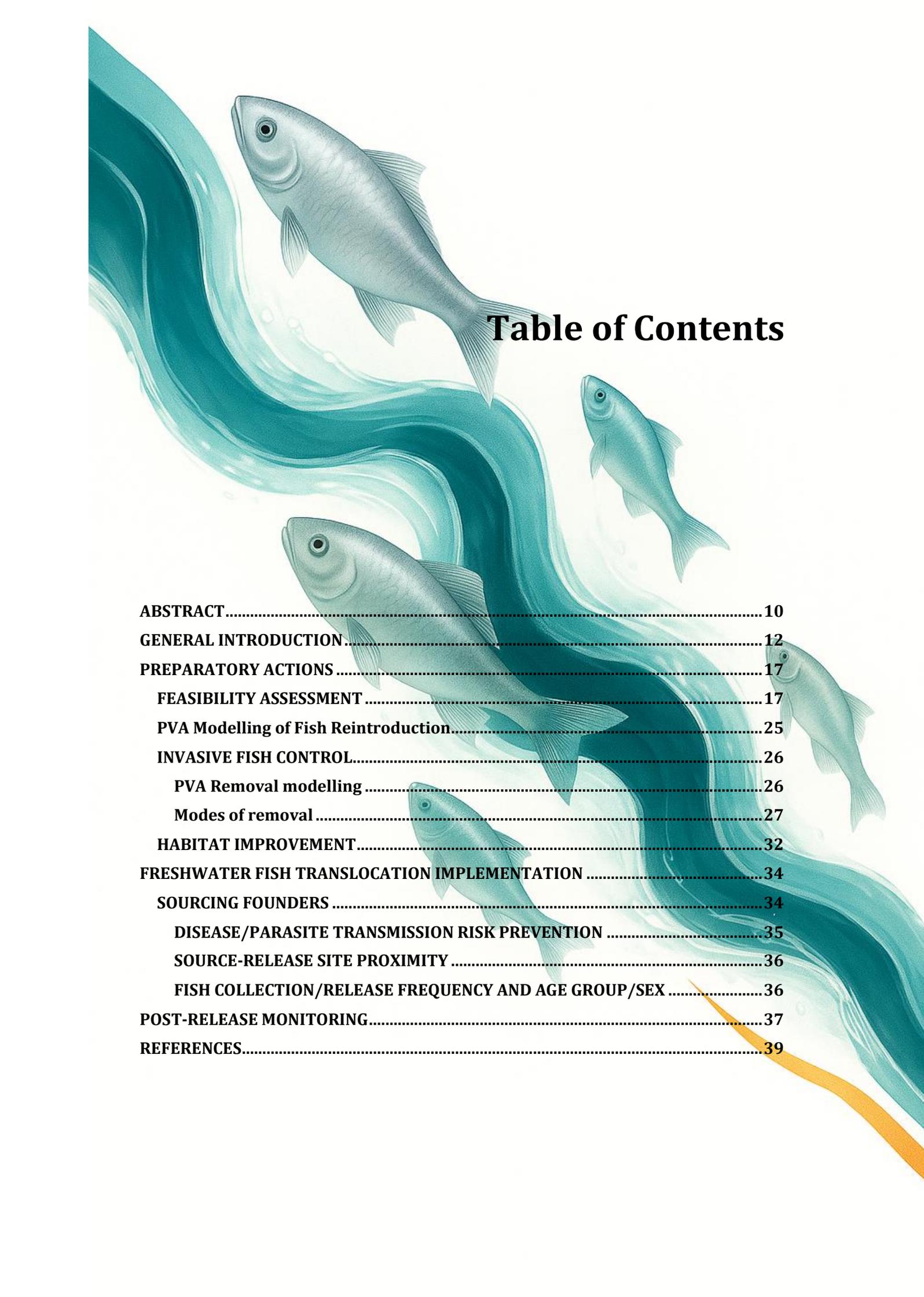
Purpose and audience

This manual provides practical guidance for the planning, implementation, and evaluation of conservation translocations of native freshwater fishes in Greece and the wider Mediterranean region. It focuses on reintroductions and population reinforcement actions in lotic systems (rivers, streams and associated spring-fed habitats), with particular emphasis on species and populations that are threatened, declining, or locally extirpated. The procedures, tools and decision criteria presented here are tailored to the ecological, hydro-morphological and management realities of Mediterranean freshwater ecosystems, and especially to Greek inland waters.

The manual is intended primarily for competent authorities responsible for water and biodiversity management (e.g. national ministries, river basin and regional water directorates, protected area management bodies), as well as for environmental agencies, NGOs and consultants involved in the design and delivery of freshwater fish conservation projects. It is also directed at researchers and technical experts who support these authorities through ecological assessments, feasibility studies, population modelling and monitoring programs. While the framework is designed with Greek legislation and governance structures in mind, most of the concepts, tools and recommended practices can be adapted and applied to similar conservation translocation projects elsewhere in the Mediterranean region.

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ABSTRACT

This manual provides a stepwise framework for the planning, implementation, and evaluation of freshwater fish reintroductions in Greece and the wider Mediterranean region. It integrates international guidance on conservation translocations with tools and criteria tailored to Mediterranean lotic systems. The preparatory section focuses on pre-translocation feasibility assessment, threat mitigation, and habitat restoration, with particular emphasis on invasive fishes' control and habitat degradation as key constraints on reintroduction success. A feasibility tool (REVIVE-HELLAS) described in detail in Vardakas et al. (2025), is proposed for the evaluation of release and source water bodies on the basis of ecological status, habitat quantity and quality, hydro-morphological alteration, invasive species pressure, riparian condition, land use, and anthropogenic pressures, as well as the source habitat's demographic and genetic suitability.

Implementation guidelines cover sourcing and handling of founders from wild and captive stocks, transport and acclimatisation, disease and parasite risk management, and the design of stocking strategies, informed by population viability analyses. The manual also outlines methods for invasive fish control, including depletion electrofishing and removal modelling, and details post-release monitoring protocols based on conventional sampling, underwater video, and environmental DNA (eDNA) to assess establishment, reproduction, and long-term viability of reintroduced populations. Overall, the manual aims to standardise and strengthen freshwater fish translocation practice, support evidence-based decision-making by authorities and practitioners, and promote the recovery of threatened freshwater fish assemblages under current and future environmental change.

GENERAL INTRODUCTION

While human-mediated movement of animal species has occurred for millennia, the intentional use of conservation translocations to counter biodiversity loss and restore ecosystem functions is a relatively recent development. Over the past 30–40 years, such efforts have increased dramatically, particularly in response to accelerating species declines and habitat degradation (Seddon et al., 2014).

Despite this progress, there remains a strong taxonomic bias in global translocation initiatives. Mammals and birds dominate both research and implementation, while freshwater fishes, along with amphibians and reptiles, remain substantially underrepresented—even though they are among the most threatened vertebrate groups (Fischer & Lindenmayer, 2000; Seddon et al., 2005, 2007, 2014; Berger-Tal et al., 2020). This underrepresentation is particularly concerning given that freshwater vertebrates are declining at nearly twice the rate of terrestrial or marine species and face a disproportionately high risk of extinction (Collen et al., 2009; McRae et al., 2017; Reid et al., 2019). The urgency is especially pronounced in European Mediterranean freshwater systems, which are globally recognised biodiversity hotspots. In this region, 30 to 60% of native freshwater fish species are currently classified as threatened by the IUCN (Smith & Darwall, 2006; Oikonomou et al., 2014), underscoring the need for targeted conservation measures such as reintroductions.

According to the IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN, 2013) and relevant ecological literature (Cowx, 1994; Minckley, 1995; Fischer & Lindenmayer, 2000; George et al., 2009; Cochran-Biederman et al., 2015), a species translocation typically comprises three interlinked stages. These include (a) the initial feasibility assessment and other preparatory actions, including habitat improvement, threat mitigation, and source–recipient compatibility evaluation, (b) the implementation of the translocation action, which is the physical relocation of individuals and release into the designated environment and (c) the post-release monitoring of the success of the action, which tracks ecological responses and population outcomes to inform adaptive management.

The feasibility assessment is a particularly complex and pivotal phase. It incorporates evaluations of habitat quality, ecological integrity, species-specific life history traits, and sociopolitical feasibility (Fischer & Lindenmayer, 2000; George et al., 2009; Berger-Tal et al., 2020). This step is formalised and operationalised through the REVIVE-HELLAS assessment tool, which evaluates habitat suitability, ecological integrity, biotic interactions, genetic compatibility, and threat levels (Kalogianni et al. 2023).

While a positive feasibility assessment is a necessary condition for translocation, detailed implementation planning is equally vital to success. This includes the active control of non-native species in the release habitat, if present, in order to reduce competition or predation risk to the native fauna (Kulp & Moore, 2000; Caudron & Champigneulle, 2011; Rytwinski et al., 2019; Davies et al., 2020). The estimation of optimal stocking densities is also essential and is best achieved using tools such as Population Viability Analysis (PVA), which can define the ideal number, age class, and sex ratio of released individuals. This analysis must consider both the carrying capacity of the release habitat and the replacement rate of the source population (Lacy, 1993, 2000; Lacy & Pollak, 2020; Lamothe et al., 2021). Additional planning components for the translocation include the evaluation of source population status, especially for wild-sourced individuals; strategic decisions about the timing, frequency, and spatial structure of releases; and appropriate handling, packing, transport, and acclimatisation protocols (George et al., 2009; Cowx, 1994; Healy et al., 2020). Consideration must also be given to the proximity between source and release habitats, to preserve both genetic and ecological compatibility, and to the potential for disease or parasite transmission, and domestication or developing naïve behavioural traits, particularly where founders originate from captive breeding programs.

Finally, post-release monitoring is essential to evaluate the effectiveness, ecological impact, and sustainability of the translocation. Monitoring must be structured and standardised, and should track population establishment, reproduction, dispersal, mortality and broader ecological responses such as community-level change and habitat use. Quantitative tools, such as fish-based ecological indices are particularly useful for assessing ecosystem condition before and after translocation, including the restoration of biotic integrity (Pont et al., 2007; Aparicio et al., 2011).

The **REINTRO-HELLAS** manual builds on this foundation, offering a detailed framework for implementing freshwater fish reintroductions in Greece and the broader Mediterranean region. Its "genealogy" includes the first attempt for a freshwater fish translocation in Greece, the translocation of the Corfu killifish *Valencia letourneuxi* that followed a strict protocol custom-made for the feasibility assessment, the translocation and the post-release monitoring of the focal species (Kalogianni et al. 2024).

It combines evidence-based criteria, standardised protocols, and a strong adaptive management approach, aligned with global best practices for species conservation.
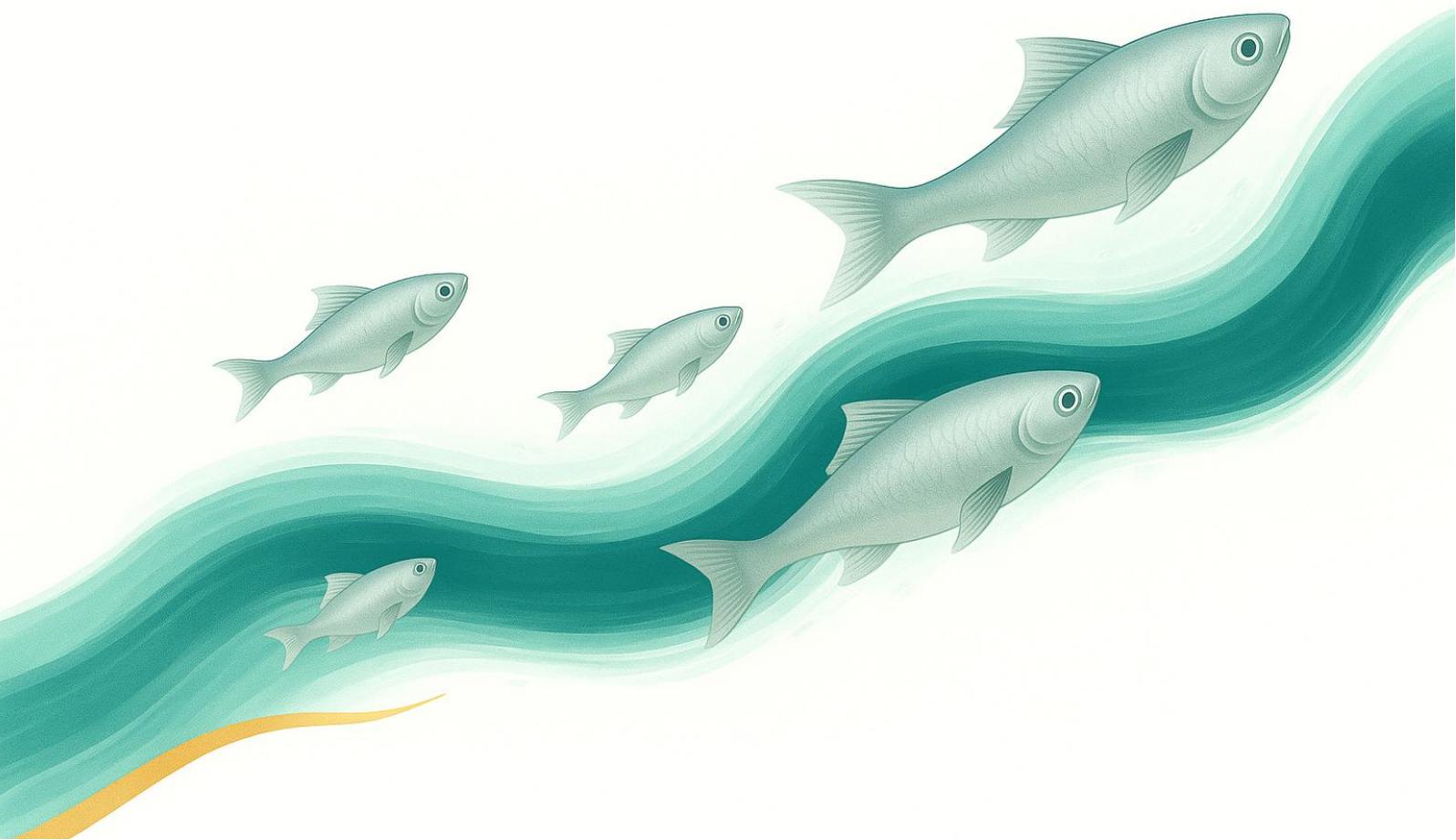
Structure of the manual

The manual is organised as a stepwise framework that follows the full cycle of a conservation translocation project (Fig. 1), from initial problem framing to long-term evaluation. It begins with preparatory actions, including clarification of objectives, review of species status and threats, and identification of candidate recipient and source water bodies. These preparatory steps culminate in a feasibility assessment using the REVIVE HELLAS tool, which evaluates ecological status, habitat suitability, hydro-morphological alteration, invasive species pressure, riparian condition, land use and future systemic pressures in recipient water bodies, alongside demographic and genetic suitability of candidate source populations.

Building on the feasibility outcome, the manual then addresses invasive fish control and habitat improvement as prerequisites for any reintroduction or reinforcement. A dedicated section presents the use of Population Viability Analysis (PVA) both for designing removal strategies in invaded systems and for planning reintroduction scenarios (e.g. number of founders, stocking frequency and duration). Subsequent sections provide implementation guidelines for sourcing and handling founders, transport, acclimatisation and release strategies, followed by a comprehensive chapter on post-release monitoring, which combines conventional sampling, removal modelling, and modern tools such as underwater video and environmental DNA (eDNA). Together, these components form a coherent workflow that supports transparent, evidence-based decision-making throughout the life cycle of a freshwater fish translocation project.
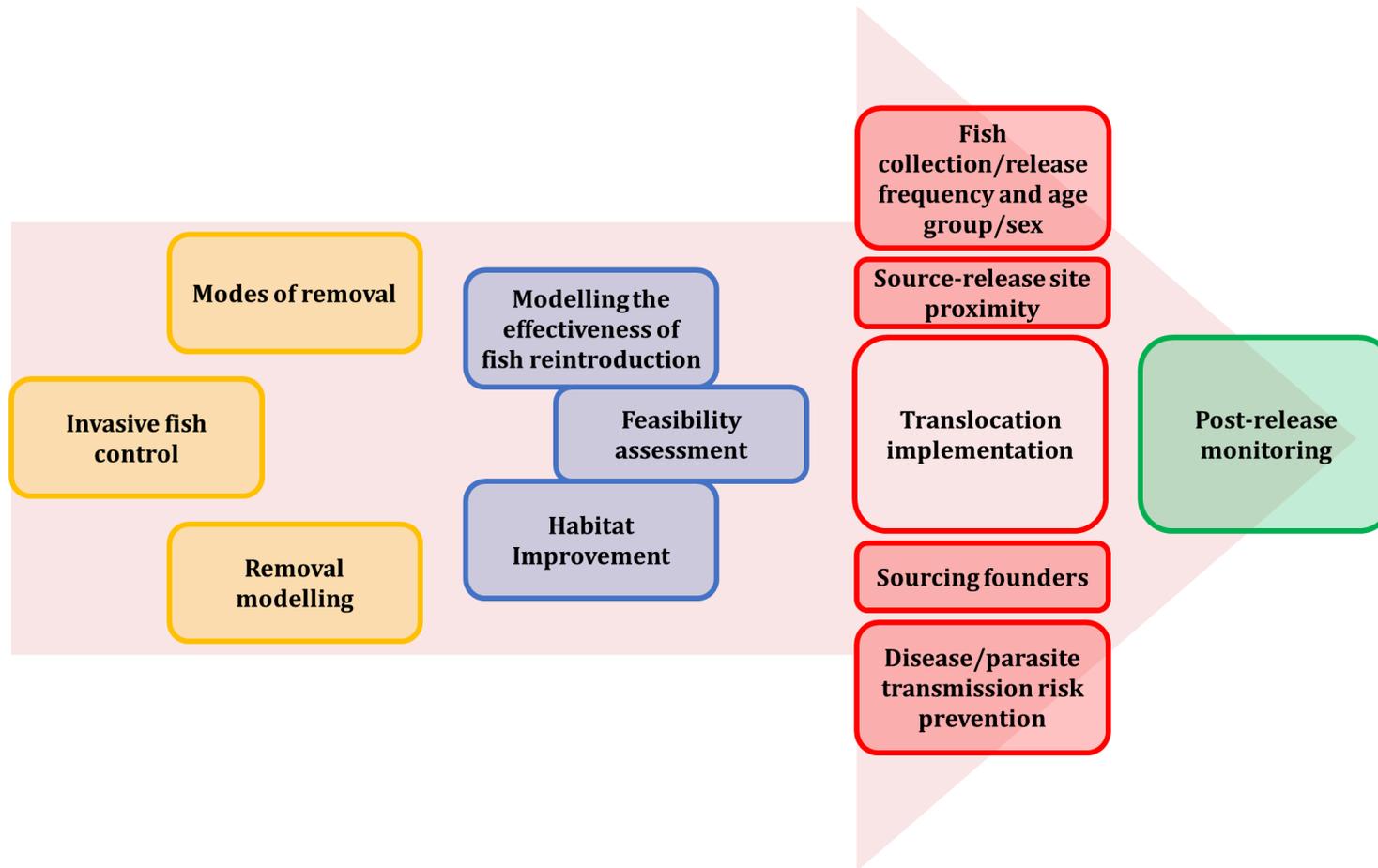
Figure 1. Workflow of the REINTRO-HELLAS framework for freshwater fish conservation translocations, from preparatory actions and feasibility assessment through invasive fish control, modelling-informed design of removal and reintroduction scenarios, implementation, and post-release monitoring, with adaptive management and feedback.

PREPARATORY ACTIONS

FEASIBILITY ASSESSMENT

A feasibility assessment tool must be applied as part of the preparatory phase of a fish translocation project, to evaluate the ecological suitability of both the potential release water bodies (R-WBs) and the source water bodies (S-WBs). This assessment is fundamental for the final selection of appropriate sites, ensuring that ecological, demographic, and genetic criteria are met before the translocation proceeds. For this purpose, the REVIVE HELLAS tool should be applied, an adaptation of the original REVIVE tool developed for freshwater fish conservation translocations in Mediterranean rivers (Kalogianni et al., 2023). The REVIVE HELLAS version retains the conceptual foundation of the original framework, while extending its application to include non-Mediterranean rivers and multiple freshwater fish groups found in Greek lotic ecosystems. Where needed, context-specific modifications can be made to reflect site conditions, species biology, or data availability.

The tool builds on international guidance and scientific literature on reintroductions and species translocations (Cowx, 1994; Minckley, 1995; Fischer & Lindenmayer, 2000; George et al., 2009; Dunham et al., 2011; Cochran-Biederman et al., 2015; IUCN, 1998, 2013; Berger-Tal et al., 2020), while incorporating additional criteria tailored to the needs of freshwater fishes in Greece. The REVIVE HELLAS tool assesses two main dimensions, (a) The capacity of candidate release water bodies (R-WBs) to support self-sustaining populations of the focal species under current and future conditions (criteria R-C1 to R-C13, **Table 1**), and (b) The suitability of potential source populations (S-WBs) in terms of demographic stability, genetic compatibility, and donor resilience (criteria S-C1 to S-C3). Several criteria include sub-criteria that evaluate distinct ecological, genetic, or environmental components.

The release water body assessment begins with **R-C1 (Current Presence),** which determines whether the target species is currently absent from the proposed R-WB. This is crucial to avoid unnecessary or ecologically inappropriate introductions. The score ranges from 1.0 (confirmed absence based on current field surveys) to 0.75 (absence confirmed by data collected within the past decade). Because absence data are inherently uncertain, the tool emphasises the use of multiple, complementary detection methods, including standardised electrofishing protocols, seine netting, and minnow traps (Lamothe & Drake, 2019), alongside novel techniques such as environmental DNA (eDNA) sampling and underwater video surveillance (Kalogianni et al., 2024). The next criterion, **R-C2 (Historical Presence),** evaluates whether the species

historically occupied the R-WB. This ensures that reintroductions align with the species' known biogeographical range and ecological niche. The score ranges from 1.0 (verified historical records within the R-WB) to 0.0 (no data or evidence of historical occurrence). The reliability of sources is critical—peer-reviewed literature, museum records, and time-series datasets are preferred. In the absence of direct records, biogeographical inference based on habitat type and species ecology may be used cautiously, with attention to potential errors caused by past misidentifications or data gaps.

The environmental suitability of the candidate release water body is evaluated **under R-C3, which includes two sub-criteria:** physico-chemical water quality (R-C3.1) and biological quality (R-C3.2). These are assessed using established ecological indices for macroinvertebrates and diatoms, following national and European water quality classification systems (Smeti & Karaouzas, 2016; Lazaridou et al., 2018; Munné et al., 2021). A site classified as having high or good ecological status receives a score of 1.0, while those with moderate, poor, or bad status are scored −1.0, reflecting their unsuitability for supporting reintroduced populations. **R-C4 evaluates the quantity of available habitat**, using measurements of wetted area, wetted length, or wetted volume, and compares these with reference sites that support viable populations of the focal species. For migratory species, this criterion is modified to incorporate longitudinal connectivity, assessing the presence or absence of upstream and downstream passage within a 10 km reach surrounding the release site.

R-C5 assesses habitat suitability in more detail by examining multiple ecological parameters: flow regime, depth, reproductive substrate availability, and trophic resources. Each parameter must align with the species-specific requirements of the focal taxon. Distinct scoring schemes are applied for small-bodied versus large-bodied species, reflecting their different ecological needs and sensitivities. Relevant data for these sub-criteria should be derived from well-documented habitats supporting stable populations, as well as from hydrological time series and species-specific ecological literature on spawning habitats and diet.

The REVIVE HELLAS tool also incorporates criteria for evaluating anthropogenic pressures that may reduce habitat quality and translocation success. **R-C6 addresses hydrological perturbation**, while **R-C7 examines channel morphological alteration**, with both criteria scored from 1.0 (natural or low perturbation) to −1.0 (high disturbance or artificial modification). These criteria account for stressors such as flow regulation, channelisation, or bank reinforcement that can affect spawning habitats and juvenile survival. **R-C8 evaluates invasive alien species (IAS)** pressure, which is scored from 1.0 (documented absence of invasive species) to −1.0 (high abundance of invasive species with demonstrated or likely negative impacts on the focal taxon). The detection of IAS should ideally be based on quantitative

field data, including relative and absolute abundance estimates, and should incorporate multiple sampling gears and eDNA-based tools to minimise false negatives (Pritt & Frimpong, 2010; Lamothe & Drake, 2019; Rees et al., 2014; Kalogianni et al., 2023). **Riparian vegetation structure (R-C9) and riparian lignified extent (R-C10)** are adapted from the River Habitat Survey (RHS v.2003) (Raven et al., 1998; Environment Agency, 2003) with modifications, and provide an indication of the structural complexity, canopy cover, and ecological integrity of the riparian corridor. These features contribute to shading, bank stability, nutrient cycling, and terrestrial invertebrate input into the aquatic food web, all of which are critical for fish habitat quality**. Land use** (**R-C11**) within a 30-meter buffer along both banks is also assessed. Water bodies surrounded by natural or semi-natural land use (e.g., forests, wetlands, low-impact grasslands) receive higher scores, while those dominated by intensive agriculture, suburban, or urban development are penalised due to their contribution to habitat degradation, pollution, and hydrological stress. **R-C12 examines the threat landscape of the potential release water body**, including both current and anticipated future pressures. It considers whether these threats are well understood, whether they can be effectively mitigated, or whether they pose persistent and unmanageable risks to population viability. Scoring reflects a combination of risk clarity and mitigation feasibility, ensuring that future scenarios are integrated into the feasibility framework. **R-C13 (Systemic Future Pressures)** assesses long-term, macro-scale drivers, such as climate change, land-use change, and socioeconomic shifts, that may affect the ecological integrity and management feasibility of the R-WB over time. Unlike R-C12, which focuses on local, site-specific threats, R-C13 incorporates regional vulnerability and adaptive capacity under future scenarios. Scores range from 1.0 (low projected risk and strong institutional/ecosystem resilience) to −1.0 (high risk from drought, urbanisation, or governance instability, with no mitigation options). A score of 0.0 reflects uncertainty due to limited or inconsistent data. Scoring should be based on downscaled climate models, demographic and land-use projections, and consultation with regional planning experts. This criterion ensures that reintroduction sites are not only viable today but also resilient to long-term systemic change.

The **source water body assessment** includes three criteria (Table 2). The first, **S-C1**, determines whether the proposed source population is wild or captive-bred. This is critical due to the potential genetic bottlenecks, domestication effects, and disease risks associated with hatchery-origin individuals. Wild populations score higher due to their generally greater genetic integrity and adaptability to natural conditions.

The second, **S-C2,** evaluates genetic compatibility between the source and release populations. Founders must belong to the same evolutionary lineage as historical populations in the release

water body. In the absence of detailed genetic data, biogeographical information may be used cautiously to infer compatibility, although this introduces additional uncertainty. This is a mandatory ("on-off") criterion, i.e., if genetic compatibility cannot be established, the source population is deemed unsuitable. This criterion should not be applied however in the case of conservation introductions/ecological replacement. The IUCN considers ecological replacement, i.e., the introduction of a species *outside* its indigenous range to replace a closely related species that has gone extinct, or to introduce a highly threatened species to a suitable habitat, not available in its original range, as highly controversial carrying significant risks, such as the introduced species becoming invasive or disrupting existing ecosystems. That said, the IUCN incorporates this concept within its broader guidelines for rewilding, identifying it as a potentially necessary conservation tool in the face of rapid climate change and biodiversity loss. The Species Survival Commission (SSC) guidelines, however, stress the need of a clear, science-based justification for why the replacement/introduction is necessary, of a thorough risk assessment, and of strict long-term monitoring to address potential negative impacts.

The third criterion, **S-C3,** applied when founders are to be sourced from wild populations, assesses whether enough individuals can be removed without endangering the source population. It incorporates a minimum for numerical abundance (≤20 individuals) or relative abundance (≤5%) (Pritt & Frimpong, 2010), and considers population structure, age classes, and reproductive strategy. As with S-C2, this is also an exclusionary criterion: if the source population is too small and demographically fragile, it is excluded from consideration. A population viability analysis (PVA) is strongly recommended to estimate a sustainable removal number and to assess potential impacts on source system resilience.

Each criterion in the REVIVE-HELLAS tool is scored on a scale from −1 to 1, with 0 representing uncertainty or data deficiency. Where sub-criteria are present, their values are averaged to produce the final score for the main criterion. The overall feasibility score is calculated as the mean of all main criteria scores. Values close to 1.0 indicate high feasibility for translocation, while values near −1.0 suggest that the site or source population is unsuitable under current conditions. This scoring system provides a transparent, repeatable, and ecologically grounded framework for comparing potential translocation scenarios and for prioritising actions with the highest likelihood of success.

Table 1. Summary of REVIVE HELLAS criteria for candidate recipient water bodies (R-WBs). Each criterion is scored from −1 to +1, with 0 indicating uncertainty or data deficiency. Sub-criteria are averaged to obtain the main criterion score.

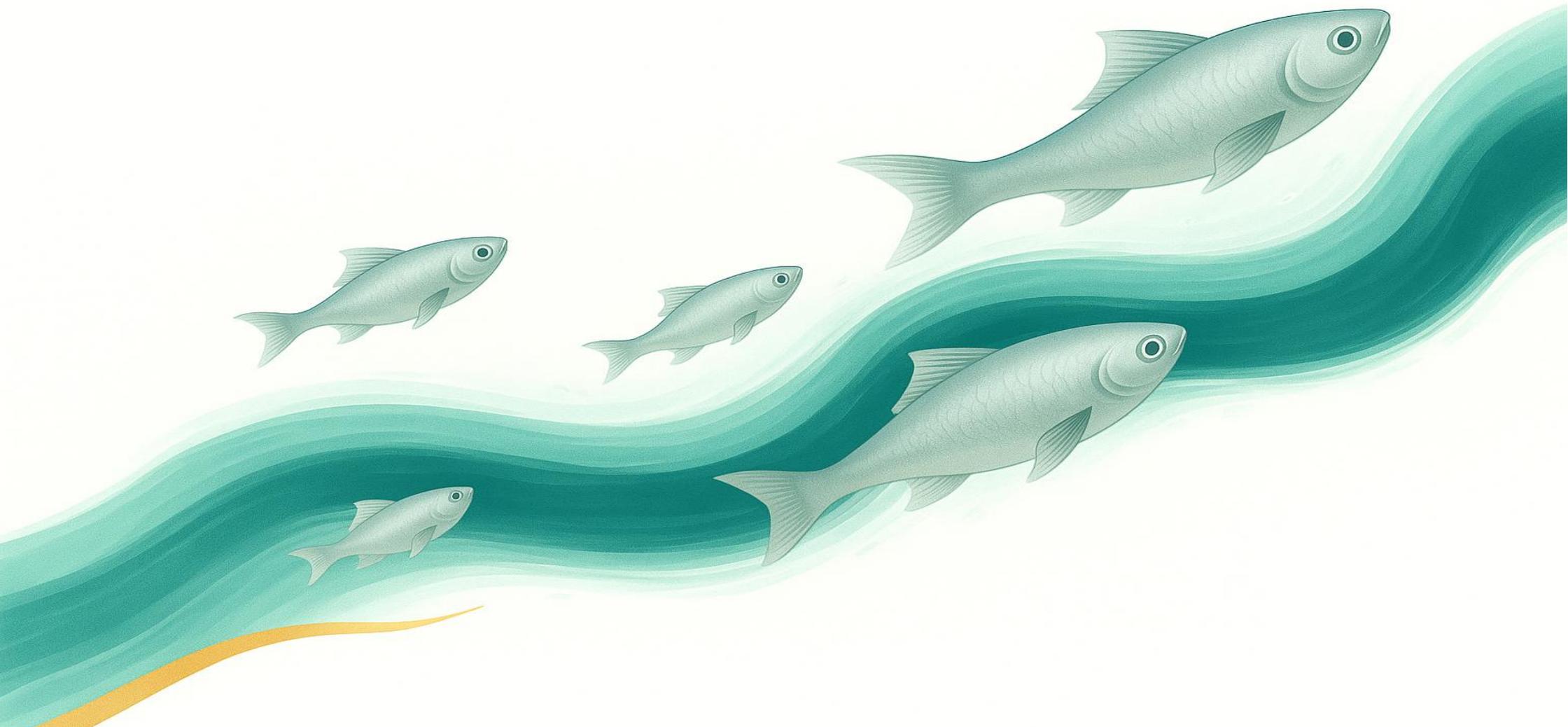| Criterion	Focus	Key data required	Typical methods	Example scoring rule (adjust as needed)	Interpretation (for feasibility)
R-C1 Current presence	Is the focal species currently present or absent in the R-WB?	Recent fish community data for focal species (and congeners if risk of confusion)	Standardised electrofishing, nets/traps, eDNA, underwater video; repeated surveys	**+1.0** = confirmed absence from multi-method surveys (pre-assessment phase); **+0.75** = absence supported by good data last 10 years	High scores mean reintroduction is justified and not redundant.
R-C2 Historical presence	Did the species historically occur in this water body/reach?	Historical fish records, museum specimens, literature, expert knowledge, biogeographic data	Review of historical datasets, distribution maps, expert consultation	**+1.0** = clear historical records in R-WB; **+0.75** = inferred from nearby basins/biogeography; **0** = no information	Ensures alignment with natural range and avoids "assisted invasion".
R-C3 Environmental quality (status) (R-C3.1 physico-chemical, R-C3.2 biological)	Overall ecological status of the site	Water quality parameters, macroinvertebrate & diatom indices, national WFD class	Routine physicochemical monitoring; macroinvertebrate and diatom sampling and indices	**+1.0** = high/good/moderate ecological status; **−1.0** = /poor/bad	Low/negative scores normally exclude the site unless restoration is planned and funded before reintroduction.
R-C4 Habitat quantity	Amount of suitable habitat available (space, length, area, volume, connectivity for migrants)	Wetted length/area/volume, mapped habitat units, barriers within ±10 km	Field mapping, GIS, hydrological data	**+1.0** = habitat quantity comparable to or greater than reference viable populations; **−1.0** = clearly insufficient habitat quantity	Indicates whether the system can support a self-sustaining population in the long term.
R-C4 CON Habitat Connectivity (if migratory species)	Connectivity for migrants	Barriers within ±10 km	Field mapping, GIS, barrier inventories	**+1.0** = no barriers within ±10 km; **+0.5** = minor barrier that are passable by the species some periods; **−1.0** = unpassable barriers within species range	Indicates whether the system can support a self-sustaining population in the long term.
R-C5 Habitat suitability (flow regime and species habitat requirements) R-C5.1 – R-C5.5	Match between habitat characteristics and species' life-history needs	Flow regime, depth, substrates, cover, spawning/nursery areas, food resources	Habitat surveys, hydraulic measurements, substrate mapping, macroinvertebrate sampling; reference sites	**+1.0** = strong match to requirements (all key life stages); **−1.0** = major mismatches (e.g. no spawning substrate)	Determines whether the physical and trophic environment fits the biology of the focal species (small vs large-bodied).

Criterion	Focus	Key data required	Typical methods	Example scoring rule (adjust as needed)	Interpretation (for feasibility)
R-C6 Hydrological perturbation	Degree of flow alteration and water abstraction	Flow records, abstraction data, dam/WEIR operation, environmental flow assessments	Hydrological time series, hydrological modelling, expert judgement	**+1.0** = natural/near-natural flow, limited abstraction; **0.5** = moderate alteration but manageable; **−1.0** = severe regulation, chronic low flows, drying	Reflects risk of flow-related failure (e.g. drying, spawning disruption).
R-C7 Channel morphological alteration	Physical modification of channel and banks	Channelisation, bank reinforcement, bed incision/aggradation, presence of structures	River Habitat Survey (RHS) or similar; geomorphological surveys; aerial imagery	**+1.0** = natural/mildly modified; **0.5** = minor alteration; **-0.5** = moderate alteration; **−1.0** = heavily engineered, simplified channel	Low/negative values indicate reduced habitat complexity and resilience.
R-C8 Invasive species pressure	Current and potential impact of invasive fishes	Species list and abundance of invasive fish etc., evidence of impacts	Standard fish sampling, eDNA, dedicated invasive species surveys; literature on impacts	**+1.0** = no invasive species detected; **−0.5** = invasive species locally rare; **−1.0** = high invasive species abundance or documented severe impacts	High invasive species pressure usually requires intensive control before any reintroduction.
R-C9 Riparian vegetation structure	Structural complexity and condition of riparian zone	Riparian cover, vegetation types, continuity, disturbance	RHS or similar riparian assessment; field mapping	**+1.0** = structurally diverse, near-natural riparian zone; **−0.5** = moderately degraded, patchy cover; **−1.0** = highly simplified or absent riparian cover	Healthy riparian zones support shading, bank stability, organic inputs and refugia.
R-C10 Riparian lignified extent	Extent of woody riparian vegetation	% of bank length with trees/shrubs, large woody debris	RHS metrics, field surveys, aerial imagery	**+1.0** = extensive woody riparian cover; **0** = patchy; **−1.0** = almost no woody vegetation	Indicates potential for bank stability, shelter, and organic inputs.
R-C11 Bankside land use	Land use within ~30 m of both banks	Land cover categories (forest, grassland, agriculture, urban, industry)	GIS land-use maps, orthophotos, field checks	**+1.0** = predominantly natural/semi-natural land use; **0.5** = herbaceous vegetation dominates; **0.25** = mixed or mosaic land use; **−0.25** = intensive agriculture dominance; **−0.5** = intensive urban/industrial or suburban dominance	Strong predictor of diffuse pollution, disturbance and future degradation.
R-C12 Understanding of threats to species' viability and potential for alleviation	Current and near-future local stressors and their manageability	Known pressures (pollution sources, new infrastructure, extraction, recreation plans) and planned mitigation	Planning documents, EIA reports, stakeholder consultation, expert judgement	**+1.0** = threats low or clearly manageable, measures planned/funded; **0** = uncertain; **−1.0** = high, unmanageable, or poorly understood threats	Avoids investing in sites where local threats will likely overwhelm the reintroduction.

Criterion	Focus	Key data required	Typical methods	Example scoring rule (adjust as needed)	Interpretation (for feasibility)
R-C13 Systemic future pressures	Basin-/region-level long-term risks (climate, land-use, governance)	Downscaled climate projections, land-use scenarios, demographic trends, institutional capacity	Review of climate/land-use models, regional plans, expert workshop	**+1.0** = low projected risk and strong adaptive capacity; **0** = uncertain; **−1.0** = high projected risk (e.g. severe drought, urbanisation) with poor mitigation options	Ensures sites are resilient under future change, not only in present-day conditions.

Table 2. Summary of REVIVE HELLAS criteria for candidate source water bodies (S-WBs). S-C2 and S-C3 are exclusionary criteria: if they are not satisfied, the source is rejected irrespective of the mean score.

Criterion	Focus	Key data required	Typical methods	Example scoring rule	Interpretation
S-C1 Origin of source population	Wild vs captive-bred origin and associated genetic/health risks	Information on population origin, breeding history, domestication, health screening	Review of hatchery records, genetic studies, health certificates, field knowledge	**+1.0** = Wild Sourced Founders: **0.5** + Captive or Hatchery-Origin Founders	Prioritises wild and genetically robust populations; low/negative scores trigger closer scrutiny or rejection.
S-C2 Genetic compatibility *(mandatory "on/off")*	Evolutionary and genetic match between source and historical/recipient population. N/A in conservation introductions	Population genetics data (markers), phylogeographic information, historical records	Genetic analyses (e.g. mtDNA, microsatellites, SNPs), biogeographic inference, expert judgement	**ON** = same evolutionary lineage; **OFF** = uncertain compatibility or clearly different lineage or non-native taxon	If **+1.0** cannot be demonstrated, the source should be considered **unsuitable** (exclusionary criterion).
S-C3 Donor resilience & removability *(mandatory "on/off")*	Can founders be removed without jeopardising the donor population?	Abundance estimates, population structure (age classes), trends, life history, PVA outputs	Fish survey data, mark-recapture, depletion sampling, long-term monitoring, PVA (VORTEX)	**ON** = removal of planned number/percentage has minimal impact (e.g. ≤5% of total abundance, PVA shows stable donor); **OFF** = uncertain or donor small or declining, removal likely unsustainable	If donor cannot safely supply founders, the source is **excluded** and alternative sources or captive breeding should be considered.

PVA Modelling of Fish Reintroduction

A Population Viability Analysis (PVA) should be also undertaken to inform realistic stocking densities and predict the long-term persistence of the reintroduced population under site-specific demographic and environmental conditions. This approach ensures that translocation efforts are not only biologically and logistically justified, but also ecologically viable, contributing meaningfully to the conservation and recovery of threatened species. Among available tools, the VORTEX software package is one of the most widely used and validated platforms for PVA modelling in conservation contexts. VORTEX is an individual-based, stochastic simulation model that projects the fate of each individual within a population over time, under the influence of natural and anthropogenic pressures. The model incorporates the effects of deterministic factors, along with demographic, environmental, genetic, and random (stochastic) events that shape population trajectories across multiple generations (Lacy, 1993, 2000; Lacy & Pollak, 2021). VORTEX simulates a population passing through key life-cycle events—such as reproduction, mortality, dispersal, and supplementation—over successive annual cycles. When applied to fish reintroductions, the model helps estimate extinction probabilities, long-term population viability, and the effects of different release strategies under site-specific environmental conditions.

Effective scenario building in VORTEX requires the input of baseline demographic and life-history parameters, including age-specific survival rates, fecundity, sex ratio, carrying capacity, maximum lifespan, and age at first reproduction. These parameters should be derived from peer-reviewed literature, field data, or expert consultation. Where data are limited, parameter estimates may be informed by ecologically similar species, with associated uncertainty clearly acknowledged (Lacy, 1993; 2000; Lacy & Pollak, 2021). PVA modelling serves a dual purpose. First, it predicts the minimum number and composition of individuals required for a successful founding population, guiding decisions on release size, sex ratio, and age structure. Second, it informs the design of the release strategy—including the number and timing of release events—to ensure that the introduced population can overcome demographic and environmental stochasticity and achieve self-sustaining growth. Recent applications of PVA in Mediterranean freshwater fish reintroductions (e.g., Kalogianni et al., 2023) have demonstrated its value in identifying critical thresholds for viability and resilience in fragmented or degraded river systems. Ultimately, the use of PVA modelling enhances the scientific rigor, transparency, and predictive power of reintroduction programs. It supports adaptive management by allowing practitioners to evaluate alternative strategies under realistic constraints and to anticipate risks and trade-offs associated with long-term population persistence in dynamic freshwater habitats.

INVASIVE FISH CONTROL
PVA Removal modelling

To assess and optimise the effectiveness of non-native fish removal efforts, a Population Viability Analysis (PVA) should be conducted prior to the implementation of physical removal campaigns. This modelling approach enables practitioners to estimate the minimum percentage of the invasive population that must be removed to achieve meaningful suppression or local eradication, while accounting for the species' reproductive potential, survival rates, and environmental variability.

One widely used and validated tool for this purpose is the VORTEX software package (Lacy, 1993, 2000; Lacy & Pollak, 2021). VORTEX is an individual-based, stochastic simulation model that projects the long-term fate of wildlife populations by simulating the effects of demographic, environmental, genetic, and anthropogenic factors. The model proceeds by simulating a series of discrete events in the life cycle of a sexually reproducing species, such as reproduction, mortality, dispersal, harvesting, and supplementation, over multiple time steps. It allows for the incorporation of both deterministic and random elements, including environmental fluctuations and demographic stochasticity, thereby mimicking the unpredictability inherent in natural systems.

By adjusting model parameters to reflect the life-history traits of the target non-native species and the ecological characteristics of the invaded system, VORTEX can be used to build a series of removal scenarios. These scenarios estimate the population-level outcomes of different removal intensities (e.g., 30%, 50%, 70% annual removal) and help determine whether single or repeated interventions are required to reach population suppression thresholds. Inputs can be grouped into species-specific and habitat/system-specific parameters. Key species-specific inputs typically include age at first reproduction, fecundity (and brood/litter-size variation), sex ratio, age-specific mortality, the proportion of adults breeding, and—in some cases—the mating system and maximum reproductive age. Key habitat/system-specific inputs include carrying capacity (and potential temporal variation), initial population size, dispersal probability (and dispersal mortality, where relevant), and the form and strength of density dependence, including the possibility of reduced performance at very low densities. Where available, these values should be drawn from peer-reviewed literature, technical reports, and/or local field data. In the absence of species-specific data, conservative estimates or proxy values from ecologically similar species may be used, with appropriate justification and uncertainty acknowledged (Fig. 2).
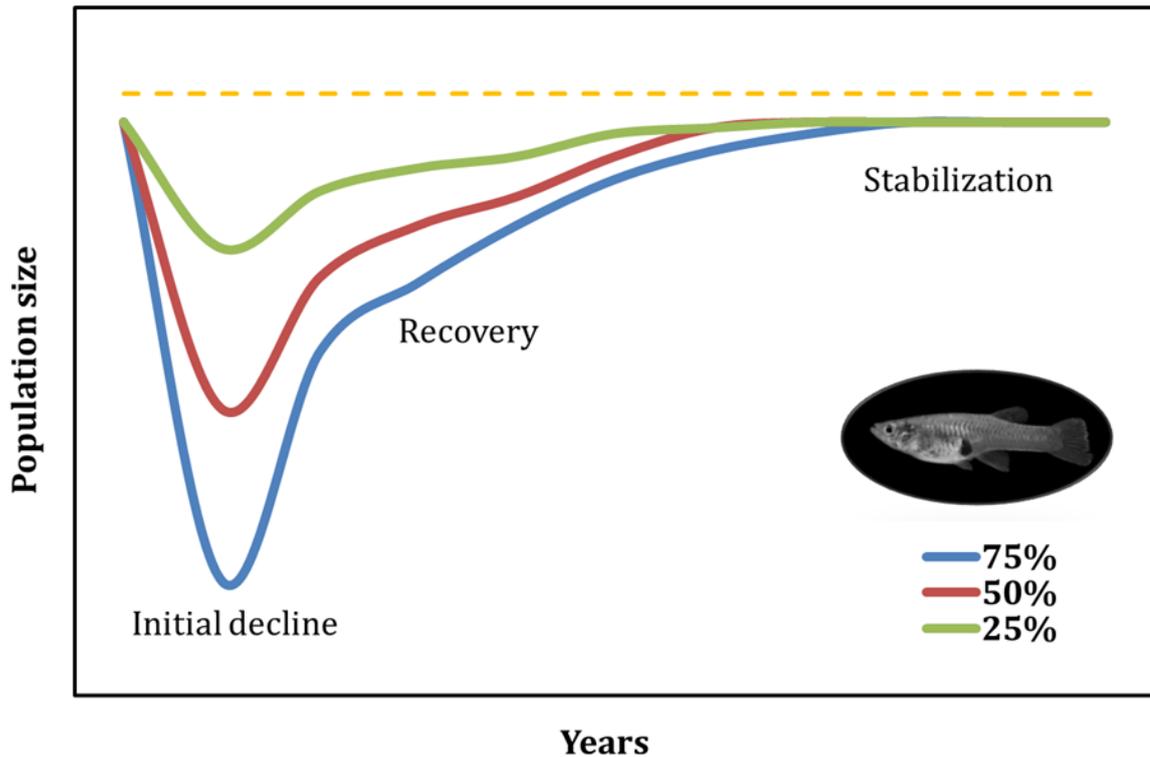
Figure 2. Conceptual population trajectories using PVA population modelling for the active removal of the invasive mosquitofish *Gambusia holbrooki* under three removal scenarios of removing 25%, 50% and 75% of the initial population.

Importantly, PVA does not merely inform the technical feasibility of removal but also supports risk assessment, by quantifying the probability of recolonisation or population rebound under different management scenarios. It thus provides a decision-support framework that links empirical data with forward-looking conservation planning. The use of PVA modelling in the context of invasive species control is increasingly recognised as best practice (Lamothe et al., 2021; Vardakas et al., 2026), especially in freshwater systems where removal efforts are resource-intensive and must be precisely targeted to ensure cost-effectiveness and ecological benefit.

For detailed guidance on model parameterisation and scenario building in VORTEX, readers are referred to Lacy (1993, 2000) and Lacy & Pollak (2021), as well as recent applications in freshwater fish management.

Modes of removal

Invasive species are among the most pervasive and damaging threats to global biodiversity, particularly in freshwater ecosystems. Their introduction, often facilitated by human activities such as global trade, aquaculture, ornamental aquarium release, or recreational fisheries, has

been linked to widespread declines in native species, degradation of ecosystem function, and collapse of trophic networks. Invasive species can outcompete natives for resources, predate on vulnerable life stages, interfere with reproductive behaviour, and alter key ecological processes such as nutrient cycling, sedimentation, and habitat complexity (Scott & Helfman, 2001; Dudgeon et al., 2006; Olden et al., 2010; Reid et al., 2019).

Within the broader context of ecological restoration and conservation translocation, the effective control of invasive species is not optional, it is a prerequisite. Without mitigating the pressures exerted by these species, efforts to re-establish native freshwater fish populations often fail, regardless of the quality of the reintroduction protocol. Numerous studies have highlighted the failure of conservation translocations due to unchecked alien species, highlighting their role as a leading limiting factor in the recovery of threatened taxa (Cochran-Biederman et al., 2014; Berger-Tal et al., 2019; Ennen et al., 2021).

Invasive species pressures are especially acute in Mediterranean river systems, which combine high endemism with increasing vulnerability to biological invasions. In these lotic environments, invasive species exert not only trophic impacts (via competition and predation) on other fish species but also non-trophic effects, including behavioural aggression, reproductive interference, negative impacts also on the flora, affecting biological/flaural composition and integrity, as well as long-term alterations to hydrological, geomorphological regimes.

From a management perspective, eradicating or suppressing non-native fish in flowing systems presents a major technical challenge. Rivers and streams are open, connected, and hydrologically variable, allowing invasive species to recolonise quickly following partial removal. The difficulty is further compounded when dealing with small-bodied, fast-reproducing species, which can rapidly recover due to high fecundity and cryptic behaviour (Nico & Walsh, 2011; Rytwinski et al., 2019). As a result, successful invasive species control often requires sustained, multi-pronged approaches, tailored to the ecological characteristics of both the target invader and the riverine system.

Invasive species management must therefore be integrated into every stage of translocation planning, from initial feasibility assessment to pre-release preparation, as a cornerstone of an ecosystem-based conservation strategy (Rytwinski et al., 2019; Davies et al., 2020). Only by reducing or eliminating the impact of invasive species can restored habitats provide the ecological conditions necessary for native populations to re-establish, persist, and evolve.

Efforts to eradicate invasive fish species in freshwater systems have historically relied on non-selective or broad-spectrum interventions, particularly in lentic environments (e.g., lakes, reservoirs, and ponds) where spatial containment is more feasible. The most widely used method in these contexts has been the application of chemical piscicides, particularly rotenone,

which can achieve near-total eradication of fish populations when properly deployed (Nico & Walsh, 2011; Tiberti et al., 2019; 2021). Similarly, habitat drying and subsequent disinfection has been used in small, isolated water bodies to eliminate invasive taxa.

However, the use of chemical removal techniques in lotic systems (rivers and streams) is far more problematic. These methods lack selectivity and often pose unacceptable risks to non-target species, including threatened native fish taxa, invertebrates, and amphibians. They also carry low public and regulatory acceptance, particularly in biodiversity-sensitive or protected areas (Britton et al., 2011; Tonkin et al., 2014; Rytwinski et al., 2019). As a result, their use is increasingly restricted to emergency containment scenarios or systems without conservation value.

In response to these limitations, attention has shifted toward more targeted and ecologically sensitive control strategies. Among the most promising are genetic and reproductive suppression techniques, which aim to reduce the long-term viability of invasive populations by interfering with their reproduction. For instance, reproductive interference, i.e., maladaptive sexual behaviour directed toward heterospecifics, can reduce reproductive success in targeted invasive species through mismating or hybridisation-related mechanisms (Tsurui-Sato et al., 2019). Experimental genetic biocontrol methods, such as sex-ratio distortion or gene-drive technologies, are also under development, particularly in systems where traditional methods have failed or are ethically and ecologically unacceptable (Gutierrez et al., 2006; Bax & Thresher, 2009). While promising, these approaches remain largely experimental, and their ecological safety and social acceptability remain under review.

In parallel, physical removal methods have become increasingly common in riverine systems, particularly in areas where chemical controls are impractical or prohibited. Among these, electrofishing is the most widely applied technique, especially for the removal of large-bodied invasive species such as non-native trout or cyprinids (Knapp & Matthews, 1998; Bosch et al., 2019; Bernery et al., 2022; Kalogianni et al., 2025). Although effective in some contexts, electrofishing campaigns often yield mixed results, due to the high mobility, cryptic behaviour, and rapid reproductive turnover of many invasive fish species (Kulp & Moore, 2000; Meyer et al., 2006; Card et al., 2020). Moreover, the technique is labour-intensive and requires repeated applications to achieve sustained population suppression.

In lentic or semi-lentic systems, gill netting, trap fishing, and barrier-based approaches have also been used with varying success. However, these techniques generally lack the precision required for selective removal and may inadvertently affect native species unless carefully managed. Ultimately, the success of any invasive species removal campaign depends also on site-specific factors, including habitat structure, flow regime, species behaviour, and accessibility. Effective

programs often integrate multiple removal techniques, coupled with monitoring and adaptive management, to reduce recolonisation risk and sustain ecological gains.

In wadable riverine environments, depletion electrofishing remains the most effective and ecologically appropriate method for the selective removal of non-native fish species. This approach should follow standardised protocols, such as those of the FAME project guidelines, adapted for Mediterranean streams (Schmutz et al., 2007), with additional refinements developed by IMBRIW-HCMR (2013) for Mediterranean streams https://imbriw.hcmr.gr/inland-water-fish-monitoring-operations-manual-electrofishing/.

Prior to sampling, the total stream length to be covered should be measured and divided into workable sections (e.g., 50-meter sections) based on the time available, staffing capacity, access constraints, and habitat complexity. In each section, a minimum of two electrofishing passes should be conducted to maximise capture efficiency and ensure meaningful population depletion. Stop-nets must be installed at the upstream and downstream boundaries of each section, where applicable (as water velocity, depth, and habitat barriers, all impact the functionality of a stop net), to prevent fish movement during the sampling process. Electrofishing should be conducted using either backpack electrofishing units, which may be battery- or fuel-powered, land-based generators, which are preferable in deeper or higher-flow habitats. Sampling should proceed upstream, starting from the lowest feasible point of the target reach, following best-practice protocols to minimise harm to native fauna. A fully staffed field team typically includes three to five trained personnel. One person operates the electrofishing anode, while two netters capture stunned fish. If non-portable equipment is used, a fourth person is needed to operate the electrofishing device and/or record field data. A fifth person may be assigned to fish handling, particularly when live storage or euthanasia procedures are required.

A note on fish welfare

Why is it important to consider fish welfare? It is likely that fish are sentient and able to experience emotions. They have the neural capability to experience emotion, evidenced by having a similar neural organisation to other vertebrates. Many fishes show complex cognitive abilities, including long-term memory storage, and have been found to have strong preferences between resource types. Fish exhibit behaviours that demonstrate experiencing pain. Mellor identifies five domains of animal welfare including: nutrition, environment, health, behaviour, and the mental domain. For health, this includes the desire to be free from illness, injury, or abnormality (Mellor, et al. 2020). During capture, handling, holding, transporting and release, fish are likely to experience emotion in the form of stress and reintroduction processes, if not done correctly, can lead to injury and subsequent illness. For this reason, paying attention to fish welfare during any translocation exercise is vital to ensure the viability and long-term success of the project.

All captured fish must be identified to species level in the field. Native and non-native individuals should be separated immediately upon capture and placed in clearly labelled, species-designated holding containers (e.g., different colours). During the first depletion pass, native fish should be held temporarily in tanks of sufficient volume to prevent overcrowding or injury and fitted with continuous aeration to minimise stress; to avoid recapture, native individuals should be returned to the river only after completion of the second pass. Non-native fish should be retained in a separate, secure container for processing and disposal under the approved protocol. Individuals should be euthanised as soon as practicable using a "humane" method consistent with recognised veterinary guidance -most commonly an overdose of an approved immersion anaesthetic - with death confirmed and, where required by ethics approval or species characteristics, followed by a secondary physical method to ensure irreversibility. Accepted methods include buffered clove oil solution, Aquacen Benzocaine, or Tricaine Pharmaq, as approved by the National Organization for Medicines of Greece. Where permitted under the research authorisation, a subset of non-native individuals may alternatively be retained for scientific purposes (e.g., genetic analyses, diet/stable-isotope studies, age and growth, disease screening). Carcasses not retained for research should then be sealed in leak-proof, labelled bags or containers and stored for the minimum time necessary (refrigerated or frozen as appropriate) prior to transfer to an authorised disposal route (e.g., approved incineration, rendering, or other legally permitted stream). Disposal and transport must follow biosecurity principles to minimise disease and spread risk, including secure storage away from waterbodies, appropriate documentation/traceability where required, and cleaning and disinfection of tanks, nets, and other equipment before moving between sites. Where staffing allows, a designated handler should oversee both holding systems and the processing workflow (native holding versus non-native processing) to maintain strict separation, preserve water quality and oxygenation in both containers, and minimise handling time for all fish. All field activities, including section coordinates, fish data, and environmental variables, should be recorded using standardised datasheets to support post-removal evaluation and monitoring.

This method offers a selective and repeatable approach for controlling invasive fish populations in wadable stream habitats. While resource-intensive in terms of labour and equipment, it avoids the non-target impacts associated with chemical control methods and is particularly well suited to ecologically sensitive systems, such as those hosting endemic or threatened species. Nevertheless, success depends on appropriate timing, frequency, modelling the life history of the species, having well-trained personnel, and consideration of flow conditions, especially in Mediterranean streams prone to seasonal intermittency and spatial fragmentation.

HABITAT IMPROVEMENT

Preparatory habitat improvement actions prior to a fish reintroduction in a lotic system are often necessary, as habitat-related factors are major drivers of species decline for many freshwater taxa, including fish (Bubac et al., 2019). These actions aim to address the ecological limitations that may have contributed to the original extirpation of the species or that would otherwise compromise the success of the reintroduction. Interventions may include increasing the structural complexity in the release habitat to improve depth, flow variability, and trophic suitability for the focal species; substrate modification to better support spawning and early life stages; or pollution control measures to reduce chronic stressors. These pressures are often intensified by water stress, resulting from both over-abstraction and natural drought cycles.

More specifically, preparatory habitat actions are essential because habitat degradation, hydrological alteration, and simplification of river structure are widely recognised as key limiting factors for fish recovery. In many cases, these factors interact with biological pressures such as invasive species, further reducing the chances of population re-establishment. Restoring habitat quality in advance can significantly enhance the survival, reproductive success, and long-term persistence of the reintroduced population by ensuring that the physical environment meets the species' life-history requirements.

Actions to increase habitat structural complexity may include the placement of large woody debris, boulders, or the creation of artificial riffle–pool sequences to enhance depth variation, shelter availability, and hydraulic diversity. These features create favourable microhabitats for different life stages, reduce predation risk, and improve trophic conditions by promoting macroinvertebrate diversity and prey abundance (O'Connor et al., 2023). Similarly, substrate improvement may be necessary, especially for species with specific spawning requirements, such as those dependent on coarse gravel beds. Restoration activities may involve gravel augmentation or the removal of fine sediments that clog interstitial spaces, reduce oxygenation, and increase egg mortality. These modifications directly influence the reproductive potential and recruitment success of reintroduced populations.

Water quality enhancement is also a key component of habitat improvement in Mediterranean lotic systems. In many Greek rivers, nutrient enrichment, chemical pollution, and urban runoff degrade spawning and nursery habitats, impede larval development, and increase the risk of sublethal stress. These impacts are exacerbated by untreated wastewater discharges, widespread over-abstraction for irrigation, and increasingly frequent drought events (Skoulikidis et al., 2011). Therefore, pollution mitigation measures should be included in any preparatory restoration plan. To address these interconnected stressors, integrated river

restoration approaches are increasingly advocated. These combine catchment-level interventions, such as improving wastewater infrastructure and reducing diffuse pollution sources, with riparian vegetation recovery and adaptive flow management to ensure ecologically sufficient baseflows, particularly during critical reproductive and recruitment periods.

Overall, preparatory habitat restoration is not only critical for the initial success of fish reintroduction, but also for ensuring the long-term ecological resilience of the restored population and ecosystem. This is especially true in heavily modified and climatically vulnerable Mediterranean river systems, where the success of reintroduction depends on both biological and hydro-morphological recovery.

FRESHWATER FISH TRANSLOCATION IMPLEMENTATION

SOURCING FOUNDERS

Sourcing founders from wild populations as opposed to captive bred ones is a decision that requires careful consideration (Fischer & Lindenmayer, 2000). While *ex situ* breeding for reintroduction purposes has become a crucial conservation tool, implementing successful captive propagation programmes presents numerous biological, technical, and logistical challenges, mainly due to the difficulty of replicating the complexity of natural habitats under controlled conditions (George et al., 2009).

To enhance the survival, fitness, and behavioural competence of fish prior to release, as well as to prevent domestication over subsequent generations in captivity the captive environment must be naturalised as much as possible. This includes simulating appropriate hydrological conditions, suitable spawning substrates, shelter or cover, and a diet that mimics natural food resources at *ex-situ* units, as well as possibly a transition phase from *ex-situ* to *in-situ* facilities for adaptation to the wild prior to the release Achieving these conditions in captivity often demands significant infrastructure, intensive maintenance, and thus high human and financial resources.

A common issue when sourcing founders, whether from wild populations or captive breeding, is the risk of genetic bottlenecks, especially when the number of founders is limited. Such bottlenecks may reduce genetic diversity and adaptive potential over successive generations, unless there is regular supplementation to maintain genetically viable populations. This implies substantial labour and cost, related to the capture (in the case of wild founders), transport, health screening, and acclimatisation of individuals. The minimum number of founders must also be considered to ensure successful establishment of the population. A greater number of founders will ensure greater genetic diversity and a wider range of survival traits transferred to the population. The individuals selected should be collected from multiple sources and/or seasons/years if possible, to reduce the likelihood of single season broods and related individuals forming the entire source population. A mix of age classes ensure that at least some of the fish are unlikely to be cohorts or siblings and at least a proportion will be of reproductive age. An ideal source number is 100 individuals but if this isn't possible the largest number available that doesn't impact the source population can be used. A minimum guide is to aim for at least 30 individuals of an equal sex ratio; 15 males and 15 females, that breed and contribute to the subsequent generation.

With 30 founders, theoretically, 98.4% of the source population's genetic diversity could be maintained; with 60 founders, his increases to 99.2%; with 90 founders, this increases to 99.4%. (Zimmerman, 2021).

Founders can be collected from the source habitat(s) using various methods, such as electrofishing or netting, with major emphasis on minimising fish stress during and after capture. Fish should be measured (total length, TL), sexed (if possible), and placed in large containers equipped with aeration and plant material for cover. They should then be transferred to the release site with care.

Alternatively, if the fish are small-bodied and transport times are long, it is advisable to transfer them in separate containers filled with source habitat water and oxygen supplied from a portable oxygen concentrator. The ratio of water to oxygen should be 1:3 (1 water to 3 oxygen), ammonia neutralising agents should be added and ideally the fish should be left to fast for 24 hours prior to packing for long-term transfer. This prevents them fouling the shipping water and causing ammonia spikes and oxygen depletion during transport. Ice packs should be used to prevent an acute rise in water temperature during transport. Prior to fish release, acclimatisation to the release habitat temperature and/or salinity should be conducted by gradual water exchange between the transport container and the receiving water, alongside concomitant physicochemical measurements (e.g., temperature, conductivity, oxygen). Fish handling should be kept to a minimum to avoid excessive stress and protect the mucous membrane and to reduce mortality during and after the collection and transport process. All hands/equipment in direct contact with the fish should be wet to avoid dry abrasive contact which will compromise the mucous membrane and allow pathogens and parasites an opportunity to reach the fish's skin and scales.

DISEASE/PARASITE TRANSMISSION RISK PREVENTION

Relevant guidelines recommend quarantining and prolonged holding of fish in dedicated facilities to examine them for parasites and diseases before translocation. However, this approach can cause significant stress to the fish and involves high economic costs. Research has demonstrated that parasite treatment of hatchery-reared fish intended for translocation can effectively prevent disease transmission to release habitats and improve the success of reintroduction efforts (Healy et al., 2020). Nevertheless, as highlighted in the IUCN (2013) guidelines, the benefits of quarantine and disease management should be evaluated on a case-by-case basis and compared with less invasive alternatives, such as conducting health screenings directly in the field during the implementation phase, particularly when transferring wild stocks within their native ranges, where the risks of pathogen introduction are generally lower

(Minckley, 1995). In such cases, the extent and intensity of quarantine should be proportional to the estimated likelihood of pathogen occurrence and the potential severity of its impact on the recipient ecosystem. A risk-based approach ensures that resources are allocated effectively and that fish welfare is not compromised unnecessarily. Finally, an alternative or complimentary to full quarantine may involve sacrificing a small number of individuals from the focal species, or from a closely related species from the same source habitat, to assess the presence of external and internal pathogens (Minckley, 1995; Kalogianni et al., 2023). This method can provide valuable insights into disease risks while minimising handling and stress for the remaining individuals. Pre-transfer top level disease screening for skin scrapes, or gill crushes to check for external parasites could be applied. Also, prophylactic treatment for internal parasites, e.g. for cestodes, prior to the release should be also considered.

SOURCE-RELEASE SITE PROXIMITY

Sourcing fish from geographically proximate localities minimises both transport-related stress and the risk of introducing novel parasites or diseases to the release environment (IUCN, 2013). Shorter travel times reduce handling duration, temperature fluctuations, and oxygen depletion, factors known to impair fish condition during translocation. In addition, spatial proximity generally implies greater ecological and evolutionary similarity between source and release habitats, increasing the likelihood of post-release adaptation and long-term survival. For these reasons, local or regional source populations are preferred wherever feasible, especially when moving wild stocks within their native range.

FISH COLLECTION/RELEASE FREQUENCY AND AGE GROUP/SEX

An effort should be made to ensure that the translocated population includes a balanced mix of males and females (if the species exhibits sexual dimorphism permitting their sexing), as well as a range of age classes, including both juvenile and adult individuals, in order to maximise post-release survival and establishment of a self-sustaining population. A heterogeneous age and sex structure increase the probability of successful reproduction in the short term and supports demographic resilience over the long term. It also reduces the risk of founder effects associated with releasing a narrow segment of the population. The collection and release frequency can be determined based on Population Viability Analysis (PVA) conducted prior to the translocation. The PVA helps define the optimal number of individuals, their age/sex composition, and whether releases should occur in a single pulse or at multiple events to improve establishment success and minimise ecological disruption.

POST-RELEASE MONITORING

Post-release monitoring is essential for evaluating the sustainability, dispersal, and viability of the translocated population, including parameters such as abundance, age structure, and recruitment success. A range of monitoring methods can be applied, and their selection, or combination, should be based on the specific characteristics of the release habitat(s), as well as on cost-effectiveness in terms of both economic and human resources, as well as on the invasiveness of each method. The main options include conventional fish sampling, underwater camera recording, and environmental DNA (eDNA) monitoring.

Fish sampling can involve semi-quantitative methods, such as hand netting, seine netting, or the use of traps, as well as quantitative approaches, including electrofishing by wading or boat electrofishing, each with differing levels of detectability. Underwater video surveys can be conducted using high-definition cameras, deployed following defined protocols concerning area coverage and video capture duration. Although less invasive, this method has limitations related to cost, site accessibility, and the detectability of the focal species. For species-specific detection, the eDNA method, as detailed by Mauvisseau et al. (2020) is recommended. This molecular-based approach is particularly useful for detecting rare or low-abundance species and has shown greater sensitivity compared to conventional electrofishing or optical surveys (Goldberg et al., 2018; Sepulveda et al., 2019; Beng & Corlett, 2020; Brys et al., 2020; Dimond et al., 2022; Rojahn et al., 2021; Meulenbroek et al., 2022).

In selecting the appropriate fish sampling method, habitat variation is a major determinant; in wadable freshwater habitats (depth < 1.5 m), electrofishing can be performed following the standard methodology of the Comité Européen de Normalisation (CEN, 2003), as adapted in the HCMR fish sampling protocol https://imbriw.hcmr.gr/inland-water-fish-monitoring-operations-manual-electrofishing/ . This involves a portable or shore-based DC electrofishing unit operated while wading upstream over a 100 m stretch, with a field shock diameter of approximately 2 m. In non-wadable habitats (depth > 1.5 m), boat electrofishing can be applied using a similar sampling design. Alternatively, bank electrofishing by a team on foot, D-shaped scoop nets, or seine netting can be employed where appropriate.

Regarding underwater camera use, it is important to recognise its limitations in terms of cost, deployment logistics, and species detection capability, especially for cryptic or benthic taxa. Similarly, while eDNA monitoring is increasingly used due to its non-invasive nature and cost-effectiveness, it is also subject to methodological biases (Ficetola et al., 2015; Jerde, 2021; Mathieu et al., 2020; Burian et al., 2021). These include false negatives (non-detection of species

known to be present) and, less frequently, false positives (detection of species not present at the site). To reduce such uncertainties, monitoring should be conducted with regular sampling efforts across the river, using an adequate number of biological and technical replicates (Burian et al., 2021).

Finally, it should be emphasised that only a combination of complementary methods, such as those described above, coupled with targeted surveys during the reproductive season for the detection of larvae (where identifiable), can increase the probability of verifying the establishment of a self-reproducing, viable population at the release habitat.
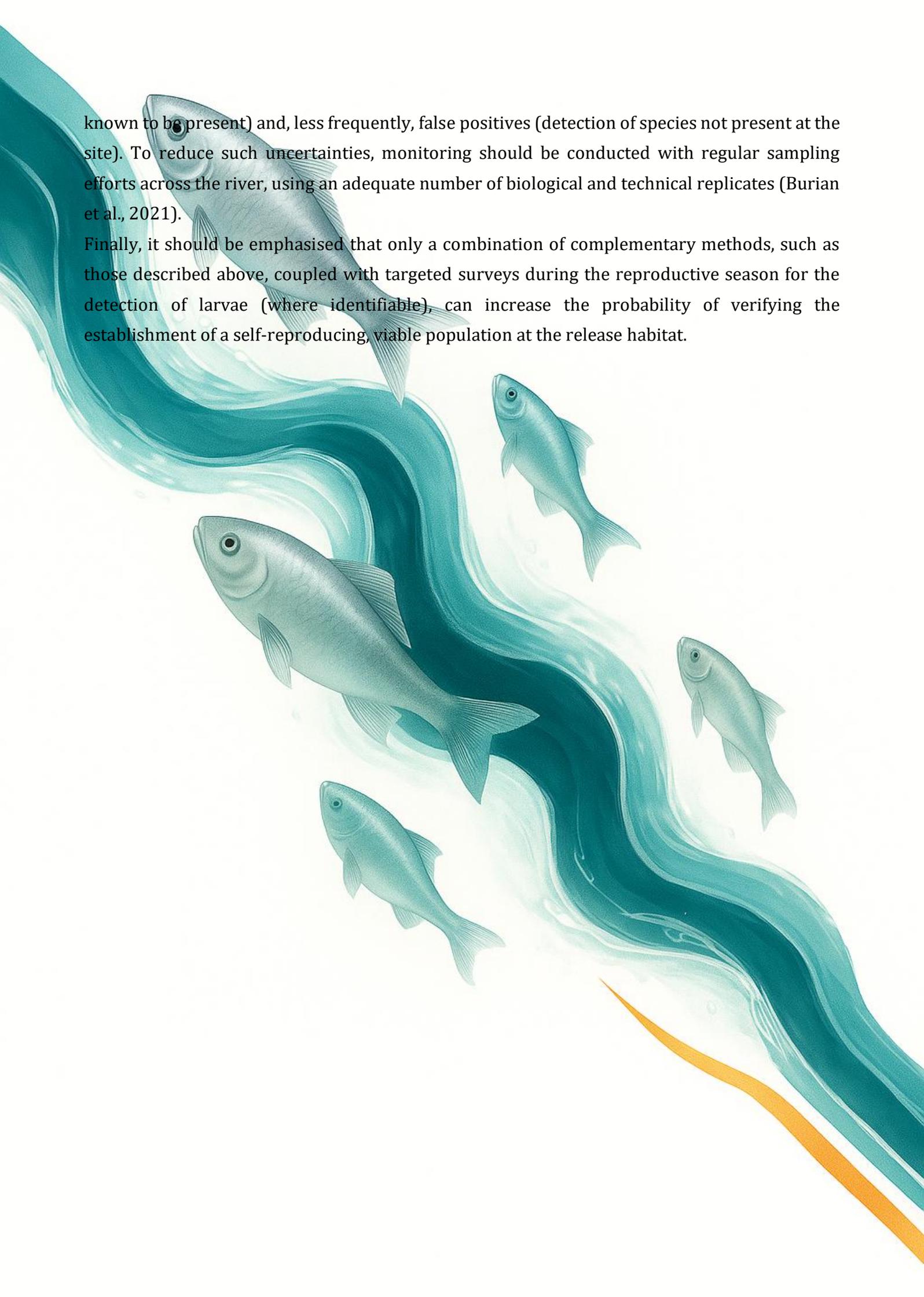
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