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Recommendation of scientific fish husbandry: Sulawesi ricefishes (Beloniformes, Adrianichthyidae)

Empfehlungen für die wissenschaftliche Fischhaltung: Tropische Reisfische aus Sulawesi (Beloniformes, Adrianichthyidae)

Julia Schwarzer[1*], Alina Schüller[1], Jan Möhring[1], Tobias Spanke[1], Arne W. Nolte[2] & Fabian Herder[1]

[1]Museum Koenig, Leibniz Institute for the Analysis of Biodiversity Change, Adenauerallee 127, 53113 Bonn, Germany; E-Mail: j.schwarzer@leibniz-lib.de (*corresponding author)
[2]Carl-von-Ossietzky-Universität Oldenburg, AG Ökologische Genomik, Carl-von-Ossietzky-Str. 9-11, 26111 Oldenburg, Germany.

Summary: Ricefishes (Teleostei: Adrianichthyidae) are a family of small fresh- and brackish water fishes with ~ 40 species belonging to the genera *Oryzias* and *Adrianichthys*. They are widespread in South, Southeast, and East Asia. Most ricefish species live in tropical environments, and more than 50% of the species are endemic to Sulawesi, Indonesia. Medaka, which is a species complex of *Oryzias latipes* and closely related ricefishes from Japan, East Korea and China, is a well-established model organism in medical and developmental science. In scientific laboratories, Medaka and other small ricefish species are usually kept in mid-sized recirculating freshwater systems, with 2 L tanks each containing a breeding pair or up to ten adults. Medaka can easily be kept in the same aquatic system as zebrafish (*Danio rerio*), as they require similar conditions with respect to water quality, temperature and light cycle. These conditions are, however, not suitable for most other ricefish species. Making a single general recommendation for keeping all ricefishes appears inappropriate, as the species come from various environmental backgrounds and evolved very different ecological adaptations, behaviors and life histories. Here, we give recommendations for keeping and breeding tropical ricefish species from Sulawesi, with emphasis on their specific needs.

Keywords: Ricefishes, animal husbandry, *Oryzias, Adrianichthys*, pelvic brooding, Sulawesi, freshwater, parental care

Zusammenfassung: Reisfische (Teleostei: Adrianichthyidae) sind eine Familie kleiner Süß- und Brackwasserfische mit ~40 Arten in den Gattungen *Oryzias* und *Adrianichthys* und einer Verbreitung in Süd-, Südost- und Ostasien. Die meisten Reisfischarten leben in tropischen Umgebungen und mehr als 50 % der Arten sind auf Sulawesi, Indonesien, endemisch. Medaka, ein Artenkomplex aus *Oryzias latipes* und eng verwandten Reisfischen aus Japan, Ostkorea und China, ist ein etablierter Modellorganismus in Medizin und Entwicklungsbiologie. In wissenschaftlichen Laboren werden Medaka und andere kleine Reisfischarten in der Regel in mittelgroßen Süßwasser-Kreislaufsystemen gehalten, wobei in 2-Liter-Aquarien jeweils ein Brutpaar oder bis zu zehn ausgewachsene Tiere gehalten werden. Medaka können problemlos in den selben Aquariensystemen wie Zebrafische (*Danio rerio*) gehalten werden, da sie ähnliche Ansprüche an Wasserqualität, Temperatur und Lichtzyklus stellen. Diese Bedingungen sind jedoch für die meisten anderen Reisfischarten nicht geeignet. Eine allgemeine Empfehlung für die Haltung aller Reisfische zu geben erscheint nicht angemessen, da die Arten unter verschiedenen Umweltbedingungen vorkommen und sich sehr unterschiedliche ökologische Anpassungen, Verhaltensweisen und Lebensgeschichten entwickelt haben. Hier geben wir Empfehlungen für die Haltung und Zucht tropischer Reisfischarten aus Sulawesi, wobei wir den Schwerpunkt auf ihre spezifischen Bedürfnisse legen.

Schlüsselwörter: Reisfische, Tierhaltung, *Oyrzias, Adrianichthys*, Bauchbrüten, Sulawesi, Süßwasser, Brutpflege

1. Introduction

Ricefishes (Teleostei: Adrianichthyidae) are small fresh- and brackish water fishes with a wide distribution across Asia. The family Adrianichthyidae comprises ~40 species that belong to the two genera *Oryzias* and *Adrianichthys* (Parenti 2008). While *Oryzias* species are rather small (2 – ~ 8 cm), the genus *Adrianichthys* includes larger ricefish species (>6 – 20 cm) all endemic to Lake Poso in Sulawesi, Indonesia (Fig. 1). Ricefishes are well known to a wider scientific audience for the medaka (*Oryzias latipes* species complex), a popular model in biological and medical disciplines for over 100 years (Wittbrodt et al. 2002; Kirchmaier et al. 2015; Hilgers & Schwarzer 2019). Their large transparent eggs and embryos made them a promising model especially in developmental biology. The natural distribution is mainly Japan, but also Korea and China. As medaka species inhabit temperate zones, they can tolerate a wide range of temperatures (from ~4 up to 40°C) both as adults and embryos (Sampetrean et al. 2009).

Medaka are easy to breed, and are usually kept in large, fully automated, aquarium systems that resemble setups used for zebrafish in scientific labs (Westerfield 2007). They are also famous in the aquarium and pond hobby, with numerous and frequently colorful strains that were bred mainly in Japan (Kinoshita et al. 2009). Other ricefish species (Fig. 1; Tab. 1) — the wild relatives of medaka — are diverse and show, besides morphological differences (Parenti 2008), varying degrees of evolutionary adaptations to salinity (Takehana et al. 2020) and different temperature regimes, as well as the repeated evolution of miniaturized species (Parenti 2008). All ricefishes belong to the reproductive guild of so-called external bearers (Wootton & Smith 2014), meaning that they carry their eggs or offspring on the outside of their body. In ricefishes, unlike many other fish species (Wootton & Smith 2014), females are the carrying sex. Already in the ovary, long attaching filaments develop at the vegetative pole of the egg chorion and eggs are laid as a cluster of up to 70 eggs. The egg cluster remains attached to the female through the filaments which partly remain inside the female's gonoduct. A male instantly fertilizes the eggs when they are spawned. Most species strip off the eggs on plants or other substrate after a short amount of time (several hours to one day), which is called transfer brooding. Some species, all endemic to Sulawesi, carry the cluster of fertilized eggs until the embryos hatch, for which Kottelat (1990a) suggested the term "pelvic brooding". During pelvic brooding, a tissue known as the "plug" forms around the attaching filaments within the female gonoduct, thereby anchoring the eggs (Iwamatsu et al. 2008; Schüller et al. 2022) (Fig. 1A).

Ricefish diversity peaks on the island of Sulawesi (Indonesia), which harbors ~24 endemic to micro-endemic species in the two genera *Oryzias* and *Adrianichthys* (Hilgers & Schwarzer 2019; Herder et al. 2022; Möhring et al. 2025a). Many of them are threatened and three of the four described *Adrianichthys* species are possibly already extinct in the wild (*Adrianichthys kruyti*, *A. roseni*, *A. poptae*; Parenti 2008; Herder et al. 2022). On Sulawesi, pelvic brooding evolved in two distantly related ricefish lineages comprising at least six species. Research on Sulawesi ricefishes benefits from their relatedness with medaka, making a wealth of already available resources accessible (Kirchmaier et al. 2015). They are used to study e.g., the evolution of adaptive divergence and trait evolution, (i.e. coloration, feeding ecology, reproductive strategies), making them promising models in evolutionary biology (Hilgers & Schwarzer 2019; Sutra et al. 2019; Ansai et al. 2021; Ansai et al. 2022; Flury et al. 2022; Hilgers et al. 2022; Montenegro et al. 2022). Originating from various tropical freshwater and brackish habitats (Fig. 1B), the known husbandry conditions for species such as the medaka correspond to some extent, but not fully with the requirements of many Sulawesi ricefish species. Here, we recommend optimal conditions for keeping and breeding tropical ricefishes from Sulawesi, with emphasis on *Oryzias* species.

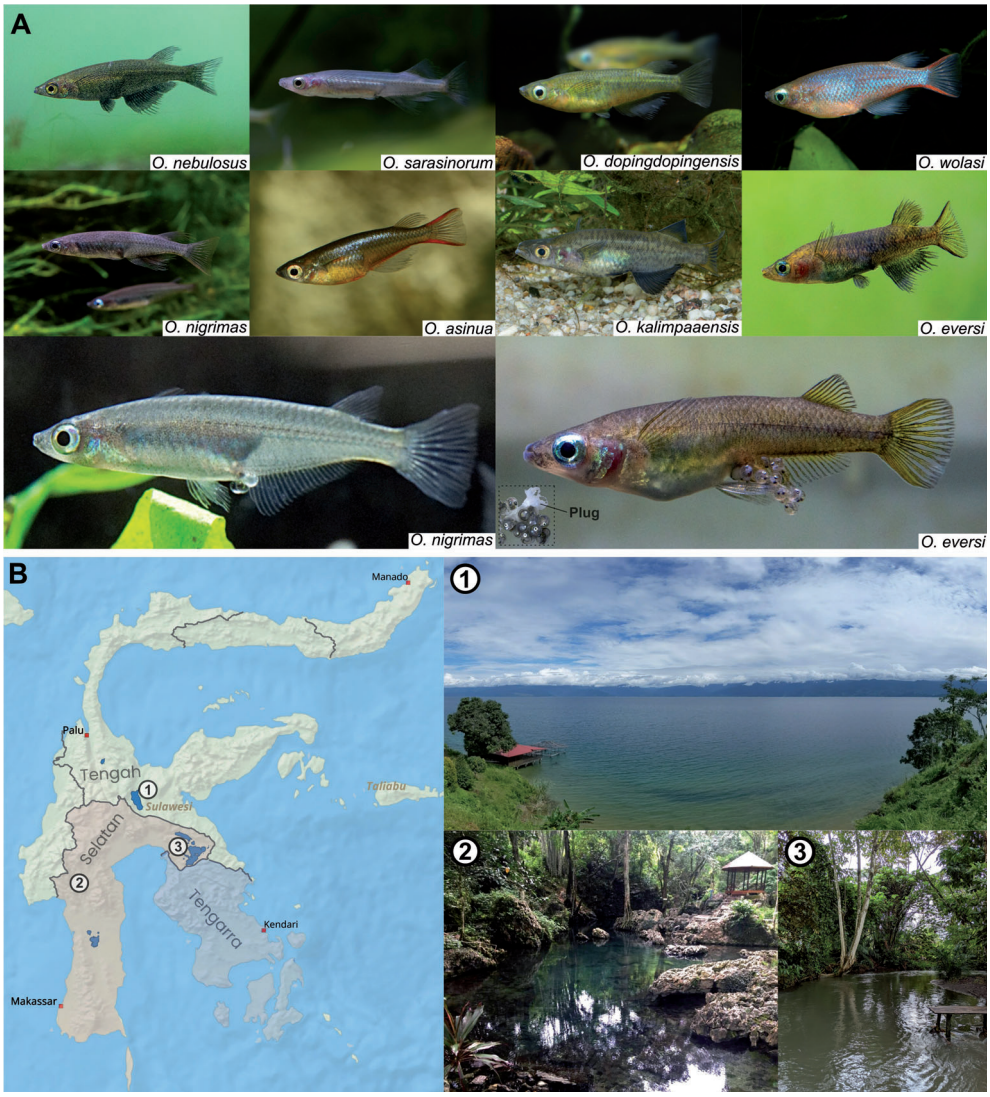


Fig. 1: Pictures of live Sulawesi ricefishes and their habitats. **A** (top and middle row) Males of selected Sulawesi ricefish species; Bottom: Egg-carrying females of transfer brooding *Oryzias nigrimas* (left) and pelvic brooding *O. eversi* (right) with well-developed eggs. The plug (lower left corner) is a tissue that develops inside the female during brooding. **B** A selection of habitats where Sulawesi ricefishes are endemic. Left: Map of Sulawesi showing the 3 habitats illustrated: B1 Lake Poso, home to an adaptive radiation of *Oryzias* ricefishes and the only habitat of *Adrianichthys* species. B2 Tilanga Pond, only known habitat of *O. eversi*. B3 Dopingdoping River, home of *O. dopingdopingensis*.

Abb. 1: Bilder von lebenden Sulawesi-Reisfischarten und ihren Lebensräumen. **A** (Obere und mittlere Zeile): Bilder von ausgewählten männlichen Sulawesi-Reisfischarten; (untere Zeile): Eitragende Weibchen der transferbrütenden Art *Oryzias nigrimas* (links) und der bauchbrütenden Art *O. eversi* (rechts) mit weit entwickelten Eiern. Der Plug (unten links) ist ein Gewebe, das sich während des Brütens innerhalb des Weibchens bildet. **B** Auswahl endemischer Lebensräume von Sulawesi-Reisfischarten. Links: Karte von Sulawesi mit drei im Folgenden illustrierten Fundorten: B1 Der Poso-See, Heimat einer adaptiven Radiation von *Oryzias*-Reisfischen und einziger Lebensraum der *Adrianichthys*-Arten. B2 Tilanga-Pool: einziger bekannter Lebensraum von *O. eversi*. B3 Dopingdoping-Fluss: Heimat von *O. dopingdopingensis*.

Tab. 1: General information on the biology of Sulawesi ricefishes; *see Tab. 2 for more detailed information.
Tab. 1: Allgemeine Informationen zur Biologie von Reisfischen aus Sulawesi. * Für weitere Informationen siehe Tab. 2.

valid name*	*Oryzias* spp; *Adrianichthys* spp.
common name	"Ricefishes": common name for both genera
systematics	Teleostei – Beloniformes – Adrianichthyidae
forms	high variation in coloration and body forms
similar species	*Oryzias* species from Japan and Asian mainland
distribution*	endemic to Sulawesi, Indonesia – all, except two species (*O. javanicus*, *O. celebensis*)
habitat	freshwater; springs, streams and rivers, various types of lakes depending on species; one euryhaline species in both freshwater and brackish waters (*O. javanicus*)
water	habitats and water requirements vary substantially among species
size	most species between ~30–50 mm; exceptions up to 200 mm
adult behavior	diurnal, free swimming, upper and medium water column, some species facultative shoal building, females of all species carry eggs after external fertilization, most stripe them off after short time (transfer brooding species), some carry the eggs with developing embryos until the larvae hatch (pelvic brooding species)
adult diet	phyto- and zooplankton, larger species: insect larvae
references	PARENTI 2008; HILGERS & SCHWARZER 2019; KOTTELAT 1990a

2. Aquarium and water parameters

Ricefish species endemic to Sulawesi tend to be larger than those from outside the island (PARENTI 2008; MÖHRING et al. 2025a). The smallest Sulawesi species (i.e. from the *Oryzias woworae* group or *O. soerotoi* – all ~30 mm standard length (SL)) are comparable in size or slightly smaller than Medaka (*Oryzias latipes* group – up to 35 mm SL), which is the largest non-Sulawesi species. While the majority of non-Sulawesi species stay below 30 mm SL, most Sulawesi species reach sizes of > 40 mm SL (Tab. 2; PARENTI 2008; MÖHRING et al. 2025a), which means that larger aquarium sizes (Fig. 2A) are required for those species compared to Medaka (*Oryzias latipes*) and other non-Sulawesi ricefishes (KINOSHITA et al. 2009). The recommended aquarium size and fish density for Sulawesi ricefishes varies between species and depends on several factors (Tab. 2). For Sulawesi ricefishes, smaller aquariums with a water volume of 30 L (min base area 0.12 m^2) or less are only suitable for juveniles or small species, i.e. those belonging to the *Oryzias woworae* group, *O. soerotoi* or *O. eversi*. Most Sulawesi species should be kept in aquaria with a minimum water volume of 45 L and a minimum base area of 0.16 m^2. Large ricefishes (> 60 mm SL) should generally be kept in larger aquariums with a minimum of 120 L and 0.4 m^2 base area. Some parameters depend on the activity levels of each species. For example, *O. sarasinorum* and *O. nigrimas* are open-water dwelling species that require sufficient space to swim, and should preferably be kept in aquaria with a minimum length of 80 cm (*O. nigrimas*) or 100 cm (*O. sarasinorum*). A higher water volume is also recommended for *O. polylepis*, a ricefish with very active and fast swimming behavior that results from its rheophilic ecology, and for *O. kalimpaaensis*, the largest *Oryzias* species (> 65 mm). Fish stocking densities are species-specific, reflecting differences in behavior and body size, and range from approximately 1 L per individual up to 8 L per individual, the latter estimated as a requirement for the large *Adrianichthys oophorus* (Tab. 2). Our recommendations of minimum aquarium size parameters (see Tab. 2 for a gene-

Tab. 2: General information on the biology and keeping of tropical ricefish from Sulawesi. List of all Sulawesi ricefish species currently known to science with their distribution. Suggestions for the minimum aquarium size apply to the husbandry of adult fishes; a minimum aquarium length is only given if it is relevant in permitting natural swimming behavior. They differ for larvae, juveniles to subadults or when setting up breeding pairs (see more detailed information in the text). *: Species kept and bred by the authors; [cc]: critically endangered species; †: likely extinct in the wild. Suggestions for species not kept by the authors are derived from experience with related species or from species living in similar habitats, and may have to be adjusted once actual experience is available.

Tab. 2: Generelle Informationen zur Biologie und zur Haltung von tropischen Reisfischen aus Sulawesi. Liste aller derzeit wissenschaftlich bekannten Sulawesi-Reisfischarten sowie deren Verbreitung. Die Angaben zur empfohlenen Mindestbeckengröße beziehen sich auf die Haltung ausgewachsener Tiere; eine Mindestlänge für Aquarien wird nur angegeben, wenn diese von Relevanz ist, um ein natürliches Schwimmverhalten zu ermöglichen. Sie unterscheiden sich für Larven, Jungfische bis Subadulte oder beim Ansetzen von Zuchtpaaren (siehe detailliertere Informationen im Text). *: Arten, die von den Autoren gehalten und vermehrt wurden; [cc]: vom Aussterben bedrohte Arten; †: wahrscheinlich in der Natur ausgestorben. Empfehlungen zu Arten, die von den Autoren nicht selbst gepflegt wurden, basieren auf Erfahrungen mit verwandten Arten oder solchen aus ähnlichen Lebensräumen und müssen gegebenenfalls angepasst werden, sobald praktische Erfahrungen vorliegen.

Species	Distribution	Reproduction	Approximate adult size (SL)	Minimum Aquarium size
Adrianichthys oophorus (KOTTELAT, 1990a)	Lake Poso, Sulawesi Tengah	Pelvic brooder	70 mm or larger	240 L 120 cm length 0.48 m² 8 L / individuum
A. poptae (WEBER & DE BEAUFORT, 1922) †	Lake Poso, Sulawesi Tengah	Pelvic brooder	200 mm or larger	
A. kruyti (WEBER, 1913) †	Lake Poso, Sulawesi Tengah	Pelvic brooder?	110 mm or larger	
A. roseni (PARENTI & SOEROTO, 2004) †	Lake Poso, Sulawesi Tengah	Pelvic brooder	90 mm or larger	
Oryzias asinua (PARENTI et al., 2013) *	Streams in Asinua River basin, Sulawesi Tenggara	Transfer brooder	25 – 35 mm	30 L 0.12 m² 1 L / individuum
O. wolasi (PARENTI et al., 2013) *	Streams in Anduna basin, Sulawesi Tenggara	Transfer brooder	25 – 35 mm	30 L 0.12 m² 1 L / individuum
O. woworae (PARENTI & HADIATY, 2010) *	Forest streams on Muna island, Sulawesi Tenggara	Transfer brooder	25 – 35 mm	30 L 0.12 m² 1 L / individuum
O. moramoensis (UTAMA et al., 2024) *	Streams in Moramo basin, Sulawesi Tenggara	Transfer brooder	25 – 35 mm	30 L 0.12 m² 1 L / individuum
O. celebensis (WEBER, 1894) *	Rivers and streams in SW Sulawesi, Sulawesi Selatan	Transfer brooder	25 – 40 mm	45l L 0.12 m² 1 L / individuum
O. sarasinorum (POPTA, 1905) *	Lake Lindu, Sulawesi Tengah	Pelvic brooder	60 mm or larger	120 L 100 cm length 0.40 m² 5 L/ individuum
O. eversi (HERDER et al., 2012) *, [cc]	Tilanga Pool, Sadang River basin, Tana Toraja, Sulawesi Selatan	Pelvic brooder	25–40 mm	30 L 0.12 m² 1.5 L / individuum
O. landangiensis (UTAMA & YAMAHIRA, 2022)	Cerekang River, Sulawesi Selatan	Transfer brooder	25–40 mm	45 L 0.12 m² 1 L / individuum
O. dopingdopingensis (MANDAGI et al., 2018) *	Doping-doping River, Sulawesi Selatan	Transfer brooder	25–40 mm	45 L 0.12 m² 1 L / individuum
O. kalimpaaensis (GANI et al., 2022) *	Lariang River basin, Sulawesi Tengah	Pelvic brooder	65 mm or larger	120 L 0.40 m² 5 L / individuum
O. bonneorum (PARENTI, 2008)	Lake Lindu, Sulawesi Tengah	Pelvic brooder	50–60 mm	45 L 0.16 m² 1 L / individuum
O. polylepis (MÖHRING et al., 2025b) *	Lariang River basin, Sulawesi Tengah	Transfer brooder	55 mm or larger	120 L 100 cm length 0.40 m² 5 L / individuum

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

Species	Distribution	Reproduction	Approximate adult size (SL)	Minimum Aquarium size
O. matanensis (AURICH, 1935) *	Lake Matano, Sulawesi Selatan	Transfer brooder	50 mm	45 L 0.16 m² 1 L / individuum
O. marmoratus (AURICH, 1935) *	Lakes Towuti, Mahalona, Lantoa, Sulawesi Selatan	Transfer brooder	40 mm	45 L 0.16 m² 1 L / individuum
O. loxolepis (KOBAYASHI et al., 2023)	Lake Towuti, Sulawesi Selatan	Transfer brooder	40 mm or larger	45 L 0.16 m² 1 L / individuum
O. profundicola (KOTTELAT, 1990b)	Lake Towuti, Sulawesi Selatan	Transfer brooder	50 mm	45l 0,16 m² 1 L / individuum
O. hadiatyae (HERDER & CHAPUIS, 2010)	Lake Masapi, Sulawesi Selatan	Transfer brooder	50 mm	45 L 0.16 m² 1 L / individuum
O. nigrimas (KOTTELAT, 1990a) *	Lake Poso, Sulawesi Tengah	Transfer brooder	50 mm	45 L 80 cm length 0.16 m² 1 L / individuum
O. orthognathus (KOTTELAT, 1990a)	Lake Poso, Sulawesi Tengah	Transfer brooder	50 mm or larger	120 L 0.40 m² 5 L / individuum
O. nebulosus (PARENTI & SOEROTO, 2004) *	Lake Poso, Sulawesi Tengah	Transfer brooder	30–40 mm	45 L 0.16 m² 1 L / individuum
O. soerotoi (MOKODONGAN et al., 2014) *	Lake Tiu, Sulawesi Tengah	Transfer brooder	25–30 mm	25 L 0.1 m² 1 L / individuum
O. javanicus (BLEEKER, 1854) *	Brackish coastal waters	Transfer brooder	25–35 mm	30 L 0.12 m² 1.5 L / individuum

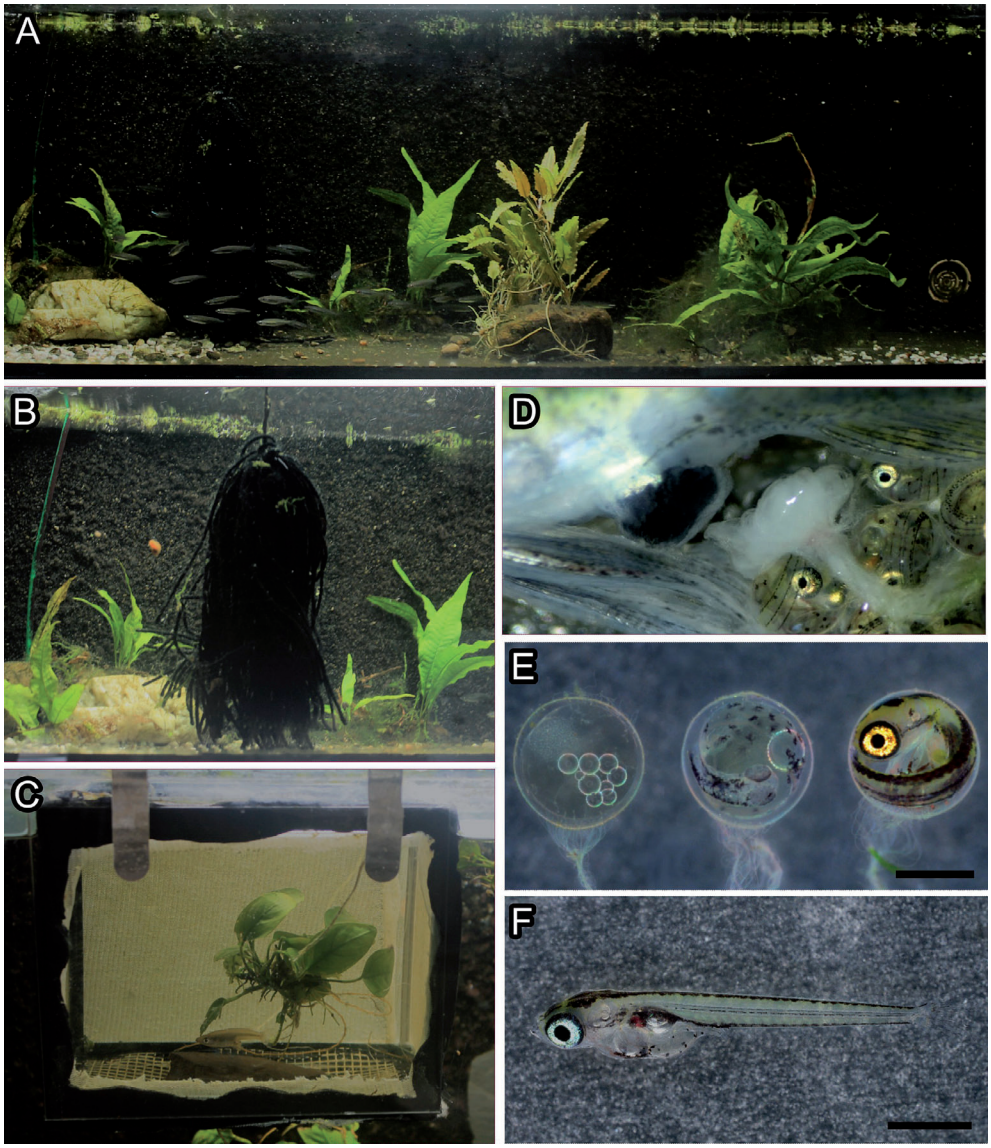
ral overview) and fish densities aim at a sustainable, long-term husbandry of adult fishes. Note that juveniles may be reared in more confined spaces and at higher densities. Likewise, adults may be temporarily isolated into smaller aquaria to protect brooding females or to cross specific pairs, without compromising animal welfare. Regardless of maximum length, a larger base area will result in calmer and less shy behavior in most species. Moreover, the decoration and

Fig. 2: Rearing and breeding Sulawesi ricefishes. **A** Picture of a suitable aquarium for Sulawesi ricefishes. The thin layer of substrate (medium-grained gravel), larger stones, and plants provide the fish with a semi-natural habitat offering shelter. **B** To breed transfer brooding species, we use this so called "spawning mop", a bundle of acrylic yarn mimicking aquatic plants. A small piece of cork or foam is attached as a floater to keep it buoyant. **C** Females of small pelvic brooding species, like *O. eversi*, are less stressed when they are separated in a "box" during brooding. This also avoids predation of conspecifics on the developing eggs. In the box displayed, the bottom was exchanged with a wider mesh, so that hatchlings can escape potential predation by the mother. **D** Plug of *O. eversi*. **E** Stages of embryo development in *O. dopingdopingensis*. **F** Freshly hatched larva of *O. dopingdopingensis*. Scale bars: 1 mm.

Abb. 2: Aufzucht und Zucht von Sulawesi-Reisfischen. **A** Bild eines geeigneten Aquariums für Sulawesi-Reisfische. Die dünne Schicht Substrat (mittelkörniger Kies), größere Steine und Pflanzen, bieten den Fischen einen semi-natürlichen Lebensraum mit Verstecken. **B** Zur Zucht von transferbrütenden Arten verwenden wir diesen sogenannten „Laichmopp", ein Bündel aus Acrylgarn, das Wasserpflanzen imitiert. Ein kleines Stück Kork oder Schaumstoff wird als Schwimmkörper angebracht, um ihn über Wasser zu halten. **C** Weibchen kleiner bauchbrütender Arten wie *O. eversi* sind weniger gestresst, wenn sie während der Brutzeit in einer „Box" getrennt gehalten werden. Dadurch wird auch die Prädation der sich entwickelnden Eier durch Artgenossen vermieden. Bei der abgebildeten Box wurde der Boden durch ein breiteres Netz ersetzt, damit die Jungtiere einer möglichen Prädation durch die Mutter entkommen können. **D** Plug von *O. eversi*. **E** Einige Stadien der Embryonalentwicklung in *O. dopingdopingensis*. **F** Frisch geschlüpfte Larve von *O. dopingdopingensis*. Maßstäbe: 1 mm.

arrangements of structural elements such as plants and free swimming space can play a critical role for the well-being of Sulawesi ricefish species. When structural elements are appropriate and well-placed, the fish will display a calm, self-assured and relaxed behavior, which is a hallmark of appropriate husbandry conditions. Likewise, as most ricefish species tend to form groups or loose swarms in nature, it is beneficial for their well-being to keep them in groups (≥ 5 individuals). While most species can be kept pairwise without problems, we have noted that swarm-forming open water species such as *O. nigrimas* tend to be particularly shy and stressed if they are not kept in larger groups (> 10 individuals). In the absence of a sufficiently large group of the respective species, the addition of swarm mates of other species will clearly reduce stress for swarm forming *Oryzias* species.

Most ricefish species come from warm waters, especially those that are endemic to the large ancient lakes of Sulawesi (Tab. 2), where water



temperatures reach > 30°C in some places. For these, we generally recommend a range between 25–30°C. For riverine species, recommended temperature ranges from 24–28°C. It should be considered good practice to avoid extended drops of temperature below 24°C. Some selected species, namely *O. eversi* and *O. kalimpaaensis*, occur in waters with temperatures as low as 20°C and can (but do not have to) be kept at temperatures below 24°C. Extended periods of water temperatures around 30°C, e.g. during heatwaves in summer, are generally tolerated well by all species kept so far by the authors. In most habitats on Sulawesi, pH ranges between 7 and 8.5, and clean, well-buffered (carbonate hardness > 4 °dKH; can be increased using crushed coral, aragonite sand or crushed shells in filter as substrate) and oxygenated (> 5 mg/l) freshwater does usually not require special treatment for keeping Sulawesi ricefishes. At least 50% of the water should be exchanged over the course of two weeks; in aquariums with very low stocking densities (> 10 L/individual) 25% may be sufficient. Also aggregating sludge should be removed on these occasions.

3. Feeding and regular care

Habitats of ricefishes in Sulawesi range from springs, streams and rivers, to small and large lakes (Hilgers & Schwarzer 2019). One species, *O. javanicus*, also inhabits brackish water estuaries. The Sulawesi ricefish radiation, which includes all Sulawesi species except *O. javanicus*, is considered in part adaptive (Möhring et al. 2025a), and different feeding ecologies are therefore to be expected. Data from the wild describing the trophic ecology of Sulawesi ricefishes is, however, very restricted. Rinandha et al. (2020) found that lake-dwelling *Oryzias* (incorrect species assignment to *O. matanensis*; this species is endemic to Lake Matano and does not occur at the Lake Towuti sites investigated) contained unspecified insects, as well as phytoplankton. Nursyahran et al. (2022) classified *O. profundicola* from Lake Towuti as omnivorous, based on dissections that revealed, among other contents, diatoms, cyanobacteria and insects. Kakioka et al. (2021) found that the *Oryzias* endemic to Lake Poso (*O. nebulosus*, *O. nigrimas*, *O. orthognathus*) differ in stable isotope signatures and concluded that they likely feed on different planktonic organisms, but did not further test this hypothesis. Feces of freshly caught *O. polylepis* contained remains of phytoplankton, whereas sympatric *O. kalimpaaensis* are apparently active predators foraging on larger items like shrimps and small fishes (Möhring et al. 2025b). Own preliminary observations, as well as so far unpublished gut content data (JM, FH), suggest that Lake Poso's endemic *Oryzias* feed on both zoo- and phytoplankton.

Feeding Sulawesi ricefishes in captivity: Sulawesi ricefishes are generally not very selective in food intake and comparatively easy to feed. Most species have a small mouth, with limited gape width, which restricts suitable food size (exceptions exist, e.g., *O. kalimpaaensis*). Feeding should always reflect the known feeding ecology of the respective species. The amount of food added to an aquarium should be limited to a point where all food is consumed rapidly, to prevent decaying food items from affecting water quality.

Suitable food types: Brine shrimp (*Artemia salina*) nauplii are very well suited for feeding nearly all ricefish species. *Artemia* eggs (cysts) are commercially available and hatch after ~24 h in salt water; they are small, pathogen-free, widely available and readily accepted (see Kinoshita et al. 2009 for protocols and further remarks). Commercial powdered, granulated or flaked foods are usually well accepted, provided the particle size matches the fishes' mouth size, which is especially important in smaller species and juveniles. Most ricefishes prefer floating or slowly sinking food items. Larger species also take frozen foods (e.g., mosquito larvae, adult *Artemia* and various life food such as *Daphnia*, Copepods, mosquito larvae, or *Tubifex* worms. As the benthic *Tubifex* are rather fatty, and may accumulate toxins, they should not be fed regularly.

Feeding management: Food quantities should be limited so that all food is eaten quickly, preventing decay and water quality deteriora-

tion. Adult ricefishes thrive on a diverse diet and should be fed once or twice daily; a weekly fasting day helps to avoid overfeeding. Planktivorous open-water species such as *O. nigrimas* are an exception: with their small mouths and low intake per feeding event, they benefit from several small meals per day. Regular visual checks of body condition and gut filling help to establish an appropriate feeding protocol.

4. Breeding and rearing

Sulawesi ricefishes are oviparous with external fertilization. The courtship display and the preferred time for mating varies between species (Sumarto et al. 2020; Firmansyah et al. 2021). During mating, male and female swim side by side and the male wraps his dorsal and anal fins around the female as she releases her eggs, which are then fertilized by the male (Ono & Uematsu 1957; Kakioka et al. 2021). Despite the presence of seasonal breeding patterns, ricefishes can be consistently bred in captivity (Iwasaki et al. 2009), when taking species-specific differences and reproductive strategy into account. Reproduction can be encouraged by simulating rainy conditions using water changes of >25% of the aquarium volume, ideally with slightly cooler water (2–3°C lower compared to previous water temperature in aquarium). As eggs and larvae are preyed upon by conspecifics, the chances of survival in a mixed tank are low. Conspecific larvae of similar ages can be kept together in the same tank.

In transfer brooding species (Tab. 2) a "spawning mop" placed in the tank as an artificial substrate for stripping the eggs off (Fig. 2B) has proven to work well. The collected eggs can be reared in three ways: (i) in a net spawning box placed inside the tank (Fig. 2C); (ii) in a separate container of water; or (iii) in "Embryo culture medium" (Kinoshita et al. 2009). To increase the hatching rate, we advise to check the eggs at least once a day and remove unfertilized eggs (with no perivitelline space), dead embryos and fungal eggs (cloudy looking egg surface) in order to prevent mold from spreading. In pelvic brooding species, the eggs remain attached to the female until the embryos hatch, on average between two to three weeks (Iwamatsu et al. 2007; Herder et al. 2012). Eggs cannot be removed without risking to injure the female, as a tissue (called "plug") anchors the eggs while brooding (Fig. 2D, Iwamatsu et al. 2008; Schüller et al. 2022). Separating brooding females in a modified net-spawning box (Fig. 2B) is beneficial for breeding smaller pelvic-brooding species, such as *O. eversi*. Here, the base of the net-spawning box has been replaced with larger-mesh fabric, allowing the hatched larvae to escape through the bottom of the box and develop in the aquarium. For larger pelvic brooding species, such as *O. sarasinorum* or *O. kalimpaaensis*, it is also possible to separate a female shortly before hatching of the eggs and collect the freshly hatched larvae from the water surface or remove the female from the tank shortly after the larvae hatched. Egg sizes range from 0.75 mm for *O. orthognathus* to 2.19 mm for *O. kalimpaaensis* (Kakioka et al. 2021; Gani et al. 2022). For *O. latipes*, it is also known that the number of eggs per spawning event varies (from eight to 48; Leaf et al. 2011). A characteristic of the ricefish egg is its transparency, which facilitates observation of embryonic development (Fig. 2E, Iwamatsu 2004; González-Doncel et al. 2005). Embryonic development varies from one to three weeks until hatching, depending on temperature and species. For instance, *O. woworae* hatches in as little as seven days at 27°C (Firmansyah et al. 2021), *O. dopingdopingensis* hatches after 11–13 days at 26°C (Mandagi et al. 2018) and hatching of embryos in *O. sarasinorum* occurs within 18–19 days at 25°C (Iwamatsu et al. 2007). In *O. latipes*, hatching success is influenced by rearing density (Rosemore & Welsh 2012).

Ricefish larvae (Fig. 2F) need to be fed soon after hatching (best within 24h, but not later than two days after hatching; Kinoshita et al. 2009; Bitter 2018). Mouth sizes are small in most species, thus food smaller than freshly hatched brine shrimp is required during the first days, such as live *Paramecium* or powdered dry food (Kinoshita et al. 2009). So-called "green water" (that is, cultures of microalgae containing a mixture of micro-organisms) is

successfully used for raising various fish species in aquaculture (Palmer et al. 2007), and is commonly used in commercial medaka breeding facilities. Green water can be produced by exposing pond or aquarium water, enriched with some fertilizer, to bright (sun)light. As an alternative, larvae can also be transferred to and raised in tanks where conditions permit growth of planktonic algae - that is slight aeration instead of filtration, strong light conditions (e.g., full-spectrum white LED), and (moderate) addition of fertilizer (e.g., F/2 fertilizer, Guillard 1975 or any type of phosphate-rich fertilizer). *Artemia nauplii* are taken after ca. five days in medaka, later in smaller species. For a few days, parallel feeding is recommended before switching to mainly *Artemia*. Consider that *Artemia* die in freshwater after a few hours. Depending on the type of food, young ricefishes are fed two times per day, or even more frequently. Food not consumed must be removed from the tank ~30 min after feeding.

5. Further remarks

In general, Sulawesi ricefish species are not highly susceptible to diseases or parasites in captive conditions. Daily checks should be conducted to ensure well-being and to detect early signs of stress or disease. We provide a blueprint for a care sheet in the appendix, which provides scores for the general observation of diseases and stress. It should be further adapted and refined based on the type and purpose of the husbandry. For euthanasia, standard protocols for medaka or zebrafish apply (e.g., following Mocho et al. 2025). An overdose of MS-222 is commonly used, but cold-water baths (also called chilled-water or ice baths, kept between 0 °C and 4 °C) could be another option for small tropical fish, including most Sulawesi ricefish species (see Mocho et al. 2025 & Bierbach et al. 2024 for a more detailed discussion of this topic). Please always ensure to adhere to legal recommendations of local authorities to decide which protocol to use.

Strategy for captive breeding: Access to wild ricefish strains can be limited. Reasons can be that natural populations are declining and/or limits by legislation. This necessitates not only successful breeding and the maintenance of stocks, but also a long-term strategy to prevent excessive inbreeding that can result in genetic degeneration of stocks. Consequences of inbreeding may include reduced viability, fertility or deviation from traits that are common in wild type populations. It has long been suggested that approaches like following a circular mating scheme fulfil the primary goals of a captive breeding program in that they preserve genetic diversity (Theodorou & Couvet 2010). However, the breeding history of most captive *Oryzias* stocks remains undocumented and is not controlled to date. We have successfully maintained closed aquarium stocks of *O. nigrimas* and *O. eversi* at the Max-Planck-Institute of Evolutionary Biology in Plön (stocks later moved to the University of Oldenburg) and at the Museum Koenig in Bonn since we described *O. eversi* (Herder et al. 2012). We have been keeping *O. sarasinorum*, *O. asinua*, *O. dopingdopingensis* and *O. nebulosus* for almost as long in our current facilities at the University of Oldenburg and the Museum Koenig. For *O. eversi* and *O. nigrimas*, this corresponds roughly to 12 generations of reproduction without the addition of any new breeding stock from the wild. We did not observe any indications for inbreeding depression in our stocks, despite the fact that we did not follow a controlled breeding scheme in the past. In the future, we will rely on a more systematic approach to preserve genetic diversity in captivity. This currently relies on the exchange of stocks between our facilities or with additional members of a captive breeding network as well as a circular mating scheme.

Stress, companion fish, enrichment and aquaria decoration: The decoration of aquaria with plants and substrates contributes to improve water quality, but its primary purpose with respect to fishes is to create a species-appropriate environment that facilitates natural behavior and reduces stress. Behavioral indicators for stress that should be continuously monitored include whether the fish display a calm and natural

swimming behavior, if they actively use most of the available space according to species-specific preferences, feed without hesitation and, finally, whether fish interact naturally and engage in reproductive behavior. Otherwise, particularly when the fish tend to panic, hide and do not move freely, the setup and decoration of the aquarium should be reevaluated. Unlike many benthic fishes, ricefishes rarely interact directly with the substrate (sand, gravel; but consider rock-spawning *O. nebulosus* as an exception). There is no general need for using substrate or natural plants in ricefish aquaria, though both can be beneficial, and should be selected with care for the respective species. Substrate and/or plants can contribute to create optimal conditions especially for species from spatially restricted environments. Both are less relevant in species dwelling in open waters such as *O. nigrimas*, *O. matanensis*, *O. sarasinorum* and *O. marmoratus*, species that prefer to swim in unobstructed space. Increased efforts for maintaining clean tanks and thus high water-quality, including efficient removal of food remains, can be arguments against using substrate under lab conditions. In this case, we recommend using a dark rather than a light or even transparent aquarium floor, to provide visible limitation of the aquarium space. Decoration with small plants that do not severely restrict swimming space or cover the surface is beneficial to create possibilities to retreat and to deposit eggs. Natural plants as spawning substrate, and also shelter, can however be replaced by artificial spawning mops, also in order to efficiently collect ricefish eggs (Fig. 2B).

For evaluating if the aquarium setting serves the needs of ricefishes, we recommend regular monitoring of their behavior. If indications for stress are recorded on a regular manner, several measures can be taken: (i) In case the aquarium is highly structured, rearrangement of plant decoration can be done to create more open space; (ii) brightly illumination can stress ricefishes; less light and/or shelter may help; (iii) substrate, preferably rather dark, or plant shelter may help calming down fishes; (iv) non-aggressive companion fishes such as *Poecilia reticulata* can be added to increase swarm size. Clearly, more research on the best suited companion fishes for *Oryzias* is warranted.

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Appendix 1: Score sheet for daily control of health condition.
Anhang 1: Bewertungsbogen für die tägliche Kontrolle des Gesundheitszustands.

Observe each tank once a day for one minute. Indicate with a SCORE 0 if none of the following symptoms are detectable. According to the classification of the symptoms (Score 1 or Score 2), give the corresponding tank, and the fish kept in it, the correct score. Document scores for your records.

Symptoms	**Health Score**
Small injuries (missing scales, small damages on skin, mouth or fins) visible in single fish	1
Substantial injuries (> 10% of body surface injured or fins torn or missing) in single fish	2
Permanently folded fins in single or some (few) fish	1
No appetite	1
ALL FISH gasp for air below the water surface (Aquatic Surface Respiration, ASR)	1
Behavioral abnormalities, such as restlessness (hectic movement) or circling, loss of motion control	2
Special disease symptoms	2

Instructions for Care Sheet:
Score 0: Feeding schedule and check-ups remain unchanged.
Score 1: Check the tank 3-4 times daily (each 1 min) and mark the tank with a red tape/indicate in the database. If necessary, proceed with an immediate water change. If no improvement after 48hrs is detectable, check for treatment or euthanize the affected fish.
Score 2: Treatment or if necessary, immediately euthanize single individuals painlessly, e.g. following recommendations for zebrafish or medaka from MOCHO et al. (2025).