# REVIVE-HELLAS

## A FRESHWATER FISH TRANSLOCATION FEASIBILITY ASSESSMENT TOOL
Version 1.0

### HELLENIC CENTRE FOR MARINE RESEARCH
INSTITUTE OF MARINE BIOLOGICAL RESOURCES AND INLAND WATERS

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**HELLENIC CENTRE FOR MARINE RESEARCH**

INSTITUTE OF MARINE BIOLOGICAL RESOURCES AND INLAND WATERS

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

The Institute of Marine Biological Resources and Inland Waters (IMBRIW) of the Hellenic Centre for Marine Research (HCMR) is the main public research body in Greece specialising in fisheries science and inland aquatic ecosystems. With an active role in the Mediterranean and within the European Union, IMBRIW generates the scientific knowledge and practical tools needed for the sustainable management of aquatic resources.

IMBRIW's mission is to support the conservation and sound management of aquatic biological resources, habitats and ecosystems; to provide independent scientific advice and specialised services to national authorities, Mediterranean and EU institutions and other international organisations; and to enhance public awareness of issues related to the protection of aquatic environments.

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.

IMBRIW's activities extend beyond Greece to most European countries, the Middle East and North Africa. Its overarching goal is to advance understanding of the structure and functioning of inland aquatic ecosystems and the higher trophic levels of marine ecosystems, including fisheries, and to apply this knowledge to integrated river-basin and coastal-zone management, ecosystem-based fisheries management and biodiversity conservation.

A key component of IMBRIW's work is the development and application of state-of-the-art tools for ecological monitoring, weather and hydrometeorological forecasting, and water-quality and ecological modelling. These tools provide robust scientific support for policy development, management decisions and adaptation to environmental change.

## Purpose and audience

This tool is tailored for the feasibility evaluation of freshwater fish conservation translocations in Greece and the wider Mediterranean region. It focuses on lotic systems (rivers, streams and associated spring-fed habitats), and incorporates the ecological, hydrological, and biogeographical variation of these systems.

This robust multicriteria tool 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.

# The REVIVE-HELLAS Tool

The REVIVE-HELLAS tool represents a specialised adaptation and significant enhancement of the REVIVE feasibility assessment tool, which was initially designed to evaluate the feasibility of conservation translocations of freshwater fish in Mediterranean-type river systems (Kalogianni et al., 2023a). In its turn, the REVIVE tool developed from a custom-made feasibility tool built for the first feasibility assessment for a freshwater fish translocation in Greece, the translocation of the critically endangered Corfu killifish *Valencia letourneuxi* (Kalogianni et al. 2023a). The more widely applicable REVIVE-HELLAS tool was developed to address the diverse ecological, hydrological, and biogeographical conditions of Greek and Mediterranean lotic systems and the threats they face (Gasith, & Resh, 1999; Smith . & Darwall, 2006'; Darwall et al, 2014; 2018; Skoulikidis et al., 2017; Reid et al., 2019; Su et al., 2021), while retaining the robust and structured methodological approach of its predecessor. Finally, an offshoot of the original REVIVE tool was the REVIVE-POLIS feasibility analysis tool, developed for urban release habitats and thus incorporating conventional ecological criteria to urban specific criteria, such as e.g. surrounding land use (Vardakas et al. 2026).

The design of the original REVIVE tool (Kalogianni et al., 2023) was informed by a broad body of scientific literature, particularly studies that provide guidelines and recommendations for conservation translocations applicable across a wide spectrum of taxa (Cowx, 1994; Minckley, 1995; Fischer and Lindenmayer, 2000; George et al., 2009; Seddon et al., 2007, 2010, 2012, 2014; Dunham et al., 2011; Cochran-Biederman et al., 2015; IUCN, 2013; Malone et al., 2018; Berger-Tal et al., 2020, Novak et al., 2021; Morris et al., 2021; Soorae, 2010, 2018, 2021).

In addition, a considerable body of knowledge focusing on freshwater fish-specific translocation and stocking practices was incorporated to refine the tool (Hayes & Banish, 2017; Healt et al., 2020; Kalogianni et al., 2024).

The REVIVE-HELLAS tool builds upon this foundation, integrating additional criteria deemed essential for freshwater fish species inhabiting both Mediterranean-type rivers (characterised by highly seasonal hydrology), while also extending its applicability to non-Mediterranean-type rivers and all fish species groups occurring in Greek inland waters.

In essence, REVIVE-HELLAS goes beyond a simple replication of the REVIVE tool. It modifies, reorganises, and expands the assessment criteria to capture the wide

range of specific ecological complexities, anthropogenic pressures, and habitat heterogeneity present in Greek rivers.

The ultimate goal is to ensure that translocation efforts not only address species recovery but also contribute to the long-term ecological integrity and resilience of freshwater aquatic ecosystems.

# The REVIVE-HELLAS Components

Similar to the structure of the original REVIVE tool (Kalogianni et al., 2023), the REVIVE-HELLAS tool is divided into two principal components:

## Assessment of the Release Water Bodies (R-WBs)

This component evaluates the potential of potential release water bodies to support the successful establishment and long-term viability of a population of the focal species. The criteria range from R-C1 to R-C13 (as expanded in Table 1). These criteria examine various ecological, physical, and biological aspects of the release habitat, including historical and current species presence, habitat quality and quantity, hydrological stability, and the influence of invasive species.

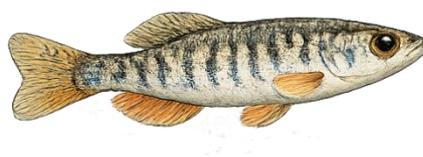## Assessment of the Source Water Bodies (S-WBs)

This component addresses the suitability of potential source populations (wild or captive bred) as sources of individuals for translocation. This includes an evaluation of genetic compatibility, population size and health, and, in the case of wild founders, the ability to harvest individuals without compromising the sustainability of the source population. These criteria are summarised as S-C1 to S-C3.

- An important feature of the REVIVE-HELLAS tool is that several of its main criteria include multiple sub-criteria, allowing for a more flexible scoring system. Each sub-criterion is evaluated on a quantitative or semi-quantitative scale, providing a transparent and reproducible framework for decision-making.
- To visualise the relationships among data sources (e.g., field data, expert judgment, bibliographic data), evaluation criteria, methodological limitations (e.g., imperfect detection, misidentification, inadequate typology), and corresponding robustness adaptations (e.g., methodology standardisation, local manager assessment), an Alluvial diagram (implemented as a Sankey plot) was constructed (Fig. 1).

**Table 1.** The criteria to assess potential release water bodies based on REVIVE-HELLAS.

| Criteria | Interpretation |
|---|---|
| R-C1 | **Current absence** |
| | *Documented absence in release habitat (pre-assessment phase)* |
| | *Apparent absence in release habitat (past 10 years)* |
| R-C2 | **Historical absence** |
| | *Direct historical data on the target species' presence in the release habitat* |
| | *Direct historical data on the target species' presence in the basin* |
| | *No information available for either release habitat or the wider basin* |
| R-C3 | **Release Habitat quality** |
| R-C3.1 | **Physicochemical water quality** |
| | *High/good/moderate* |
| | *Poor/bad* |
| R-C3.2 | **Biological quality based on macro and diatoms** |
| | *High/good/moderate* |
| | *Poor/bad* |
| R-C4 | **Habitat quantity** |
| | *Sufficient habitat quantity* |
| | *Non-sufficient habitat quantity* |
| R-C4 CON | **Habitat Connectivity (if the focal species migratory)** |
| | *Complete longitudinal connectivity* |
| | *Partial connectivity* |
| | *Full disconnection* |
| R-C5 | **Habitat suitability (flow regime & species habitat requirements)** |
| R-C5.1 | **Flow regime** |
| | *Perennial flow* |
| | *Intermittent flow* |
| R-C5.2 | **Flow requirements** |
| | *Within optimal range* |
| | *Outside optimal range* |
| R-C5.3 | **Depth requirements** |
| | *Within optimal range* |
| | *Outside optimal range* |
| R-C5.4 | **Reproductive habitat requirements** |
| | *Availability* |
| | *Non availability* |
| R-C5.5 | **Trophic requirements** |
| | *availability* |
| | *Non availability* |
| R-C6 | **Hydrological perturbation** |
| | *None/low* |
| | *Moderate* |
| | *High* |
| R-C7 | **Channel Morphological Perturbation** |
| | *None/low* |

| | | |
|---|---|---|
| | | *Minor* |
| | | *Moderate* |
| | | *Major* |
| **R-C8** | **Pressure of invasive species** | |
| | | *Absent* |
| | | *Locally rare* |
| | | *Locally abundant* |
| **R-C9** | **Riparian Structure** | |
| | | *Complex Vegetation Structure* |
| | | *Uniform Vegetation* |
| | | *Absence of Vegetation* |
| **R-C10** | **Riparian Lignified Extent** | |
| | | *Semi-Continuous or Continuous Cover* |
| | | *Moderate, Patchy Cover* |
| | | *Minimal or Absent Cover* |
| **R-C11** | **Dominant Land Use** | |
| | | *Natural or Semi-Natural Dominance* |
| | | *Herbaceous Vegetation Dominance* |
| | | *Mixed or Mosaic Land Use* |
| | | *Agricultural Dominance* |
| | | *Urban or Suburban Dominance* |
| **R-C12** | **Understanding of threats to species' viability & alleviation potential** | |
| **R-C12.1** | **Current Threats** | |
| | | *Well understood, easily alleviated* |
| | | *Poorly understood* |
| | | *Well understood, difficult to alleviate* |
| **R-C12.2** | **Future Threats** | |
| | | *Well understood, easily alleviated* |
| | | *Poorly understood* |
| | | *Well understood, difficult to alleviate* |
| **R-C13** | **Systemic Future Pressures** | |
| | | *High Resilience to Systemic Pressures* |
| | | *Uncertain or Poorly Characterised Trends* |
| | | *High Risk and Low Mitigation Potential* |

**Source Data**

Bibliographical data

Climate change scenario/population/economy data

Current field data

Expert judgment

Time series field data

Time series map data

**Criteria**

R-C1
R-C8
R-C3.1
R-C3.3
R-C5.1
R-C3.4
R-C4
R-C3.2
R-C3.5
R-C2
R-C5.2
R-C5.3
R-C5.4
R-C5.5
R-C6
R-C12
R-C7
R-C9
R-C10
R-C11
R-C13

**Constraints**

Imperfect detection

Inadequate representation of a certain riverine type

Limited data availability

Misidentification

Narrow time frame of data acquisition

non-quantitative data

**Adaptations**

"one out, all out" principle not applicable

Application of the "one out, all out" principle

Local manager assessment

Local manager consultation

Multiple gear use, novel detection methods use, standartization sampling methodology

Use of data for related species

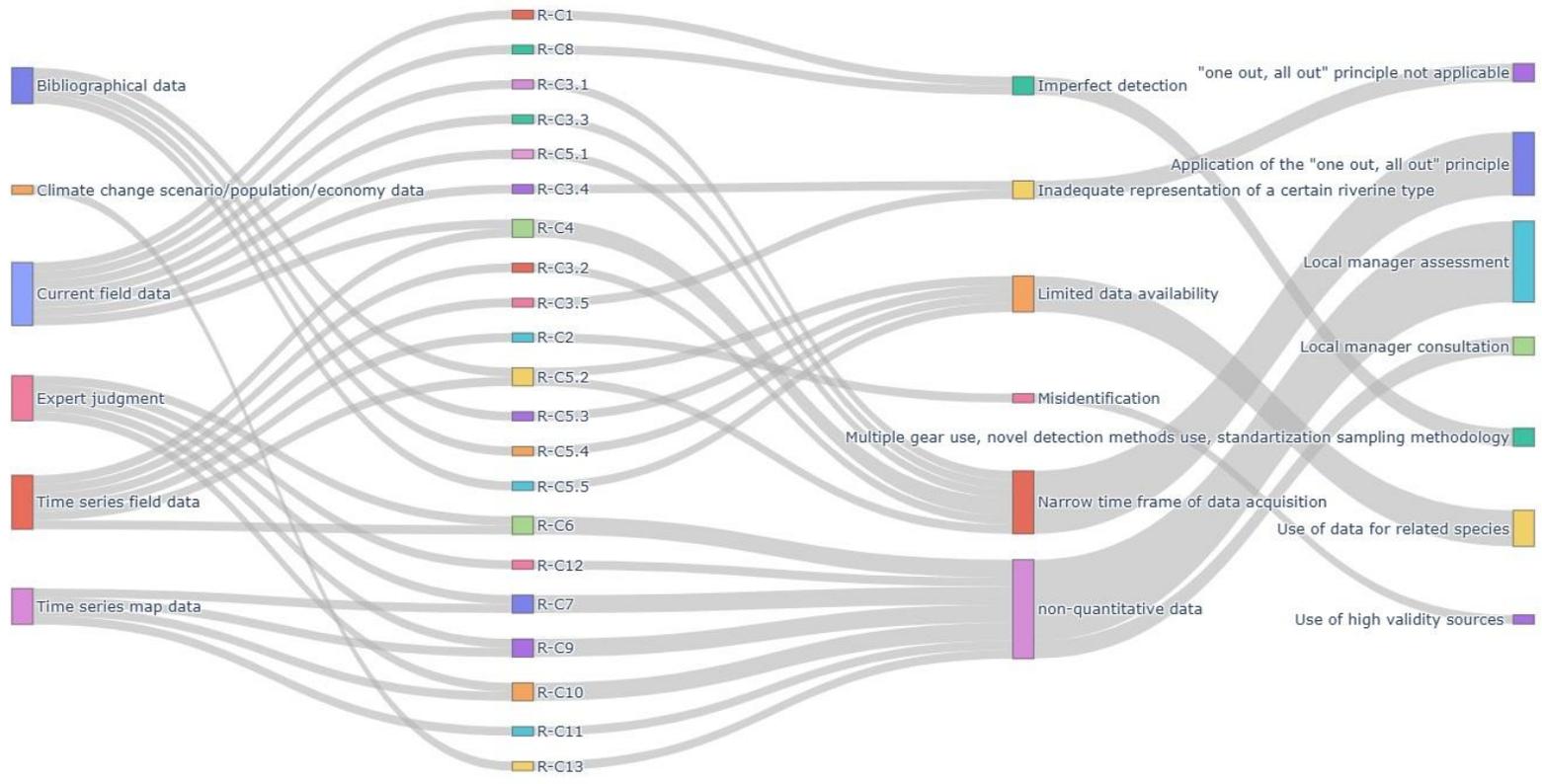Use of high validity sources

**Fig. 1.** Alluvial diagram visualising the relationships among data sources, evaluation criteria, methodological limitations, and corresponding robustness adaptations.

# Release Water Body (R-WB) Assessment Criteria

The evaluation of R-WBs is crucial for determining whether the release habitat can sustain a translocated population that will be self-reproducing in the long term.

Each criterion is designed to assess a specific ecological dimension, and the overall feasibility score for a given R-WB is derived by averaging the individual scores of the main criteria. Below is an expanded description of the main criteria:

## Current Presence (R-C1)

The R-C1 criterion is fundamental in assessing the feasibility of a freshwater fish translocation and serves to ensure that the focal species is genuinely absent from the candidate release water body (R-WB) prior to reintroduction. Its primary rationale is twofold: (1) to avoid redundancy, i.e., translocating individuals to an already occupied site offers no clear conservation benefit and could divert resources from higher-priority locations, and (2) to prevent unintended genetic mixing between existing and transferred populations, which could compromise locally adapted gene pools or disrupt ongoing evolutionary processes.

This criterion is binary in nature, yet it incorporates a tiered confidence structure to reflect the quality and recency of data available:

**Score 1.0**: Confirmed absence of the focal species based on recent (current) field surveys conducted as part of the translocation feasibility assessment prior to the planned release.

**Score 0.75**: Apparent absence based on surveys conducted within the past decade (≤10 years), assuming methods and effort were sufficient to detect the species if present.

Given the well-documented issue of imperfect detection in aquatic monitoring (e.g., rare or cryptic species, seasonal variability), this criterion emphasises the need for high-confidence data. Therefore, it is recommended that multiple, complementary sampling techniques be applied, including electrofishing, seine netting, and minnow traps, ideally across seasons and under varying flow conditions, to reduce false negatives (Lamothe & Drake, 2019).

Where feasible, the use of emerging detection tools such as environmental DNA (eDNA) assays (Galloway et al, 2016, Deiner et al, 2017; Beng & Corlett, 2020; Mauvisseau et al., 2020; Carraro et al., 2020) and underwater video or sonar systems should be incorporated to increase detection probability (Kalogianni et al., 2024).

# R-C1 – CURRENT PRESENCE

CONFIRMING THE ABSENCE OF THE FOCAL SPECIES IN THE RELEASE WATER BODY
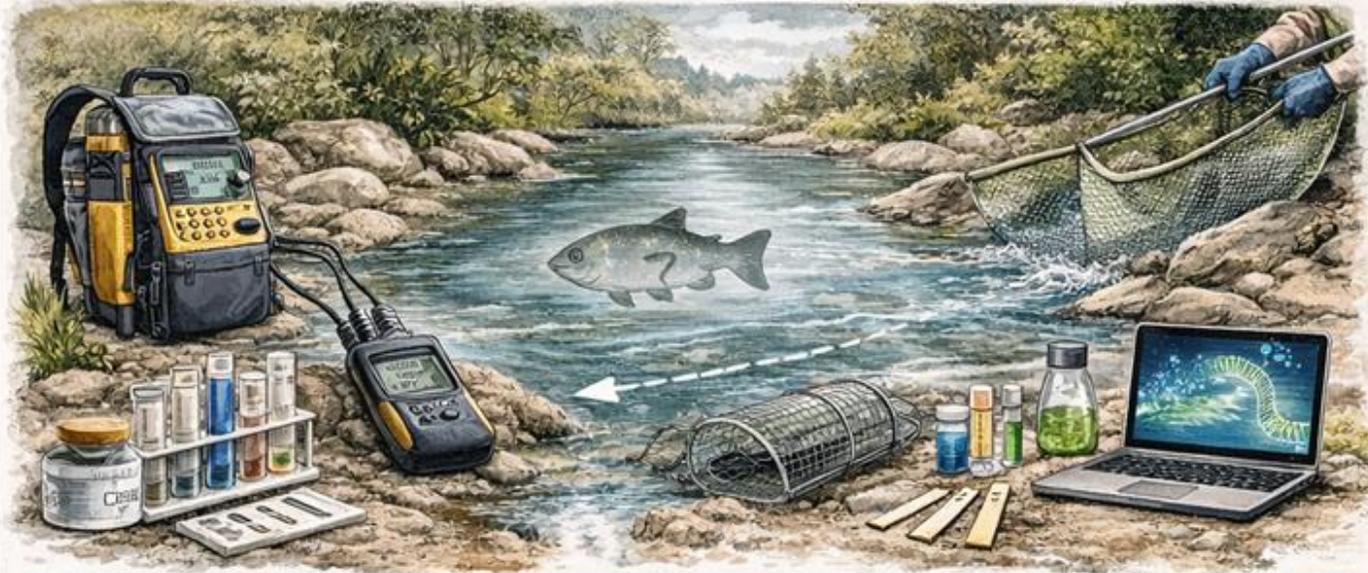
✓ **CONFIRMED ABSENCE**
RECENT FIELD SURVEY

❓ **APPARENT ABSENCE**
0.75  SURVEY WITHIN ≤ 10 YEARS

# Historical Presence (R-C2)

The R-C2 criterion assesses whether the focal species historically occurred within the potential R-WB, thereby evaluating the ecological and biogeographical appropriateness of the release habitat. This criterion is rooted in the principle that reintroductions should aim to restore species to areas where they were once native, thereby aligning with conservation goals such as ecological restoration, genetic integrity, and historical continuity.

The rationale behind this criterion is twofold:

**Ecological justification** — species introductions into historically unoccupied habitats may result in unpredictable ecological interactions, such as competition with other native species, alteration of trophic dynamics, or habitat incompatibility.

**Biogeographical validity** — selecting release habitats within the species' former range ensures that reintroductions contribute to restoring natural distributions rather than artificially expanding them, though recently there is a debate on the need for conservation introductions, i.e., introducing the species outside its historical range, if suitable habitats within its range are lacking.

A tiered scoring system is employed to reflect confidence in historical occurrence:

**Score 1.0**: Confirmed historical presence within the R-WB, based on reliable past data such as museum specimens, historical ecological surveys, or peer-reviewed literature.

**Score 0.75**: Probable historical presence inferred from records in the wider sub-catchment or river basin, suggesting ecological plausibility but lacking site-specific documentation.

**Score 0.0**: No available historical records or credible indirect evidence to support previous occurrence in the region.

In the absence of direct historical records, biogeographical inference methods can offer supplementary insight. However, caution must be exercised to avoid drawing conclusions from erroneous or ambiguous sources, particularly given the risk of historical misidentification or inconsistent taxonomic treatment. Triangulation of multiple evidence types (e.g., habitat matching, range continuity, paleo-ecological data) is recommended to increase inferential robustness.

*R-C2 serves as a safeguard against ecologically unjustified translocations and supports a strategic and ecologically sound prioritisation*

# R-C2 – HISTORICAL PRESENCE

DOCUMENTING THE HISTORICAL OCCURRENCE OF THE FOCAL SPECIES IN THE RELEASE
WATER BODY
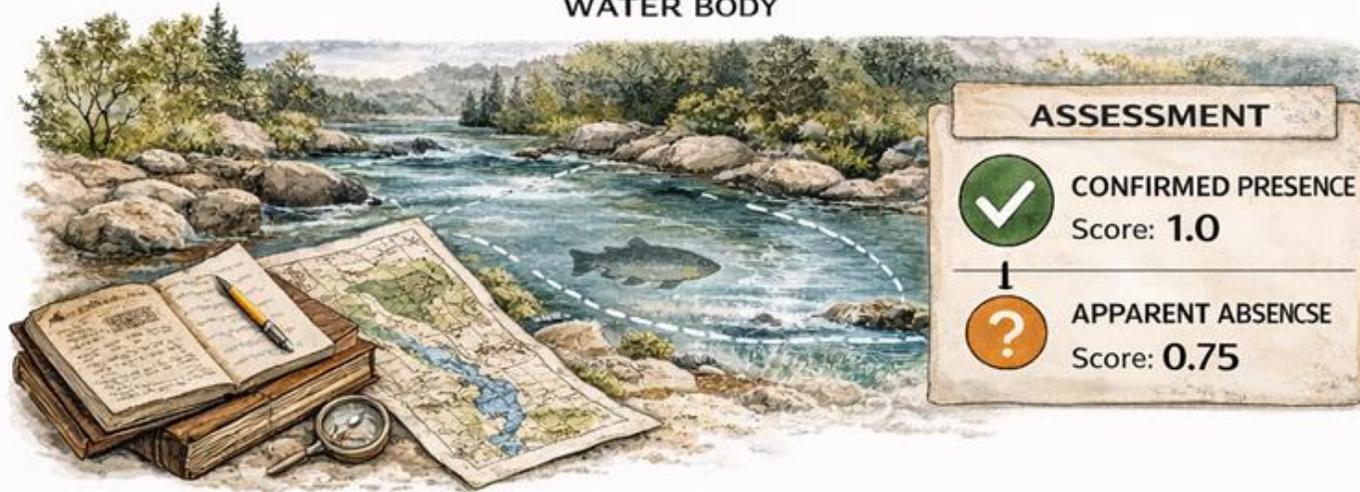


## ASSESSMENT

**CONFIRMED PRESENCE**
Score: **1.0**

1

**APPARENT ABSENCSE**
Score: **0.75**

**CONFIRMED ABSENCE**
**1.0** RECENT FIELD SURVEY

**APPARENT ABSENCE**
**0.75** SURVEY WITHIN ≤ 10 YEARS

**NO RECORDS**
**0.0**

# Release Habitat Quality (R-C3)

The R-C3 criterion evaluates whether the abiotic and biotic conditions of the potential R-WB are ecologically suitable to support a viable population of the focal species in the long term, following translocation. Release habitat quality is central to reintroduction success, influencing both short-term survival and long-term persistence. Unsuitable conditions, whether water chemistry or biological integrity, can reduce fitness, reproduction, or recruitment, undermining restoration goals.

R-C3 is subdivided into two complementary components, both of which are required to characterise the habitat's ecological condition:

**R-C3.1** – **Physico-chemical water quality**: Assesses parameters such as nutrients, dissolved oxygen, conductivity, and pH, interpreted via national and EU water quality systems.

**R-C3.2** – **Biological quality**: Evaluates taxonomic composition, diversity, and ecological condition of benthic macroinvertebrate and diatom communities, based on nationally validated indices for Greece (Smeti & Karaouzas, 2016; Lazaridou et al., 2018; Munné et al., 2021) aligned with the Water Framework Directive (WFD).

A binary scoring scheme is applied to both components:

**Score 1.0**: The R-WB exhibits high or good ecological status based on the respective quality indicators.

**Score -1.0**: The R-WB is classified as moderate, poor, or bad ecological status, indicating that essential ecological functions may be compromised.

Time-series monitoring data are strongly recommended, as they average seasonal variability. Where unavailable, the "one out, all out" principle assigns the lowest class observed, ensuring no impairment is overlooked. However, ecological context is essential: in karstic spring-fed systems with naturally low macroinvertebrate diversity, indices may underestimate quality. Here, expert judgment or site-specific indicators should supplement, to avoid false negatives in classification.

*R-C3 ensures the viability and establishment of the translocated population*

**RELEASE HABITAT QUALITY (R-C3)**

ECOLOGICAL SUITABILITY OF THE RELEASE WATER BODY FOR LONG-TERM POPULATION PERSENCE

R-C3.1 — PHYSICO-CHEMICAL WATER QUALITY
- NUTRIENTS
- DISSOLVED OXYGEN
- CONDUCTIVITY
- pH

R-C3.2 — BIOLOGICAL QUALITY
MACROINVERTEBRATES

GOOD ECOLOGICAL STATUS
RECENT FIELD SURVEY

POOR ECOLOGICAL STATUS
Score: −1.0

## Release Habitat Quantity (R-C4)

The R-C4 criterion assesses whether the spatial extent of the R-WB is sufficient to support the long-term persistence of a viable population of the focal species. Habitat size plays a critical role in determining ecological carrying capacity, influencing factors such as resource availability, home range accommodation, dispersal potential, and population resilience to demographic and environmental fluctuations. This criterion is especially important in the context of founder population dynamics. Small or fragmented habitats are more likely to support populations that are vulnerable to genetic drift, inbreeding, stochastic events, and Allee effects, i.e., the decrease of a population's growth rate at low population densities due to ecological and behavioural processes, all of which can undermine translocation success.

The assessment is based on direct measurements of:

- Water body length (m) for linear systems
- Wetted area (m²) for surface-based systems
- Water volume (m³) for lentic or deep habitats
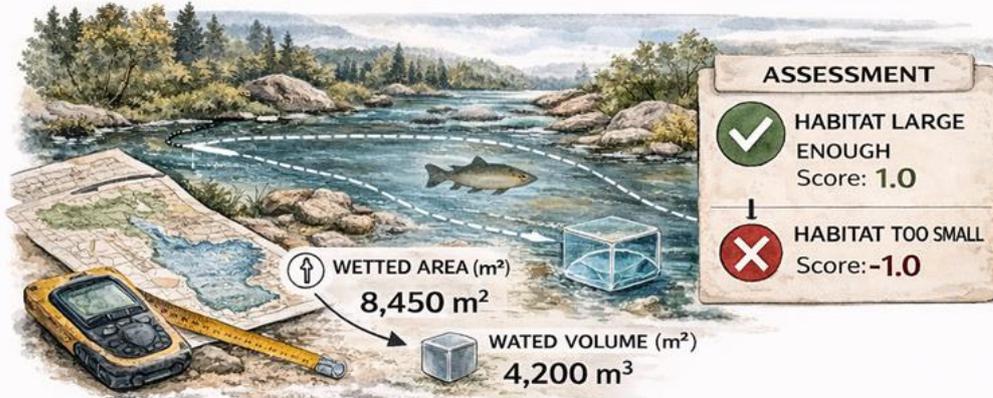
Scoring is assigned as follows:

**Score 1.0**: The R-WB is of sufficient size to support the focal species, if comparable to or exceeding that of a reference habitat where stable populations are known to persist.

**Score -1.0**: The R-WB is significantly smaller to a reference habitat where stable populations are known to persist or highly fragmented, if indicating limited capacity to support a viable or genetically diverse population over time.

## RELEASE HABITAT QUANTITY (R-C4)

IS THE HABITAT LARGE & CONNECTED ENOUGH TO SUPPORT A VIABLE POPULATION?

**ASSESSMENT**

✓ HABITAT LARGE ENOUGH
Score: **1.0**

✗ HABITAT TOO SMALL
Score: **-1.0**

⬆ WETTED AREA (m²)
**8,450 m²**

WATED VOLUME (m²)
**4,200 m³**

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### Adaptation for Migratory Species

For species with obligatory migratory behaviour (e.g., potamodromous or diadromous fishes), spatial adequacy is reinterpreted through the lens of longitudinal connectivity, rather than static habitat size. In these cases, the effective habitat includes access to upstream and downstream reaches critical for life-cycle completion (e.g., spawning, foraging, overwintering).

Connectivity is assessed within a 10 km fluvial radius of the R-WB, considering anthropogenic or natural barriers (e.g., dams, weirs, culverts). The scoring scheme is as follows:

**Score 1.0**: Complete longitudinal connectivity; no barriers impede upstream or downstream movement.

**Score -0.5**: Partial connectivity; one direction (typically upstream) is impeded, while the other remains open.

**Score -1.0**: Full disconnection; barriers prevent movement in both directions, effectively isolating the R-WB.

# Release Habitat Suitability (R-C5)

The R-C5 criterion evaluates the degree to which the environmental conditions of the R-WB match the ecological niche requirements of the focal species. Ensuring such alignment is essential for increasing the likelihood of establishment, reproduction, and long-term persistence of reintroduced populations.

Species reintroduction efforts often fail not due to the absence of a relatively ecologically healthy release habitat, but because critical ecological components for completing its life cycle are suboptimal or missing. Therefore, this criterion is structured into five sub-criteria (Table 2), each reflecting a key dimension of habitat suitability, based on published habitat selection studies, species-specific ecological models, and/or data from closely related taxa.

*R-C5 ensures that translocation is considered only where the habitat provides the fundamental ecological requirements for the species to complete its life cycle and establish a self-sustaining population*

**Table 2**. Scoring framework for the R-C5 criterion, assessing how well release water body (R-WB) conditions match the focal species' ecological requirements.

| Subcriteria | Score 1.0 | Score -1.0 |
|---|---|---|
| **R-C5.1 – Flow Regime** | | |
| This sub-criterion distinguishes between perennial and intermittent flow conditions in the R-WB. Most freshwater species are adapted to specific flow regimes that influence oxygen availability, habitat stability, and access to seasonal microhabitats. | Perennial flow maintained year-round, suitable for species requiring continuous aquatic habitat. | Intermittent or ephemeral flow; likely unsuitable for species requiring stable hydrological conditions. |
| **R-C5.2 – Flow Requirements** | | |
| Assesses whether the flow patterns of the R-WB align with the species' preferred flow velocities, derived from flow-habitat suitability curves (e.g., Vardakas et al., 2017). These preferences influence swimming performance, energy expenditure, and habitat selection. | Flow falls within the species' optimal flow range during key life stages. | Flow deviates significantly from species-specific tolerances. |
| **R-C5.3 – Depth Requirements** | | |
| Assesses water depth availability, referencing depth-habitat suitability models (e.g., Vardakas et al., 2017) or empirical studies. Depth influences foraging behaviour, predator avoidance, and spawning site selection. | Depth range supports species' habitat preferences across seasons. | Depths are persistently too shallow or too deep to meet ecological requirements. |
| **R-C5.4 – Reproductive Substrate Availability** | | |
| Evaluates the presence of suitable spawning substrates. Where species-specific reproductive data are unavailable, closely related species can be used as proxies. | Preferred substrates are abundant (>25%) and accessible in the R-WB. | Reproductive substrates are absent, degraded, or unsuitable. |
| **R-C5.5 – Trophic Resource Availability** | | |
| Examines the availability of suitable prey or other dietary resources. For generalist feeders, a broader trophic spectrum is acceptable; for specialists, the presence of target prey taxa is critical. | Key dietary resources of the species are present in sufficient abundance (>20 inds in semi-quantitative sampling) | Trophic base is depauperate or mismatched with species' needs. |

# RELEASE HABITAT SUITABILITY (R-C5)

| Subcriteria | Score 1.0 | Score −1.0 |
|---|---|---|

### R-C5.1 – FLOW REGIME



Perennial flow maintained year-round, suitable for species requiring continuous aquatic habitat.

### R-C5.2 – FLOW REQUIREMENTS



Intermittent or ephemeral flow; likely unsuitable for species requiring stable hydrological conditions.

### R-C5.2 – FLOW REQUIREMENTS



0.6ms
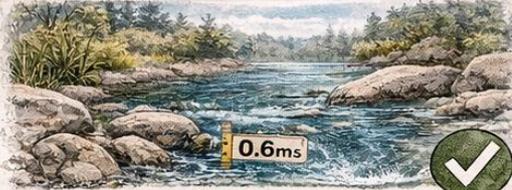
Flow falls within the species' optimal flow range during key life stages.

### R-C5.3 – DEPTH REQUIREMENTS



0.1ms
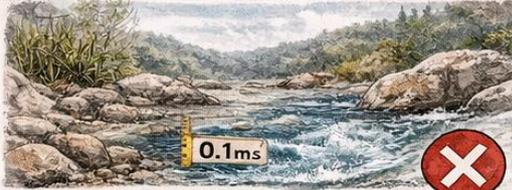
Flow deviates significantly from species-specific tolerances.

### R-C5.4 – DEPTH REQUIREMENTS



0.5m.

Depth range supports species' habitat preferences across seasons.

### R-C5.5 – REPRODUCTIVE SUBSTRATE AVAILABILITY



0.1ms

Depths are persistently too shallow or too deep to meet ecological requirements.

### R-C5.5 – TROPHIC RESOURCE AVAILABILITY



Key dietary resources of the species are present in sufficient abundance (>20 inds in semi-quantitative sampling)

### R-C5.5 – TROPHIC RESOURCE AVAILABILITY



Trophic base is depauperate or mismatched with species' needs.

# Hydrological Perturbation (R-C6)

The R-C6 criterion evaluates the extent to which the natural flow regime of a release water body (R-WB) has been altered by anthropogenic activities (e.g., flow regulation, abstraction, and/or artificial releases). Flow variability is a key driver of ecological integrity in lotic systems, shaping sediment transport, channel morphology, nutrient cycling, and habitat availability. For freshwater fishes, deviations from the natural hydrological regime can disrupt critical life history processes, such as spawning, recruitment, and foraging (Dudgeon et al., 2006).

This criterion recognises that though species may be adapted to natural seasonal flow variability, they can be particularly sensitive to anthropogenic hydrological disturbance (Cid et al., 2017). Therefore, assessing the degree of anthropogenic flow alteration is essential for determining whether a given R-WB can support a viable, self-sustaining population post-translocation. Scoring is based on the intensity and ecological impact of hydrological perturbation:

**Score 1.0** – **None or Low Perturbation**: The hydrological regime closely mimics natural seasonal variability. Minor alterations, such as small-scale abstractions or occasional dam releases, may be present but presumed to exert negligible ecological effects. Natural high and low flow pulses remain largely intact.
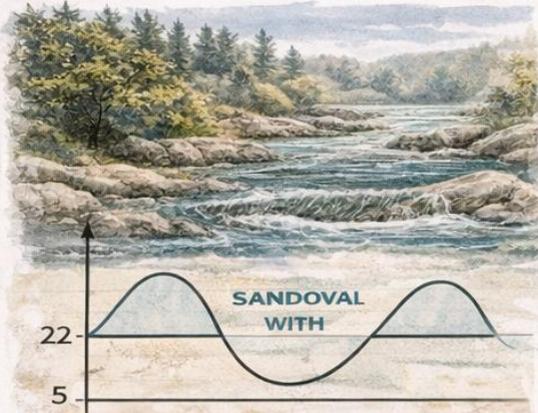
**Score 0.5** – **Moderate Perturbation**: The flow regime is moderately regulated. This may include predictable artificial flow peaks (e.g., for irrigation or hydropower), reduced seasonal variation, or partial disconnection from natural floodplain dynamics. While some ecological functions persist, they are compromised in duration or magnitude.

**Score -1.0** – **High Perturbation**: Severe modification of the hydrological regime is evident, often due to intense water abstraction, hydropeaking, or long-term flow suppression. Natural variability is overridden by engineered flow schedules, resulting in biologically unseasonal floods, extended low-flow periods, or abrupt flow changes that affect habitat stability and species fitness.

Where available, quantitative time-series datasets (e.g., discharge records, dam operation logs, abstraction volumes) should be used to inform scoring. These provide objective, longitudinal evidence of hydrological pressure. In the absence of robust flow data, assessments should rely on: (a) Repeated field observations of hydrological patterns and infrastructure, (b) Expert judgment from hydrologists, ecologists, or basin managers with site-specific knowledge, (c) Historical context regarding past or ongoing alterations to the system.

*R-C6 serves to screen out habitats with chronically unstable or ecologically inappropriate flow regimes, safeguarding against translocations into systems where hydrological disturbance may jeopardize long-term population viability*
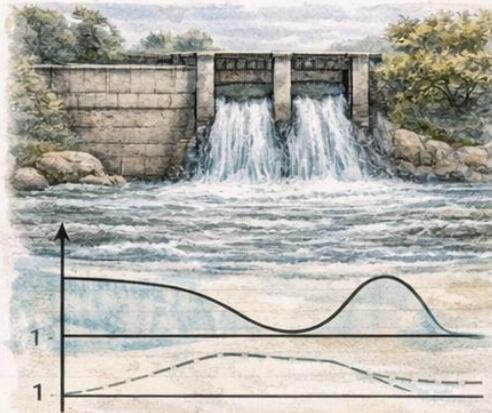
# HYDROLOGICAL PERTURBATION (R-C6)
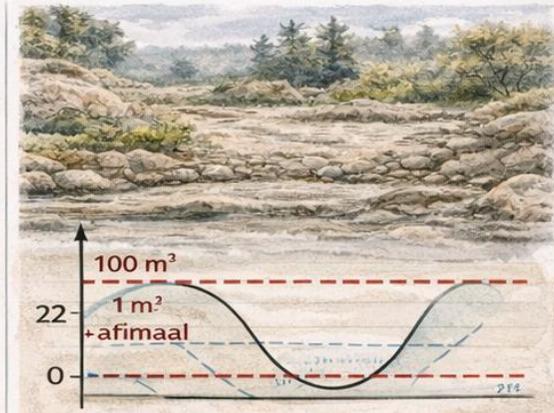


Natural flow variability intact

Score: **1.0**

Artificial flood pulses, reduced natural variability

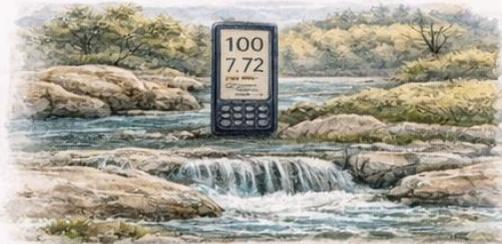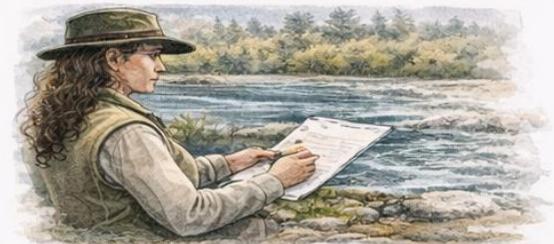Score: **0.5**

Biologically unseasonal flood

Score: **−1.0**

Natural discharge data

Dam operations & flow logs

Field observations & expert judgment

# Channel Morphological Perturbation (R-C7)

The R-C7 criterion evaluates the degree of physical alteration to the river channel, with particular attention to how these changes influence habitat structure, sediment transport, and hydrodynamic processes. Natural morphological features, such as meanders, pools, riffles, and diverse substrate types, create the heterogeneity needed to support different life stages of freshwater fish. Altered channel forms can reduce this complexity, simplify flow paths, and disrupt key ecological processes such as spawning, feeding, and refuge use. Anthropogenic modifications (e.g., channelisation, embankment, substrate armouring, and hydraulic structures) can lead to the loss of hydraulic diversity, sediment imbalance, and functional disconnection from floodplains or backwaters. These effects are especially detrimental to species requiring structurally complex habitats.

Scoring is based on the intensity and ecological impact of morphological modification along a representative (a minimum 100-150 m) reach of the R-WB, within a realistic context that recognises that almost all systems in human inhabited areas of the world have been subjected to some degree of habitat modification:

**Score 1.0** – **None or Low Modification**: Less than 50% of the reach shows signs of channel alteration (e.g., straightening, deepening, or embankment), and no artificial materials (e.g., concrete, riprap) are used. Natural channel features, such as sinuous planform, variable depths, and substrate composition, are well preserved.

**Score 0.5** – **Minor Modification**: Over 50% of the reach has been modified in form (e.g., straightened or deepened), but without the use of artificial substrate or hard engineering. Although some morphological complexity is lost, the riverbed remains natural, and ecological functionality is partially retained.

**Score -0.5** – **Moderate Modification**: Artificial substrates (e.g., gabions, concrete slabs, riprap) are present but cover less than 50% of the riverbed. The reach may also contain small-scale, permeable, and reversible hydraulic structures, such as low weirs or wooden barriers, that locally affect flow and habitat connectivity without fully impeding ecological processes.

**Score -1.0** – **Major Modification**: More than 50% of the riverbed is armoured with artificial materials, and the channel includes permanent, impermeable structures such as high weirs, sluices, or locks. These interventions severely alter hydrodynamics, flow depth, sediment transport, and lateral connectivity, resulting in substantial habitat degradation.

Assessments should be grounded in direct field observations across multiple points along the representative reach. Where available, drone-based imagery (Fig. 2), high-resolution orthophotos, i.e., aerial or satellite images that have been geometrically

*R-C7 ensures that reintroductions are not directed toward highly engineered or structurally simplified systems, where ecological functions and habitat niches necessary for population persistence may be significantly compromised*
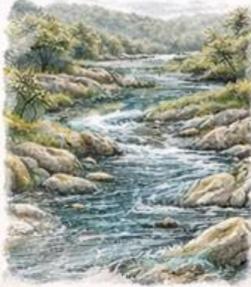
corrected ("orthorectified") so that the scale is uniform across the entire image, or GIS-based hydro-morphological datasets can enhance accuracy and provide a broader spatial perspective. Such remote sensing tools are particularly useful in identifying features that are not easily detectable on foot, such as large-scale embankments or channel infilling upstream or downstream.

**Fig. 2**. Aerial photo used to assess the extent of physical alteration in the release water body (R-WB) for a killifish translocation site near a settlement in western Greece (Vardakas et al., 2026).

# CHANNEL MORPHOLOGICAL PERTURBATION (R-C7)

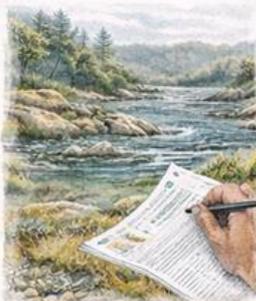| NONE OR LOW MODIFICATION | MINOR MODIFICATION | MODERATE MODIFICATION | MAJOR MODIFICATION |
|---|---|---|---|
| ✅ | ➕ | ➖ | ❌ |
| Score: **1.0** | Score: **0.5** | Score: **−0.5** | Score: **−1.0** |
| Field observations | Drone imagery | High-resolution orthophotos | High-resolution orthophotos |
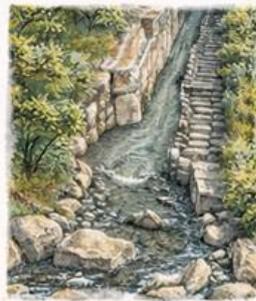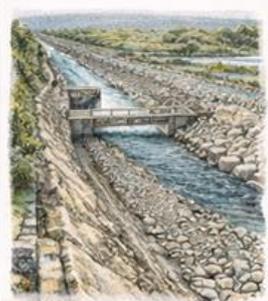
# Invasive Alien Species Pressure (R-C8)

The R-C8 criterion evaluates the ecological pressure of invasive alien species (IAS) within the potential R-WB, that can compromise the success of native species translocations. IAS are among the leading drivers of freshwater biodiversity decline globally, through mechanisms such as predation, competition, hybridisation, and disease transmission (Ribeiro & Leunda, 2011; Reid et al., 2019; Bernery et al. 2022; Arianoutsou et al., 2023). This criterion aligns with the IUCN Guidelines for Reintroductions and Other Conservation Translocations, which explicitly recommend that recipient habitats be assessed for invasive species presence and IAS risk prior to any release.

The ecological rationale behind R-C8 is that even low-density populations of IAS can exert disproportionate negative effects on native biota, particularly during critical life stages (e.g., spawning, juvenile recruitment). Therefore, both presence and relative IAS abundance to contextualise the functional risk of IAS, are considered in scoring (Abundance thresholds used for classification are informed by empirical studies e.g., Pritt & Frimpong, 2010; Kalogianni et al., 2023):

**Score 1.0** – **Absence of IAS**: No documented presence of IAS in the R-WB. The habitat is considered free of known predatory, competitive, or disease-vector non-native taxa, based on recent and reliable survey data.

**Score -0.5** – **Low Abundance Presence**: IAS are present but occur only at low numerical abundance (≤ 20 individuals) **and** low relative abundance (≤ 5% of the fish community), in a 100 m reach, with no significant ecological disruption presumed. Risk is considered latent but manageable.

**Score -1.0** – **High Abundance Presence**: IAS are present at high density **and/or** represent a substantial portion of the fish community (> 20 individuals **and/or** >5%). There is clear empirical or inferred evidence that their ecological impact could jeopardise translocation success, either by direct predation, competitive exclusion, or interference with reproductive behaviours.
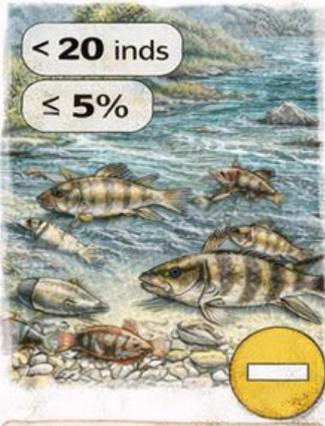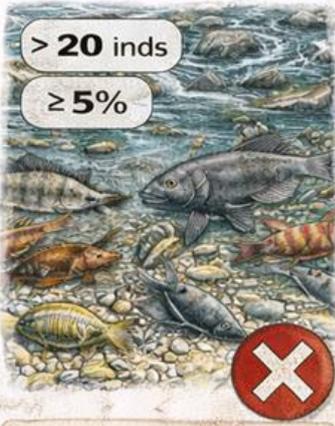
> Quantitative assessment of IAS pressure should be based on:
> - Multi-method field sampling using electrofishing, seine nets, and/or traps;
> - Abundance metrics, including absolute counts and relative proportions of total fish biomass or individuals;
> - Scientific literature on the known impacts of specific invasive species in similar ecological contexts;
> - Molecular tools such as environmental DNA (eDNA) screening, which improve detection of cryptic or low-density IAS and help address issues of imperfect detection (Lamothe & Drake, 2019; Rees et al., 2014).

*R-C8 acts as a critical filter to prevent the translocation of focal species in habitats where invasive alien species pose a substantial ecological threat—one that could undermine the objectives of the translocation program or introduce new conservation conflicts*

# INVASIVE ALIEN SPECIES PRESSURE (R-C8)

ECOLOGICAL PRESSURE OF INVASIVE ALIEN SPECIES WITHIN THE POTENTIAL REINTRODUCTION WATER BODY:

| ABSENCE OF 1AS | LOW ABUND REMDANCE PRESENCE | HIGH ABUNDANCE PRESENCE |
|---|---|---|
| | < 20 inds <br> ≤ 5% | > 20 inds <br> ≥ 5% |
| ✔ | ➖ | ✖ |
| No invasive alien species detected | IAS present at low abundance | IAS present at high abundance |
| Score: **1.0** | Score: **−0.5** | Score: **−1.0** |

# Riparian Structure (R-C9)

The R-C9 criterion assesses the structural complexity of riparian vegetation along the bank face and bank top of the candidate R-WB, recognising its essential role in maintaining ecological integrity and supporting both aquatic and terrestrial species. Riparian zones act as ecological interfaces that contribute to habitat heterogeneity, nutrient cycling, shade regulation, and organic matter input (e.g., leaf litter, woody debris, terrestrial prey), all of which are critical for the persistence of freshwater fish populations.

This criterion is adapted from the River Habitat Survey (RHS v. 2003) methodology (Raven et al., 1998), with a focus on vegetation stratification, coverage, and microhabitat provision **within the first three (3) meters** from the bank edge **and for a 100 m reach (one bank).**

Scoring is assigned as follows:

**Score 1.0** – **Complex Vegetation Structure**: Two or more distinct vegetation types are present within both the bank face and bank top (up to 3 m width, as mentioned above). These may include combinations of herbaceous species (e.g., bryophytes, sedges, reeds, grasses), shrubs, and mature trees. This vertical stratification provides diverse ecological niches and supports high functional diversity.

**Score -0.5** – **Uniform Vegetation**: Only a single type of herbaceous vegetation is present, with no shrub or tree cover. While some ground-level cover exists, vertical diversity and associated ecological functions (e.g., shading, detritus input, refuge provision) are substantially reduced.
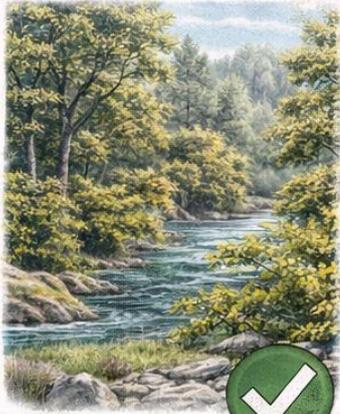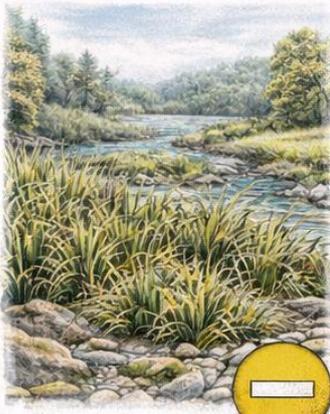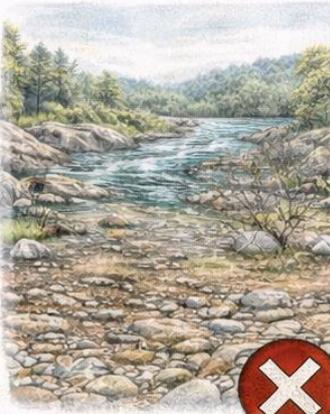
**Score -1.0** – **Absence of Vegetation**: The bank face and top are devoid of any vegetation. Exposed soil, rock, or artificial substrates dominate the riparian zone, resulting in poor habitat conditions and a lack of ecological buffering functions.

Assessment should be conducted via standardised field surveys, carried out by trained personnel using visual inspection protocols. Ideally, evaluations should be performed during the growing season when vegetation is fully developed, and supported, where possible, by photographic or drone imagery for documentation and inter-observer consistency.

*R-C9 serves to ensure that potential release habitats possess sufficient riparian vegetation complexity to support multi-trophic interactions, microclimate regulation, and ecosystem resilience, thereby enhancing the long-term viability of translocated populations*

**NOTE:** In REVIVE-HELLAS, we make no distinction between native and alien riparian vegetation, but there can be indirect effects on hydrology, soil chemistry, and fire regimes by alien, invasive species (Waterworth, 2015); thus, the tool could be modified accordingly, if this is an issue to consider for the focal fish species.

# RIPARIAN STRUCTURE (R-C9)

RIPARIAN VEGETATION ALONG THE BANK FACE AND BANK TOP

| COMPLEX VEGETATION STRUCTURE | UNIFORM VEGETATION | ABSENCE OF VEGETATION |
|---|---|---|
| Complex vegetation Structure | Only a single typs of herbacessrs nag | Less than 10% of bank top area |
| Score: **1.0** | Score: **−0.5** | Score: **−1.0** |

Field observations

Photography

Drone imagery

# Riparian Lignified Extent (R-C10)

R-C10 complements the structural vegetation assessment in R-C9 by evaluating the longitudinal continuity of woody riparian cover, specifically tree and shrub presence along the bank top of the candidate R-WB. Continuous or semi-continuous riparian canopies provide essential ecological functions, including bank stabilisation, thermal regulation through shading, leaf litter input, and habitat for terrestrial invertebrates that serve as prey for many freshwater fish species. Woody vegetation corridors also facilitate ecological connectivity, supporting the movement of riparian fauna and buffering the aquatic ecosystem from adjacent land-use pressures. Disruption or fragmentation of tree/shrub cover can impair these services and reduce the ecological suitability of the release habitat.

This criterion is adapted from the "extent of trees" metric in the River Habitat Survey (RHS v.2003), with scoring based on the proportion of riparian zone (bank top within 3 m) occupied by trees and shrubs:

**Score 1.0** – **Semi-Continuous or Continuous Cover**: Trees and/or shrubs line more than 50% of the bank top (within a 3 m buffer). This configuration provides strong ecosystem support functions, including persistent canopy cover, root bank reinforcement, and consistent organic matter input.

**Score -0.5** – **Moderate, Patchy Cover**: Trees and shrubs are regularly spaced, covering 10% to 50% of the bank top area. While ecological functions are partially maintained, the system is more exposed to thermal variability and may suffer from spatial discontinuities in detrital input and shade.

**Score -1.0** – **Minimal or Absent Cover**: Less than 10% of the bank top area is occupied by trees or shrubs. The riparian corridor lacks woody vegetation, reducing habitat quality, thermal buffering, and food web complexity.

Assessment should be conducted via systematic field surveys, with visual estimation of cover extent by trained observers. Where feasible, field-based observations should be supplemented by high-resolution aerial imagery or drone-based orthophotos, which offer consistent measurement of linear vegetation continuity, particularly over longer reaches.

*R-C10 ensures that riparian conditions not only provide vertical structural complexity but also deliver spatial continuity—a key requirement for the support of self-sustaining aquatic populations*

REVIVE-HELLAS tool

# RIPARIAN LIGNIFIED EXTENT (R-C10)

LONGITUDINAL CONTINUITY OF WOODY RIPARIAN COVER ALONG THE BANK TOP
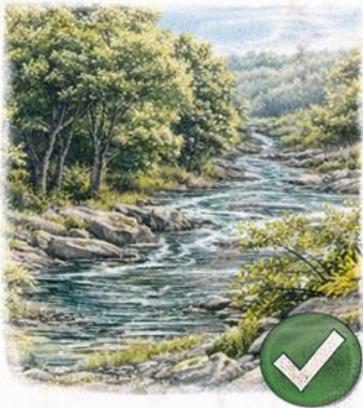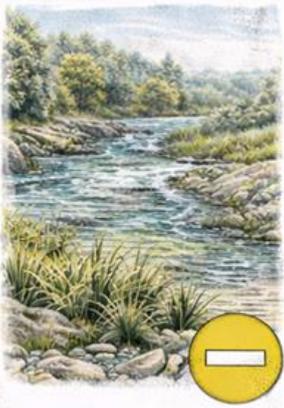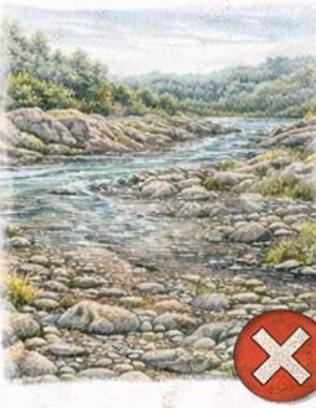
| SEMI-CONTINUOUS OR CONTINOUS COVER | MODERATE, PATCHY COVER | MINIMAL OR ABSENT COVER |
|---|---|---|
|  |  |  |
| Semi-continuous or continuous cover | Trees and/chrubs 10% to 50% | Less than 10% of bank top area |
| Score: **1.0** | Score: **−0.5** | Score: **−1.0** |



Field observations



High-resolution orthophotos



Drone imagery

# Dominant Land Use (R-C11)

The R-C11 criterion evaluates the dominant land use (Table 3), acknowledging the critical role of adjacent landscapes in shaping instream habitat conditions, sediment dynamics, water quality, and riparian ecological processes. Land use within this buffer can directly influence the nutrient loads, pesticide runoff, and thermal regimes of the R-WB, as well as affect riparian vegetation integrity and wildlife corridor function. For freshwater species translocations, a natural or semi-natural buffer zone acts as a protective filter, maintaining ecosystem integrity and improving the likelihood of successful population establishment and persistence. Conversely, intensive agricultural or urbanised land use contributes to physical disturbance, nutrient enrichment, and hydrological instability.

*R-C11 serves as a proxy for assessing external pressures on the aquatic environment and helps identify release habitats where buffer zone integrity supports habitat stability, and long-term translocation success*

**Table 3.** Scoring framework for the R-C11 criterion, assessing dominant land use within a 30-meter buffer zone on both sides of the R-WB.

| Criteria | Scoring |
|---|---|
| **Natural or Semi-Natural Dominance** | |
| Over 50% of the buffer zone is occupied by natural habitats, such as forests, wetlands, shrublands, or native grasslands. These landscapes support ecological resilience and minimise external pressures on the aquatic environment. | **Score 1.0** |
| **Herbaceous Vegetation Dominance** | |
| Low herbaceous vegetation or managed grassland (e.g., extensively grazed pasture) dominates >50% of the buffer. Though not entirely natural, these systems exert relatively low pressure and maintain some ecological functionality. | **Score 0.5** |
| **Mixed or Mosaic Land Use** | |
| No single land use class dominates. The buffer consists of a heterogeneous mixture (e.g., 30% forest, 30% agriculture, 40% pasture), often resulting in fragmented ecological conditions and variable pressure levels. | **Score 0.25** |
| **Agricultural Dominance** | |
| More than 50% of the buffer zone is occupied by intensive cropland or orchard use, increasing the risk of nutrient runoff, pesticide inputs, and physical disturbance to the riparian zone. | **Score -0.25** |
| **Urban or Suburban Dominance** | |
| The buffer is primarily composed (> 50%) of artificial surfaces (e.g., roads, buildings, paved infrastructure), representing a high level of anthropogenic impact, loss of habitat connectivity, and altered hydrological dynamics. | **Score -0.5** |

Assessment should be carried out using GIS-based spatial analysis, incorporating high-resolution land cover datasets (e.g., CORINE, Copernicus, or Sentinel-2 imagery). All scoring should be referenced to a consistent 30-meter buffer on both banks. To improve reliability, field verification is recommended, especially in cases of ambiguous or rapidly changing land use mosaics.

# DOMINANT LAND USE (R-C11)

Assesses broader macro-scale pressures affecting the long term viability of freshwater fish translocations in candidate R-WBs.

### Natural or Semi-Natural Dominance

Over 50% of the buffer zone is natural habitat (forest, wetlands, shrubland, or native grassland).

Score: **1.0**

### Herbaceous Vegetation Dominance

Over 50% of the buffer zone is managed grassland (extensively grazed pasture or herbaceous vegetation).

Score: **0.25**

### Agricultural Dominance

Over 50% of the buffer zone is intensive cropland or orchard.

Score: **−0.25**

### Mixed or Mosaic Land Use

Score: 1.0

### Buffer zone is a heterogeneous mixture

Score: 0.25

### Urban or Suburban Dominance

Score: −0.5

GIS and Satellite imagery

Field Verification

Drone Surveys

Photographic Documentation

# Understanding of Threats to Species' Viability & Alleviation Potential (R-C12)

The R-C12 criterion evaluates both current and anticipated future threats to the candidate R-WB, with a particular focus on their degree of understanding, ecological severity, and practical imitability. This dual-component assessment is fundamental to ensuring that reintroduced populations are not placed into environments where unresolved stressors will undermine their persistence.

The criterion is divided into two sub-components:

1)  **R-C12.1** – **Current Threats**: This sub-criterion examines existing stressors that are already impacting the R-WB, such as:

*   Water pollution and eutrophication,
*   Flow regime alteration (e.g., hydropeaking, abstraction),
*   Presence of invasive species,
*   Physical habitat fragmentation (e.g., barriers, embankment),
*   Riparian degradation.

Scoring reflects both the quality of knowledge available and the feasibility of mitigation:

**Score 1.0** – **Well Understood and Easily Mitigated**: Threats are clearly identified and quantified, with effective, practical mitigation measures already available (e.g., riparian buffer planting, invasive species control, dam management protocols). Their ecological severity is low to moderate, and solutions are feasible within current governance or funding frameworks.

**Score 0.0** – **Poorly Understood or Documented**: There is insufficient or outdated information on the presence, the extent, or the ecological effects of key threats. Risk assessment is impaired, and planning cannot proceed with confidence.

**Score -1.0** – **Well Understood but Difficult to Alleviate**: Existing threats are known and well-characterised, but technical, ecological, or socio-political barriers prevent effective mitigation. These may include chronic pollution from non-point sources, systemic flow alteration, or entrenched land use conflicts.

R-C12 ultimately helps to ensure that translocation efforts are not only ecologically justified but also resilient to change, by proactively identifying and addressing risks to population establishment and persistence

2) **R-C12.2** – **Future Threats**: This sub-criterion focuses on projected or emerging pressures over a 10-year horizon, such as:

- Climate change (e.g., warming, drought frequency),
- Land-use intensification or urban expansion,
- Infrastructure development (e.g., new dams, roads),
- Hydrological regime shifts.

These threats may not yet be present but could jeopardise translocation success if not accounted for in feasibility planning. Scoring follows the same structure:

**Score 1.0** – **Predictable and Manageable**: Future threats are foreseeable (e.g., based on climate models, spatial planning data), and adaptive management strategies exist to address them. Governance structures are in place to implement mitigation if needed.

**Score 0.0** – **Poorly Characterised or Uncertain**: Scenario forecasting is either unavailable or highly uncertain. No clear strategies exist to address possible future impacts.

**Score -1.0** – **Intractable or Inevitable Impacts**: Major threats are projected with high confidence (e.g., large-scale water abstraction, irreversible urban development) and cannot be realistically mitigated, even with long-term planning or intervention.

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**Methodological Considerations**

Robust scoring for both R-C12.1 and R-C12.2 should be based on:

- Expert workshops with local managers, hydrologists, ecologists, and planners
- Historical time-series datasets (e.g., flow, land use, pollution)
- Peer-reviewed ecological assessments and vulnerability models
- Stakeholder consultation and local knowledge, especially in basins with complex governance

# UNDERSTANDING of THREATS TO SPECIES' VIABILITY & ALLEVIATION POTENTIAL

Evaluates understanding and manageability of current and anticipated threats to candidate R-WBs.

## R-C12.1 – CURRENT THREATS

**CURRENTLY IMPACTING R-WB**

| Well Understood and Easily Mitigated | Poorly Understood or Documented | Well Understood but Difficult to Alleviate |
|---|---|---|
| Threats are clearly identified, feasible mitigation measures in place. Ecological severity is low to moderate. | Insufficient or outdated information impairs risk assessment. Planning cannot proceed with confidence. | Existing threats are known, but barriers to effective mitigation exist. Threats have moderate to high severity. |
| Score: **1.0** | Score: **0.0** | Score: **−1.0** |

## R-C12.2 – FUTURE THREATS

| Predictable and Manageable | Poorly Characterised or Uncertain | Intractable or Inevitable Impacts |
|---|---|---|
| Field Surveys | Monitoring Data | Scientific Reports |
| Score: 1.0 | Score: 0.5 | Score: −0.5 |

Field Surveys    Monitoring Data    Satellite & Aerial Imagery    Scientific Reports

# Systemic Future Pressures (R-C13)

The R-C13 criterion assesses broader macro-scale pressures that are likely to affect the ecological integrity and management feasibility of the candidate R-WB over the long term. Unlike R-C12, which focuses on water-body-specific threats, R-C13 addresses regional and systemic drivers—including climate change (demographic shifts, and socioeconomic transformations—that may indirectly impact the success of freshwater fish translocations. By integrating this foresight-based criterion, planners can ensure that selected sites are not only suitable under current conditions but also resilient to long-term change.

**Scoring Categories**:

**Score 1.0** – **High Resilience to Systemic Pressures**: The R-WB is located in a region where future climatic and socioeconomic changes are projected to be moderate, and adaptive capacity (e.g., governance, funding, infrastructure) is high. Catchment-wide integrated water management frameworks are in place, and ecological buffers (e.g., natural forests, protected areas) enhance resilience.

**Score 0.0** – **Uncertain or Poorly Characterised Trends**: Available models and demographic projections are unclear, outdated, or contradictory. It is uncertain how regional changes will affect the R-WB's suitability for long-term population persistence. No clear policies exist to monitor or mitigate these trends.

**Score -1.0** – **High Risk and Low Mitigation Potential**: The R-WB is situated in a vulnerable region (e.g., prone to water scarcity, desertification, high urbanisation pressure). Climate change models project severe alterations to hydrology or temperature regimes, and no feasible measures are in place to buffer these effects (But et al. 2021).

> **Methodological Considerations**
>
> Scoring should rely on:
>
> - Downscaled regional climate models (e.g., RCP scenarios, CMIP6),
> - Population and land-use change projections from national statistics or global databases (e.g., SSPs, UN-WPP, Copernicus),
> - Scenario planning frameworks integrating environmental, economic, and policy drivers,
> - Consultation with regional planning authorities, conservation experts, and social scientists.
>
> Ideally, a multi-criteria vulnerability assessment should be performed to synthesise exposure, sensitivity, and adaptive capacity, ensuring that the R-WB is not exposed to unmanageable risk under future scenarios.

*R-C13 enhances the robustness of translocation planning by acknowledging that species reintroductions must be embedded within long-term socio-ecological trajectories. Integrating systemic foresight allows practitioners to avoid maladaptive investments and prioritize sites where populations are more likely to persist and thrive into the future*

# SYSTEMIC FUTURE PRESSURES (R-C13)

Assesses broader macro-scale pressures affecting long-term viability of candidate R-WBs.

## Scoring Categories:

| HIGH RESILIENCE TO SYSTEMIC PRESSURES | UNCERTAIN OR POORLY CHARACTERISED TRENDS | HIGH RISK AND LOW MITIGATION POTENTIAL |
|---|---|---|
| High Resilience to Systemic Pressures | Uncertain or Poorly Characterised Trends | High Risk and Low Mitigation Potential |
| Future changes moderate, adaptive governance capacity high. | Future trends unclear, outdated models and projections. | Future risks severe, no feasible measures in place. |
| Score: 1.0 | Score: 0.0 | Score: −1.0 |
| Climate Change Models | Demographic Projections | Catchment Studies |

# Source Water Body (S-WB) Assessment Criteria

In contrast to the original REVIVE tool, the REVIVE-HELLAS tool introduces a more detailed framework for evaluating the suitability of source populations for species translocation. This refinement is designed to account for potential genetic or epidemiological risks introducing captive-bred source populations. The tool is built on three primary criteria, which collectively aim to ensure that translocation efforts are both ecologically sustainable and genetically viable, with the second and third criterion being on-off criteria.

## Sourcing of Founders (C-SC1)

This criterion evaluates the origin and genetic integrity of the founder population, with the aim of ensuring that individuals selected for translocation are ecologically fit, genetically robust, and unlikely to introduce pathogens into the recipient ecosystem. The founder source directly influences translocation success by determining the adaptive potential, demographic resilience, and long-term viability of the reintroduced population. The rationale behind this criterion lies in the comparative conservation value of wild versus captive-bred sources

Wild populations, particularly those that are naturally reproducing and free from anthropogenic genetic interference, typically maintain:

- Higher levels of genetic diversity,
- Natural adaptive traits aligned with local environments, and
- Lower prevalence of artificially selected phenotypes or latent disease

Individuals sourced from captive breeding programmes, hatcheries, or artificial propagation facilities may be affected by:

- Inbreeding depression or genetic drift due to small effective population sizes,
- Domestication selection, which can reduce survival in the wild,
- Unnatural rearing conditions, leading to maladaptive behaviours or pathogen exposure (Frankham et al., 2011; Araki et al., 2007).
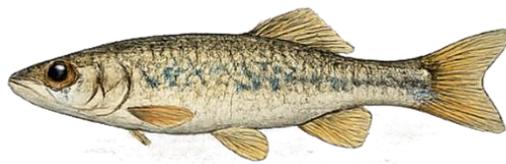
**Scoring System:**

**Score 1.0** – **Wild Sourced Founders**: Individuals are sourced from naturally reproducing wild populations, ideally from a single, ecologically appropriate water body. These populations should be genetically representative of the species' native diversity and not subjected to recent admixture with captive-bred individuals.

**Score 0.5** – **Captive or Hatchery-Origin Founders**: Individuals are sourced from captive breeding facilities, fish farms, or hatcheries. While such populations may offer logistical convenience or demographic buffering, they pose a higher risk of genetic bottlenecks, reduced fitness, and domestication effects (Robert, 2009). Their use may be acceptable under limited or emergency scenarios, but with appropriate genetic monitoring.

> **Methodological Guidance**
>
> Genetic screening (i.e., microsatellite markers, Single Nucleotide Polymorphisms (SNPs), mitochondrial haplotypes) should be used where possible to assess genetic diversity, effective population size, and relatedness. Health assessments should be conducted to screen for pathogens, especially in captive-sourced individuals. Documentation of source history is essential, including breeding protocols and population origin if the source is not wild. By prioritising genetically robust and ecologically adapted founder stock, practitioners can avoid common pitfalls such as founder effects, loss of adaptive traits, and reintroduction failure due to poor individual performance in natural habitats.
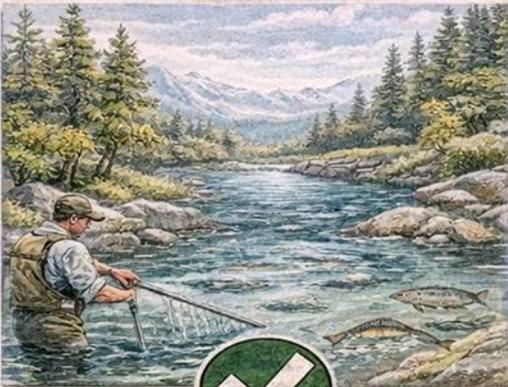
# SOURCING OF FOUNDERS (C-SC1)

Evaluates the suitability of source populations for species translocation.

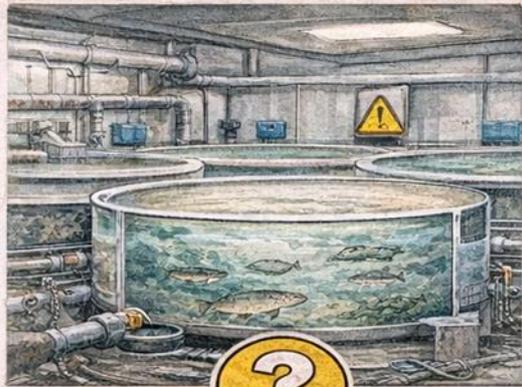## SOURING of FOUNDER (C-SC1)

### Wild-Sourced Founders



**Wild Sourced Founders**

Individuals sourced from naturally. reproducing wild populations from ecologically appropriate water bodies

**Score: 1.0**

### Captive or Hatchery-Origin Founders



**Captive or Hatchery-Origin Founders**

Individuals sourced from hatcheries or fish farms, higher risk of genetic bottlenecks and domestication effects.

**Score: 0.5**



Field Surveys

Lab Tests

Epidemiological Records

# Genetic Compatibility to the Release Habitat (C-SC2)

This criterion assesses whether the source population—regardless of whether it originates from the wild or a captive breeding facility—is genetically compatible with the population that historically or currently inhabits the candidate R-WB. Compatibility is defined by shared evolutionary lineage, genetic continuity, and an absence of long-term reproductive or geographic isolation that could result in maladaptive gene flow or disruption of local adaptations. The translocation of individuals from genetically distinct populations poses significant ecological and evolutionary risks.

Genetic compatibility is a non-negotiable prerequisite for translocation planning and functions as a binary exclusion criterion: populations that fail to meet this requirement must be excluded from consideration.

**Scoring System**:

**YES – Genetically Compatible**: The source population belongs to the same evolutionary lineage as the historical or extant population of the focal species in the R-WB. This may be confirmed through molecular data (e.g., mitochondrial DNA, microsatellites, SNPs) or through robust indirect evidence such as regional biogeography or natural dispersal barriers.

**NO – Genetically Incompatible**: The source population shows evidence of long-term geographic or reproductive isolation from the R-WB population, such that genetic divergence is likely or confirmed. This includes cases where historical or phylogeographic data indicate that the two populations evolved under different selective pressures or environmental conditions.

### Methodological Considerations

Where available, molecular genetic analyses should be prioritised to confirm lineage equivalence. Appropriate markers include:
- Mitochondrial haplotypes for maternal lineage tracing,
- Microsatellites or SNPs for assessing genetic distance and diversity.

In the absence of genetic data, compatibility may be inferred (with caution) based on:
- Historical species distribution records,
- Hydrographic connectivity,
- Biogeographical regionalisation (e.g., shared ecoregions or sub-drainages),
- while explicitly acknowledging the associated uncertainty.

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*C-SC2 criterion reflects a precautionary approach to translocation and is essential for maintaining the evolutionary integrity of natural populations. It ensures that conservation actions do not inadvertently disrupt existing genetic structures or undermine local adaptations critical for long-term population persistence*

**NOTE:** 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.
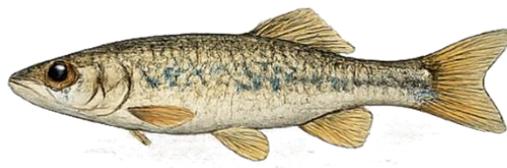
# GENETIC COMPATIBILITY TO THE RELEASE HABITAT (C-SC2)

## GENETICALLY COMPATIBLE



### YES – Genetically Compatible

Shared evolutionary lineage, compatible genetic markers confirmed.

**SCORE: YES - Genetically Compatible**
Shared evolutionary lineage, compatible genetic markers confirmed.

SCORE: YES - Genetically Compatible

## GENETICALLY INCOMPATIBLE



### NO - Genetically Incompatible

**SCORE: NO - Genetically Incompatible**
Long-term genetic isolation, divergent genetic markers identified

SCORE: NO - Genetically Incompatible

# Provision of Sufficient Propagules with no Population Viability risk (C-SC3)

This criterion is applied exclusively when the translocation involves wild-sourced founders and is intended to safeguard the demographic integrity and genetic viability of the source population. It evaluates whether the removal of individuals for translocation would impose undue demographic pressure or reduce the genetic diversity of the source population, thereby compromising its long-term sustainability. While the use of wild individuals is often preferred due to their genetic fitness and ecological competence (see C-SC1), it also presents conservation risks. Overharvesting from small or isolated populations can lead to:

- Demographic decline, particularly when reproductive adults are removed,
- Genetic bottlenecks, reducing allelic richness and increasing inbreeding,
- Disruption of age or sex structure, especially in K-selected or slow-reproducing species.

Therefore, this criterion functions as a conservation safeguard: any source population that does not meet minimum thresholds of abundance and demographic resilience must be excluded from donor consideration.

**Scoring System**:

**YES** – **Demographically and Genetically Sustainable**: The source population is locally abundant, with sufficient demographic buffering capacity to tolerate the removal of individuals without compromising its long-term viability. This status is supported by empirical data or repeated field surveys, indicating healthy population size, balanced age/sex structure, and stable recruitment.

**NO** – **Demographically or Genetically Vulnerable**: The source population is rare, declining, or demographically fragile, and therefore unsuitable for translocation sourcing. Use of such a population may cause irreversible damage to its viability.

**Assessment Criteria and Thresholds**:

**Numerical Abundance**: Populations with ≤20 individuals in a 100 m reach are considered uncommon/rare, following rather strict conservation thresholds adapted from Pritt & Frimpong (2010) OR **Relative Abundance** OR **Biomass**: Populations contributing ≤5% to the total species count or biomass within the habitat are considered low-density and vulnerable to harvesting impacts.

**Population Structure**: Consideration must be given to:

- Age-class distribution (juveniles vs. adults),

*C-SC3 aligns with the principle of "do no harm" and reinforces the dual responsibility of species conservation: supporting at-risk populations while protecting those that remain viable in the wild*

- Sex ratio balance, especially in sexually dimorphic species,
- Reproductive strategy (e.g., K-selected species with low fecundity require higher caution) and seasonality.

> **Methodological Requirements**
> - Repeated field surveys should be conducted using standardised sampling methods (e.g., electrofishing, mark-recapture, visual counts).
> - Demographic monitoring should include basic population metrics (e.g., N, age structure, sex ratio).
> - In cases of uncertainty or data scarcity, expert consultation and a precautionary approach should be adopted to avoid overexploitation.
> - For species with limited known distribution, range-wide assessments may be necessary to prevent cumulative impacts from multiple sourcing events.



**PROVISION OF SUFFICIENT PROPAGULES WITH NO POPULATION VIABILITY RISK (C-SC3)**

DEMOGRAPHICALLY AND GENETICALLY SUSTAINABLE

SCORE: YES - Demographically and Genetically Sustainable
Source population is locally abundant; harvesting poses no viability risk

SCORE: YES - Demographically and Genetically Sustainable

DEMOGRAPHICALLY OR GENETICALLY VULNERABLE

NO - Demographically or Genetically Vulnerable

SCORE: NO - Demographically or Genetically Vulnerable
Rare, declining, or fragile population, at risk from removal

SCORE: NO - Demographically or Genetically Vulnerable

# Scoring and Final Feasibility Assessment

Each criterion within the REVIVE-HELLAS tool is scored on a continuous or categorical scale ranging from −1 to +1, reflecting the degree to which a given water body (R-WB or S-WB) meets the ecological, genetic, and management-related requirements for translocation.

- **Score +1** indicates full compliance with the criterion and reflects favourable conditions.
- **Score 0** represents partial compliance, uncertainty, or moderate suitability.
- **Score −1** denotes non-compliance and indicates conditions that are likely to hinder successful translocation.

For criteria composed of multiple sub-components (e.g., R-C3, R-C5, R-C12), the mean of sub-criterion scores is calculated to derive a single, integrated score for the overall criterion.

Binary "ON/OFF" criteria (e.g., C-SC2, C-SC3) serve as exclusion filters: if the condition is not met (i.e., score = "NO"), the corresponding candidate site or source is disqualified from further consideration, regardless of the cumulative score.

**Overall Feasibility Score**

The final feasibility score for each potential R-WB is calculated as the mean of all applicable main criterion scores, excluding binary exclusion criteria. This quantitative approach allows for the ranking and prioritisation of candidate sites based on ecological, biological, and management considerations.

Feasibility classes are interpreted as follows:

| Mean Score Range | Interpretation |
|---|---|
| +1.0 to +0.5 | High feasibility — site is strongly suitable for translocation |
| +0.49 to 0.0 | Moderate or uncertain feasibility — may require further investigation or mitigation |
| < 0.0 | Low feasibility — site is not recommended for translocation |

This scoring framework promotes objectivity, transparency, and comparability across multiple candidate R-WBs, supporting evidence-based decision-making in freshwater fish conservation planning. The structured use of weighted criteria, tiered confidence levels, and binary safeguards increases the likelihood of both short-term translocation success and long-term population persistence.
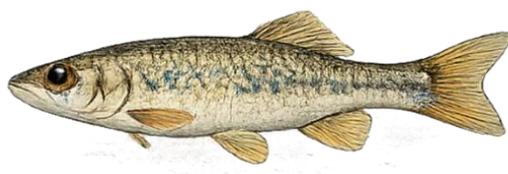
# Trial application of the REVIVE-HELLAS tool

Using the REVIVE-HELLAS we assessed the suitability as potential R-WBs of the threatened Evrotas chub (*Squalius keadicus*), five streams of the Evrotas/ Vassilopotamos basin (Southern Greece), which were previously assessed with the REVIVE tool (see Kalogianni et al., 2023). Fig. 3 is a visualisation of the scoring (in brackets) in radar graphs for each of the criteria and subcriteria applied for the five potential release water bodies R-WBs.

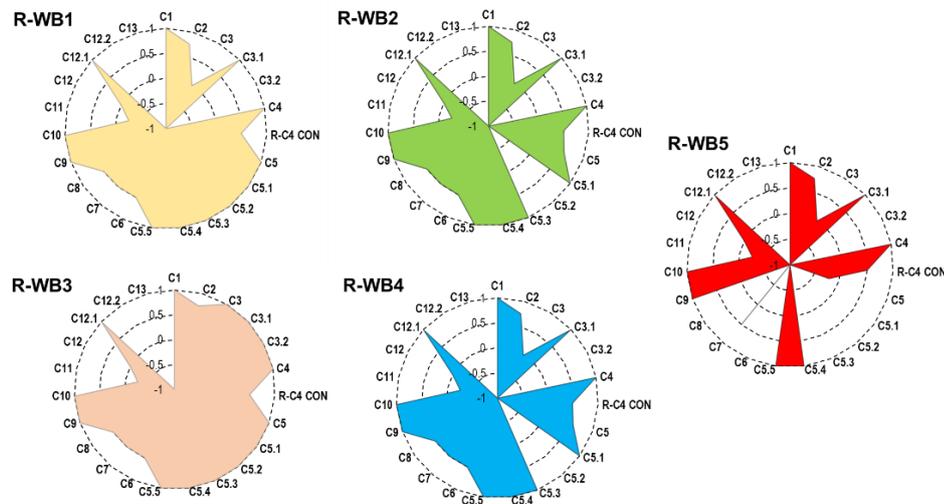*The validation of REVIVAL-HELLAS indicated its robustness*



**Fig. 3** Radar graphs with the scoring of each of the criteria and subcriteria applied for the five potential release water bodies R-WBs. Range of scoring −1 (centre) to 1 (periphery). The largest the area of the circle covered by the shaded arachnoid diagram, the higher the suitability score.

All potential R-WBs scored positively, but on the lower end, as only one R-WB scored more than 0.5 (0.54). Most however, scored close to 0.5 (0.43 – 0.54), with the exception of R-WB5 that scored close to 0, indicating low potential for Evrotas chub translocation. When compared with the assessment of these R-WBs using the REVIVE HELLAS this pattern of the low potential of R-WB5 is also evident. However, when assessed with REVIVE the rest of the R-WBs scored much higher (53-78); this is due to the incorporation of several additional criteria in REVIVE HELLAS related to dominant land use, riparian vegetation and systemic pressures to which all R-WBs scored poorly.

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