water

MDPI

Review

An Overview of Ecological Indicators of Fish to Evaluate the Anthropogenic Pressures in Aquatic Ecosystems: From Traditional to Innovative DNA-Based Approaches

Maurizio Pinna [1,2,3,*], Francesco Zangaro [1,2], Benedetta Saccomanno [1], Chiara Scalone [1], Fabio Bozzeda [1,2], Lucia Fanini [1,2] and Valeria Specchia [1,3]

[1] Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, S.P. Lecce-Monteroni, 73100 Lecce, Italy
[2] Research Centre for Fisheries and Aquaculture of Aquatina di Frigole, DiSTeBA, University of Salento, 73100 Lecce, Italy
[3] National Biodiversity Future Center (NBFC), 90133 Palermo, Italy
* Correspondence: maurizio.pinna@unisalento.it

Abstract: In order to halt the loss of global biodiversity and considering the United Nations Decade for Ocean Conservation Agenda, increasing efforts to improve biomonitoring programs and assessment of ecosystem health are needed. Aquatic environments are among the most complex to monitor, requiring an array of tools to assess their status and to define conservation targets. Although several parameters need to be considered for a comprehensive ecological status assessment, it is important to identify easy-to-apply high-resolution monitoring methods. Shifts in fish composition and abundance are often good indicators of ecosystem health status in relation to anthropogenic activities. However, traditional monitoring methods are strictly related to the habitat under study and cannot be applied universally. This review summarizes the importance of ecological indicators for aquatic environments subjected to anthropogenic stressors, with a particular focus on fish communities and transitional water ecosystems. We describe the main characteristics of both traditional and novel methods for fish monitoring, highlighting their advantages and shortcomings in an attempt to identify simple and reliable ways for a correct evaluation of the dynamics of aquatic ecosystems.

Keywords: ecological indicators; biomonitoring plans; fish diversity; environmental DNA; DNA metabarcoding; aquatic ecosystems

check for updates

Citation: Pinna, M.; Zangaro, F.; Saccomanno, B.; Scalone, C.; Bozzeda, F.; Fanini, L.; Specchia, V. An Overview of Ecological Indicators of Fish to Evaluate the Anthropogenic Pressures in Aquatic Ecosystems: From Traditional to Innovative DNA-Based Approaches. *Water* **2023**, *15*, 949. https://doi.org/10.3390/w15050949

Academic Editor: Jun Yang

Received: 20 January 2023
Revised: 24 February 2023
Accepted: 26 February 2023
Published: 1 March 2023

1. Introduction

One of the greatest challenges in the management of aquatic ecosystems, from oceans to coastal and transitional waters, is to find simple ways to evaluate their condition [1]. To find these methods, it is important to choose the most appropriate indicators, metrics, and evaluation tools to use in order to measure most of the properties of the ecosystem under study. In this context, ecological indicators are markers representing the ecosystem's integrity and durability in relation to anthropogenic activities [2].

Undeniably, the choice of the appropriate indicator or set of indicators depends not only on the questions that are addressed but also on the characteristics of the ecosystem under study [3]. Indicators span across all biodiversity levels, and the selection of these metrics should be properly calibrated for each case study [1].

This review aims to describe the main characteristics of ecological indicators and to offer a synthesis of the most commonly applied ecological indicators, with particular emphasis on fish individuals and fish communities, to assess the status of aquatic ecosystems. Particular attention has been paid to indicators that are generally applied in the management of transitional water ecosystems. A description of their strengths, weaknesses, and possible applications and interpretations is also provided, together with recent advancements in the field.

2. Importance of Ecological Indicators

Ecological indicators are defined as biological assemblages of species or taxa which, due to their presence or condition, can give information on the integrity of an ecosystem and their variation can be representative of the anthropogenic and natural impacts, and pressures acting on the ecosystems at different spatial and temporal scales [4]. Ecological indicators are important for understanding the ecosystem characteristics and for summarizing large quantities of information about its status to inform management and direct nature protection actions towards conservation and livelihood sustainability [5].

In particular, ecological indicators provide a tool for evaluating the integrity of an ecosystem, and by doing so, they help to identify changes in ecosystem conditions and predict the direction and possible magnitude of impacts or responses to stress. Ecosystems represent a complex network of biotic and abiotic factors, and, for this reason, assessing the disturbances over time can be quite challenging, also concerning the abiotic spatial features [6]. From this side, ecological indicators describe an ecosystem in simple terms by splitting it into its main features, whose changes reflect the alteration of the ecosystem structure and functioning itself [7,8]. Hence, the main contribution of ecological indicators is to allow the assessment of the impact that human activities have on the ecosystem. However, this does not automatically translate into the identification of the factors causing it [9–12].

Ecological indicators can be based on both qualitative and quantitative metrics, but the latter is probably more useful in management. If we consider marine ecosystems, there are many factors leading to biodiversity loss which are either directly or indirectly related to human activities, such as agricultural waste mismanagement [13], unsustainable coastal development [14], the introduction of alien and invasive species [15,16], overexploitation of marine resources [17,18], and ocean warming and acidification [19,20]. This explains why it is important to assess the entity of pressures and how to implement amelioration measures. There is a growing concern about the critical status of many aquatic ecosystems and the need to define, test, and work with robust indicators to track the ecosystem's status and make informed management decisions [21].

It is important to underline the complexity of aquatic ecosystems and how just one biological metric is often not enough to describe the complexity and dynamics of these ecosystems [22]. The contextual use of multiple indicators or the synthesis of many metrics in a multi-metric index is highly recommended when assessing the status of an ecosystem. The simultaneous use of both "descriptive indicators", such as taxa composition and richness, and "functional indicators", measuring the interaction between trophic levels and the cycling of nutrients [23–25], should generally be preferred. Even though most of the impacts of human activities on aquatic ecosystems are well known, it is still difficult to properly quantify all of them [26] due to the high natural variability and poor knowledge of these ecosystems. This spurred the interest of the scientific community in finding effective indicators to assess ecosystem status and guide management plans.

In Europe, the publication of the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) have encouraged researchers to identify a set of descriptors of status of European water bodies. This resulted in an increasing number of publications describing different indicators aimed to depict the ecosystem status [1]. It is time to review ecological indicators of aquatic ecosystems, considering the global targets proposed by the 17 Sustainable Development Goals (SDG), and the opening of the United Nations Decade for Ocean Conservation.

3. Characteristics of a Good Ecological Indicator

As stated above, indicators have an important role in monitoring and assessing the status of an ecosystem, in quantifying the intensity and the extent of human pressures, and in informing management to allow the preservation of habitats and biodiversity. For this reason, the set of indicators must be chosen wisely.

To be cost-effective and provide clear management directions, the suites of indicators should be kept as small as possible, avoiding redundancy but still fulfilling the needs of all users [27]. From a management perspective, Rice and Rochet [27] provided a seven-steps guide:

- Step 1: determine user needs, identifying who the managers and the stakeholders interested in the data are;
- Step 2: develop a list of candidate indicators that are effective in measuring the ecosystem status relative to the objectives;
- Step 3: determine screening criteria, such as concreteness, theoretical basis, public awareness, costs, measurement, historical data, sensitivity, responsiveness, and specificity;
- Step 4: score candidate indicators against the screening criteria;
- Step 5: summarize the scoring results;
- Step 6: decide how many indicators are needed;
- Step 7: make the final selection and report the chosen suite of indicators.

A good indicator must reflect ecosystem dynamics (physical, biological) and their possible evolution. It is, therefore, an expression of the connection between different ecosystem compartments and must reflect the changes occurring between them.

4. Why Use Fish as Ecological Indicators?

Ichthyofauna composition and anthropogenic impacts are tightly connected. Biological monitoring is preferred over chemical monitoring because the latter often fails to detect many of the human-induced disturbances in aquatic ecosystems, such as habitat degradation or resource overexploitation [28]. The fish assemblages of both resident and migrant species associated with an aquatic ecosystem are potentially impacted by many human activities. Such pressures can alter their diversity and biomass, their spawning rate, their food sources, and even their behaviour and survival rates.

Many different fish species have been successfully used as indicators of environmental quality changes in a wide variety of aquatic habitats [29,30]. For Whitfield and Elliott [28], their success in being good indicators relies on numerous characteristics, such as:

- Their presence in almost all aquatic systems, apart from the highly polluted waters;
- The extensive information on life history and environmental response available for most species;
- Fishes are relatively easy to be correctly identified, and non-destructive sampling can be applied;
- Fish communities usually include a range of species that represent a variety of trophic levels;
- Fishes are long-lived species compared to other aquatic organisms and, therefore, can provide a long-term record of environmental stress;
- They contain many life forms and functional guilds and, thus, are likely to cover several components of aquatic ecosystems affected by anthropogenic disturbance;
- Fish species can be both sedentary and migratory and, thus, can reflect stressors within one area or provide information about broader effects;
- Fishes hold a higher value compared to invertebrates or aquatic plants to the general public, meaning that stakeholders might be more interested and invest more in collecting data about the condition of the fish community because of the economic, aesthetic, and conservation values associated with fish.

The use of fish as indicators of ecosystem status shows some difficulties given to intrinsic factors, such as [5]:

- Sensitive fish species may spontaneously avoid stressful environmental conditions, reducing their exposure to toxic or other harmful conditions;
- Spatial variability is also very high, meaning that an extensive sampling effort is necessary to adequately characterize the fish assemblage;
- Their life cycle and behaviour must be considered, including reproductive and overwintering migrations;
- Some fish are extremely habitat selective, and their habitats may not be easy to sample.

The use of fish species as indicators of ecological changes in aquatic ecosystems assumes that fish communities are sensitive to changes within these systems and that fish abundance and species diversity can provide a good measure of the impacts and pressures to which a particular system is exposed [31,32].

Fish provide a unique and still overly underexploited asset in understanding changes in the aquatic environment, even on a long temporal scale, through the comparison of contemporary data with archaeological ones [33]. This approach can be best achieved through the integration of both qualitative and quantitative data from a suite of sources ranging from organism to ecosystem levels. The use of a wide range of criteria is likely to be the best strategy to relax the above-mentioned constraints [5] and fully exploit the potential of fish as ecological indicators.

5. Ecological Indicators for Fish

Even though multiple indicators would be generally preferable, single indicators are sometimes chosen because they are more cost-effective than using several and multi-metric ecological indicators, and they might be sufficiently sensitive to detect changes in the ecosystem over natural and anthropogenic variations [34]. Fish communities constitute a relevant tool for the evaluation of the ecological status of aquatic ecosystems [31,35]. Several types of fish-based indices exist for fish species. One main difference between them is represented by singular indicators and multi-metric indicators. Singular indicators are based on only one criterion (see, e.g., the Community Degradation Index (CDI) [36] and the Biological Health Index (BHI) [37]), while multi-metric indicators consist of a combination of several metrics, such as Indices of Biotic Integrity (IBI) [31,38].

A metric is defined as "a measurable factor that represents various aspects of a biological assemblage, structure, function, or any other community component" [39,40]. In light of this, multi-metric indices are expected to provide more comprehensive information on different aspects of fish assemblages. Multi-metric indices suit a more holistic, integrative, and functional approach [38], providing robustness and avoiding biases in consideration of complex environments [34].

5.1. Population Features as an Indicator

One of the ecological indicators of historical change in a given environment is the variation in the abundance of the population of one or more species. This can be measured as a decrease or increase in the number of individuals, their biomass, their average size and age, as well as an expansion or contraction of their distribution range over time [7,41]. This type of indicator was applied, for example, in the island of Sardinia based on the biometric parameters of lobsters, whose results highlighted changes in food availability linked to some physical environmental features, such as temperature and salinity [41].

The binary data given by a species' presence or absence in a particular ecosystem can also be used as an indicator. By pairing presence/absence information with the species' ecological function, scenarios can be depicted. A common scenario is that the disappearance of a fish species with unique ecological functions might cause declines in overall species abundance and diversity, as well as shifts to a different taxonomic composition. For example, the decline of large predators may cause an increase in the abundance or size of their prey and competitors, triggering trophic cascades and affecting several lower trophic levels [42,43]. Such changes to species interactions, community dynamics, food-web processes, and ecosystem functions were efficiently applied as indicators in the USA [7].

5.2. Species Diversity

Species diversity is a property of the community organization level [44]. It can be investigated at different spatial and temporal scales [45]:

- α diversity: the mean species diversity referred to spatially defined units, such as defined assemblages or within a habitat;

- β diversity: the ratio between regional and local species diversity, reflecting biotic changes or species replacement between different areas and/or habitats or turnover phenomena;
- γ diversity: the total species diversity at the landscape scale;
- ε diversity: the total species diversity of a group of areas of γ diversity, referred to as the total species diversity within a biogeographic province;
- δ diversity: the change in species composition and abundance between areas of γ diversity, which occur within an area of ε diversity. It is referred to as differentiation diversity over wide geographic areas.

For monitoring purposes, the species diversity index refers to the α diversity, whereas its increase is generally assumed to indicate more pristine environment conditions [46]. Species diversity can be partitioned into two components: richness and evenness [47]. Species diversity indices can express one or both components.

5.3. Changes in Species Traits: Body Size and Trophic Ecology

In addition to changes in the occurrence and abundance of aquatic species, historical data can also give insight into changes in phenotypical traits. Human activities (e.g., fishing, tourism) were found to affect different specific traits [7]. An example is the case of high fishing pressure affecting fish body size and trophic ecology [48].

Data related to fish body mass and length can be used to analyse trends in the size-frequency of a population or species over time [33]. Such data are relatively easy to find in records of fishery landings [49] and environmental monitoring [50]. When recent records are combined with archaeological samples, this allows for the detection of shifts in the size-frequencies of the sampled species over different time scales [33]. Changes in the size-frequency of a population provide a metric of change in population abundance as a density-dependent response to exploitation [51] and also to environmental conditions in change [52].

From a trophic point of view, the feeding habits of a marine organism provide information on its feeding preferences, while its trophic position places it in relation to other organisms in the food web based on its diet. The trophic position of an organism can be expressed in terms of trophic level (TL) [41,53]. In general, algae, plants, and detritus are commonly considered to have a TL = 1. Therefore, the organisms feeding on them (the so-called primary consumers) have a TL = 2, the organisms that feed on primary consumers have a TL = 3, and so on. Since marine species feed on a range of organisms that occupy different trophic positions in the marine food web, their trophic levels are expressed as fractional numbers (e.g., TL = 3.25 or 4.5) [7].

5.4. Tropho-Dynamic Indicators

In understanding the biological organization of an ecosystem, it is really useful to know the food web structure and the trophic links that constitute it. That is why Lindeman, in 1942 [53], proposed the introduction of tropho-dynamics as "the dynamics of nutrition or metabolism". Tropho-dynamics depict the energy flow through the food webs and the relationships between the different components of an ecosystem. It describes the functional role of a certain species in an ecosystem [54,55], whereas the trophic level describes the species' positions in a food web [56,57].

Originally, tropho-dynamics were not designed for aquatic environments, but researchers successfully applied this concept to marine food webs, e.g., in studies carried out in coral reef ecosystems [58,59]. In particular, Cury et al. [60] identified six indicators to detect ecosystem-level patterns in a conservative way, i.e., they slowly respond to large structural changes in the ecosystem. These are:

- Mean trophic level (TL or TLm) of fisheries landings;
- Fishing in Balance (FiB) index;
- Relative change in species (or functional group) composition;
- Primary production required (PPR) to support catches;

- Mixed trophic impact (TI);
- Proportion of production by different components.

Trophodynamic indicators allow us to measure the entity of the interactions between different trophic compartments [60] and the structural changes in an ecosystem as a response to its exploitation (e.g., fishing activities) [60]. They are reported below, along with notes on their applicability and limitations.

5.4.1. Mean Trophic Level (TLm)

The Mean Trophic Level (TLm) is the mean value of the trophic levels of the caught fish in a certain area. The TLm of fisheries landings can be used as an index of sustainability. It represents a community-level or ecosystem-level indicator targeting shifts in the food web structure [61]. TLm is considered a good indicator to quantify the effects of fishing on food webs. However, both economic and technological factors can alter the TLm value of catches, generating bias in estimating the fishing impact [62,63].

5.4.2. Fishing in Balance (FiB)

The Fishing in Balance (FiB) index is an extension of the TLm. It incorporates a known constant value of trophic level transfer efficiency between trophic levels of the food web, therefore measuring the "trophic level balance" [64]. FiB is useful for monitoring the expansion and contraction of fishing fleets over time, as shown by the trophic level of catches, and allows a comparison among different ecosystems. However, FiB requires stringent data and complex modelling [65,66]. In addition, both TLm and FiB require a large amount of data, which limits their application [48].

5.4.3. Relative Change in Species (or Functional Group) Composition

The relative change in species (or functional group) composition within the caught or surveyed community can be quantified by means of biomass ratios (preferable to catch ratios) to characterize ecosystem changes (e.g., piscivorous, zooplanktivorous fish). These ratios are easy to understand and measure and are often (but not exclusively) sensitive to fishing. However, finding theoretical foundations to set the reference points is difficult, and these should be empirically defined based on historical data [60].

5.4.4. Primary Production Required (PPR)

The Primary Production Required (PPR) is the estimated amount of primary production that is able to support the biomass harvested by fisheries. It is an ecosystem-level indicator of the impact of fishing at the lowest trophic level of the ecosystem.

5.4.5. Mixed Trophic Impact (TI)

Mixed trophic impact (TI) is a measure of the relative impact of a change in the biomass of trophic compartments. The analysis is based on an input-output method used to assess direct and indirect interactions [60]. TI quantifies the net effects of one species on every other species in an ecosystem, considering the positive effects of a prey species on its predator species (weighted relative to its proportion in the predator diet), negative effects of a predator species on its prey species (weighted according to the fraction of the production of prey that is consumed by the predator), and the indirect effects that one species can have on others through trophic interactions. Matrices of relative net impacts of each group on every other are constructed, scaled between -1 and 1. The trophic structure is assumed to remain constant, implying that TI should not be used in a predictive sense but rather as a sensitivity analysis to identify those groups that may have large trophic impacts on others and so might be suitable indicators for monitoring fisheries effects across an ecosystem [60].

5.4.6. Proportion of Production by Different Trophic Compartments

The proportion of production by different trophic compartments, and the proportion of the total consumption of each prey by each predator group, can be used to quantify the relative importance of prey or predators [60]. The importance of predation and/or fishing mortality relative to total mortality may be helpful in monitoring changes in trophic structure within or among systems. Predation mortality results are often larger than fishing mortality, but their relative importance can change over time or differ between systems. Fishing effects will result most apparent in cases of tight trophic coupling, such as between forage fish subject to heavy fishing pressure and seabird predation [60].

5.5. Index of Biological Integrity (IBI)

In 1981, Karr [31] introduced the Index of Biological Integrity (IBI) using stream fish communities. This index puts together different parameters (Table 1) regarding species richness and composition, other than further ecological factors, to provide a classification system for fish communities (Table 2), along with suggested boundaries for the environmental quality classification (Table 3).

Table 1. List of parameters and ecological traits used in the assessment of fish communities; modified from [31].

Species Composition and Richness
Number of Species.
Presence of Intolerant Species.
Species Richness and Composition of Darters.
Species Richness and Composition of Suckers.
Species Richness and Composition of Sunfish (except Green Sunfish).
The proportion of Green Sunfish.
Ecological Traits
Number of Individuals in Sample.
The proportion of Omnivores (Individuals).
Proportion of Insectivorous Cyprinids.
Proportion of Top Carnivores.
Proportion, along with Disease, Tumours, Fin Damage, and Other Anomalies' presence.
Proportion of Hybrid Individuals.

Table 2. Biotic integrity classes used in fish communities' assessment with general descriptions and attributes; modified from [31].

Class	Attributes
Excellent	Comparable to the best situations without human influence. All regionally expected species are present with a full array of age and sex classes, including the most intolerant forms, according to habitat and stream size. Balanced trophic structure.
Good	Species richness is below expectation, especially due to the loss of most intolerant species. Some species have less than optimal abundances or size distribution. The trophic structure shows some signs of stress.
Fair	Signs of additional deterioration include fewer intolerant species and a more skewed trophic structure (e.g., increasing frequency of omnivores). Older age classes of top predators may be rare.
Poor	Dominated by omnivores, pollution-tolerant species, and habitat generalists. Few top carnivores. Growth rates and condition factors are commonly depressed. Hybrids and diseased fish are often present.
Very Poor	Few fish are present, mostly introduced or very tolerant species. Hybrids are commonly present. Disease, parasites, fin damage, and other anomalies are regular.
No Fish	Repetitive sampling fails to turn up any fish.

Table 3. IBI values corresponding to each class of biotic integrity.

Ecological Quality Class	Ecological Indicator Value
Excellent (E)	57–60
E-G	53–56
Good (G)	48–52
G-F	45–47
Fair (F)	39–44
F-P	36–38
Poor (P)	28–35
P-VP	14–27
Very Poor (VP)	≤13

In 1981, the IBI was proposed as a preliminary index, with indications for its use mainly as a summary of the individual metrics on which it is based [31]. Hence, the metrics considered in this index are flexible and can be modified and adapted depending on the study area, the species present, and the boundaries for the quality classes [67,68].

5.6. Marine Fish Community Index (MFCI)

The Marine Fish Community Index (MFCI) incorporates functional and structural information about marine fish communities, including information about the abundance and size structures of each type of substrate [69].

The use of this indicator assumes that substrate type and depth are the main factors responsible for habitat complexity and, so, drivers of the marine fish assemblages [70–74]. For this reason, MFCI has been separated into four different typologies based on the considered kind of substrate:

- Rocky Subtidal (RS–permanently submerged rocky reefs down to a depth of 30 m);
- Shallow Soft-bottom (SS–sandy or muddy substrate down to 20 m deep);
- Intermediate Soft-bottom (IS–sandy or muddy substrate 20 to 100 m deep);
- Deep Soft-bottom (DS–sandy or muddy substrate 100 to 200 m deep).

The metrics selected to compute the MFCI were further divided into four major attributes: (1) diversity and composition, (2) abundance, (3) nursery function, and (4) trophic integrity. The choice of the core metrics (Table 4) for each substrate typology was made considering the most informing metrics based on the specific features of each substrate typology, their ecological meaning, and their response to environmental changes [69]. As a result, The MFCI returns a score within five quality classes that describe the ecological status as Bad, Poor, Moderate, Good, and Excellent, spanning from 0 to 1.

The MFCI metrics are representative of the typical communities of each substrate typology and sensitive to the main impacts acting on the marine environment. MFCI has been demonstrated to effectively incorporate functional and structural information of marine fish communities, thus being an efficient tool to convert ecological information into ecological status with potential applications for conservation and management purposes [69].

5.7. Fish Assemblage Indices Applied in Estuaries

It is possible to use the previous indices by considering all the due corrections and the constraints of estuaries. Specifically, the following fish community indices were implemented for estuaries [28]:

- Estuarine Community Degradation Index (CDI);
- Estuarine Biological Health Index (BHI);
- Estuarine Biotic Integrity Index (EBI);
- Estuarine Fish Recruitment Index (FRI);

5.7.1. Estuarine Community Degradation Index (CDI)

The Estuarine Community Degradation Index (CDI) is based on the comparison between the current fish community within an aquatic ecosystem and the (estimated) pristine one in the absence of any pressure or the community that existed before the degradation [36]. This index assumes that differences between the potential community and the actual ones are due to habitat degradation. As a consequence, CDI shows some advantages in monitoring an estuary recovery, as well as documenting the degradation of an estuary over time and assisting in the identification of estuary types where the fish communities are most threatened [28].

Table 4. Selected metrics for the MFCI divided by the correspondent substrate typologies; modified from [69]. TNS = total number of species; RUS = number of rare or uncommon species; PDS = pelagic/demersal ratio (in number of species); TA = total abundance; MAS = number of species that make up 90% of the abundance; PRS = proportion of resident species; CNC = commercial/non-commercial ratio (in proportion of species); GS = gregarious species; LVL = proportion of low and very low resilience species; BAS = proportion of benthic-associated species; IOM = proportion of individuals over maturity size; ABC = ABC curves; PSS = proportion of spawning species; CSL = cryptic, *Symphodus* sp. and *Labrus* sp. Species; SJP = number of species with juveniles present or proportion of juveniles; PIS = proportion of invertivore species; POS = proportion of omnivore species; PMS = proportion of piscivore and macrocarnivore species; PZS = proportion of zooplanktivorous species.

Attribute	Rocky Subtidal	Shallow Soft-bottom	Intermediate Soft-bottom	Deep Soft-bottom
Diversity and composition	TA RUS/TNS	TNS RUS/TNS	TNS RUS/TNS PDS	TNS RUS/TNS PDS
Abundance	TA MAS PRS CNC	TA [ln(n + 1)] MAS excluding GS LVL BAS IOM ABC	TA [ln(n + 1)] MAS excluding GS LVL IOM ABC	TA [ln(n + 1)] MAS excluding GS LVL IOM ABC
Nursery function	PSS excluding CSL Proportion of CSL SJP.	PSS SJP	PSS SJP	PSS SJP
Trophic integrity	PIS POS PMS	PMS PIS PZS	PZS PIS PMS	PMS PIS

5.7.2. Estuarine Biological Health Index (BHI)

The Estuarine Biological Health Index (BHI) is a modification of CDI. BHI incorporates a measure of the degree of similarity between the pristine community and the actual community [37]. BHI ranges from 0 (poor) to 10 (good). Reference communities are usually determined by establishing the normal range of fish community components (e.g., presence and absence of taxa in the most unimpaired waters representative of the considered area or region). Although BHI results in a useful tool for condensing information on estuarine fish assemblages into a single numerical value, it is based exclusively on presence/absence data and does not consider the relative proportions of the various species present. In particular, BHI incorporates measures of "health" and "importance" of an estuary and combines them into a single index. "Health" is the measure of the degree to which the current condition of an estuary deviates from the reference conditions; "importance" measures how much the current condition of an estuary contributes to the whole region's condition [28].

5.7.3. Estuarine Biotic Integrity Index (EBI)

The Estuarine Biotic Integrity Index (EBI) measures the impact of anthropogenic alterations on the ecosystem in relation to the status of higher trophic levels [28,75]. The EBI includes eight metrics:

- Total number of species;
- Dominance;
- Fish abundance (number or biomass);
- Number of nursery species;
- Number of estuarine spawning species;
- Number of resident species;
- Proportion of benthic-associated species;
- Proportion of abnormal or diseased fish.

5.7.4. Estuarine Fish Recruitment Index (FRI)

The Estuarine Fish Recruitment Index (FRI) uses information on fish populations to assess changes in habitat suitability, especially the availability of nursery areas for marine migrant fish. The FRI is a management-directed index based on the integration of three key information sets. The first set scores information on the dependency of marine fishes on a particular estuarine habitat and whether any of those fishes is endemic to the considered region or not. The second information set is the highest peak in the immigration period for a particular species (optimal recruitment score). The third information set incorporates known environmental requirements for the recruitment of young of the year (YOY) marine fish [28].

5.8. Other Potential Ecological Indicators

In the literature, hundreds of potential ecological indicators can be found. One example is the New Index of the Ecological Status of Fish Communities (NISECI) defined by the Italian Higher Institute for Protection and Environmental Research (ISPRA). In particular, this is a multi-metric index based on the following three metrics [76]:

- Presence/absence of indigenous species;
- Biological condition of native species populations;
- Presence of alien or hybrid species, structure of the related populations, and numerical ratio with respect to indigenous species.

Here, we summarized the most commonly used fish ecological indicators to assess changes in populations and ecosystems (Table 5). Their application (see examples in Section 5.7) spans from research to management, from impact assessment to recruitment sources identification and provides outcomes as both scientific papers and scientific reports, sustaining knowledge advances, information, and management.

Table 5. Summary table of the main indicators considered, their respective original references, and target environment.

Ecological Indicator	Original Reference	Target Environment
Fish population features	[42]	all
Species diversity	[45]	all
Body size and Trophic ecology	[53]	all
Mean Trophic level (TLm)	[61]	all
Fishing in balance (FiB)	[64]	pelagic
Primary production Required (PPR)	[18]	pelagic
Biological Integrity Index (IBI)	[31]	pelagic
Marine Fish Community Index (MFCI)	[69]	pelagic
Estuarine Community Degradation Index (CDI)	[36]	estuarine
Estuarine Biological Health Index (BHI)	[37]	estuarine
Estuarine Biotic Integrity Index (EBI)	[75]	estuarine
Estuarine Fish Recruitment Index (FRI)	[28]	estuarine
New Index of the Ecological Status of Fish Communities (NISECI)	[76]	freshwater

6. Qualities of Ecological Indicators

Challenges in the evaluation of the ecosystem status are:

- Characterizing the features of a pristine ecosystem objectively;
- Unavailability of historical data to examine long-term trends;
- Restricted spatial extent of studies;
- Separating natural fluctuations in the system from other long-term trends;
- Lack of appropriate analytical methods, which can all be highly subjective because they are different for every case and based on the researchers' skills.

In practice, it is difficult to identify benchmarks and critical levels [77]. The use of reference values is often required, such as in the Water Framework Directive [78]. The reference conditions should be determined by a physical and ecological comparison with (i) pristine control areas, which are often hard to find due to the widespread human footprint; (ii) by hind-casting, which requires adequate past data; (iii) by predictive modelling, which requires adequate empirical or stochastic models; (iv) by expert judgement, however subjective and difficult to quantify [28].

In the absence of any distinct and fixed reference levels for ecosystem indicators, reference directions can be considered for an approximate evaluation of recent ecosystem patterns [79]. When using ecological indicators applied to fisheries, a major weakness of most indicators is the difficulty in distinguishing the response to fishing impacts from other ecological and biological stressors that can cause a response in the indicator [20]. Indicators must have an evident and demonstrable cause-effect link with the investigated impact, but it is not always easy to identify such a link, especially in community-level indicators [80]. Consequently, a progressive rank of indicators [81] or 'holistic suite' of indicators [82] which apply to various levels of the ecological organization—species, population, community, and ecoregion—have to be considered in conjunction with measures about fishing impacts on the ecosystem.

Regarding the feasibility of developing indicators for the management of marine resources, during the Paris Symposium in 2004, many conclusions have been drawn [60,83]:

- Defining and implementing indicators can be achieved with present knowledge, data, and frameworks;
- No single indicator can describe all aspects of an ecosystem and its dynamics; a suite of indicators covering different datasets, fish groups, and ecological processes should be used;
- Global effects of environmental change (e.g., regime shifts) on higher trophic levels are not well captured by the individual use of most indicators, further highlighting why the use of indicators suites is highly recommended;
- High trophic level indicators (e.g., birds, marine mammals) summarize changes in fish communities, with top-down effects that can be quantified using tropho-dynamic indicators;
- Size-based indicators are useful to characterize fish community dynamics in the context of overexploitation;
- Ecosystem-based indicators are conservative, meaning they can detect changes only if the ecosystem is strongly affected, while trends and rapid changes must be evaluated by research and monitoring in the study area;
- Interpretation of indicators requires scientific expertise due to potential mistakes and biases in the analyses. Strong feedback between scientific experts and decision-makers is necessary to improve indicators and their practical use.

Aiming to develop more sustainable fisheries and to integrate the exploitation effects on ecosystems, the elaboration of an ecosystem approach to fisheries (EAF) needs appropriate guidelines to evaluate the status of a disturbed ecosystem.

The IndiSeas workgroup, in 2010 [79], drew the steps that the scientific community as a whole should take to make EAF a reality at a global level:

- Combining and integrating multi-disciplinary indicators, including indicators of climate, ecological, and human dimensions, would provide a complete characterization of change in the studied area. Integration should be quantitative to compare, classify, and rank the status of exploited marine ecosystems. It should also be graphical and easy to obtain in order to communicate the ecosystem status to a broad spectrum of stakeholders and the public;

- Developing a synergy between model- and data-based approaches in order to know the performance of an indicator, its sensitivity, specificity, and its reference levels. This will also allow ecosystem indicators to be tested in changing scenarios of global change and fisheries management;
- Using research survey data.

7. Molecular Tools Advances

Despite the robustness and reliability of all the aforementioned indicators, the urgent need to intensify and expand the range of monitoring programs led to the investigation of new and innovative tools for monitoring the ichthyofauna. Environmental DNA (eDNA) metabarcoding is now considered extremely promising in biomonitoring thanks to its ease of applicability and reproducibility. This method consists in sequencing the DNA extracted from environmental samples, such as water or sediment, to identify the species inhabiting that environment through molecular markers. Such a technique was already applied in the past, though restricted to the identification of microbial and fungal communities. The turning point was the realization that also eukaryotes, by shedding cells and releasing biological material into the environment, can be identified through this technique [84–86].

7.1. The Use of eDNA for Large-Scale Biomonitoring

eDNA metabarcoding is being increasingly used for biomonitoring [87,88], tracking alien and rare species [89–91], and assessing trophic levels [92,93]. There are several reasons why this method substitutes or complements traditional monitoring methods, as summarised in Table 6.

The application of molecular barcoding is characterized by three main aspects: the choice of one or more target genes, the choice of suitable primers, and the choice of sequencing platforms.

The key feature in the application of eDNA metabarcoding for ecological status assessment was the identification of the so-called molecular barcodes and the creation of the Barcode of Life database. Barcodes are defined as short molecular fragments of conserved genes that allow the identification up to, and sometimes beyond, species level, and they are available to any user worldwide via public repositories such as GenBank and BOLD Systems [94,95]. A suite of different barcodes can be selected depending on the target organism. For example, a region of about 650 base pairs of the mitochondrial Cytochrome Oxidase subunit I (COI) gene is widely used as a barcode for animals. The commonly used marker gene for fish is the ribosomal gene 12S. Other marker genes are the ribosomal genes 16S for prokaryotes, the nuclear ribosomal internal transcribed spacers 1 and 2 (ITS) for fungi, and two plastid genes—the maturase-coding gene (matK) and the large subunit of ribulose 1,5-bisphosphate carboxylase-coding gene (rbcL)—for plants [96]. These target genes can be used either independently or simultaneously to improve resolution.

The second aspect of the application of molecular identification is the proper choice of primers for the amplification of the marker gene. Usually, universal primers are preferred over species-specific primers because they allow the amplification of different target organisms and, thus, the detection of multiple species at once. Universal primers have the peculiarity of being composed of a partially degenerated sequence that can bind to different templates in spite of a few mismatches. The choice of primers strongly influences the depth and scale of the results. Therefore, primers selection is tightly tangled to the environment and species under study. For this reason, increasing efforts in the scientific community have been put into validating different primers and publishing their sequences together with the resulting barcodes [97–99].

Table 6. Main advantages of eDNA metabarcoding over traditional monitoring methods.

Rapid and non-destructive sampling	It overcomes the problem of finding hard-to-detect species and the risk of disturbing the species under study.
Straightforward identification	The uniqueness of the genetic material allows reasonable species identification, which can often be skewed by morphological traits, overcoming the difficulty in identifying different life stages.
Standardization of methods	It can be applied universally across different taxa within environments. Samples are extracted and processed independently from the species under study, overcoming species-specific sampling and detection methods.
Fast tool for biomonitoring	It requires only a few days for field sampling and lab-based work, such as DNA extraction, polymerase chain reaction (PCR) and high-throughput sequencing (HTS), by a single researcher.
Historical data estimation	DNA is known to persist for a long time in the environment, mainly when incorporated in sediment or ice cores and is not exposed to UV light. Therefore, it can provide information on ancient and extinct species, allowing to answer ecological questions on shifts in community composition, even when historical data are lacking.

The third aspect is linked to a relevant decrease in HTS cost, making it a more accessible diagnostic tool for everyday research. In the past, molecular data generation was a very costly and time-consuming task, which prevented its application, especially in metabarcoding studies. Nowadays, a plethora of different HTS platforms, such as Illumina, PacBio, and Ion-torrent, among others, can be chosen depending on the study aim [100,101]. Currently, Illumina MiSeq is the most used platform, especially in fish diversity studies, thanks to its depth and resolution [102–104]. Furthermore, the millions of sequences that are generated can be filtered and refined with the use of publicly available software packages, such as QIIME and OBITools. This process allows the clustering of sequences into molecular operational taxonomic units (MOTUs) or amplicon sequence variants (ASVs) and subsequent automated taxon annotation [105].

7.2. eDNA Metabarcoding for Assessing Fish Diversity and Abundance

Thomsen et al. [103] were the first to apply eDNA metabarcoding to infer fish diversity in a marine environment by amplifying a 100 bp fragment of the cytochrome b codifying gene. A few years later, Yamamoto et al. [106], to monitor *Trachurus japonicus* in Maizuru Bay, demonstrated that eDNA quantity proportionally correlates to fish biomass detected through echo sounder technology. From that moment on, eDNA metabarcoding has been widely applied in coastal marine environments to infer fish species richness, abundance, and distribution [107–110]. A wide range of studies has also been performed to identify primer sets suitable for fish identification by eDNA metabarcoding. Specifically, a set of universal PCR primers called MiFish-U/E was developed. These primers target a hypervariable region of the 12S rRNA gene and were designed using an alignment of sequences from 880 different fish species [111]. This primer set has been efficiently used in eDNA studies in both freshwater and marine systems [112–116]; instead, in specific estuarine sites, the 12S primers called Riaz_12S primer set were the most effective for eDNA metabarcoding of fishes [117].

Interestingly, eDNA studies were able to recover seasonal variation in marine fish communities [118], facilitate fish biodiversity surveys in the deep ocean [119], monitor the effects of river barrier removal on fish community composition [120], and unveil fish community shifts in temperate lotic ecosystems [121].

The main controversy that still remains to be solved is whether eDNA can quantitatively reflect fish abundance, which is important, especially in monitoring specific environments like those subjected to fishing activities. In fact, some studies show that eDNA proportionally correlates with or even improves traditional monitoring methods [122–125], while others found no correlation or discrepancies in species composition [126–129]. This is probably due to different factors affecting both molecular and traditional survey methods. On the one hand, eDNA rates could be affected by abiotic factors, such as temperature and acidity [130,131], but also by the size of the target organism, which can influence DNA excretion rates [132]. On the other hand, traditional monitoring is not always able to detect less abundant or hard-to-detect species, is often limited to specific physic-chemical zones and can fail to provide early warnings [133]. Altogether, both methods have unique capabilities that need to be thoroughly addressed before deciding if they can be applied individually or simultaneously when assessing the fish assemblage of a given ecosystem.

7.3. Current Limitations and Future Prospects

Molecular identification is subjected to some limitations.

(1) Incompleteness of DNA barcode reference libraries. Despite large molecular data being generated in metabarcoding studies, the sequences generated can be associated with a given taxon only if reference sequences are in repositories. Several studies have quantified the gaps in relation to different taxa and environments to improve molecular identification [21,134,135]. Weigand et al. [136] revealed that coverage differs among geographical areas and taxa, with fishes being the most barcoded and molluscs and diatoms the least. However, the majority of barcodes for fish are represented by the COI sequence, which is less reliable compared to 12 s in metabarcoding studies [116,137].

(2) Inability to provide information on phenotypical traits such as size, sex, age, and viability. However, the scientific community is already exploring new ways to overcome this problem. For example, targeting sex chromosome markers could potentially estimate the sex of individuals and their ratio; targeting methylation sites could give insights into the age of the target organisms and into the presence of environmental stressors; and targeting eRNA instead of eDNA could narrow the identification only to organisms that are alive at the sampling time. The main shortcomings of these approaches are that not all organisms present a chromosome-dependent sexual determination, that methylation patterns vary among tissue types, and that eRNA is more difficult to retrieve due to its high instability and fast degradation rate [138]. Nonetheless, the possibility of fine-tuning these techniques could broaden the application of molecular detection not only to the estimation of species composition and abundance but also to physiological features.

8. Accessibility to Information Repositories

General and accessible repositories are needed. Open access information is currently required by several research funding agencies, including the EU. Yet, the development of species and communities' data lies well behind the molecular repositories. Programs for legacy data rescue have been launched [139], but efforts are unfortunately limited in this sense. Although the request for Findable, Accessible, Interoperable, and Reusable (FAIR) data will surely support the background of work on fish indicators, the use of standards remains a milestone in data provision and storage. It is important to develop standardized methods in order to connect past, present, and future records and to apply ecological indicators in a meaningful way. Current major standards are represented by those proposed in the Darwin Core approach for biodiversity, which also applies to specific issues, such as alien species in FishBase. The path towards establishing FAIR data is still long, yet the vision backing those repositories represents a milestone in the approach to biodiversity records for the benefit of the broadest range of users, including managers and policy-makers at all levels.

9. Adoption and Inter-Calibration of Ecological Indicators on Fish Fauna in European Countries

The European Union Water Framework Directive—WFD (2000/60/EC) refers to monitoring guidelines for coastal and transitional water ecosystems and includes indices based on fish for transitional waters only. The WFD generated a suite of tools for assessment, reviewed by Birk et al. [140]. Nevertheless, the principles framing the use of fish indexes are still valid, also a few decades after their definition.

Following the international frameworks, the information from fish-based indices can be channelled into two different categories: surveillance and monitoring. Surveillance targets the compliance of water bodies within the EU with reference conditions [141].

In these contexts, no new fish indices were developed, though (1) the implementation of the directive allowed single countries to strengthen monitoring tools [142], and (2) the integration with other groups, and the use of multiple, combined indices allows to better tackle the intrinsic variability of systems. Along with phytoplankton, macroalgae, seagrasses, and macroinvertebrates, fish represent biological quality elements (BQEs) to be scored in an integrative approach to water quality assessment. They can successively be mainstreamed into models, such as the Driver-Pressure-State Change-Impact-Response, again with the goal of making the implementation of the directive effective in the assessment of the EU aquatic ecosystems [143].

Different EU directives can build on synergies, and, in this context, fish-based indices were found to be suitable for specific applications, e.g., towards conservation, bridging the WFD with the habitat directive (92/43/CEE) [144]. The different conditions across EU countries led to the harmonization of the WFD via Geographical Intercalibration Groups (GIGs), defined by the similarity of the water bodies of countries within the same GIG. As in the JRC report 88342 [145], results from intercalibrations became legally binding for member states in 2008 and 2013: "Member States should apply the results of the intercalibration exercise to their national classification systems in order to set the boundaries between high and good status and between good and moderate status for all their national types". Intercalibration exercises include a compilation, country by country, of the fish fauna assessment method [145] and a check of the methods at the national level for compliance at the European Union level.

10. Conclusions

Scientists and decision-makers need simple yet scientifically reliable methodologies to measure human pressures and recovery processes. The capability to evaluate the status of complex systems accurately and easily is key to effectively informing stakeholders.

Management of aquatic ecosystems raised the need for simple and reliable indicators of ecosystem status and functioning. Many indicators and evaluation tools have been selected, tested, and used to detect and track the properties and the dynamics of an ecosystem in a simple yet reliable way to inform management in different areas.

Every single index or ecological indicator has its own strengths and limitations that may make it useful or not, depending on the habitat under study and its features and components. It is unlikely that just one biological metric could be able to adequately capture the complexity and dynamics of any aquatic ecosystem, and that is why it is highly recommended to use a suite of different indicators.

Author Contributions: Conceptualization, M.P. and V.S.; Formal analysis F.Z.; Funding acquisition, M.P. and V.S.; Methodology, M.P.; Resources, M.P.; Supervision, M.P. and V.S.; Writing—original draft, M.P. and C.S.; Writing—review and editing, All Authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union Programme Interreg ADRION 2014–2020-ImPrEco Project (project n. 450) awarded to M. Pinna, by the Italian Ministry of University and Research Funding of basic Research Activities (FFABR) 2017 awarded to M. Pinna and V. Specchia, and by the project "Dipartimenti di Eccellenza" awarded to DiSTeBA.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rombouts, I.; Beaugrand, G.; Artigas, L.F.; Dauvin, J.C.; Gevaert, F.; Goberville, E.; Kopp, D.; Lefebvre, S.; Luczak, C.; Spilmont, N.; et al. Evaluating marine ecosystem health: Case studies of indicators using direct observations and modelling methods. *Ecol. Indic.* **2013**, *24*, 353–365. [CrossRef]
2. Rapport, D.J.; Hildén, M. An evolving role for ecological indicators: From documenting ecological conditions to monitoring drivers and policy responses. *Ecol. Indic.* **2013**, *28*, 10–15. [CrossRef]
3. Galuppo, N.; Maci, S.; Pinna, M.; Basset, A. Habitat types and distribution of benthic macroinvertebrates in a transitional water ecosystem: Alimini Grande (Puglia, Italy). *Transit. Waters Bull.* **2007**, *1*, 9–19.
4. Zhang, N.; Shang, G.; Dai, Y.; Zhang, Y.; Ding, S.; Gao, X. Testing the Sensitivity and Limitations of Frequently Used Aquatic Biota Indices in Temperate Mountain Streams and Plain Streams of China. *Water* **2021**, *13*, 3318. [CrossRef]
5. Ponti, M.; Vadrucci, M.R.; Orfanidis, S.; Pinna, M. Biotic indices for ecological status of transitional water ecosystems. *Transit. Waters Bull.* **2009**, *3*, 32–90.
6. Fonnesu, A.; Pinna, M.; Basset, A. Spatial and temporal variations of detritus breakdown rates in the river Flumendosa basin (Sardinia, Italy). *Int. Rev. Hydrobiol.* **2004**, *89*, 443–452. [CrossRef]
7. Coll, M.; Lotze, H.K. Ecological indicators and food-web models as tools to study historical changes in marine ecosystems. In *Perspectives on Oceans Past*; Schwerdtner Máñez, K., Poulsen, B., Eds.; Springer: Dordrecht, The Netherlands, 2016; pp. 103–132.
8. Pinna, M.; Marini, G.; Cristiano, G.; Mazzotta, L.; Vignini, P.; Cicolani, B.; Di Sabatino, A. Influence of aperiodic summer droughts on leaf litter breakdown and macroinvertebrate assemblages: Testing the drying memory in a Central Apennines River (Aterno River, Italy). *Hydrobiologia* **2016**, *782*, 111–126. [CrossRef]
9. Davis, W.S.; Scott, J. *Mid-Atlantic Highlands Streams Assessment: Technical Support Document*; Region 3, Office of Research and Development, Mid-Atlantic Integrated Assessment Program; US Environmental Protection Agency: Washington, DC, USA, 2000.
10. Dale, V.H.; Beyeler, S.C. Challenges in the development and use of ecological indicators. *Ecol. Indic.* **2001**, *1*, 3–10. [CrossRef]
11. Pinna, M.; Sangiorgio, F.; Fonnesu, A.; Basset, A. Spatial analysis of plant detritus processing in a Mediterranean River type: The case of the River Tirso Basin, Sardinia, Italy. *J. Environ. Sci.* **2003**, *15*, 227–240.
12. Niemi, G.J.; McDonald, M.E. Application of Ecological Indicators. *Annu. Rev. Ecol. Evol. Syst.* **2004**, *35*, 89–111. [CrossRef]
13. Beman, J.M.; Arrig, K.R.; Matson, P.A. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. *Nature* **2005**, *434*, 211–214. [CrossRef]
14. Defeo, O.; Elliott, M. The 'triple whammy' of coasts under threat-Why we should be worried! *Mar. Pollut. Bull.* **2021**, *163*, 111–832. [CrossRef]
15. Airoldi, L.; Beck, M. Loss, status and trends for coastal marine habitats of Europe. *Oceanogr. Mar. Biol.* **2007**, *45*, 345–405.
16. Bax, N.; Williamson, A.; Aguero, M.; Gonzalez, E.; Geeves, W. Marine invasive alien species: A threat to global biodiversity. *Mar. Policy* **2003**, *27*, 313–323. [CrossRef]
17. Coll, M.; Libralato, S.; Tudela, S.; Palomera, I.; Pranovi, F. Ecosystem overfishing in the Ocean. *PLoS ONE* **2008**, *3*, e3881. [CrossRef]
18. Pauly, D.; Christensen, V.; Dalsgaard, J.; Fröese, R.; Torres, J.F. Fishing down marine food webs. *Science* **1998**, *279*, 860–863. [CrossRef]
19. Greene, C.H.; Pershing, A.J. Climate drives sea change. *Science* **2007**, *315*, 1084–1085. [CrossRef]
20. Orr, J.C.; Fabry, V.J.; Aumont, O.; Bopp, L.; Doney, S.C.; Feely, R.A.; Gnanadesikan, A.; Gruber, N.; Ishida, A.; Joos, F.; et al. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **2005**, *437*, 681–686. [CrossRef]
21. Zangaro, F.; Saccomanno, B.; Tzafesta, E.; Bozzeda, F.; Specchia, V.; Pinna, M. Current limitations and future prospects of detection and biomonitoring of NIS in the Mediterranean Sea through environmental DNA. *NeoBiota* **2021**, *70*, 151–165. [CrossRef]
22. Buchs, W. Biodiversity and agri-environmental indicators: General scopes and skills with special reference to the habitat level. *Agric. Ecosyst. Environ.* **2003**, *98*, 35–78. [CrossRef]
23. Bunn, S.E.; Davies, P.M. Biological processes in running waters and their implications for the assessment of ecological integrity. *Hydrobiologia* **2000**, *422*, 61–70. [CrossRef]
24. Elliott, M.; Quintino, V. The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Mar. Pollut. Bull.* **2007**, *54*, 640–645. [CrossRef] [PubMed]
25. Xu, F.L.; Jørgensen, S.E.; Tao, S. Ecological indicators for assessing freshwater ecosystem health. *Ecol. Model.* **1999**, *116*, 77–106. [CrossRef]
26. Liu, Y.; Chen, C.; Yang, S. Assessment of anthropogenic impact versus climate change on the succession of the diatom community in Lugu Lake (Yunnan-Guizhou plateau, China) using the sedimentary record of geochemical elements. *Water* **2019**, *11*, 655. [CrossRef]
27. Rice, J.C.; Rochet, M. A framework for selecting a suite of indicators for fisheries management. *ICES J. Mar. Sci.* **2005**, *62*, 516–527. [CrossRef]
28. Whitfield, A.K.; Elliott, M. Fishes as indicators of environmental and ecological changes within estuaries—A review of progress and some suggestions for the future. *J. Fish Biol.* **2002**, *61*, 229–250. [CrossRef]
29. Whitfield, A.K. Fishes and the environmental status of South African estuaries. *Fish. Manag. Ecol.* **1996**, *3*, 45–57. [CrossRef]

30. Dabuleviciene, T.; Jucevicius, D.; Zolubas, T.; Vaiciute, D.; Nika, N. The Effect of Short-Term Upwelling Events on Fish Assemblages at the South-Eastern Coast of the Baltic Sea. *Water* **2023**, *15*, 452. [CrossRef]
31. Karr, J.R. Assessment of biotic integrity using fish communities. *Fisheries* **1981**, *6*, 21–27. [CrossRef]
32. Zhang, Y.; Wang, X.N.; Ding, H.Y.; Dai, Y.; Ding, S.; Gao, X. Threshold responses in the taxonomic and functional structure of fish assemblages to land use and water quality: A case study from the Taizi River. *Water* **2019**, *11*, 661. [CrossRef]
33. Izzo, C.; Doubleday, Z.A.; Schultz, A.G.; Woodcock, S.H.; Gillanders, B.M. Contribution of water chemistry and fish condition to otolith chemistry: Comparisons across salinity environments. *J. Fish Biol.* **2015**, *86*, 1680–1698. [CrossRef]
34. Fulton, E.A.; Fuller, M.; Smith, A.D.M.; Punt, A.E. *Ecological Indicators of the Ecosystem Effects of Fishing: Final Report*; Report No.: R99/1546; CSIRO Div. of Marine Research//Australian Fisheries Management Authority: Hobart, Australia; Canberra, Australia, 2004.
35. Delpech, C.; Courrat, A.; Pasquaud, S.; Lobry, J.; Le Pape, O. Development of a fish-based index to assess the ecological quality of transitional waters: The case of French estuaries. *Mar. Pollut. Bull.* **2010**, *60*, 908–918. [CrossRef]
36. Ramm, A.E.L. The community degradation index: A new method for assessing the deterioration of aquatic habitats. *Water Res.* **1988**, *22*, 293–301. [CrossRef]
37. Cooper, J.A.G.; Ramm, A.E.L.; Harrison, T.D. The Estuarine Health Index: A new approach to scientific information transfer. *Ocean Coast. Manag.* **1994**, *25*, 103–141. [CrossRef]
38. Roset, N.; Grenouillet, G.; Goffaux, D.; Pont, D.; Kestemont, P. A review of existing fish assemblage indicators and methodologies. *Fish. Manag. Ecol.* **2007**, *14*, 393–405. [CrossRef]
39. USEPA. *Estuarine and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance*; US Environmental Protection Agency Report; US Environmental Protection Agency: Washington, DC, USA, 2000.
40. Coates, S.A.; Colclough, S.R.; Robson, M.A.; Harrison, T.D. *Development of an Estuarine Classification Scheme for the Water Framework Directive. Phases 1 & 2—Transitional Fish Component*; R&D Technical Report E1-131/TR; Environment Agency: Bristol, UK, 2004; p. 55.
41. Tidu, C.; Sardà, R.; Pinna, M.; Cannas, A.; Meloni, M.F.; Lecca, E.; Savarino, R. Morphometric relationships of the European spiny lobster *Palinurus elephas* from Northwestern Sardinia. *Fish. Res.* **2004**, *69*, 371–379. [CrossRef]
42. Myers, R.A.; Baum, J.K.; Shepherd, T.D.; Powers, S.P.; Peterson, C.H. Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science* **2007**, *315*, 1846–1850. [CrossRef]
43. Ferretti, F.; Worm, B.; Britten, G.; Heithaus, M.; Lotze, H. Patterns and ecosystem consequences of shark declines in the ocean. *Ecol. Lett.* **2010**, *13*, 1055–1071. [CrossRef]
44. Begon, M.; Harper, J.L.; Townsend, C.R. *Ecology, Individuals, Populations and Communities*; Blackwell Scientific Publications: Hoboken, NJ, USA, 1986.
45. Whittaker, R.H. Evolution and measurement of species diversity. *Taxon* **1972**, *21*, 213–251. [CrossRef]
46. McClure, E.C.; Richardson, L.E.; Graba-Landry, A.; Loffler, Z.; Russ, G.R.; Hoey, A.S. Cross-shelf differences in the response of herbivorous fish assemblages to severe environmental disturbances. *Diversity* **2019**, *11*, 23. [CrossRef]
47. Simpson, E.H. Measurement of diversity. *Nature* **1949**, *163*, 688. [CrossRef]
48. Rochet, M.J.; Trenkel, V.M. Which community indicators can measure the impact of fishing? A review and proposals. *Can. J. Fish. Aquat. Sci.* **2003**, *60*, 86–99. [CrossRef]
49. Genner, M.J.; Sims, D.W.; Southward, A.J.; Budd, G.C.; Masterson, P.; Mchugh, M.; Rendle, P.; Southall, E.J.; Wearmouth, V.J.; Hawkins, S.J. Body size-dependent responses of a marine fish assemblage to climate change and fishing over a century-long scale. *Glob. Chang. Biol.* **2010**, *16*, 517–527. [CrossRef]
50. Trach, Y.; Chernyshev, D.; Biedunkova, O.; Moshynskyi, V.; Trach, R.; Statnyk, I. Modeling of Water Quality in West Ukrainian Rivers Based on Fluctuating Asymmetry of the Fish Population. *Water* **2022**, *14*, 3511. [CrossRef]
51. Ziegler, P.E.; Lyle, J.M.; Haddon, M.; Ewing, G.P. Rapid changes in life-history characteristics of a long-lived temperate reef fish. *Mar. Freshw. Res.* **2007**, *58*, 1096–1107. [CrossRef]
52. Neuheimer, A.; Thresher, R.; Lyle, J.; Semmens, J. Tolerance limit for fish growth exceeded by warming waters. *Nat. Clim. Chang.* **2011**, *1*, 110–113. [CrossRef]
53. Lindeman, R.L. The trophic-dynamic aspect of ecology. *Ecology* **1942**, *23*, 399–418. [CrossRef]
54. Cruz-Escalona, V.H.; Abitia-Cardenes, L.A.; Campos-Davila, L.; Galvan-Magaña, F. Trophic interrelations of the three most abundant fish species from Laguna San Ignacio, Baja California Sur, Mexico. *Bull. Mar. Sci.* **2000**, *66*, 361e373.
55. Hajisamae, S.; Chou, L.M.; Ibrahim, S. Feeding habitats and trophic relationships of fishes utilizing an impacted coastal habitat, Singapore. *Hydrobiologia* **2004**, *520*, 61e71. [CrossRef]
56. Froese, R.; Palomares, M.L.D.; Pauly, D. *Draft User's Manual of FishBase, a Biological Database on Fish (Version 1.0)*; ICLARM Software; ICLARM: Manila, Philippines, 1992; p. 7.
57. Sosa-López, A.; Mouillot, D.; Do Chi, T.; Ramos-Miranda, J. Ecological indicators based on fish biomass distribution along trophic levels: An application to the Terminos coastal lagoon. *ICES J. Mar. Sci.* **2005**, *62*, 453–458. [CrossRef]
58. Paine, R.T. Food webs: Linkage, interaction strength and community infrastructure. *J. Anim. Ecol.* **1980**, *49*, 667–685. [CrossRef]
59. Ryther, J.H. Photosynthesis and fish production in the sea. The production of organic matter and its conversion to higher forms of life vary throughout the world ocean. *Science* **1969**, *166*, 72–76. [CrossRef]

60. Cury, P.M.; Shannon, L.J.; Roux, J.-P.; Daskalov, G.M.; Jarre, A.; Moloney, C.L.; Pauly, D. Trophodynamic indicators for an ecosystem approach to fisheries. *ICES J. Mar. Sci.* **2005**, *62*, 430–442. [CrossRef]
61. Jennings, S.; Kaiser, M.J.; Reynolds, J.D. *Marine Fisheries Ecology*; Blackwell Science: London, UK, 2001.
62. Caddy, J.F.; Csirke, J.; Garcia, S.M.; Grainger, R.J.R. How pervasive is fishing down marine food webs? *Science* **1998**, *282*, 1383–1385. [CrossRef]
63. Caddy, J.F.; Garibaldi, L. Apparent changes in the trophic composition of world marine harvests: The perspective from the FAO capture database. *Ocean Coast. Manag.* **2000**, *43*, 615–655. [CrossRef]
64. Pauly, D.; Christensen, V.; Walters, C. Ecopath, Ecosim and Ecospace as tools for evaluating ecosystem impact of fisheries. *ICES J. Mar. Sci.* **2002**, *57*, 697–706. [CrossRef]
65. McAllister, M.; Orr, P. *A Review of Modelling Methods of Indicators for the Identification of Fishing Impacts on Marine Ecosystems*; Institute for European Economic Policy (IEEP): London, UK, 2004.
66. Degnbol, P.; Jarre, A. Review of indicators in Fisheries Management—A development perspective. *Afr. J. Mar. Sci.* **2004**, *26*, 303–326. [CrossRef]
67. An, K.G.; Yeom, D.H.; Lee, S.K. Rapid bioassessments of Kap stream using the index of biological integrity. *Korean J. Environ. Biol.* **2001**, *19*, 261–269.
68. Gonino, G.; Benedito, E.; Cionek, V.D.M.; Ferreira, M.T.; Oliveira, J.M. A fish-based index of biotic integrity for neotropical rainforest sandy soil streams—Southern Brazil. *Water* **2020**, *12*, 1215. [CrossRef]
69. Henriques, S.; Pais, M.P.; Costa, M.J.; Cabral, H. Development of a fish-based multimetric index to assess the ecological quality of marine habitats: The Marine Fish Community Index. *Mar. Pollut. Bull.* **2008**, *56*, 1913–1934. [CrossRef]
70. Sapounidis, A.S.; Koutrakis, E.T. Development of a fish-based multimetric index for the assessment of Lagoons' ecological quality in northern Greece. *Water* **2021**, *13*, 3008. [CrossRef]
71. Demestre, M.; Sánchez, P.; Albelló, P. Demersal fish assemblages and habitat characteristics on the continental shelf and upper slope of the north-western Mediterranean. *J. Mar. Biolog. Assoc. UK* **2000**, *80*, 981–988. [CrossRef]
72. Pihl, L.; Wennhage, H. Structure of fish assemblages on rocky and soft bottom shores on the Swedish west coast. *J. Fish Biol.* **2002**, *61*, 148–166. [CrossRef]
73. Letourneur, Y.; Ruitton, S.; Sartoretto, S. Environmental and benthic factors structuring the spatial distribution of a summer infralittoral fish assemblage in the north-western Mediterranean Sea. *J. Mar. Biolog. Assoc. UK* **2003**, *83*, 193–204. [CrossRef]
74. Prista, N.; Vasconcelos, R.; Costa, M.J.; Cabral, H.N. The demersal fish assemblage of the coastal area adjacent to the Tagus estuary (Portugal): Relationships with environmental conditions. *Oceanol. Acta* **2003**, *26*, 525–536. [CrossRef]
75. Deegan, L.A.; Finn, J.T.; Ayvazian, S.G.; Ryder-Kieffer, C.A.; Buonaccorsi, J. Development and validation of an Estuarine Biotic Integrity Index. *Estuaries* **1997**, *20*, 601–617. [CrossRef]
76. Macchio, S.; Rossi, G.L.; Rossi, G.; De Bonis, S.; Balzamo, S.; Martone, C. Nuovo indice dello stato ecologico delle comunità ittiche (NISECI). In *Manuali e Linee Guida*; ISPRA: Ozzano dell'Emilia, Italy, 2017; p. 159.
77. Link, J.S. Translating ecosystem indicators into decision criteria. *ICES J. Mar. Sci.* **2005**, *62*, 569–576. [CrossRef]
78. European Union. *Parliament and Council Directive 2000/60/EC of 23rd October 2000. Establishing a Framework for Community Action in the Field of Water Policy*; Official Journal PE-CONS 3639/1/00 REV 1; European Union: Brussels, Belgium, 2000.
79. Shin, Y.J.; Bundy, A.; Shannon, L.J.; Simier, M.; Coll, M.; Fulton, E.A. Can simple be useful and reliable? Using ecological indicators to represent and compare the states of marine ecosystems. *ICES J. Mar. Sci.* **2010**, *67*, 717–731. [CrossRef]
80. Keough, M.J.; Quinn, G.P. Causality and the choice of measurements for detecting human impacts in marine environments. *Aust. J. Mar. Freshw. Res.* **1991**, *42*, 539–554. [CrossRef]
81. Chabanet, P.; Adjeroud, M.; Andréfouët, S.; Bozec, Y.-M.; Ferraris, J.; Garcia-Charton, J.; Schrimm, M. Human-induced physical disturbances and their indicators on coral reef habitats: A muti-scale approach. *Aquat. Living Resour.* **2005**, *18*, 215–230. [CrossRef]
82. Rochet, M.J.; Trenkel, V.M. Factors for the variability of discards: Assumptions and field evidence. *Can. J. Fish. Aquat. Sci.* **2005**, *62*, 224–235. [CrossRef]
83. Perry, R.I.; Livingston, P.; Fulton, E.A. Ecosystem indicators. In Report of Working Group 19 on Ecosystem-based Management Science and its Application to the North Pacific. *PICES Sci. Rep.* **2010**, *37*, 83–89.
84. Ficetola, F.; Miaud, C.; Pompanon, F.; Taberlet, P. Species detection using environmental DNA from water samples. *Biol. Lett.* **2008**, *4*, 423–425. [CrossRef]
85. Foote, A.; Thomsen, P.; Sveegaard, S.; Wahlberg, M.; Kielgast, J.; Kyhn, L.; Salling, A.; Galatius, A.; Orlando, L.; Thomas, M.; et al. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS ONE* **2012**, *7*, e4178. [CrossRef]
86. Thomsen, P.; Kielgast, J.; Iversen, L.; Wiuf, C.; Rasmussen, M.; Gilbert, M.; Orlando, L.; Willerslev, E. Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.* **2012**, *21*, 2565–2573. [CrossRef]
87. Leese, F.; Altermatt, F.; Bouchez, A.; Ekrem, T.; Hering, D.; Meissner, K.; Mergen, P.; Pawlowski, J.; Piggott, J.; Rimet, F.; et al. DNAqua-Net: Developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe. *Res. Ideas Outcomes* **2016**, *2*, e11321. [CrossRef]
88. Stat, M.; Huggett, M.J.; Bernasconi, R.; DiBattista, J.D.; Berry, T.E.; Newman, S.J.; Harvey, E.S.; Bunce, M. Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment. *Sci. Rep.* **2017**, *7*, 12240. [CrossRef]

89. Sigsgaard, E.E.; Carl, H.; Møller, P.R.; Thomsen, P.F. Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biol. Conserv.* **2015**, *183*, 48–52. [CrossRef]
90. Smart, A.S.; Tingley, R.; Weeks, A.R.; van Rooyen, A.R.; McCarthy, M.A. Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecol. Appl.* **2015**, *25*, 1944–1952. [CrossRef]
91. Chucholl, F.; Fiolka, G.; Segelbacher, L.S. eDNA detection of native and invasive crayfish species allows for year-round monitoring and large-scale screening of lotic systems. *Front. Environ. Sci.* **2021**, *9*, 639380. [CrossRef]
92. Djurhuus, A.; Closek, C.J.; Kelly, R.P.; Pitz, K.J.; Michisaki, R.P.; Starks, H.A.; Walz, K.R.; Andruszkiewicz, E.A.; Olesin, E.; Hubbard, K.; et al. Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nat. Commun.* **2020**, *11*, 254. [CrossRef] [PubMed]
93. Kang, Y.; Kwak, I.; Kang, C. Co-occurrence of Bacillariophyceae-based and Cryptophyceae-based planktonic food webs in a temperate estuarine ecosystem revealed via eDNA. *Estuar. Coast. Shelf Sci.* **2022**, *264*, 107661. [CrossRef]
94. Sayers, E.W.; Beck, J.; Bolton, E.E.; Bourexis, D.; Brister, J.R.; Canese, K.; Chan, J.; Comeau, D.C.; Connor, R.; Funk, K.; et al. Database resources of the national center for biotechnology information. *Nucleic Acids Res.* **2021**, *49*, D10. [CrossRef] [PubMed]
95. Ratnasingham, S.; Hebert, P.D. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Mol. Ecol. Notes* **2007**, *7*, 355–364. [CrossRef]
96. CBOL Plant Working Group. A DNA barcode for land plants. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 12794–12797. [CrossRef]
97. Cannon, M.; Hester, J.; Shalkhauser, A.; Chan, E.R.; Logue, K.; Small, S.T.; Serre, D. In silico assessment of primers for eDNA studies using PrimerTree and application to characterize the biodiversity surrounding the Cuyahoga River. *Sci. Rep.* **2016**, *6*, 22908. [CrossRef]
98. Piñol, J.; Senar, M.A.; Symondson, W.O. The choice of universal primers and the characteristics of the species mixture determine when DNA metabarcoding can be quantitative. *Mol. Ecol.* **2019**, *28*, 407–419. [CrossRef]
99. Zhang, S.; Zhao, J.; Yao, M. A Comprehensive and Comparative Evaluation of Primers for Metabarcoding eDNA from Fish. *Methods Ecol. Evol.* **2020**, *11*, 1609–1625. [CrossRef]
100. Quail, M.A.; Smith, M.; Coupland, P.; Otto, T.D.; Harris, S.R.; Connor, T.R.; Bertoni, A.; Swerdlow, H.P.; Gu, Y. A tale of three next generation sequencing platforms: Comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genom.* **2012**, *13*, 341. [CrossRef]
101. Frey, K.G.; Herrera-Galeano, J.E.; Redden, C.L.; Luu, T.V.; Servetas, S.L.; Mateczun, A.J.; Mokashi, V.P.; BishopLilly, K.A. Comparison of three next-generation sequencing platforms for metagenomic sequencing and identification of pathogens in blood. *BMC Genom.* **2014**, *15*, 96. [CrossRef]
102. Evans, N.T.; Olds, B.P.; Renshaw, M.A.; Turner, C.R.; Li, Y.; Jerde, C.L.; Mahon, A.R.; Pfrender, M.E.; Lamberti, G.A.; Lodge, D.M. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Mol. Ecol. Resour.* **2016**, *16*, 29–41. [CrossRef]
103. Li, Y.; Evans, N.T.; Renshaw, M.A.; Jerde, C.L.; Olds, B.P.; Shogren, A.J.; Deiner, K.; Lodge, D.M.; Lamberti, G.A.; Pfrender, M.E. Estimating fish alpha- and beta-diversity along a small stream with environmental DNA metabarcoding. *Metabarcoding Metagenom.* **2018**, *2*, e24262. [CrossRef]
104. Nakao, R.; Inui, R.; Akamatsu, Y.; Goto, M.; Doi, H.; Matsuoka, S. Illumina iSeq 100 and MiSeq exhibit similar performance in freshwater fish environmental DNA metabarcoding. *Sci. Rep.* **2021**, *11*, 15763. [CrossRef]
105. Thomsen, P.; Kielgast, J.; Iversen, L.; Moller, P.; Rasmussen, M.; Willerslev, E. Detection of a diverse marine fauna using eDNA from seawater samples. *PLoS ONE* **2012**, *7*, e41732. [CrossRef]
106. Yamamoto, S.; Minami, K.; Fukaya, K.; Takahashi, K.; Sawada, H.; Murakami, H.; Tsuji, S.; Hashizumel, H.; Kubonaga, S.; Horiuchi, T.; et al. Environmental DNA as a 'snapshot' of fish distribution: A case study of Japanese jack mackerel in Maizuru Bay, Sea of Japan. *PLoS ONE* **2016**, *11.3*, e0149786.
107. DiBattista, J.D.; Coker, D.J.; Sinclair-Taylor, T.H.; Stat, M.; Berumen, M.L.; Bunce, M. Assessing the utility of eDNA as a tool to survey reef-fish communities in the Red Sea. *Coral Reefs* **2017**, *36*, 1245–1252. [CrossRef]
108. Sigsgaard, E.E.; Nielsen, I.B.; Carl, H.; Krag, M.A.; Knudsen, S.W.; Xing, Y.; Holm-Hansen, T.H.; Moller, P.R.; Thomsen, P.F. Seawater environmental DNA reflects seasonality of a coastal fish community. *Mar. Biol.* **2017**, *164*, 128. [CrossRef]
109. Yamamoto, S.; Masuda, R.; Sato, Y.; Sado, T.; Araki, H.; Kondoh, M.; Minamoto, T.; Miya, M. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Sci. Rep.* **2017**, *7*, 40368. [CrossRef]
110. Ushio, M.; Murakami, H.; Masuda, R.; Sado, T.; Miya, M.; Sakurai, S.; Yamanaka, H.; Minamoto, T.; Kondoh, M. Quantitative monitoring of multispecies fish environmental DNA using high-throughput sequencing. *Metabarcod. Metagenom.* **2018**, *2*, e23297.
111. Miya, M.; Gotoh, R.O.; Sado, T. MiFish metabarcoding: A high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fish. Sci.* **2020**, *86*, 939–970. [CrossRef]
112. Berger, C.S.; Hernandez, C.; Laporte, M.; Côté, G.; Paradis, Y.; Kameni, T.D.W.; Normandeau, E.; Bernatchez, L. Fine-scale environmental heterogeneity shapes fluvial fish communities as revealed by eDNA metabarcoding. *Environ. DNA* **2020**, *2*, 647–666. [CrossRef]
113. Doble, C.J.; Hipperson, H.; Salzburger, W.; Horsburgh, G.J.; Mwita, C.; Murrell, D.J.; Day, J.J. Testing the performance of environmental DNA metabarcoding for surveying highly diverse tropical fish communities: A case study from Lake Tanganyika. *Environ. DNA* **2020**, *2*, 24–41. [CrossRef]

114. Oka, S.I.; Doi, H.; Miyamoto, K.; Hanahara, N.; Sado, T.; Miya, M. Environmental DNA metabarcoding for biodiversity monitoring of a highly diverse tropical fish community in a coral reef lagoon: Estimation of species richness and detection of habitat segregation. *Environ. DNA* **2021**, *3*, 55–69. [CrossRef]
115. Antognazza, C.M.; Britton, R.J.; Read, D.S.; Goodall, T.; Mantzouratou, A.; De Santis, V.; Davies, P.; Aprahamian, M.; Franklin, E.; Hardouin, E.A.; et al. Application of eDNA metabarcoding in a fragmented lowland river: Spatial and methodological comparison of fish species composition. *Environ. DNA* **2021**, *3*, 458–471. [CrossRef]
116. McDevitt, A.D.; Sales, N.G.; Browett, S.S.; Sparnenn, A.O.; Mariani, S.; Wangensteen, O.S.; Coscia, I.; Benvenuto, C. Environmental DNA metabarcoding as an effective and rapid tool for fish monitoring in canals. *J. Fish Biol.* **2019**, *95*, 679–682. [CrossRef]
117. Kumar, G.; Reaume, A.M.; Farrell, E.; Gaither, M.R. Comparing eDNA metabarcoding primers for assessing fish communities in a biodiverse estuary. *PLoS ONE* **2022**, *17*, e0266720. [CrossRef]
118. Sigsgaard, E.E.; Torquato, F.; Frøslev, T.G.; Moore, A.B.; Sørensen, J.M.; Range, P.; Ben-Hamadou, R.; Bach, S.S.; Møller, P.R.; Thomsen, P.F. Using vertebrate environmental DNA from seawater in biomonitoring of marine habitats. *Conserv. Biol.* **2020**, *34.3*, 697–710. [CrossRef]
119. McClenaghan, B.; Fahner, N.; Cote, D.; Chawarski, J.; McCarthy, A.; Rajabi, H.; Singer, G.; Hajibabaei, M. Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. *PLoS ONE* **2020**, *15*, e0236540. [CrossRef]
120. Muha, T.P.; Rodriguez-Barreto, D.; O'Rorke, R.; Garcia de Leaniz, C.; Consuegra, S. Using eDNA metabarcoding to monitor changes in fish community composition after barrier removal. *Front. Ecol. Evol.* **2021**, *9*, 629217. [CrossRef]
121. García-Machado, E.; Laporte, M.; Normandeau, E.; Hernández, C.; Côté, G.; Paradis, Y.; Mingelbier, M.; Bernatchez, L. Fish community shifts along a strong fluvial environmental gradient revealed by eDNA metabarcoding. *Environ. DNA* **2022**, *4*, 117–134. [CrossRef]
122. Czeglédi, I.; Sály, P.; Specziár, A.; Preiszner, B.; Szalóky, Z.; Maroda, Á.; Pont, D.; Meulenbroek, P.; Valentini, A.; Erős, T. Congruency between two traditional and eDNA-based sampling methods in characterising taxonomic and trait-based structure of fish communities and community-environment relationships in lentic environment. *Ecol. Indic.* **2021**, *129*, 107952. [CrossRef]
123. Goutte, A.; Molbert, N.; Guérin, S.; Richoux, R.; Rocher, V. Monitoring freshwater fish communities in large rivers using environmental DNA metabarcoding and a long-term electrofishing survey. *J. Fish Biol.* **2020**, *97*, 444–452. [CrossRef]
124. Salter, I.; Joensen, M.; Kristiansen, R.; Steingrund, P.; Vestergaard, P. Environmental DNA concentrations are correlated with regional biomass of Atlantic cod in oceanic waters. *Commun. Biol.* **2019**, *2*, 461. [CrossRef]
125. Zou, K.; Chen, J.; Ruan, H.; Li, Z.; Guo, W.; Li, M.; Liu, L. eDNA metabarcoding as a promising conservation tool for monitoring fish diversity in a coastal wetland of the Pearl River Estuary compared to bottom trawling. *Sci. Total Environ.* **2020**, *702*, 134704. [CrossRef]
126. Cilleros, K.; Valentini, A.; Allard, L.; Dejean, T.; Etienne, R.; Grenouillet, G.; Iribar, A.; Taberlet, P.; Vigouroux, R.; Brosse, S. Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): A test with Guianese freshwater fishes. *Mol. Ecol. Resour.* **2019**, *19*, 27–46. [CrossRef]
127. Spear, S.F.; Groves, J.D.; Williams, L.A.; Waits, L.P. Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. *Biol. Conserv.* **2015**, *183*, 38–45. [CrossRef]
128. Thomsen, P.F.; Møller, P.R.; Sigsgaard, E.E.; Knudsen, S.W.; Jørgensen, O.A.; Willerslev, E. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PLoS ONE* **2016**, *11*, e0165252. [CrossRef]
129. Yates, M.C.; Fraser, D.J.; Derry, A.M. Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. *Environ.DNA* **2019**, *1*, 5–13. [CrossRef]
130. Specchia, V.; Saccomanno, B.; Zangaro, F.; Tzafesta, E.; Pinna, M. Exploring the Biodiversity of a European NATURA 2000 Mediterranean Lagoon through eDNA Metabarcoding. *Diversity* **2022**, *14*, 991. [CrossRef]
131. Seymour, M.; Durance, I.; Cosby, B.J.; Ransom-Jones, E.; Deiner, K.; Ormerod, S.J.; Colbourne, J.K.; Wilgar, G.; Carvalho, G.R.; de Bruyn, M.; et al. Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. *Commun. Biol.* **2018**, *1*, 4. [CrossRef]
132. Maruyama, A.; Nakamura, K.; Yamanaka, H.; Kondoh, M.; Minamoto, T. The release rate of environmental DNA from juvenile and adult fish. *PLoS ONE* **2014**, *9*, e114639. [CrossRef] [PubMed]
133. Pawlowski, J.; Apothéloz-Perret-Gentil, L.; Mächler, E.; Altermatt, F. *Environmental DNA Applications in Biomonitoring and Bioassessment of Aquatic Ecosystems; Guidelines*; Federal Office for the Environment, Bern. Environmental Studies; Federal Office for the Environment: Bern, Switzerland, 2020; Volume 2010, p. 71.
134. Leite, B.; Vieira, P.; Teixeira, M.; Lobo Arteaga, J.; Hollatz, C.; Borges, L.; Duarte, S.; Troncoso, J.; Costa, F. Gap-analysis and annotated reference library for supporting macroinvertebrate metabarcoding in Atlantic Iberia. *Reg. Stud. Mar. Sci.* **2020**, *36*, 101307. [CrossRef]
135. Specchia, V.; Tzafesta, E.; Marini, G.; Scarcella, S.; D'Attis, S.; Pinna, M. Gap Analysis for DNA Barcode Reference Libraries for Aquatic Macroinvertebrate Species in the Apulia Region (Southeast of Italy). *J. Mar. Sci. Eng.* **2020**, *8*, 538. [CrossRef]
136. Weigand, H.; Beermann, A.J.; Čiampor, F.; Costa, F.O.; Csabai, Z.; Duarte, S.; Geiger, M.F.; Grabowski, M.; Rimet, F.; Rulik, B.; et al. DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work. *Sci. Total Environ.* **2019**, *678*, 499–524. [CrossRef] [PubMed]
137. Collins, R.A.; Bakker, J.; Wangensteen, O.S.; Soto, A.Z.; Corrigan, L.; Sims, D.W.; Genner, M.J.; Mariani, S. Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods Ecol. Evol.* **2019**, *10*, 1985–2001. [CrossRef]

138. Sigsgaard, E.E.; Jensen, M.R.; Winkelmann, I.E.; Møller, P.R.; Hansen, M.M.; Thomsen, P.F. Population-level inferences from environmental DNA—Current status and future perspectives. *Evol Appl.* **2020**, *13*, 245–262. [CrossRef]
139. Faulwetter, S.; Pafilis, E.; Fanini, L.; Bailly, N.; Agosti, D.; Arvanitidis, C.; Boicenco, L.; Capatano, T.; Claus, S.; Dekeyzer, S.; et al. EMODnet Workshop on mechanisms and guidelines to mobilise historical data into biogeographic databases. *Res. Ideas Outcomes* **2016**, *2*, e9774. [CrossRef]
140. Birk, S.; Bonne, W.; Borja, A.; Brucet, S.; Courrat, A.; Poikane, S.; Solimini, A.; Van De Bund, W.; Zampoukas, N.; Hering, D. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. *Ecol. Indic.* **2012**, *18*, 31–41. [CrossRef]
141. Ferreira, J.G.; Vale, C.; Soares, C.V.; Salas, F.; Stacey, P.E.; Bricker, S.B.; Marques, J.C. Monitoring of coastal and transitional waters under the EU Water Framework Directive. *Environ. Monit. Assess.* **2007**, *135*, 195–216. [CrossRef]
142. Kelly, F.L.; Harrison, T.D. The Water Framework Directive: Advances in fish classification tools in Ireland. *Biol. Environ. Proc. R. Ir. Acad.* **2016**, *116*, 205–219. [CrossRef]
143. Borja, A.; Elliott, M.; Henriksen, P.; Marbà, N. Transitional and coastal waters ecological status assessment: Advances and challenges resulting from implementing the European Water Framework Directive. *Hydrobiologia* **2013**, *704*, 213–229. [CrossRef]
144. Facca, C.; Cavraro, F.; Franzoi, P.; Malavasi, S. Lagoon resident fish species of conservation interest according to the habitat directive (92/43/CEE): A review on their potential use as ecological indicator species. *Water* **2020**, *12*, 2059. [CrossRef]
145. Böhmer, J.; Arbaciauskas, K.; Benstead, R.; Gabriels, W.; Porst, G.; Reeze, B.; Timm, H. *Water Framework Directive Intercalibration Technical Report*; Central Baltic Lake Benthic Invertebrate Ecological Assessment Methods; Joint Research Centre of the European Commission: Ispra, Italy, 2014; p. 77.