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Review

The Mediterranean Killifish *Aphanius fasciatus* (Valenciennes, 1821) (Teleostei: Cyprinodontidae) as a Sentinel Species for Protection of the Quality of Transitional Water Environments: Literature, Insights, and Perspectives

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Abstract: Transitional waters are fragile ecosystems with high ecological values, representing the breeding and resting sites for rare and threatened species. They warrant particular attention in regards to protection, as they experience numerous anthropogenic threats. The present review aims to analyze the recent literature on *Aphanius fasciatus*, currently considered one of the most strictly estuarine-dependent fish species, thus affected by the degradation of lagoon habitats, and to discuss its suitability as a sentinel species for protection of the quality of transitional water environments. The analysis and discussion highlight the potential applicability of the molecular, cellular, and physiological responses of this species as diagnostic tools for detecting the subtle effects induced by environmental pollution on the biota in transitional water environments. Moreover, the suitability of the responses of this species is suggested in the wider framework of the One Health perspective, which considers human and animal health and the environmental state to be highly interconnected, sharing common aspects. To date, omics technologies show great potential in reacquiring novel knowledge on the responses of the organisms to environmental changes and to the alterations of the environmental health status. Therefore, considering the relevant potential of this organism as a sentinel species, many efforts are required in the near future to improve the quantity and quality of the omics tools that refer to *A. fasciatus*.

Keywords: killifish; *Aphanius fasciatus*; coastal lagoon; biomarker; VOSviewer; genome

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1. Introduction

Transitional waters are fragile ecosystems with high ecological values, as they represent the breeding and resting sites for rare and threatened species. According to the European Directive 92/43/EEC (Habitat Directive), transitional waters warrant particular attention in regards to protection, as they experience numerous threats, such as the impairment of water quality due to the river inputs enriched with nutrients and pollutants, the destruction or reduction of habitats due to the construction of infrastructures (such as ports), urbanization, intensive aquaculture, the introduction of alien species, etc.

In their recent review, Facca et al. [1] proposed six fish species (namely *Aphanius fasciatus*, *A. iberus*, *Knipowitschia panizzae*, *Ninnigobius canestrinii*, *Valencia hispanica*, and *V. letourneuxi*), more strictly related to salt marshes and wetlands and belonging to the guild of estuarine resident fish, as ecological bioindicators of lagoon environmental conditions, providing information on their presence and abundance in these ecosystems and thus, on their health (see also, e.g., [2]).

This work focuses on one of these six species, i.e., *A. fasciatus* (Valenciennes, 1821), a small dimorphic fish found in large-sized populations in the coastal brackish waters of the central-eastern Mediterranean Sea. *Aphanius fasciatus* is currently considered one of the most strictly estuarine-dependent fish species, and as such, it may be affected by the degradation of lagoon habitats [3].

The species is of conservation interest; it is included in the Habitat Directive Annex II (species requiring conservation measures) and spends its entire life cycle in the Mediterranean priority habitat 1150* "Coastal Lagoons". The International Union for Conservation of Nature (IUCN) Red List categories and criteria [4] includes *A. fasciatus* within the "Least Concern" category.

It is known that anthropogenic alterations of the environment may significantly contribute to population decline. In recent years, the study of the molecular and cellular responses of the organisms to anthropogenic environmental stressors (biomarkers) has seen a significant increase, as it is seen as a very robust tool contributing to the support of a variety of biodiversity conservation strategies. Biomarkers have recently been applied to several research areas of biodiversity conservation, including environmental quality monitoring of protected areas and the assessment of the health status of species at risk (for review see, e.g., [5]). Moreover, in the framework of biodiversity conservation, the research on endangered species and the detection of their responses to environmental changes can benefit significantly from the application of emerging genomic, proteomic, metabolomic, and bioinformatic technologies, which show great potential for acquiring novel knowledge concerning the responses of the organisms to environmental changes and their impact on the organisms' health status [6].

The aim of this review is to discuss the suitability of *A. fasciatus* as a sentinel species for the protection of the quality of transitional water environments, starting with a bibliometric analysis of the literature produced in recent decades. The use of its cellular and molecular responses as diagnostic tools for detecting the subtle effects induced by the environmental changes (before more dramatic effects become evident) on the distribution of the species (in terms of, e.g., abundance, reduction, or absence) could provide significant insights and help develop novel perspectives in various biodiversity conservation fields.

This study was carried out in the framework of the Project "Monitoring Natura 2000 Sites, Species and Habitats in the Apulian Region (MoSSHa)", POR Puglia FESR-FSE, included in the Natura 2000 network.

2. *Aphanius fasciatus:* Biology, Habitat Preference, Distribution, and Conservation Status

Aphanius fasciatus (class Actinopterygii, order Cyprinodontiformes, family Aphanidae, genus *Aphanius*) is a small dimorphic fish found in large populations in the coastal brackish waters of the central-eastern Mediterranean area, with the exception of the westernmost area, where it is replaced by *Aphanius iberus* and *Aphanius baeticus*, and possibly in the Aegean Sea, where it may be substituted by *Aphanius almirensis*. In the south-eastern Mediterranean region, *A. fasciatus* partially overlaps *Aphanius dispar*. It also colonizes the Suez Canal and Red Sea. The distribution area of the species, as reported by the International Union for Conservation of Nature (IUCN), includes all the

Mediterranean region countries, with the exception of the Iberian Peninsula, and is limited to the coastal areas. It is also found in several Mediterranean islands [7].

The genus is defined ante-Lessepsian because it is considered to have thrived in the ancient Tethys gulf before its closure at the end of Miocene epoch (Messinian, 7 Mya), and it survived the consequent drought of the Mediterranean Basin [8]. A highly adjustable physiology likely allowed the ancient species to survive the intense habitat variations of the Messinian period.

Aphanius fasciatus shows several distinctive morphological characteristics (Figure 1) when compared to the other species of the genus *Aphanius* found in Europe, such as the presence of light yellow to dark yellow fin in males, with a broad dark submarginal bar in some populations; 8–15 dark blue to gray bars on a silvery background, usually of regular shape; females with 11–17 short dark brown bars on the sides, with a faint greyish mediolateral stripe; 24–29 scales in a mediolateral series on the body; and a pectoral fin with 14–15 rays [4].

Figure 1. Representative image of a male (**A**) and a female (**B**) specimen of *Aphanius fasciatus.*

Aphanius fasciatus tolerates harsh environmental conditions, including high temperatures and salinity, as well as low oxygen levels. Reproduction takes place from April to September, with life history traits including external fertilization, demersal eggs deposited on benthic vegetation, a short generation time (~3.5 years), a high reproductive rate, and rapid population turnover. Its diet is made of invertebrates, mainly crustaceans and insect larvae. *A. fasciatus* is typified by habitat fidelity in its adult phase, and the lifestyles of the hatchlings resemble those of the adult population [1].

Despite its tolerance to wide variations in environmental physicochemical parameters, several aspects of the biology of the species confer vulnerability to environmental changes. For example, the intra-population component of the genetic variation of the species is much smaller than the inter-population component [9], a characteristic that determines a lower evolutionary potential in natural populations [10].

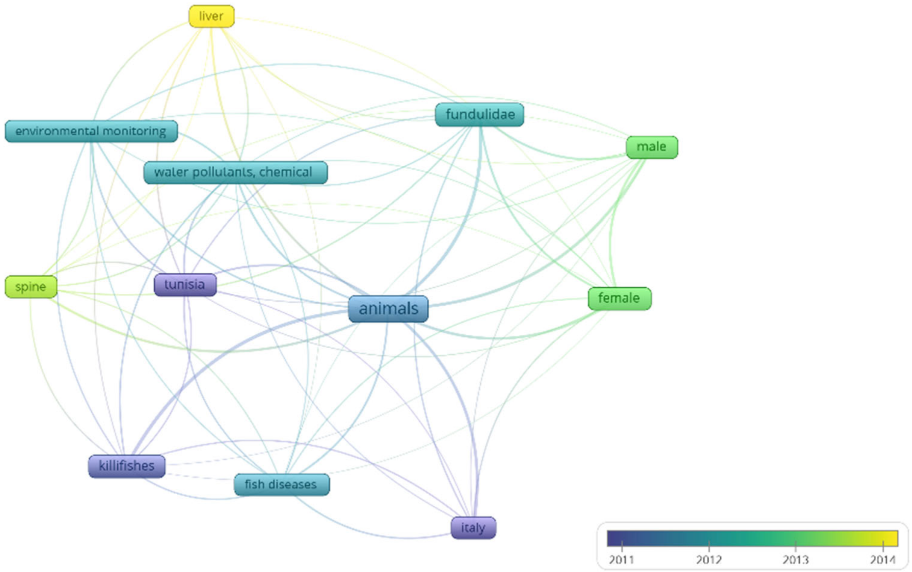
Despite its sporadic presence in coastal marine waters, possibly related to floods or dystrophic crises, the most common habitats of this species are transitional waters and a variety of fresh and brackish waters, including small weedy ponds and ditches. This contributes to a very restricted gene flow that may occur only between adjacent populations. Mainly because of the virtually complete isolation of brackish-water habitats and the highly selective environmental features, *A. fasciatus* populations are highly divergent (see, e.g., [11] and the literature cited therein). In Italy, two main clusters with a similar genetic structure were identified, the North Tyrrhenian populations above about latitude 41° N, and the southern Tyrrhenian and the Adriatic populations [12].

In recent years the interest in the conservation of this species has grown due to the documented decline, and in some cases, even extinction, of many *A. fasciatus* populations at several sites. The causes of the decline have to be found to be the alteration and deterioration of natural biotopes and the loss of habitats due to the increased pressure of human activities on coastal areas. In addition, competition with alien species such as *Gambusia holbrooki* Girard, 1859, a poecilid fish introduced in southern Europe to control the proliferation of malaria-carrying mosquitoes, caused a strong reduction in *A. fasciatus* over time [11]. This decline of the species has stimulated conservation actions, represented by, e.g., the inclusion of *A. fasciatus* on lists of protected species. This species is now included in Annex II of the Habitats Directive of the European Union (Protocol ASPIM 92/43/EEC) and in Appendices II and III of the Bern Convention (Bern/Berne, 19.IX.1979). Currently, the conservation status of this species in the Mediterranean region is defined as "unfavourable-inadequate" [13].

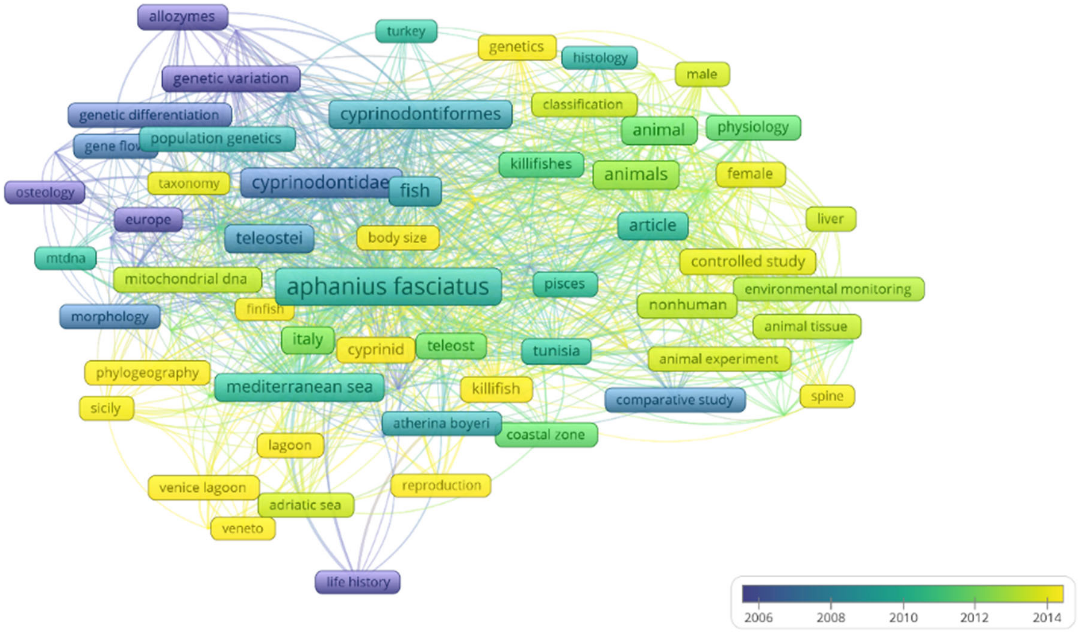
In Italy, the historical analysis of the distribution of *A. fasciatus* along the Italian coasts has been carried out by Valdesalici et al. [11], based on the census of natural populations along the Italian coasts and bibliographic and museum material. From the historical analysis of the distribution of the species, the authors concluded that *A. fasciatus* showed a reduction in its distribution along the Italian coasts in the 1990s, with a recovery in the following years. They outlined the presence of stable populations along the central Tyrrhenian, and northern Adriatic, Sardinian, and Apulian coasts [11]. In particular, along the Apulian coasts, the presence of populations of *A. fasciatus* has been documented in the Pond of Acquatina (LE) [14,15], Cesine (LE) [15], Alimini (LE) [16], Palude del Capitano—S. Isidoro (LE) [15,17], Mar Piccolo of Taranto (TA) [18–20], salt marshes of Margherita di Savoia (FG) [18], Lago di Lesina (FG) [9,21–26], and Lago di Varano (FG) [18,26]. In November 2022, during the scientific activities of the above-mentioned project, the presence of the species was confirmed in Acquatina (LE) (IT9150003 Natura 2000 site) and Cesine (IT9150032 Natura 2000 site).

3. *Aphanius fasciatus* as Sentinel Species for the Ecotoxicological Risk of Coastal Lagoons

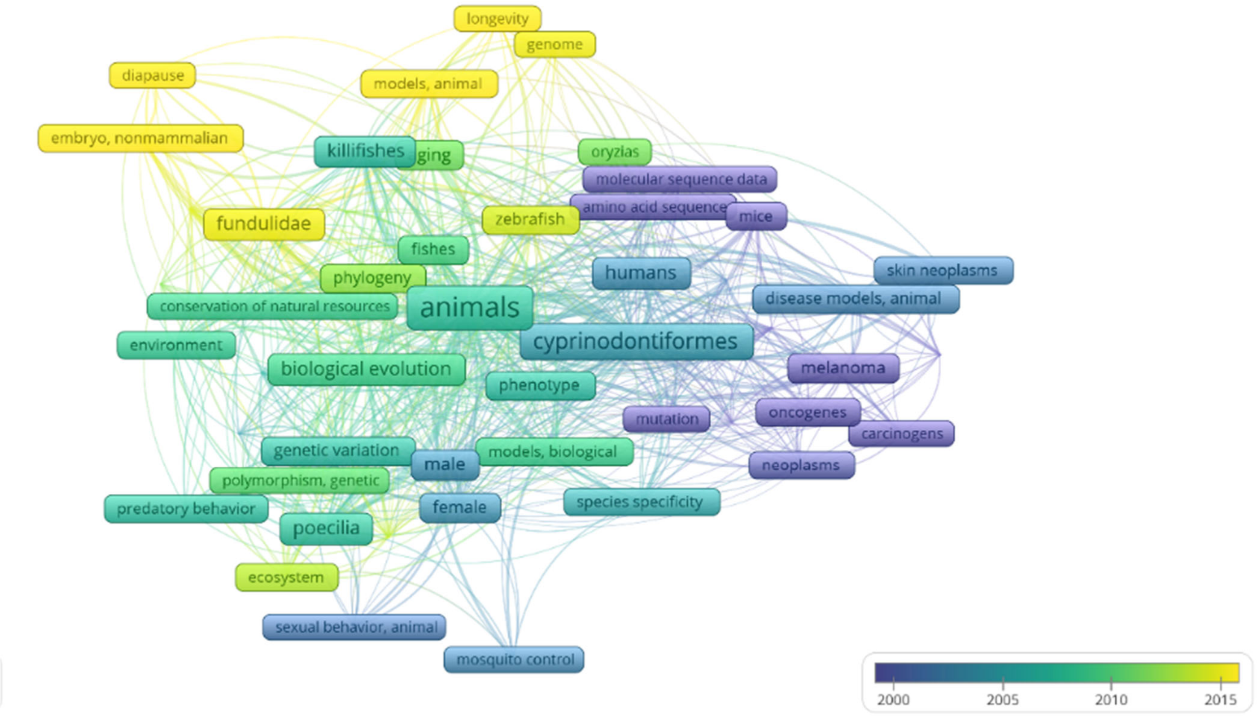
The scientific interest in *A. fasciatus* is documented by the increasing number of related publications produced in the last decades, with 121 papers in *Scopus* reporting "*Aphanius fasciatus*" in the "Article title", "Abstract", or "Keywords" fields starting in 1962, and 32 papers in *PubMed* reporting the same query in "All Fields" starting in 1979 [Last database consultation carried out on June 13, 2023] (see Figure 2). A bibliometric analysis, carried out in a comparative manner by using the two data sources *PubMed* (Figure 2A) and *Scopus* (Figure 2B), and the mapping tool VOSviewer [27], highlights the state-of-the-art research on *A. fasciatus* and identifies the emerging research trends in regards to this species in the larger context of Cyprinodontiformes biology (for comparison, see Figure 2C,D). It is rather evident that the information included in the *Scopus* database, which includes a wider variety of disciplines, is much more consistent and detailed when compared to that reported in a biomedicine-oriented database such as *PubMed*. In any case, the analysis carried out on these databases clearly indicates that over the years, research on the *A. fasciatus* species has changed, moving from the general analysis of the biology and genetics of the organism to a study of the biochemical, biomolecular, toxicological, and environmental facets of this species. The occurrence in more recent years of keywords such as "environmental monitoring" and "water pollutants, chemical" (see Figure 2A) or "environmental monitoring", "phylogeography", "coastal zone", and "lagoon" (Figure 2B) suggests the recent interest in this species as a bioindicator of coastal lagoons. Moreover, many keywords refer to Tunisia (because of the activity of a strong research group focusing on *A. fasciatus* in that country) and to a variety of Italian geographic areas, suggesting that there is a specific interest in this species in this country as well. Last but not least, "*Aphanius fasciatus*" links to certain keywords such as "fish diseases" and "spine" (Figure 2A) or "osteology", "spine", "animal experiment", "controlled study", "comparative study", and "physiology", among others (Figure 2B), showing the increasing interest in this species as an animal model in the study of certain pathophysiological states. Therefore, the following discussion strictly reflects the available information on selected major research topics. *A. fasciatus* has been studied in relation to its potential use as a sentinel species for the ecotoxicological risks of coastal lagoons.



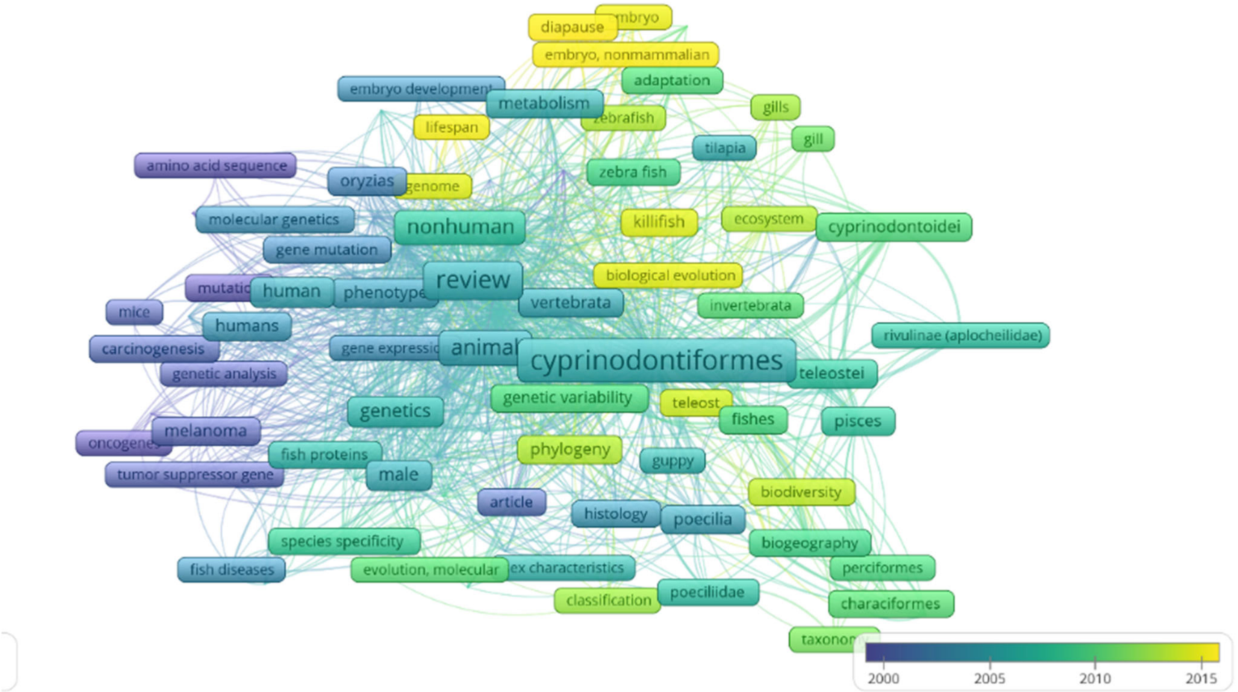
(**A**)



(**B**)



(C)



(D)

Figure 2. (**A**,**B**) Visualization of the main terms contained in "All Fields" after querying *PubMed* (**A**), and in the "Title", "Abstract", and "Keywords" fields after querying *Scopus* (**B**) literature collections, as obtained by using the two (combined) keywords "*Aphanius fasciatus*" and the bibliometric mapping tool VOSviewer [Please note: for (**A**), number of papers retrieved in *PubMed*: 32; minimum number of occurrences of a (MeSH) keyword: 5; of the 163 (MeSH) keywords, 12 met the threshold;

for **B**, number of papers retrieved in *Scopus*: 121, minimum number of occurrences of a keyword: 5; of the 1096 keywords, 80 met the threshold]. (**C**,**D**) Visualization of the main terms contained in "All Fields" after querying *PubMed* (**C**), and in the "Title", "Abstract", and "Keywords" fields after querying *Scopus* (**D**) literature collections, as obtained by using the keyword "Cyprinodontiformes" filtered for "Review" and VOSviewer [Please note: for (**C**), number of papers retrieved in *PubMed*: 127; minimum number of occurrences of a (MeSH) keyword: 5; of the 504 (MeSH) keywords, 54 met the threshold; for (**D**), number of papers retrieved in *Scopus*: 127, minimum number of occurrences of a keyword: 5; of the 1741 keywords, 104 met the threshold]. The range of colors from blue to yellow indicates the temporal range of the keywords, from those associated with older to those associated with more recent texts [last database consultation carried out on 13 June 2023].

A. fasciatus as Sentinel Species for Metal Pollution

Coastal lagoons are important areas between the land and the sea as a habitat for living organisms, representing highly productive environments and delivering several ecosystem services, as well as ecological, cultural, and socioeconomic benefits [28–30]. However, due to their location between land and sea, they are subject to several anthropogenic pressures due to tourism, intensive aquaculture, and the input from their catchment areas, receiving urban, agricultural, and/or industrial effluents.

Pollution remains one of the major environmental issues in coastal lagoons, and its impact is exacerbated by global warming and more frequent torrential rains due to climate changes. Common pollutants found in Mediterranean coastal lagoons are trace metals from mining and industrial activities [30,31], polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorinated pesticides (OCPs) from industrial and agricultural effluents [32,33].

Pollutant-induced alterations at the molecular, cellular, and physiological levels in the organisms (biomarkers) have been extensively used to detect the relationships between the chemical contamination of the environmental matrices, internal levels of tissue contamination, and effects on the organisms [34,35]. Alterations measured at the molecular or cellular level have thus been proposed as sensitive "early warning" tools in environmental quality assessment, as they allow for the detection of the pollution-induced effects in target biota prior to the appearing of more integrated effects at higher levels of biological organization [5,36]. In this context, it is worth noting that: (i) "exposure biomarkers" are early biochemical responses related to the exposure of the organism to pollutants, often represented by the detoxification responses of the organism to pollutant exposure; (ii) "effect biomarkers" are represented by the toxicological effects exerted on the organisms by the exposure to pollutants and are directly related to possible health impairment or disease; and (iii) "susceptibility biomarkers" are intrinsic or acquired abilities of an organism that account for increased sensitivity to the effects of an environmental pollutant [5].

Fish species are recognized as key elements for the assessment of the quality of aquatic ecosystems, thanks to their high ecological relevance due to their influence on the food web structure, nutrient cycling, and energy transfer. Indeed, fish species are widely used for aquatic health assessment through the biomarker approach, but the choice of the fish bioindicator species must take into account several issues, including the potential pathways of exposure to the contaminant of concern and the detectable biological responses that they are able to develop [37].

In the case of *A. fasciatus*, some characteristics of its biology and ecology, such as the benthivore nature of the adults and their preference for the terminal sections of rivers and closed zones, make this species susceptible to exposure to chemical pollutants. The benthivorous diet exposes the animals to high doses of chemical pollutants because benthic invertebrates are known to concentrate contaminants [38] from sediments, which are the sink of contamination present in the water column. Moreover, estuarine environments are known to receive pollutants through industrial discharges, yard and street runoff, agricultural lands, and storm drains; moreover, closed zones are more polluted than the surrounding areas due to low hydrodynamism. In this regard, the sedentary behavior and the high territoriality of *A. fasciatus* makes this species highly suitable for pollution monitoring in transitional water environments.

Regarding the biomarker responses of *A. fasciatus*, some studies are already available in the literature and are mainly related to metal contamination. For example, in environments contaminated by heavy metals, Kessabi et al. [39] have found a relationship between metal concentrations in the water, sediment, and tissues of *A. fasciatus* natural populations from the Gulf of Gabes (Tunisia)—particularly for Cd, Zn, and Cu—thus suggesting its ability to bioaccumulate trace metals from the environment. Moreover, metallothionein induction has been observed in the liver of metal-exposed animals. Metallothioneins are cysteine-rich metal-binding proteins involved in the regulation of the metabolism of trace metals and protection against heavy metal toxicity and oxidative stress in a wide range of organisms [40–42]. Therefore, the results reported by Kessabi et al. [39] indicate the ability of the species to develop a detoxification response after metal exposure. The *A. fasciatus* natural populations from the Gulf of Gabes (Tunisia) exposed to metal contamination also show histological liver alterations, as well as deformities such as compressed vertebral discs and a general distortion of the vertebral column, accompanied by vertebral lesions [39]. It is known that metals can interfere with calcium homeostasis [43]; thus, the alteration reported by the authors could be attributed to the toxicological mechanism(s) directly or indirectly affecting calcium homeostasis. This study suggests that the species is responsive to metal exposure, showing bioaccumulation and the activation of exposure and effect biomarker responses. Indeed, *A. fasciatus* can accumulate heavy metals in its tissues at higher levels than the toxic concentration present in its environment, presumably due to active absorption along the gill and gut epithelium. Also, the species shows metallothionein induction, which represents a widely used metal exposure biomarker. Moreover, *A. fasciatus* shows effect biomarkers, such as liver histological alterations and skeletal deformities, when the contamination exposure exceeds the detoxification capacity of the organism.

Spinal deformities had previously been detected by Messaoudi et al. [44] in the *A. fasciatus* natural populations from the Gulf of Gabes, with kyphosis, scoliosis, and lordosis frequently co-occurring at varying degrees of severity. This condition paralleled the high frequencies of spinal deformities in wild specimens of other teleost fish species in polluted environments [45–47]. As found by Kessabi et al. [39], the skeletal deformities observed in natural populations are related to the ability of *A. fasciatus* to accumulate large amount of Cd. The occurrences of skeletal deformity in fish have thus been proposed by Messaoudi et al. [44] as a good and practical response to assessing environmental quality, i.e., as an effect biomarker. The responses observed in *A. fasciatus* are similar to the symptoms described in the human species in the case of itai itai disease caused by cadmium accumulation [48]. This suggests the suitability of the responses of this species for consideration in the wider framework of the One Health concept, which considers human and animal health and the environmental state to be highly interconnected, sharing common aspects that can be applied globally in these three components. In this perspective, a broader vision of the use of biomarkers of risk assessment is developing, bridging environmental health and human health according to a more global vision [49,50]. Therefore, the suitability of biomarker responses for both environmental and human biomonitoring is of great usefulness for the development of this emerging approach.

Annabi et al. [51] detected an accumulation of trace metals in the liver and gonads in natural populations of *A. fasciatus* exposed to environments characterized by trace metal pollution. The bioaccumulation resulted in a parallel disturbance of the reproductive status in these natural populations in terms of, e.g., sex ratio unbalance and the alteration of sexual hormone blood concentrations. Thus, *A. fasciatus* is also able to express effect biomarkers related to alterations in reproduction.

Mosesso et al. [52] assessed the suitability of *Aphanius fasciatus* as a sentinel organism to detect complex genotoxic mixtures in a coastal lagoon ecosystem through the comet assay, which represents one of the most used assays for the evaluation of genotoxic damage. Following optimization and validation of the assay under laboratory conditions, fish were collected in the Orbetello lagoon (Tuscany, Italy), which is considered a significantly polluted site. The results showed statistically significant increases in tail DNA (%) compared to the values observed in the erythrocytes of fish caught in the unpolluted reference site Saline di Tarquinia. The physicochemical parameters of the water (i.e., salinity, pH, and oxygen content) did not significantly influence the induction of DNA damage. These results indicate that the comet assay provides a reliable parameter, and that *A. fasciatus* is a promising sentinel organism to detect the genotoxic impact of complex mixtures in coastal lagoon ecosystems.

Sebbio et al. [53] also found accumulation of metals in *A. fasciatus* natural populations from the Civitavecchia area and, in parallel, genotoxic damage was noted, as assessed by comet assay. The results obtained in the natural populations of *A. fasciatus* mirrored a trend of increasing environmental pollution across time and space, which was evident from accumulation profiles consistent with DNA damage. These results confirm *A. fasciatus* as a highly sensitive species in detecting heavy metal pollution.

4. Perspectives

The requirements for a species to be considered an optimal animal sentinel are represented by the sensitivity to environmental stressors and the ability to develop measurable responses, a home range overlapping the area to be monitored, and easy cataloging and capture. The review of the literature available on *A. fasciatus* reported in the previous section demonstrates that *A. fasciatus* meets these requirements and outlines the prospective usefulness of this species as a sentinel species for the protection of the quality of transitional water environments. In this respect, some aspects merit consideration. The species shows a high sensitivity to exposure to contamination and also exhibits the ability to develop detectable responses. However, the data available mainly focus on heavy metal contamination, while other pollutants known to be widely present in coastal lagoons, including PAHs, PCBs, and OCPs, have not been considered yet. Moreover, considering that coastal lagoons are particularly vulnerable to global climate change, the investigation of the pollutant responses of *A. fasciatus* under multiple stress conditions, e.g., related to climate change, such as high temperatures and salinity, as well as low oxygen levels, would be worth studying in order to exalt the role of this species as a sentinel organism.

To date, genomics, transcriptomics, proteomics, metabolomics, bioinformatics, and all the other emerging -omics technologies, show great potential in acquiring novel knowledge regarding the responses of the organisms to environmental changes and the alterations of the environmental health status [6]. Modern genomics offers high-throughput and information-rich approaches for characterizing the biological responses to environmental stressors and for assessing the impacts of pollutants on living organisms. Moreover, such technologies also offer very useful tools to determine the consequences of exposure to environmental pollution at the population level and for understanding the bases of deployment in population-level long-term monitoring programs [54]. These approaches can thus be relevant for environmental monitoring purposes, as well as in the framework of biodiversity conservation.

Aphanius fasciatus belongs to the order Cyprinodontiformes, which, to date, expresses as many as 66 genomes per 47 species (NCBI database last queried in May 2023) (Table 1). Of the various families of Cyprinodontiformes, at least 5 (i.e., Fluviphylacidae, Profundulidae, Pantanodontiidae, Procatopodidae, and Valencidae) still lack a sequenced genome, but all the other families include at least one species for which a reference genome is available (Table 1).

Notably, all the Cyprinodontiformes are characterized by a natural ability to adapt to extreme environments. Emblematic is the case of *Nothobranchius furzeri*, the African turquoise killifish (Table 1) typical of mud pools in the African savannah, which completes its life cycle in ~3 months and today represents an irreplaceable model for the study of aging and aging-related diseases (see, e.g., [55,56]). This also suggests the perspective of the possible use of *A. fasciatus*—like other Cyprinodontiformes—as a suitable animal model, to investigate how aquatic animals tolerate harsh environmental conditions, including high temperatures and salinity, and low oxygen levels. The knowledge in this field is also relevant in the framework of global climate changes that can exacerbate pollution conditions in vulnerable environments such as coastal lagoons.

Therefore, it is worth underscoring that in the near future, many efforts are required to improve the quantity and quality of the -omics tools relating to *A. fasciatus*. In fact, its genome is not yet available, as shown in Table 1, in which the genomes of Cyprinodontiformes catalogued at the National Center for Biotechnology Information (NCBI) are reported. However, the complete mitochondrial genome of the closely related species *Aphanius iberus* (the Spanish toothcarp) has recently been published [57] (see Table 1). In addition, while lacking an annotated and/or simply sequenced genome, only a few hundred sequences—the large majority of which refer to mitochondrial and not to nuclear DNA, RNA, and protein—are associate with *A. fasciatus*. This information is briefly summarized in Table 2.

Table 1. List of Cyprinodontiformes genomes available at NCBI (source: https://www.ncbi.nlm.nih.gov/datasets/taxonomy/28738/, accessed on 13 June 2023).

Order (Suborder)	Family	Genus	Species	Sequenced Genomes
Cyprinodontiformes (killifish and others)				66
(Aplocheiloidei)	Rivulidae (New World rivulines)	Austrofundulus	*Austrofundulus limnaeus*	1 [£]
		Kryptolebias	*Kryptolebias brasiliensis*	1 [£]
			Kryptolebias gracilis	1 [£]
			Kryptolebias hermaphroditus	1 [$]
			Kryptolebias marmoratus (mangrove rivulus)	2 [$]
			Kryptolebias ocellatus (sardinita)	1 [£]
		Nematolebias	*Nematolebias whitei (Rio pearlfish)*	1 [$]
	Nothobranchiidae	Aphyosemion	*Aphyosemion australe* (lyretail killifish)	1 [£]
		Callopanchax	*Callopanchax toddi*	1 [£]
		Nothobranchius	*Nothobranchius furzeri* (turquoise killifish)	6 [£]
			Nothobranchius kuhntae (Beira killifish)	2 [£]
	Aplocheilidae (rivulines)	Pachypanchax	*Pachypanchax playfairii* (golden panchax)	1 [£]
(Cyprinodontoidei)	Anablepidae	Anableps	*Anableps anableps* (largescale foureyes)	2 [$]
	Aphaniidae	Aphanius	*Aphanius iberus* (Spanish toothcarp)	1 [£]
	Fluviphylacidae	-	-	-
	Goodeidae (goodeids)	Girardinichthys	*Girardinichthys multiradiatus*	1 [$]
	Cyprinodontidae (killifish)	Cyprinodon	*Cyprinodon brontotheroides*	1 [£]
			Cyprinodon variegatus (sheepshead minnow)	1 [£]
			Cyprinodon nevadensis (Amargosa pupfish)	1 [£]

		Cyprinodon tularosa	1 [&]
Poeciliidae (livebearers)	Alfaro	*Alfaro cultratus*	1 [£]
	Brachyrhaphis	*Brachyrhaphis roseni*	1 [£]
		Brachyrhaphis terrabensis	1 [£]
	Gambusia	*Gambusia holbrooki* (eastern mosquitofish)	1 [£]
		Gambusia affinis (western mosquitofish)	2 [$]
	Girardinus	*Girardinus metallicus* (metallic livebearer)	1 [£]
	Micropoecilia	*Micropoecilia bifurca*	1 [£]
	Phalloptychus	*Phalloptychus januarius*	1 [&]
	Poecilia	*Poecilia mexicana* (shortfin molly)	1 [£]
		Poecilia formosa (Amazon molly)	3 [£]
		Poecilia latipinna (sailfin molly)	1 [£]
		Poecilia gillii (Gill's molly)	1 [£]
		Poecilia retiulata (guppy)	2 [$]
		Poecilia wingei	1 [£]
		Poecilia picta (swamp guppy)	2 [&]
	Poeciliopsis	*Poecilopsis turrubanensis*	1 [£]
		Poecilopsis paucimaculata	1 [£]
		Poecilopsis occidentalis (Gila topminnow)	1 [£]
		Poecilopsis infans (Lerma livebearer)	1 [£]
		Poecilopsis prolifica (blackstripe livebearer)	2 [£]
		Poecilopsis gracilis (portohole livebearer)	2 [&]
		Poecilopsis retropinna	1 [£]
		Poecilopsis turneri (blackspotted livebearer)	2 [&]
		Poecilopsis presidionis (Sinaloa livebearer)	1 [£]
	Xiphophorus	*Xiphophorus hellerii* (green swordtail)	2 [$]
		Xiphophorus couchianus (Monterrey platyfish)	1 [$]
		Xiphophorus maculatus (southern platyfish)	2 [$]
Profundulidae (Middle American killifish)	-	-	-
Pandanodontidae	-	-	-
Procatopodidae	-	-	-
Fundulidae (topminnows)	Fundulus	*Fundulus heteroclitus* (mummichog)	2 [$]
Valenciidae	-	-	-

Notes: [$] Reference genome available, chromosome level; [£] Reference genome available, scaffold level; [&] Reference genome available, contig level (database last queried: May 2023).

Table 2. *Aphanius fasciatus* sequence and literature data from the database Taxonomy https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=30736 (accessed on 13 June 2023).

Entrez Records	
Database Name	**Number of Relevant Data Available**
Nucleotide	453
Protein	211
Popset	29
PubMed Central	31
Identical Protein Groups	79
Taxonomy	1

5. Conclusions

In conclusion, the analyses and discussion of the literature regarding *A. fasciatus* highlight the suitability of this species as a sentinel organism for the protection of the quality of transitional water environments and the use of its molecular, cellular, and physiological responses as diagnostic tools for detecting the subtle effects induced on the biota by environmental pollution in these vulnerable environments. The peculiar physiological traits of this species also suggest its usefulness as a suitable animal model to investigate tolerance to harsh environmental conditions, including high temperatures and salinity, as well as low oxygen levels, with relevant implications for research on global climate change adaptation. Moreover, the increasing interest regarding *A. fasciatus* as an animal model in the study of certain pathophysiological states mainly related to metal exposure also suggests the suitability of the responses of this species in the wider framework of the One Health perspective, which considers human and animal health and the environmental state as highly interconnected, sharing common aspects.

To date, omics technologies show significant potential in reacquiring novel knowledge regarding the responses of organisms to environmental changes and the alterations in the environmental health status. Therefore, considering the relevance of this species as a sentinel species, many efforts are required in the near future to improve the quantity and quality of the -omics tools that refer to *A. fasciatus*.

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