Bulletin of Fish Biology	Volume 20	15.10.2024	35-48

Recommendations for scientific fish husbandry – a series for promoting animal welfare, reproducibility and transferability in ichthyologic research

Empfehlungen für die wissenschaftliche Fischhaltung – eine Reihe zur Förderung von Tierschutz, Reproduzierbarkeit und Übertragbarkeit in der ichthyologischen Forschung

Timo Moritz[1,2], David Bierbach[3,4], Julia Schwarzer[5] & Bianka Grunow[6]

[1] Ocean Museum Germany, Katharinenberg 14-20, D-18439 Stralsund, Germany, timo.moritz@meeresmuseum.de (corr. Author)
[2] Institute of Biological Sciences, University of Rostock, D-18059 Rostock, Germany
[3] Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, D-12487 Berlin, Germany.
[4] Faculty of Life Sciences, Albrecht Daniel Thaer Institute, Humboldt Universität zu Berlin, D-10115 Berlin, Germany.
[5] Leibniz Institute for the Analysis of Biodiversity Change (LIB), Museum Koenig Bonn, D-53113 Bonn, Germany
[6]Fish Growth Physiology, Research Institute for Farm Animal Biology (FBN), D-18196 Dummerstorf, Germany

Summary: Fishes are commonly used as model organisms in many scientific areas, which often require the maintenance of live specimens for investigation. Providing these animals with husbandry conditions required for their well-being and health is an undisputable promise in any scientific laboratory and well-based in legislative regulations. While this sounds simple, the high diversity of species and their life histories represented by more than 36,600 fish species worldwide are often overstraining evaluations of husbandry standards. There is simply no "one size fits all" husbandry protocol for all fish species and species-specific expert knowledge is needed to support (i) legislative bodies in deciding upon, (ii) administrational bodies to oversee and (iii) researchers to execute best possible husbandry conditions for this sheer number of different fish species. Therefore, the series ‚recommendations for scientific fish husbandry' is introduced. It aims to provide proposals how to house, feed, breed and raise specific fish species or species groups of similar biology taking into account the specific needs and animal welfare aspects. This lead article summarizes our need to keep live fish for research purposes, introduces the legal background set by EU and German laws, highlights the need to search for alternatives, and proposes a structure for future species-specific articles.

Keywords: Ichthyology, 3R, fishkeeping, aquarium keeping, model organisms

Zusammenfassung: In vielen wissenschaftlichen Bereichen werden Fische als Modellorganismen verwendet, was eine Haltung lebender Exemplare für Untersuchungen erfordert. Diesen Tieren die für ihr Wohlergehen und ihre Gesundheit erforderlichen Haltungsbedingungen zu bieten, ist eine unbestreitbare Notwendigkeit in jedem wissenschaftlichen Labor und in den gesetzlichen Vorschriften gut begründet. Das hört sich einfach an, doch die große Artenvielfalt und verschiedenen Lebensweisen der weltweit mehr als 36.600 Fischarten überfordern oft die Bewertung von Haltungsstandards. Es gibt einfach kein einheitliches Haltungsprotokoll für alle Fischarten, und so ist artspezifisches Fachwissen erforderlich, um (i) die gesetzgebenden Organe bei der Festlegung, (ii) die Verwaltungsorgane bei der Überwachung und (iii) die Forscher bei der Umsetzung der bestmöglichen Haltungsbedingungen für diese große Anzahl verschiedener Fischarten zu unterstützen.

Daher wird die Reihe „Empfehlungen für die wissenschaftliche Fischhaltung" eingeführt. Sie soll Vorschläge für die Haltung, Fütterung, Zucht und Aufzucht bestimmter Fischarten oder Artengruppen mit ähnlicher Biologie unter Berücksichtigung der spezifischen Bedürfnisse und Tierschutzaspekte liefern. Dieser Leitartikel fasst unseren Bedarf zur Haltung lebender Fische für Forschungszwecken zusammen, stellt den rechtlichen Hintergrund vor, der durch die EU- und die deutsche Gesetzgebung vorgegeben ist, hebt die Notwendigkeit hervor, nach Alternativen zu suchen, und schlägt eine Struktur für künftige artspezifische Artikel dieser Reihe vor.

Schlüsselwörter: Ichthyologie, 3R, Fischhaltung, Aquarienhaltung, Modellorganismus

1. Introduction

Fishes matter. They play an important role in the world's aquatic ecosystems, vertebrate evolution and species diversity, human nutrition and economy, and many other relevant fields. Yet, with more than 36,600 valid species (Fricke et al. 2024) living in virtually all aquatic habitats on our planet (e.g., Lagler et al. 1977; Helfman et al. 2009), any generalisations about their needs and requirements in human care that can be applied to all taxa are doomed to fail. This also applies to legal frameworks, which aim to secure animal welfare in fish keeping. Here lessons learned from husbandry of a few mammal species like rats and mice that represent the majority of animals used in scientific testing are often applied to 'fishes'. This approach – "one standard to fit all species" – then leads to rather vague recommendations and regulations that rarely serve as a valuable base for fish welfare at all. We thus identified the need for an accessible collection of knowledge on different fish species that could both help practitioners (researchers and animal care technicians) as well as administrative and legislative bodies (local animal welfare offices and politicians in charge) to make better – more animal-based – decisions about fish welfare in captivity.

In the scientific community as well as in the public there is a common agreement that the use of live animals in science can be problematic regarding animal welfare aspects especially when studies negatively impact health and well-being of the specimens. The 3R movement (see section 3 below) therefore postulates to minimise the use of animals for scientific purposes (Kirk 2018; Grunow & Strauch 2023). In cases where working with live animals in captivity cannot be avoided or is regarded to be appropriate due to the expected outcome, husbandry conditions have to meet certain standards, which insure that animals do not suffer (see section 2). Furthermore, it can be assumed, that studying stressed or suffering animals will produce heavily biased results. It is, however, a precondition for a scientific community that studies should allow for transferability of results, e.g. to natural conditions, as well as reproducibility and continuity of studies (see Webster & Rutz 2020).

Keeping live fishes for various scientific investigations is important for a profound understanding of the taxon's biology, like physiology, morphology, behaviour, ontogeny, species evolution and molecular mechanisms but also there is a need for live animal models in medical research (drug development, physiology, understanding the course of diseases, etc.) and human nutrition research (aquaculture, fisheries research, etc.). A great deal of our current day knowledge on heredity, development and physiology as well as on their underlying processes is derived from studies on model organisms, also called reference organisms (Müller & Grossniklaus 2010). Model organisms in fish often combine specific attributes, like easy handling and husbandry, fast development, external fertilization, transparent eggs and larvae, high and fast reproduction rate and share numerous genetic similarities with mammals, including humans. Concerning the latter issue, they may serve as models for human diseases, like heart diseases, cancer, neurological disorders, and genetic diseases (Choi et al. 2021; Lin et al. 2016). Most popular fish models are the zebrafish *Danio rerio* (e.g., Briggs 2002; Grunwald & Eisen 2002;

Khan & Alhewairini 2018) and the Medaka, i.e. the *Oryzias latipes* species complex (Wittbrodt et al. 2002). Especially, zebrafish are used for drug development and in toxicology (MacRae & Peterson 2015) as well as in monitoring and environmental research to investigate the effects of environmental pollutants on organisms (Stegemann et al. 2010). Many studies, law regulations and information concerning specific needs in fish husbandry focuss heavily on model species (Avdesh et al. 2012; Aleström et al. 2020; d'Angelo & Girolamo 2012; Lawrence 2007; Lee et al. 2022; Murata et al. 2019). However, in science, various fish species are kept for many different purposes, e.g. in basic research for studies on evolution (e.g., Meyer & Schartl 1999; Kornfield & Smith 2000; Rüber et al. 2004), behaviour (e.g., Kalueff et al. 2013; Bierbach et al. 2018; Laskowski et al. 2022), morphology (e.g., Koch et al. 2023; Moritz et al. 2023), development (e.g., Grunow et al. 2022; Hilgers et al. 2022), genomics (e.g., Braasch et al. 2015; Wang & Guo 2019), physiology (e.g., Cheng & Detrich 2007; Schüller et al. 2022), ecology (e.g., Godin & McDonough 2003; Klunzinger et al. 2012), aquaculture research (Simeanu et al. 2022; Tönissen et al. 2022; Kozłowski & Piotrowska 2023), fisheries research (e.g., Allman et al. 2016; del Mar Gil et al. 2017; Grew et al. 2024,), toxicology (e.g., Planchart et al. 2016; Hong & Zha 2019), infectiology (e.g., Padrós et al. 2001; Collet et al. 2015) and climate change (e.g., Scott & Johnston 2012; Seebacher et al. 2014; Ripley et al. 2023). Apart from a very few economically important exceptions, such as Atlantic salmon *Salmo salar* (RSPCA 2021) and rainbow trout *Oncorhynchus mykiss* (RSPCA 2020), there are no clear guidelines for keeping fishes, and legal regulations and husbandry specifications are transferred from model species to other species, even if it is not clear whether they are suited at all. Even so called near-model-species, i.e., species that are close relatives to well-studied model species, often greatly differ in their biology and thus husbandry needs. The close relatives of *Danio rerio*, i.e. Danioninae or Danionini (Tang et al. 2011; Liao et al. 2011) are widespread in various freshwater habitats in the South-East Asian tropics. They show a great variation in coloration and size differences ranging up to 9 cm (Kullander 2015) or even 15 cm (Froese & Pauly 2024) in *Danio dangila* to just over one centimetre in *Danionella* and *Paedocypris*, which are among the world's smallest vertebrates (Roberts 1986; Britz & Conway 2009). In ricefishes (Adrianichthyidae), the medaka species live in temperate regions in contrast to most other closely related species. The majority of ricefishes are distributed in a variety of tropical fresh and brackish water habitats and, even when they occur in close geographical proximity, show differences in morphology and, in some cases, even in reproductive systems (Hilgers & Schwarzer 2019). For both, zebrafish and medaka, most of the known husbandry conditions do not align with the requirements of their near-model relatives. Information on optimal husbandry conditions is rare, even for closely related species, and tends to be passed on anecdotally.

To promote ichthyology and insure/improve at the same time animal welfare, we see it as the right time to introduce a series proposing recommendations for scientific fish husbandry detailed and refined specifically for selected taxa. In this lead article, we give a brief introduction to guidelines on legal and practical aspects of scientific fish husbandry, highlight the need to search for alternatives by delineating the 3R principles, and propose an exemplary structure for species-specific articles to come.

1. Legal aspects of scientific fish husbandry

1.1. Background

According to European Union (EU) and national laws in Europe, specifically strict regulations apply where animals are used or intended to be used in procedures (= experiments for scientific purposes) or bred specifically so that their organs or tissues may be used for scientific purposes. Directives of the EU have to be implemented in national regulations, as for example the Directive 2010/63/EU was put into German law as Tierschutz-Versuchstierverordnung (TierSchVersV) in 2013. Following EU regulations, animals used for scientific purposes should be specially breed and not taken from the wild (§ 10). This is of importance as the zebrafish (*D. rerio*) is listed in Appendix I of Directive 2010/63/EU, which prohibits the use of wild-caught individuals for scientific purposes. So far other fishes are not included in that list, meaning that experiments with wild-caught individuals are not generally forbidden. Increasing restrictions on the use of wild-caught animals and growing ethical demand to use natural resources sustainable, make it of utmost importance that scientific facilities are able to breed animals intended for scientific purposes. How this breeding and keeping (=husbandry in our understanding) has to be done is also specified to some degree: Installations and equipment have to be suited to the species of animals housed (§ 22 Directive 2010/63/EU), need to be daily checked (see § 1 TierSchVersV) and Appendix III (Directive 2010/63/EU) provides general housing recommendations for fishes:

BOX 1: Recommendations for fish housing from the European Union

In September 2010, the European parliament and council published recommendations for the protection of animals used for scientific purpose: 2010/63/EU. In each country, these recommendations should be implemented in the legislation to become effectual. Nevertheless, when planning fish husbandry in a scientific context, we advise to consult also the complete directive of the EU. Section B of Annex III of the directive deals with species-specific requirements and contains a paragraph for fish in general. We give here the excerpt Annex III, Section B, Paragraph 11 'Fish' from the directive 2010/63/EU:

11.1. Water supply and quality

Adequate water supply of suitable quality shall be provided at all times. Water flow in re-circulatory systems or filtration within tanks shall be sufficient to ensure that water quality parameters are maintained within acceptable levels. Water supply shall be filtered or treated to remove substances harmful to fish, where necessary. Water quality parameters shall at all times be within the acceptable range that sustains normal activity and physiology for a given species and stage of development. The water flow shall be appropriate to enable fish to swim correctly and to maintain normal behaviour. Fish shall be given an appropriate time for acclimatisation and adaptation to changes in water quality conditions.

11.2. Oxygen, nitrogen compounds, pH, and salinity

Oxygen concentration shall be appropriate to the species and to the context in which the fish are held. Where necessary, supplementary aeration of tank water shall be provided. The concentrations of nitrogen compounds shall be kept low. The pH level shall be adapted to the species and kept as stable as possible. The salinity shall be adapted to the requirements of the fish species and to the life stage of the fish. Changes in salinity shall take place gradually.

11.3. Temperature, lighting, noise

Temperature shall be maintained within the optimal range for the fish species concerned and kept as stable as possible. Changes in temperature shall take place gradually. Fish shall be maintained on an appropriate photoperiod. Noise levels shall be kept to a minimum and, where possible, equipment causing noise or vibration, such as power generators or filtration systems, shall be separate from the fish-holding tanks.

11.4. Stocking density and environmental complexity

The stocking density of fish shall be based on the total needs of the fish in respect of environmental conditions, health and welfare. Fish shall have sufficient water volume for normal swimming, taking account of their size, age, health and feeding method. Fish shall be provided with an appropriate environmental enrichment, such as hiding places or bottom substrate, unless behavioural traits suggest none is required.

11.5. Feeding and handling

Fish shall be fed a diet suitable for the fish at an appropriate feeding rate and frequency. Particular attention shall be given to feeding of larval fish during any transition from live to artificial diets. Handling of fish shall be kept to a minimum.

These recommendations have to be met in order to obtain approval as a scientific husbandry facility in Germany ("Haltungsgenehmigung"), as they are the underlying basis for the national regulations (e.g., TierSchVersV). In addition, any facility intending to keep and/or breed fishes for scientific purposes have to specify for the used species how these recommendations are put into concrete actions. The Directive 2010/63/EU does not prescribe specific parameters but rather identifies key considerations – a factum that stems from the aforementioned sheer number of fish species present and their diverse needs and requirements.

1.2. More specific recommendations

More detailed recommendations stem from an expert report for the BMEL (Gutachten BMEL 1998) that lists specific water parameters, tank sizes, food and densities for many fish species. This report was a first and urgently needed step into a more standardized fish husbandry with more detailed measures available and its recommendations can be considered still valid. Interestingly, the strongest general recommendation in this report regards aquarium sizes and minimum volumes for certain fish species. For the smallest species category listed, the expert recommendation is given as no long-term housing of adult fish in tanks smaller than 54 L (the standard 60-cm tank). The reasoning behind is that "…the larger the water volume of an aquarium, the more stable the water quality, so the aquarium volume for permanent keeping should not be less than 60 litres. A minimum aquarium volume of 54 litres should be considered for permanent keeping." (Gutachten BMEL 1998). We largely follow these recommendations and like to point out that the water volume, not the tank size has to be in the main focus, especially for small-sized species. For example, many zebrafish breeding racks are designed as re-circulating systems with large overall water volume but small sizes of the actually holding tanks (ca. 3 L). Here the overall volume easily exceeds 300 litres while an easy control of the fish is enabled by the smaller tanks used for holding the fish.

1.3. Daily routines

Besides housing conditions suited to the species at hand, regulations focus strongly on daily check-up that aim at resource-based, management-based and animal-based measures (§1 TierSchVersV). While resource-based measures include water quality and controls of technical equipment, management-based measures cover feeding and hygiene schedules; animal-based measures aim at observable health and welfare parameters of the fishes that often involve morphological, physiological as well as behavioural traits being checked. For this purpose, care sheets that organize and standardize the daily observational routines are advocated. For some useful measures, please see AAC report (2023) as well as the check-list provided by TVT (2015).

1.4. Summary

EU and national laws provide obligatory regulations and recommendations for the husbandry of fishes used for scientific purpose. The overall aim of these regulations is to minimize pain, suffering or damage; and advocate conditions based on the total needs of the fish in respect of environmental conditions, health and welfare. Nevertheless, most recommendations and regulations stay vague as the sheer number of different fish species used contradicts detailed standards that fit all species.

2. 3R Principles in fish research

2.1. The principles

The principles of Reduce, Refine and Replace, commonly referred to as the 3R principles, established by Russel & Burch (1959) are fundamental ethical guidelines in scientific research, including fish research. These guidelines are incorporated in the Directive 2010/63/EU. They aim to minimise the use of animals, reduce their suffering, and explore alternative methods whenever possible. These principles have significant implications for improving the welfare of fish and reducing the impact of research on these animals.

2.1.1. Reduce

This principle advocates for minimizing the number of fish used in experiments. Researchers should carefully plan their studies to ensure that they use the smallest number of fish required to achieve scientifically valid results. Proper statistical design and experimental planning are essential in this regard. Additionally, an extensive literature review, including exploration of European Commission resources (ALURES data base), as well as data sharing (e.g., ECHA data base) aims to prevent the duplication of experiments across different laboratories.

2.1.2. Refine

Research methods have to be checked if any refinement is possible to reduce or eliminate any potential pain, distress or harm to fish. Researchers should adopt techniques and practices that improve the welfare of the animals involved. This also includes species-specific husbandry condition based on their biological needs, as well as using anaesthesia, analgesics, or refined handling procedures to reduce stress and discomfort, like e.g. photo identification instead of individual markings of the specimen (e.g., Barriga et al. 2015; Hook et al. 2019).

2.1.3. Replace

Researchers are encouraged to seek alternatives to live fish whenever possible. This can involve the use of in vitro models, computer simulations or other non-animal methods to achieve research goals without the need for live fish. A well-known and certified example is the use of fish cell cultures to replace the animal use in acute toxicity tests (ISO 21115:2019; Rodrigues et al. 2019; Scott & Minghetti 2020). Especially the field of in vitro models has experienced significant growth in recent years, demonstrating their application in basic and advanced science (Goswine at al. 2022). This expansion has already led to the development of increasingly complex 3D cell models based on their application (Verdile et al. 2021; Faber et al. 2021; Grunow et al. 2011, 2015).

2.2. Summary

The 3R principles in fish research reflect a commitment to ethical and responsible research practices. They aim to minimize harm to fish, reduce the number of animals used and explore alternative methods, ultimately advancing the welfare of fish in scientific research. For more detailed information on the 3R principles in fish research please read Grunow & Strauch (2023).

3. Considerations for anaesthesia and euthanasia in fish research

In ichthyological research, but also in aquaculture, there are instances where animals need to be anesthetised, e.g., for general examinations, tissue sampling, tagging, transponder implantation, or before the slaughter process (euthanasia). Guidelines have been established in the aquaculture industry that aim to ensure that fish are handled in an appropriate way during euthanasia and slaughter process (EFSA 2004; TiHO 2017; OIE 2019).

In fish research, there is a wide range of commonly used chemicals for anaesthesia, including benzocaine, diazepam, ethanol, 2-phenoxyethanol, ether, eugenol/isoeugenol (clove oil), isoflurane, ketamine hydrochloride, propofol, quinaldine sulfate, tricaine methane sulfonate, lidocaine and MS-222 (Saint-Erne et al. 2015; Martins et al. 2019). Monitoring the animals during sedation involves checking water temperature and dissolved oxygen concentration in the water, as well as using a pulse oximeter to monitor the fish's vital signs (Saint-Erne et al. 2015). However, determining the appropriate amount of anaesthetics depends on the species, age, and environmental factors like temperature (Neiffer & Stamper 2009; Collymore et al. 2015; Martins et al. 2019). Different species have varying reactions to anaesthetics and require specific concentration assessments (Matsche 2011; Machnik et al. 2018; Ferreira et al. 2022). Techniques like electroencephalography (EEG) and electrocardiography (ECG) showed that traditional methods of visually evaluating anaesthesia based on movement, eye reflex, swimming position and gill breathing are often insufficient (Rendon-Morales et al. 2005; Bowman et al. 2019). Ultimately, before subjecting fish to examinations and anaesthesia, careful consideration should be given to the necessity of the procedures, as the treatment potentially results in impairment of memory and cognitive flexibility (Fontana et al. 2021). When fish need to be killed, a variety of possible methods is known, as e.g. an overdose of anaesthetic chemicals, blow on the head, decapitation, heart puncture, cold water (for tropical fish) or electro-stunning. Yet, the appropriateness and effectiveness of several methods is heavily discussed, the legal situation is often insufficiently solved and researchers are obliged to decide depending on species, size and current knowledge to find the most "humane" solution.

4. Husbandry conditions

Well-being, growth and health of fish in captivity is largely influenced by the general keeping conditions, like water quality, tank size and setup, food and stocking density (see also Table 1). In the wild, species may be adapted to a relatively wide range of biotic and abiotic conditions, but this total range represents conditions which are not ideal but can be tolerated, at least for a certain time span. Yet, for all parameters there are species-specific optimum ranges and each species may weigh different parameters differentially. In order to promote animal welfare and finally study 'normal' specimens and not permanently stressed or chronically suffering animals, fish husbandry should aim to provide optimal conditions for the respective species (see Schreckenbach et al. 2021). We advocate orientating at the species natural habitats in order to find and define parameter ranges for most relevant conditions that have to be provided in captivity (see Table 1 for a list of highly relevant conditions).

Highest growth and maximum well-being and health of the fish can be expected in the optimum range, although some species may need annual fluctuations for permanent well-being and longevity. Such alterations e.g. in temperature, conductivity, light regime or food availability, may deviate from the actual optimum, but may be needed to simulate seasons and by this, stimulate the reproductive cycle. Conditions like food or substrate may change also during the individual ontogeny of a species and this have to be accounted as well.

Tab. 1: Important parameters in fish husbandry.
Tab. 1: Parameter, die in der Fischhaltung berücksichtigt werden müssen.

parameter	remark
tank type	Suitability of tanks (or ponds), flow-through tanks or recirculation-aquaculture systems depends on their shape and size; e.g. epipelagic species require a higher water column, while benthic species necessitate more bottom area; permanent swimmers profit from tanks without corners.
tank setup	While some pelagic species need no substrate and hardly plants or objects to feel comfortable, other species may depend strongly on a specific substrate or hiding places. Some objects, like plants, blank stones or rough gravel, may only be needed for reproductive behaviour, but nevertheless have to be taken into account. The setup should not pose any danger for injury by objects, tank mates or technical equipment.
swimming type	It is important to consider whether a species is a permanent swimmer, which benefits from permanent or occasional currents, or a more sedentary species, which tends to avoid stronger currents or currents over longer periods.

Tab. 1: Continued.
Tab. 1: Fortsetzung.

parameter	remark
social behaviour	For stocking density, one has to consider whether a species is naturally solitary, living in smaller groups or schooling. Aggressive behaviour and territoriality may be challenging for small scale husbandry, although or especially when only seasonally expressed. It may become necessary to size-sort specimens in order to minimise dominance behaviour, increase stocking density to avoid territoriality or to even isolate individuals from otherwise gregarious species. High stocking densities, if required, are challenging though as health status must be monitored even more closely, pathogens can be transmitted more quickly and water parameters may change suddenly when technical malfunctions occur.
light regime/ activity times	Light exposure should be adapted to the animal's life style. This includes appropriate day/night cycle, light spectrum and intensity.
food supply	Feeding times have to be adapted to activity times (e.g., diurnal vs. nocturnal species). Food needs to be optimized in composition, item size and amount.
temperature	Water temperature affects the metabolic rate of fish, their feeding habits, and growth. Higher temperatures usually increase metabolism, leading to greater food consumption. Temperature also influences dissolved oxygen availability.
oxygen	Sufficient oxygen content is critical and may largely differ between fish species. Besides temperature, high fish density, water pollution, and low surface agitation decrease oxygen levels. Low oxygen content can stress fish, lead to diseases, and even fish mortality.
conductivity/ salinity	Depending on fish species, the salinity/conductivity needs to be adapted. Salt may also be used to manage diseases. But salinity adjustments influence osmoregulation and can also affect pH value.
pH value	Fish species may be adapted for a certain pH value range, e.g., very low in black water fishes or very high in cichlids of East African lakes. Deviation from that range results in stress and health issues. Furthermore, pH value affects nutrient availability, effectiveness of chemicals (including medications) and impact the toxicity of certain compounds, such as ammonia. pH level drops with temperature decrease or CO_2 concentration increase.
nitrogenous compounds	Nitrate (NO_3^-) and nitrite (NO_2^-) are products of the nitrogen cycle in fish keeping systems. High levels of nitrate and especially nitrite can be harmful for fish. Metabolism of fish and bacteria also produces ammonia (NH_3/NH_4^+), which is highly toxic to fish, especially at elevated pH levels. We here advocate a (close to) zero nitrite and zero ammonia regime.
hardness and alkalinity	These parameters influence pH stability and the availability of calcium and magnesium, essential for fish skeletal and scale formation.

5. Specific accounts – a guideline

This series aims to provide articles that include recommendations for scientific fish husbandry. The articles can focus on a specific species or include several species, provided they have very similar requirements. The recommendations are in a first way aimed to laboratories and small-scale research facilities, and not explicitly for public display in large aquaria and zoos; the recommendations are not aimed for aquaculture or other economic purposes. Articles of the proposed series should follow a certain structure and aim to provide essential information for researchers and responsible persons for animal welfare in research institutions, ethic committees and governmental authorisation bodies. Statements must be supported by scientific evidence, cited from existing literature, or clearly be marked if speculative. Own experiences are encouraged, but have to be based on a routine and not on single observations.

Articles for this series should be titled "Recommendations for scientific fish husbandry –" followed by the name (either vernacular plus Latin name or only Latin name, followed by author of the species) and taxonomic grouping. All manuscripts will undergo a standard peer-review procedure and have principally to follow the guidelines for articles in the Bulletin of Fish Biology. They can be found at the end of each issue or under https://www.ichthyologie.de/wp-content/uploads/2021/06/Bulletin_Autorenhinweise_dt_engl_01_06_21-1.pdf. Articles in this series have furthermore to follow a strict structure, which is given in table 2. All sections can be supplemented with figures and tables. In cases where substantial changes or additions need to be made to previously published species accounts, short notes may be submitted to this series. It should be named "Recommendations for scientific fish husbandry – Addendum to [species] ([taxonomic grouping)]" and may not follow the initial article's strict structure.

Again, we emphasized that articles in this series will be recommendations or proposed guidelines, but no obligatory regulations for a specific field. Nevertheless, we aim to facilitate authorisation processes by enabling species-specific requirements to be developed and implemented more quickly. We hope that this series will be helpful to advance ichthyology while promoting animal welfare in scientific fish keeping at the same time.

Tab. 2: Structure of articles for the series recommendations for scientific fish husbandry.
Tab. 2: Aufbau der Artikel in der Serie ‚recommendations for scientific fish husbandry'.

Summary	Please keep it very short, below 250 words; each one or two sentences for: • the importance of the species • remarks on their biology • peculiarities for keeping the species • a summarizing remark on suitability for aquarium based studies
1. Introduction	Present the species and elaborate its importance for scientific studies. Also other important points like economically importance or history of its discovery can be mentioned here. Full sentences.
Biology and general information	Please provide a **table** giving an overview on: • valid name including authority • common names – if applicable • systematic position • forms/variability – if applicable: subspecies, colour morphs, distinct breeding lineages, etc. • similar species – only if applicable • distribution – in words, no map • habitat – details on the natural environmental parameter may be given • size – in wild and in aquaria; if known also age and weight • behaviour – for adults in wild, e.g. solitary, schooling, nocturnal, migratory, etc.

Tab. 2: Continued.
Tab. 2: Fortsetzung.

	• diet – for adults in wild • references – if applicable, select a limited number of important references which refer to a certain topic, e.g. (species description), (protective status), (physiology), (reproduction), …
2. Tank and water parameters	Please compile available information on tank design and setup, as well as on physio-chemical parameters. Own experience can be given here; please insure to clarify what are recommendations and what are experiences.
3. Feeding and regular care	This section should contain possible and suggested food items and feeding schemes for adult specimens. Also include any kind of regular needed care, like schemes for water change. This section should also include descriptions on the behaviour of the species in captivity and may be added with other keeping-related issues, like age in captivity.
4. Breeding and rearing	Here the knowledge on reproduction including behaviour in wild should be compiled. Then reproduction in captivity should be explained, including pre-requirements (e.g. special food, water parameters, light regime to prepare and induce spawning), technical setup (e.g. providing spawning substrate, specification and installation of special breeding tanks, special lights regime or water parameter), description of reproduction itself in captivity (e.g. courtship behaviour, spawning behaviour, parental care), and handling of eggs (including information on size, amount and time to hatch). Please also include information on the biology, handling and rearing of larvae and juveniles.
5. Further remarks	The content and structure of this final section is very open. It can include own experience on husbandry of the species including exemplary data on growth, breeding success, unsuccessful husbandry and breeding attempts etc., but also details on collecting the species in wild, adapting experimental setup, or species-specific doses of medication or anaesthetics. The section can also highlight a research area for which the presented species is of interest, e.g. behaviour, systematics, taxonomy, aquaculture, genetics, immunology, ecology, evolution.
Acknowledgements	Add, if applicable.
Literature	Stay to the format as usual for Bulletin of Fish Biology.
Score sheet	Please provide an exemplary score sheet for the daily control of the health condition as appendix. In cases of treating several species in an article, multiple exemplary score sheets may be added.

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Received: 27.04.2024
Accepted: 09.05.2024
Published: 13.05.2024