Freshwater Teleost Taxon Advisory Group Best Practice Guidelines for liquorice gouramis, *Parosphromenus* spp.

Edition 1.0

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Cover image: *Parosphromenus deissneri*. Photo by K. Keibel.

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Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country.

Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species

Introduction

This Best Practice Guideline has been collated from the small amount of information available on the natural history of *Parosphromenus* species, largely the papers in which the species are originally described. The majority has come from the observations and experiences of rearing this genus in captivity.

Some key points to take away from this document for the successful maintenance of this genus in captivity:

- The necessity of appropriate water quality, and particularly water chemistry, requiring soft, acidic water.
- The importance of regular feeding of live invertebrate food items.
- The need for maintaining good genetic diversity due to the size of the captive gene pool, and stringent breeding records due to historic mislabelling of imported species.

Acknowledgement

We would like to extend our thanks to all the colleagues from the Parosphromenus Project and their dedication to rearing and managing this genus in captivity; their photo resources and the condensation of visible species differences have been invaluable to this document. Particular thanks go to Benjamin Wilden for reviewing this document, Helene Schoubye Johansen for reviewing and photographs, and to Wentian Shi for reviewing the document, provision of species photographs and his expertise on map alterations.

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Section 1: Biology and field data

1.1 Taxonomy

- Order – Anabantiformes
- Suborder - Anabantoidei
- Family – Osphronemidae
- Subfamily - Macropodusinae
- Genus – *Parosphromenus (BLEEKER, 1877)*

In recent years the number of *Parosphromenus* species has increased considerably to now 23 listed species (Shi *et al*., 2021), having increased twice even during the writing of this document. Equally, however, two species, *P. alfredi* and *P. barbarae*, are thought to have become extinct in the wild during this time (Shi, pers. comm., 2023). There are likely to be further taxonomic changes in the future, as there are a number of other forms which may be local variants of existing species, or potentially new sub-species or species (Parosphromenus Project, no date a), or species such as *P. ornaticauda* or *P. parvulus* that may be re-categorised due to their behavioural differences when compared to others in the genus (Parosphromenus Project, no date b).

Scientific Name	English Name	Distribution	Max. Length (mm)	Year
Parosphromenus alfredi		Johore, Malaysia	26 SL	2005
Parosphromenus allani		Sarawak, Borneo	35 TL	1987
Parosphromenus anjunganensis		Anjungan, Borneo	26 SL	1991
Parosphromenus barbarae		Sarawak, Borneo	25.7 SL	2020
Parosphromenus bintan		Bintan/Bangka, Indonesia	26 SL	1998
Parosphromenus deissneri	Liquorice gourami	Bangka/Belitung, Indonesia	30 SL	1859
Parosphromenus filamentosus	Spiketail gourami	Banjarmasin, Borneo	40 TL	1981
Parosphromenus gunawani		Jambi, Sumatra	28 SL	2012
Parosphromenus harveyi		Selangor, Malaysia	40 TL	1987
Parosphromenus juelinae		Bangka, Indonesia	32.3 SL	2021
Parosphromenus kishii		Kalimantan Tengah, Borneo	32.1 SL	2021
Parosphromenus linkei		Sukamara, Borneo	28 SL	1991
Parosphromenus nagyi		Kuantan, Malaysia	40 TL	1985
Parosphromenus opallios		Sukamara, Borneo	25 SL	2005
Parosphromenus ornaticauda		Anjungan, Borneo	18 SL	1991
Parosphromenus pahuensis		Muara Pahu, Borneo	22 SL	2005
Parosphromenus paludicola		Terengganu, Malaysia	37 TL	1952
Parosphromenus parvulus		Kalimantan Tengah, Borneo	27 TL	1979
Parosphromenus phoenicurus		Pekanbaru, Sumatra	29 SL	2012
Parosphromenus quindecim		Sungai Pawang, Borneo	29 SL	2005
Parosphromenus rubrimontis		Perak, Malaysia	25 SL	2005
Parosphromenus sumatranus		Jambi, Sumatra	23 SL	1955
Parosphromenus tweediei		Johore, Malaysia	25 SL	2005

Table 1: List of all current described species of the genus *Parosphromenus*
Information from Shi *et al.*, 2021; Tan and Jongkar, 2020; Fishbase, 2013 and Parosphromenus Project, no date c.

1.2 Morphology

1.2.1 Measurements and morphometrics

The weight of wild adult specimens is not known at this time, though body length is usually 25 – 40mm SL (Linke, 1991), however, there is variety within the genus, as shown in **Table 1**. In addition to genus variety, Kottelat and Ng (2005) noted the difficulty in replicating measurements given the small size of specimens, and as such are easily dehydrated or damaged. Therefore, such measurements often offer little useful diagnostic data, and instead male colour pattern is a more important parameter for species recognition. The colouration of females and juveniles is not useful for species recognition as most species share the same colour pattern and the colouration is also duller (Kottelat and Ng, 2005), as such, females and juveniles of most species are easily confused.

Fin formula also varies between species, though all fall in the range of dorsal fin formula XI-XIX, 4-8; anal fin formula VII-XIII. A further breakdown of the formula into species level can be seen in **Table 2**.

Species	Dorsal fin formula	Total	Anal fin formula	Total
P. alfredi	XII-XIV, 5-6	18-19	XI-XIII, 8-10	20-22
P. allani	XI-XIII, 5-6	16-19	XII-XIII, 8-11	21-22
P. anjunganensis	XII-XIII, 6-7	18-20	XI-XIV, 9-11	21-23
P. barbarae	XI-XIV, 4-5	15-18	XI-XIII, 6-10	19-21
P. bintan	XI-XIII, 5-7	17-20	XI-XIII, 8-10	19-22
P. deissneri	XII-XIII,6	18-19	XII-XIII, 8-10	21-23
P. filamentosus	XII-XIII, 6-7	18-20	XI-XII, 10	21-22
P. gunawani	XI-XIII, 5-8	18-19	XI-XII, 8-10	20-22
P. harveyi	XI-XIII, 5-7	17-18	XI-XIII, 8-11	20-22
P. juelinae	XII-XIV, 5-7	18-21	X-XII, 9-10	19-22
P. kishii	XIII-XIV, 6-8	20-22	XII-XIII, 9-10	22-23
P. linkei	XII-XIV, 7-8	19-22	XI-XIV, 10-12	22-24
P. nagyi	XI-XIII, 5-7	16-19	XI-XIII, 8-11	20-23
P. opallios	XI-XIII, 6-8	18-19	XI-XII, 10-11	21-23
P. ornaticauda	IX-XI, 6-7	16-17	VII-IX, 10-13	18-21
P. pahuensis	XII-XIV, 5-7	19-20	XIII-XIV, 6-9	20-22
P. paludicola	XVII-XIX, 5-7	22-25	XIII-XVI, 6-9	21-23
P. parvulus	X-XI, 5-6	15-17	VIII-IX, 10-11	18-20
P. phoenicurus	XI-XIII, 6-7	17-20	XI-XIII, 8-11	21-24
P. quindecim	XIII-XV, 6-7	19-21	XIII, 9-10	22-23
P. rubrimontis	XI-XII, 5-7	16-19	XIII-XIV, 6-8	19-21
P. sumatranus	XI-XII, 6-7	17-19	X-XII, 8-10	20-22
P. tweediei	X-XII, 6-7	17-19	X-XII, 10-13	21-23

Table 2: List of dorsal and anal fin formulae for *Parosphromenus* species
Information from Shi *et al.*, 2021; Tan and Jongkar, 2020; Schindler and Linke, 2012; Kottelat and Ng, 2005.

1.2.2 Colouration

The general colouration of males involves a background of pale yellow-brown, with longitudinal stripes and head markings of dark brown to black. Male *P. ornaticauda, P. nagyi* and to some extent, *P. parvulus* are particularly noteworthy for having a horizontal two-tone breeding colouration rather than the more obvious striping of other species (Shi *et al.*, 2021; Tan and Jongkar, 2020; Ng *et al*., 1994).

Exact colouration patterns vary among the 23 *Parosphromenus* species, and while an important indicator of species, other qualities such as the body shape and shape of the caudal fin should also be considered, as some species can otherwise be confused. **Table 3** describes the main differences between visibly similar species, and species photographs can be found in **Figures 1-4**.

Species	Similar to	Differences
P. alfredi	*P. rubrimontis*, *P. tweediei*	• *P. rubrimontis* has blue, black-green or black pelvic fins compared with light blue or white pelvic fins of *P. alfredi* • *P. tweediei* does not usually exhibit large blue patches and has a different dorsal fin structure
P. allani	*P. tweediei*	• *P. tweediei* has a more regular pattern on the caudal fin
P. anjunganensis	*P. barbarae*, *P. opallios*	• *P. barbarae* has black ocellus present at base of caudal fin • *P. opallios* has dark banding on caudal and dorsal fins
P. barbarae	*P. anjunganensis*, *P. opallios*	• *P. anjunganensis* is missing the black ocellus on the caudal fin and does not have the blue pelvic fins of *P. barbarae* • *P. opallios* lacks the black caudal ocellus and usually exhibits light blue colouration on the anal fin compared with the full red of *P. barbarae*
P. bintan	*P. deissneri*, *P. gunawani*, *P. harveyi*	• *P. deissneri* have a more triangular or rhomboid caudal fin with a long black caudal filament (not visible in **Figure 4**) • *P. gunawani* are stouter in body as adults, and males have colourful parallel bands in unpaired fins, usually with more red-brown than *P. bintan* • *P. harveyi* usually lack any red colouration
P. deissneri	*All in older literature*	• *Parosphromenus* were once believed to be just one species, known as *P. deissneri*. As such, particularly older literature may be referring to other species when mentioning *P. deissneri* • *P. bintan* have rounded tails and continuous bands of colouration on unpaired fins
P. filamentosus		• Unlike many *Parosphromenus, P. filamentosus* has a clearly extended caudal filament, with red brown colouration to the inner portions and white-blue edges to the unpaired fins
P. gunawani	*P. bintan*	• *P. bintan* are usually not as stout in body as *P. gunawani* and male *P. gunawani* have colourful parallel bands on the unpaired fins
P. harveyi	*P. bintan*	• *P. harveyi* usually lacks any red portions in colouration. There is still the possibility of some confusion with variants within the Bintan complex
P. juelinae	*P. bintan*, *P. deissneri*	• *P. bintan* unpaired fin band colours are usually more continuous than *P. juelinae,* whilst *P. juelinae* usually has more obvious red colouration on the flanks • *P. deissneri* does not have the rounded caudal fin of *P. juelinae,* instead being more triangular or rhomboid with a filament. *P. juelinae* usually also has a smaller anal fin

P. kishii		• Unlike most other *Parosphromenus*, *P. kishii* has a rhomboid caudal fin with irregular turquoise patterning
P. linkei	*P. pahuensis*	• *P. linkei* has a large caudal fin with a filament as opposed to the rounded caudal of *P. pahuensis*
P. nagyi	*P. ornaticauda*, *P. parvulus*	• Breeding colouration is similar in that the lower half is blackish and the top paler, however the colouration differs with *P. nagyi* showing continuous blue bands on unpaired fins
P. opallios	*P. anjunganensis*, *P. barbarae*, *P. sumatranus*	• *P. anjunganensis* lacks banding on the unpaired fins • *P. barbarae* has a clear black ocellus on the caudal fin • *P. sumatranus* has a more clearly defined black ocellus on the rear portion of the dorsal fin, while the marks *P. opallios* does not usually present as a clear ocellus. *P. sumatranus* also exhibits head up courtship unlike *P. opallios*
P. ornaticauda	*P. nagyi*, *P. parvulus*	• *P. parvulus* has a similar 'lean' body shape, and horizontal split in breeding colouration, but *P. ornaticauda* has black unpaired fins with white spots and relatively broad white edges, as well as a characteristic red flame pattern on the caudal fin
P. pahuensis	*P. linkei*	• *P. linkei* has a more lanceolate caudal fin with filament, opposed to a rounded caudal without in *P. pahuensis*
P. paludicola		• *P. paludicola* is more elongated than other *Parosphromenus*, which is also reflected in fin structure. *P. paludicola* has long filaments on ventral fins, while caudal fins may be oval or triangular and have several filaments, or more rounded with just one longer filament ("Wakaf Tapei" local form which may be a different sub/species)
P. parvulus	*P. nagyi*, *P. ornaticauda*	• *P. parvulus* exhibits the same horizontal split whilst in breeding colouration as the other species, but colour differs, the unpaired fins of *P. parvulus* are black with red spots
P. phoenicurus	*P. tweediei*	• *P. phoenicurus* has a rhomboid caudal compared with the rounded caudal of *P. tweediei*, and the inner markings on the caudal of *P. phoenicurus* are nearly triangular
P. quindecim		• *P. quindecim* is larger but more compact than other species, and colouration of the longitudinal stripes may be interrupted
P. rubrimontis	*P. alfredi*, *P. opallios*	• *P. alfredi* has light blue to white pelvic fins compared with blue, black-green or black pelvic fins of *P. rubrimontis* • *P. opallios* has paler pelvic fins that are less often as blue as *P. rubrimontis*
P. sumatranus	*P. opallios*, *P. ornaticauda*, *P. parvulus*	• *P. opallios* lacks the clear black ocellus at the rear end of the dorsal • *P. ornaticauda* does not have a monochrome caudal fin, and is only similar in lean body shape and head up courtship • *P. parvulus* does not have a monochrome caudal fin, and is only similar in lean body shape and head up courtship
P. tweediei	*P. alfredi*, *P. allani*, *P. phoenicurus*	• *P. alfredi* has a different dorsal fin structure, but individuals of *P. tweediei* exhibiting large blue areas can be easily confused with *P. alfredi* • *P. allani* has a less regular pattern on the caudal fin • *P. phoenicurus* has a rhomboid caudal compared with the rounded caudal of *P. tweediei*, and the inner markings on the caudal of *P. phoenicurus* are nearly triangular

Table 3: Main differences between visibly similar species of *Parosphromenus.*
Information from Parosphromenus Project, no date c.

1.2.3 Species images

Peninsular Malaysian species

Figure 1: *Parosphromenus* species found on mainland Malaysia. (A) *P. alfredi* (Photo credit: J. Schmidt), (B) *P. harveyi* (Photo credit: K. Weissenberg), (C) *P. nagyi* (Photo credit: S. Rick), (D) *P. paludicola* (Photo credit: W. Shi), (E) *P. rubrimontis* (Photo credit: S. Rick), (F) *P. tweediei* 'Sri Burinan' (Photo credit: L. Kent).

Bornean species

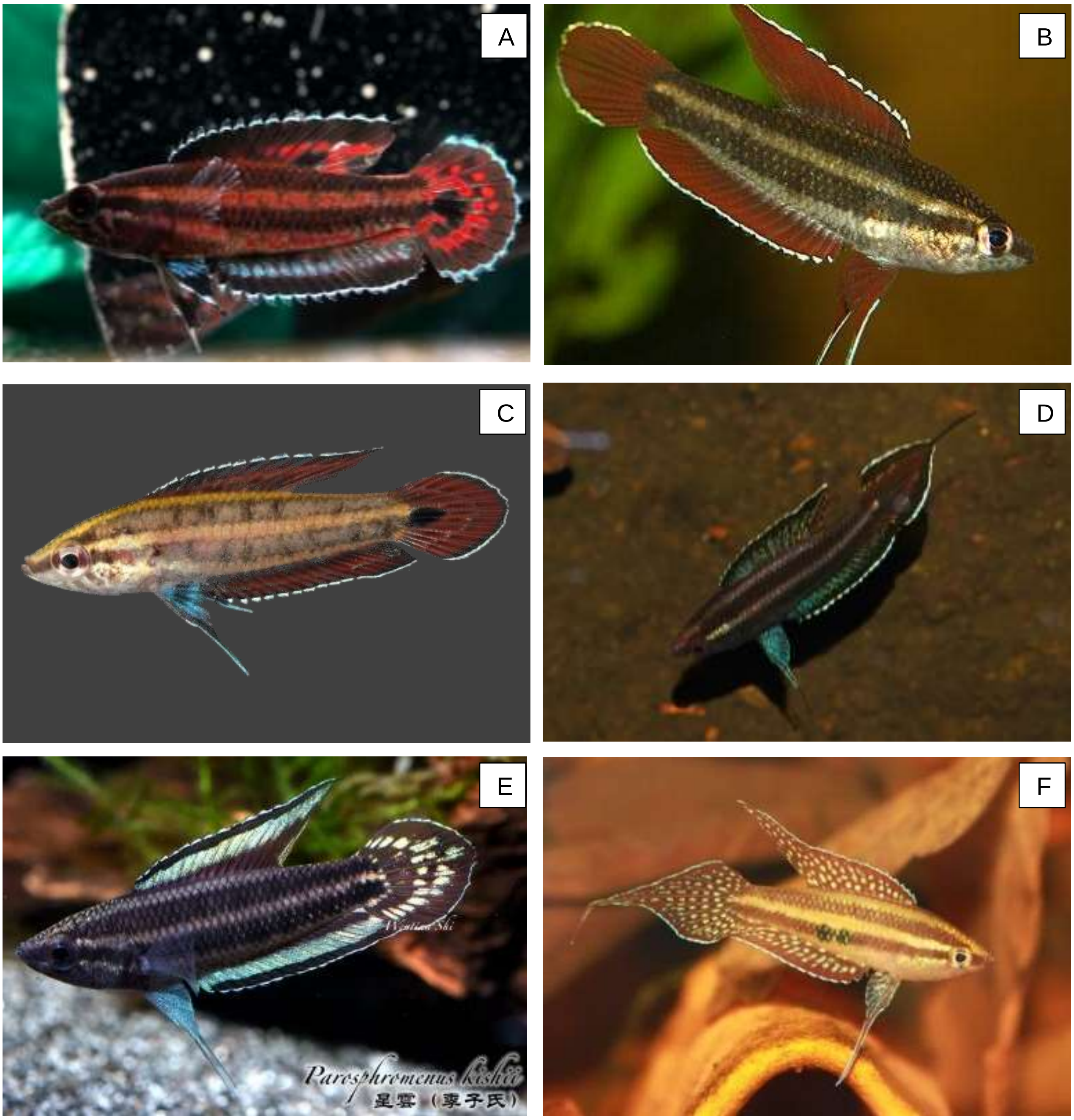

Figure 2: *Parosphromenus* species found on Borneo. (A) *P. allani* (Photo credit: W. Shi), (B) *P. anjunganensis* (Photo credit: A. Vuorela), (C) *P. barbarae* (Photo credit: from Tan and Jongkar, 2020), (D) *P. filamentosus* (Photo credit: H. Wimmer), (E) *P. kishii* (Photo credit: W. Shi), (F) *P. linkei* (Photo credit: C. Hinz).

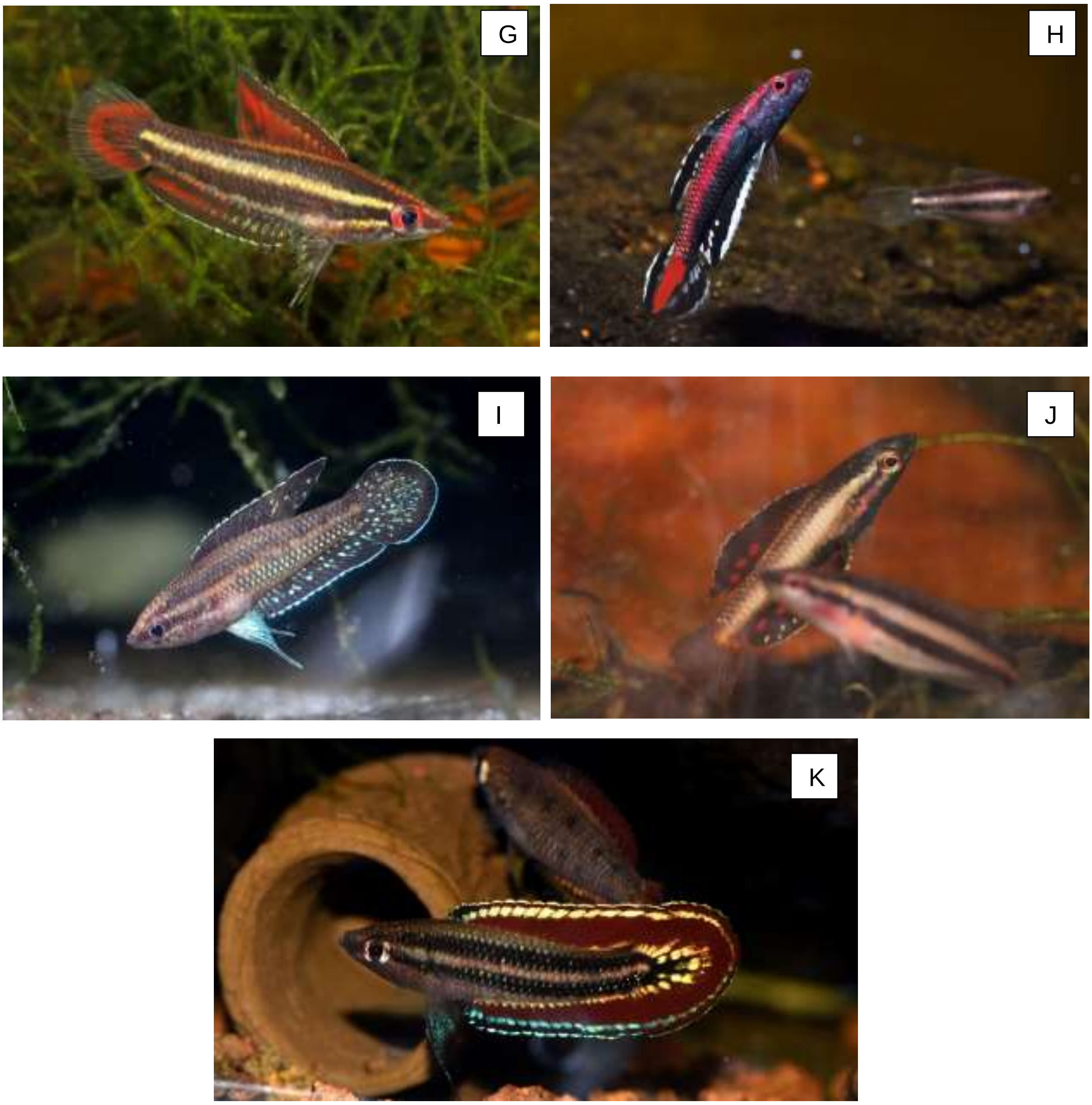

Figure 2 *cont*.: *Parosphromenus* species found on Borneo *continued*. (G) *P. opallios* (Photo credit: D. Jones), (H) *P. ornaticauda* (Photo credit: W. Shi), (I) *P. pahuensis* (Photo credit: W. Shi), (J) *P. parvulus* (Photo credit: H. Schoubye Johansen), (K) *P. quindecim* (Photo credit: W. Shi).

Sumatran species

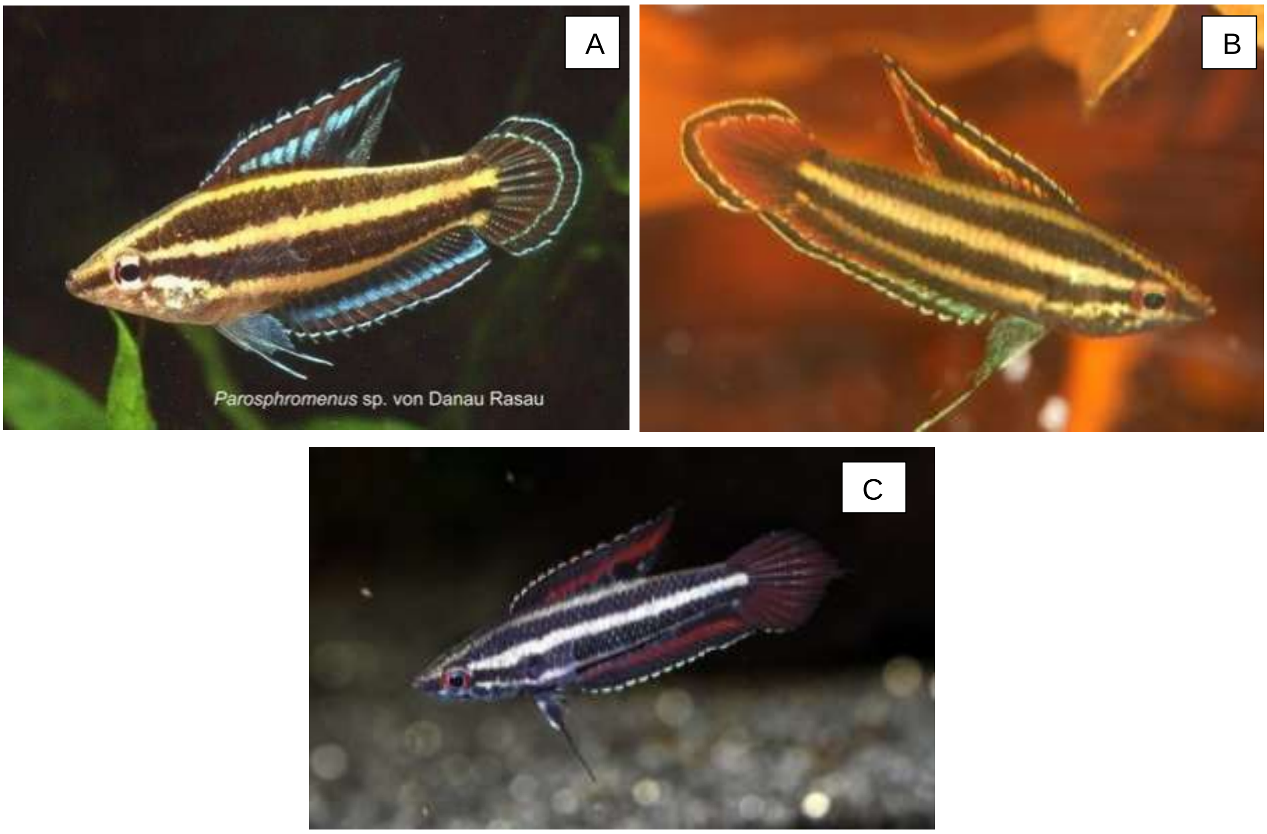

Figure 3: *Parosphromenus* species found on Sumatra. (A) *P. gunawani* (Photo credit: H. Linke), (B) *P. phoenicurus* (Photo credit: H. Schoubye Johansen), (C) *P. sumatranus* (Photo credit: W. Shi).

Bintan / Bangka / Belitung Island species

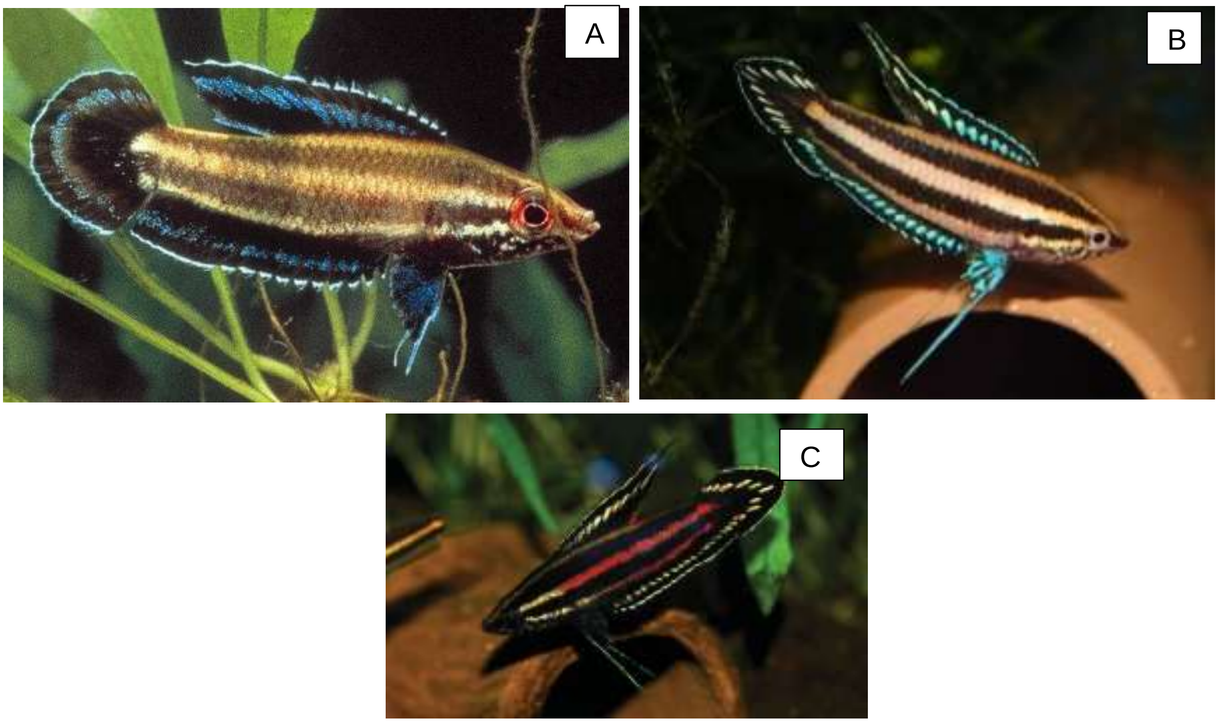

Figure 4: *Parosphromenus* species found on the islands of Bangka, Belitung and Bintan. (A) *P. bintan* (Photo credit: H. Linke), (B) *P. deissneri* (Photo credit: K. Keibel), (C) *P. juelinae* (Photo credit: W. Shi).

1.3 Physiology

Until recent years, *Parosphromenus* species had only been described from a limited number of specimens and were unavailable for osteological studies (Liem, 1963). As an anabantoid, *Parosphromenus* spp. possess a labyrinth organ allowing them to breathe atmospheric air, however, it is reported by Goldstein (2015) to be reduced to almost the point of absence. They are capable of using this organ to create bubble-nests underwater (Hubert *et al.,* 2015; Kopic, 2012; Rüber *et al*., 2006), but an experiment by Walter Foersch found that *Parosphromenus* spp. do not appear to require supplementary surface breathing under normal conditions as some other anabantoids do (Pinter, 1986; Parosphromenus Project, no date d).

1.4 Longevity

Longevity of wild specimens is unknown, but captive bred fish have been recorded to live in excess of nine years (Kopic, 2012), and remain fertile beyond their third year of life (Kopic, 2012; Pinter, 1986).

1.5 Conservation status, zoogeography & ecology

1.5.1 Distribution

Parosphromenus spp*.* are found in South-East Asia, specifically within the historic Sundaland area (Goldstein, 2015; Hubert *et al*., 2015), across Peninsular Malaysia, Sumatra, and Borneo, as well as the smaller Indonesian islands between these places. *P. paludicola* is currently the species that can be found the furthest north, with its range including southern Thailand.

Figure 5: General locality map of Parosphromenus species distribution. (Adapted from Parosphromenus Project, no date e).

At the time of writing, no *Parosphromenus* have been found in the northern reaches of Borneo, with no species found in Brunei or the Malaysian state of Sabah. *Parosphromenus* have only been found west of the Wallace Line (Goldstein, 2015; Schindler and Linke, 2012; Kottelat and Ng, 2005).

Figure 5 shows the general locality of *Parosphromenus* spp., while **Figures 6 – 8** show more detailed ranges for known *Parosphromenus* spp., with the exception of *P. kishii* whose extremely limited location is currently being withheld (Shi *et al.,* 2021). While much of this data is from IUCN, it has been updated with the aid of Wentian Shi (pers. comm., 2023). Climate data for nearby locations has also been included in **Tables 4 – 9**.

Peninsular Malaysia

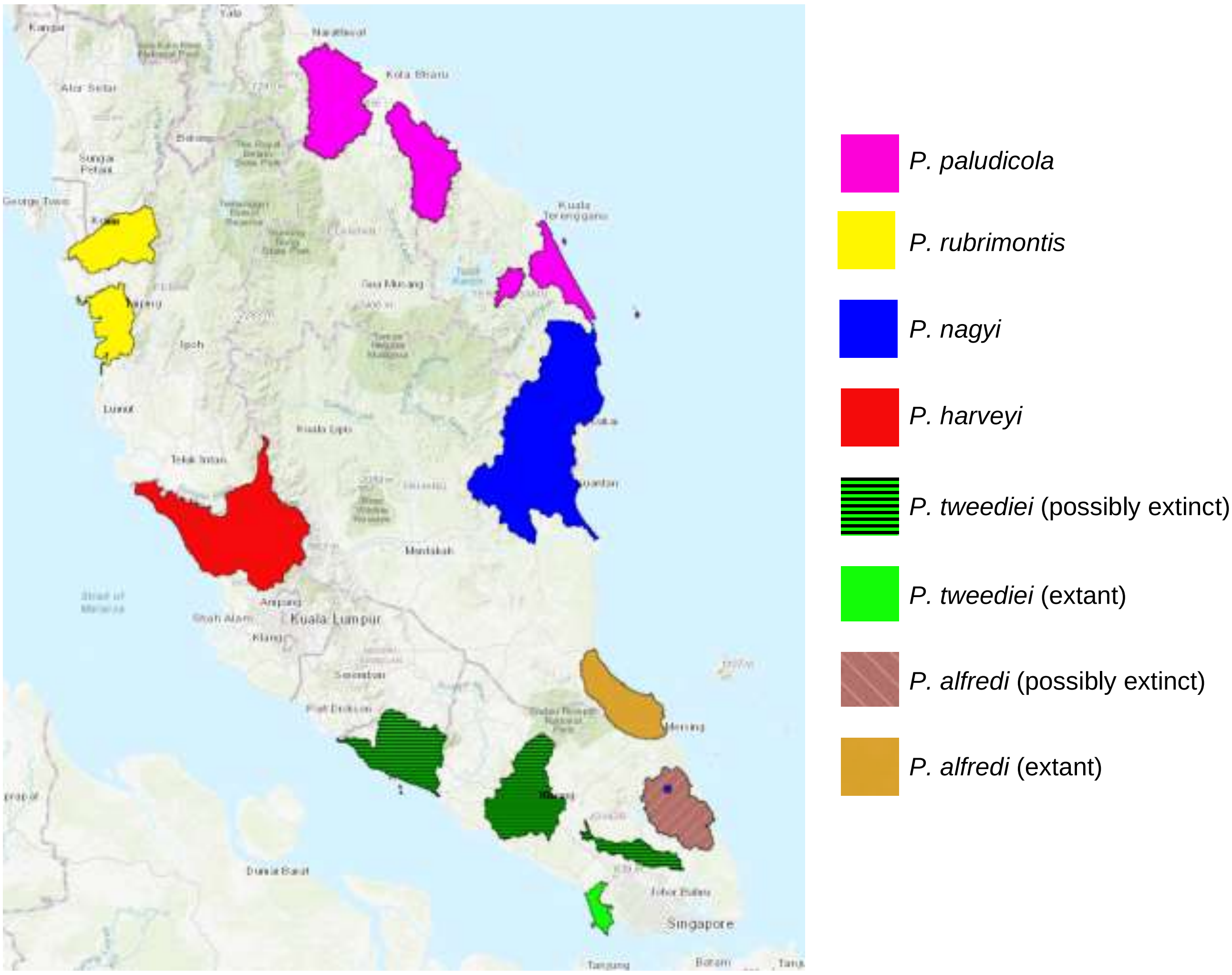

Figure 6: *Parosphromenus* spp. Ranges on Peninsular Malaysia.
(Adapted from IUCN RedList, 2023 with aid of Shi, pers. comm., 2023).

	January	February	March	April	May	June	July	August	September	October	November	December
Avg. Temperature °C (°F)	25.1 (77.2)	25.6 (78.2)	26.6 (79.8)	27.6 (81.6)	27.7 (81.9)	27.5 (81.5)	27.3 (81.1)	27.2 (81.0)	26.9 (80.4)	26.3 (79.3)	25.6 (78.2)	25.2 (77.4)
Min. Temperature °C (°F)	22.9 (73.3)	22.9 (73.3)	23.8 (74.9)	24.8 (76.7)	25.3 (77.5)	25.1 (77.1)	24.8 (76.6)	24.7 (76.4)	24.5 (76.0)	24.2 (75.5)	23.8 (74.8)	23.4 (74.1)
Max. Temperature °C (°F)	27.5 (81.6)	28.5 (83.4)	29.8 (85.7)	31.0 (87.8)	31.2 (88.2)	31.0 (87.8)	30.7 (87.3)	30.8 (87.4)	30.3 (86.6)	29.3 (84.8)	28.2 (82.8)	27.5 (81.5)
Precipitation / Rainfall mm (in)	171 (6)	87 (3)	120 (4)	108 (4)	128 (5)	116 (4)	118 (4)	126 (4)	146 (5)	271 (10)	415 (16)	445 (17)
Humidity (%)	85	82	82	81	82	83	83	82	84	87	89	88
Avg. Sun Hours (hours)	7.8	8.3	8.9	9.7	10.2	10.5	10.5	10.4	10.1	9.1	7.8	7.4

Table 4: Average weather by month in Kota Bharu.
Information from ClimateData.org, 2023.

	January	February	March	April	May	June	July	August	September	October	November	December
Avg. Temperature °C (°F)	25.4 (77.8)	25.9 (78.6)	26.4 (79.5)	26.7 (80.1)	27.0 (80.6)	26.9 (80.4)	26.6 (79.9)	26.5 (79.7)	26.5 (79.7)	26.4 (79.5)	25.9 (78.6)	25.6 (78.1)
Min. Temperature °C (°F)	23.6 (74.5)	23.7 (74.7)	24.1 (75.4)	24.6 (76.3)	25.0 (77.0)	24.9 (76.9)	24.7 (76.5)	24.6 (76.3)	24.5 (76.0)	24.3 (75.8)	24.1 (75.3)	23.9 (75.0)
Max. Temperature °C (°F)	28.3 (82.9)	29.1 (84.3)	29.7 (85.4)	29.6 (85.3)	29.5 (85.0)	29.2 (84.6)	28.8 (83.8)	28.7 (83.7)	28.9 (84.1)	29.1 (84.4)	28.8 (83.8)	28.4 (83.1)
Precipitation / Rainfall mm (in)	208 (8)	141 (5)	216 (8)	252 (9)	228 (8)	179 (7)	170 (6)	177 (6)	176 (6)	258 (10)	336 (13)	321 (12)
Humidity (%)	86	83	85	87	88	86	86	86	86	87	88	88
Avg. Sun Hours (hours)	7.8	8.1	8.0	7.8	7.9	8.3	8.2	8.2	8.3	8.2	7.7	7.8

Table 5: Average weather by month in Johor Bahru.
Information by ClimateData.org, 2023.

Sumatra and nearby islands

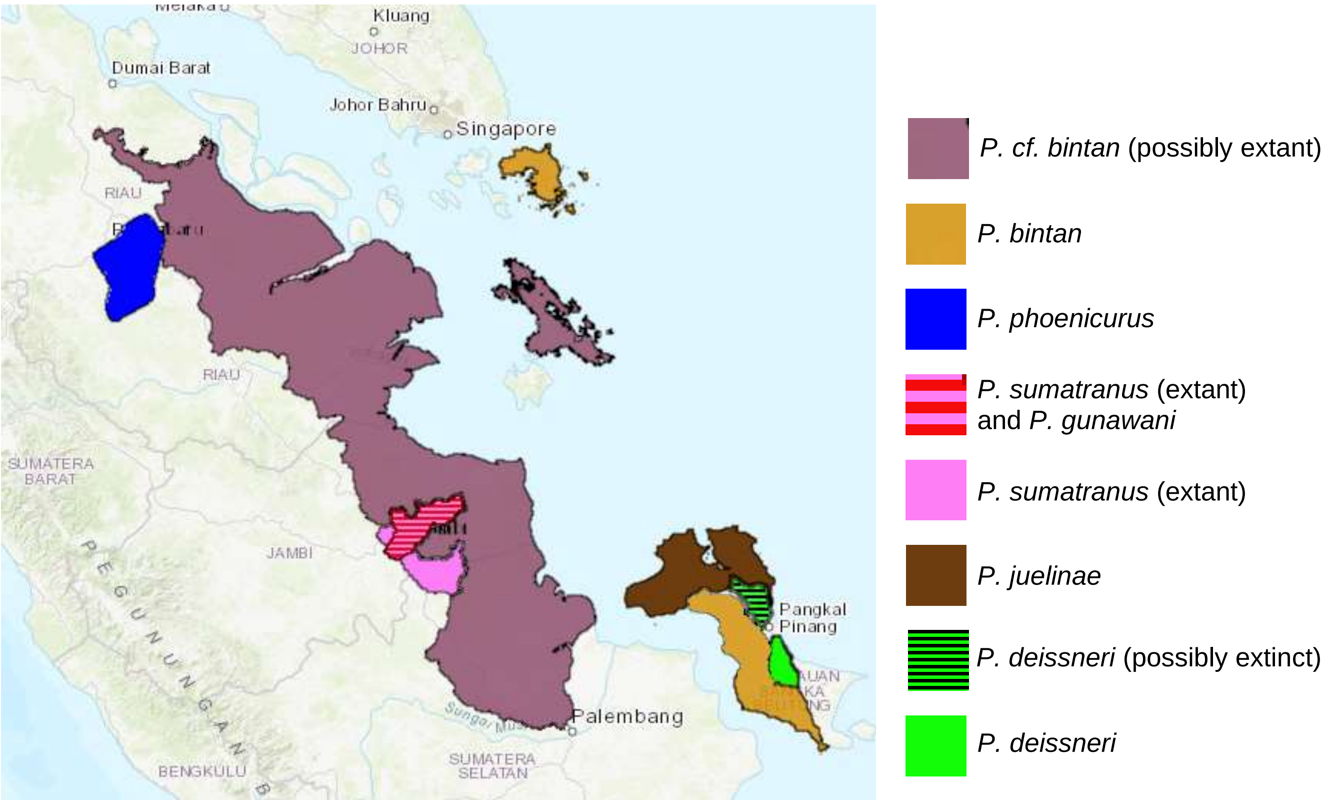

Figure 7: *Parosphromenus* spp. Ranges on Sumatra and nearby islands (Adapted from IUCN RedList, 2023 with the aid of Shi, pers. comm., 2023).

	January	February	March	April	May	June	July	August	September	October	November	December
Avg. Temperature °C (°F)	25.4 (77.7)	25.8 (78.4)	26.0 (78.8)	26.2 (79.1)	26.4 (79.5)	26.3 (79.3)	26.1 (79.0)	26.6 (79.8)	26.7 (80.1)	26.4 (79.5)	25.8 (78.4)	25.5 (77.9)
Min. Temperature °C (°F)	23.1 (73.5)	23.2 (73.7)	23.4 (74.2)	23.8 (74.8)	23.9 (75.1)	23.6 (74.5)	23.3 (73.9)	23.4 (74.2)	23.5 (74.3)	23.5 (74.4)	23.4 (74.2)	23.3 (74.0)
Max. Temperature °C (°F)	29.1 (84.4)	29.7 (85.5)	30.2 (86.3)	30.1 (86.2)	30.2 (86.3)	30.0 (86.1)	30.0 (86.0)	30.8 (87.5)	31.1 (88.0)	30.7 (87.3)	29.7 (85.4)	29.1 (84.4)
Precipitation / Rainfall mm (in)	246 (9)	208 (8)	278 (10)	280 (11)	201 (7)	125 (4)	127 (5)	125 (4)	139 (5)	242 (9)	304 (11)	302 (11)
Humidity (%)	88	86	88	90	89	87	84	80	80	84	89	89
Avg. Sun Hours (hours)	7.4	7.5	7.3	7.3	7.9	8.3	8.5	8.5	8.7	8.1	7.4	7.5

Table 6: Average weather by month for Jambi. Information from ClimateData.org, 2023.

Borneo

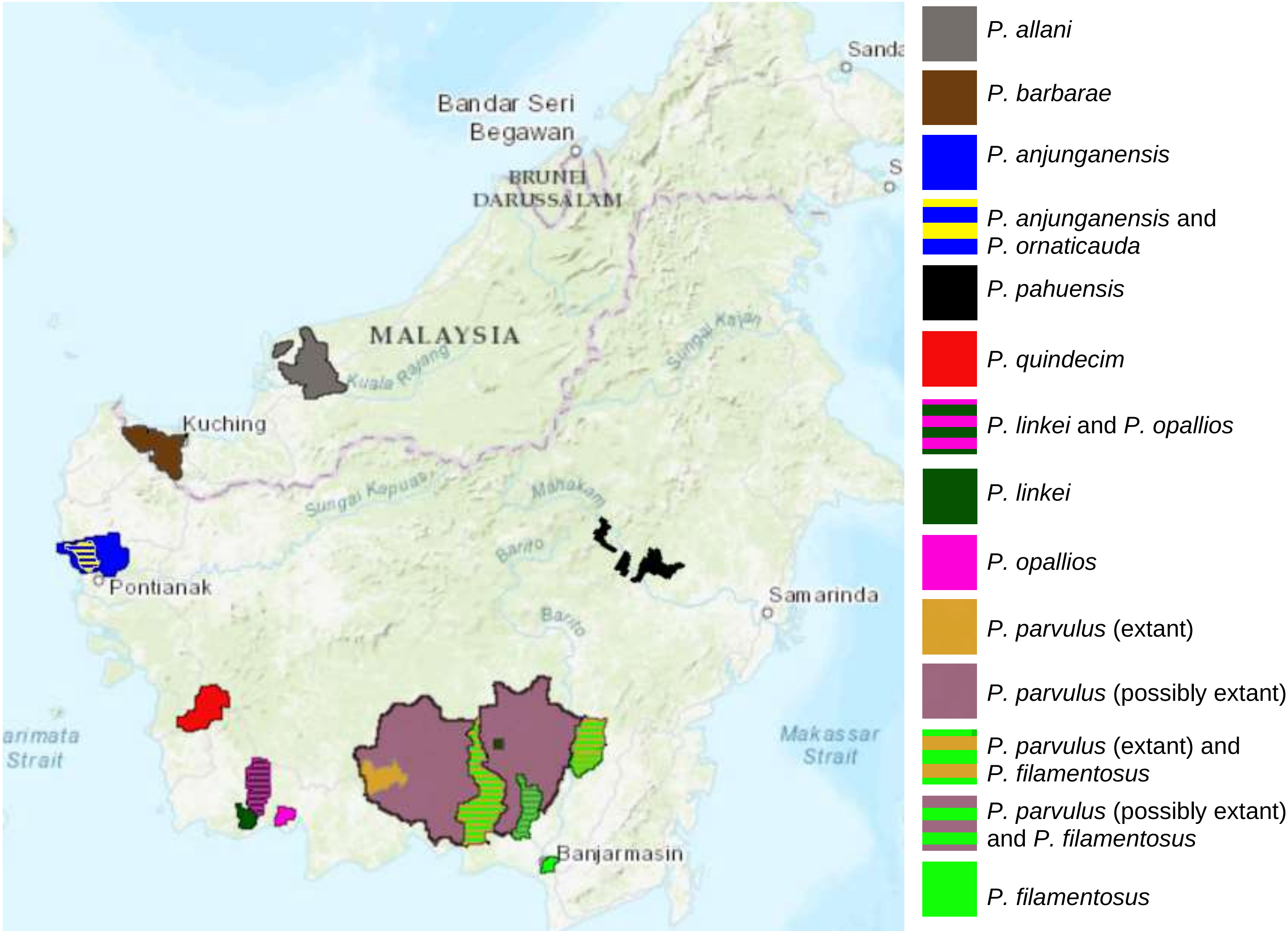

Figure 8: *Parosphromenus* spp. Ranges on Borneo, excluding *P. kishii.*
(Adapted from IUCN RedList, 2023 with aid of Shi, pers. comm., 2023).

	January	February	March	April	May	June	July	August	September	October	November	December
Avg. Temperature °C (°F)	25.2 (77.4)	25.5 (77.8)	25.9 (78.7)	26.3 (79.3)	26.6 (80.0)	26.5 (79.8)	26.4 (79.6)	26.6 (79.8)	26.2 (79.2)	25.9 (78.6)	25.6 (78.1)	25.4 (77.7)
Min. Temperature °C (°F)	23.3 (73.9)	23.4 (74.1)	23.7 (74.6)	24.0 (75.3)	24.3 (75.8)	24.1 (75.4)	23.8 (74.9)	23.8 (74.9)	23.8 (74.8)	23.7 (74.6)	23.6 (74.4)	23.4 (74.2)
Max. Temperature °C (°F)	28.3 (82.9)	28.5 (83.4)	29.3 (84.8)	29.9 (85.8)	30.1 (86.2)	30.0 (86.1)	30.1 (86.2)	30.4 (86.8)	30.0 (86.0)	29.6 (85.2)	29.1 (84.3)	28.6 (83.5)
Precipitation / Rainfall mm (in)	435 (17)	322 (12)	281 (11)	278 (10)	250 (9)	193 (7)	184 (7)	201 (7)	260 (10)	337 (13)	367 (14)	436 (17)
Humidity (%)	90	88	88	89	88	87	85	84	87	89	90	90
Avg. Sun Hours (hours)	5.6	5.8	6.8	7.4	7.9	8.5	8.7	8.7	8.0	7.7	7.1	6.4

Table 7: Average weather by month for Kuching.
Information from ClimateData.org, 2023.

	January	February	March	April	May	June	July	August	September	October	November	December
Avg. Temperature °C (°F)	25.6 (78.1)	25.8 (78.4)	25.8 (78.5)	26.0 (78.7)	26.1 (79.0)	25.8 (78.4)	25.7 (78.2)	25.9 (78.7)	26.2 (79.2)	26.2 (79.2)	25.9 (78.6)	25.8 (78.4)
Min. Temperature °C (°F)	23.2 (73.8)	23.3 (73.9)	23.3 (74.0)	23.5 (74.4)	23.7 (74.7)	23.5 (74.2)	23.2 (73.8)	23.3 (74.0)	23.4 (74.2)	23.5 (74.3)	23.4 (74.2)	23.4 (74.1)
Max. Temperature °C (°F)	29.6 (85.2)	29.8 (85.6)	29.9 (85.9)	30.0 (86.0)	29.9 (85.9)	29.5 (85.1)	29.4 (84.9)	29.9 (85.8)	30.5 (86.9)	30.5 (87)	30 (86)	29.8 (85.6)
Precipitation / Rainfall mm (in)	288 (11)	246 (9)	306 (12)	315 (12)	263 (10)	228 (8)	187 (7)	164 (6)	177 (6)	231 (9)	299 (11)	323 (12)
Humidity (%)	89	89	89	90	90	89	87	84	84	86	89	90
Avg. Sun Hours (hours)	7.1	7.3	7.1	6.8	6.9	7.1	7.2	7.5	7.6	7.3	6.9	7.1

Table 8: Average weather by month for Samarinda.
Information from ClimateData.org, 2023.

	January	February	March	April	May	June	July	August	September	October	November	December
Avg. Temperature °C (°F)	26.0 (78.9)	26.2 (79.2)	26.3 (79.4)	26.5 (79.7)	26.7 (80.1)	26.3 (79.4)	26.2 (79.1)	26.8 (80.2)	27.4 (81.3)	27.3 (81.1)	26.6 (79.8)	26.0 (78.9)
Min. Temperature °C (°F)	23.9 (74.9)	23.9 (75.0)	24.0 (75.2)	24.2 (75.5)	24.3 (75.7)	23.9 (74.9)	23.5 (74.3)	23.6 (74.5)	23.9 (75.1)	24.1 (75.4)	24.1 (75.4)	23.9 (75.1)
Max. Temperature °C (°F)	29.4 (84.8)	29.6 (85.3)	29.8 (85.6)	29.9 (85.8)	30.1 (86.2)	29.7 (85.4)	29.7 (85.4)	30.8 (87.5)	31.7 (89.1)	31.4 (88.6)	30.2 (86.3)	29.3 (84.7)
Precipitation / Rainfall mm (in)	299 (11)	255 (10)	286 (11)	240 (9)	154 (6)	119 (4)	92 (3)	57 (2)	71 (2)	160 (6)	258 (10)	342 (13)
Humidity (%)	87	86	87	87	85	84	81	74	73	77	85	88
Avg. Sun Hours (hours)	7.9	7.8	7.8	7.9	8.4	8.5	8.6	9.0	9.3	9.0	8.0	7.7

Table 9: Average weather by month for Banjarmasin.
Information from ClimateData.org, 2023.

1.5.2 Habitat

Parosphromenus are stenotopic inhabitants of acid blackwater habitats and slow flowing areas of peat swamp forests, with heavy riparian vegetation in which this genus almost exclusively resides (Shi *et al.,* 2021; Tan and Jongkar, 2020; Hallmann, 2012; Schindler and Linke, 2012; Brook *et al.,* 2003; Ng *et al.,* 1994). Vegetation can include *Utricularia* (Shi *et al*., 2021), *Macaranga* and *Pandanus* (Ng *et al*., 1994).

Figure 9: Parosphromenus habitats (A) *P. anjunganensis* habitat (Photo credit: A. Vuorela), (B) *P. paludicola* habitat (Photo credit: M. Illam Norhakim Lokman).

The water is usually clear, but coloured dark due to tannins from decomposing vegetation and the peat substrate (Shi et al., 2021; Tan and Jongkar, 2020; Beamish *et al.,* 2003). *Parosphromenus* are more often ranging around a pH value of 3.4 – 3.8 (Beamish *et al.,* 2003; Ng *et al.,* 1994), though have been found as low as 3 (Dennis and Aldhous, 2004; Ng *et al*., 1994), and as high as pH 6 (Zulkifle *et al*., 2021; Goldstein, 2015).

Water depth varies from less than 10 centimetres to over a metre (Schindler and Linke, 2012; Beamish *et al.,* 2003; Ng *et al.,* 1994), with temperature varying accordingly, measuring 29.3˚C in shallow areas and 25.2˚C in deeper waters (Schindler and Linke, 2012; Beamish *et al.,* 2003).

Air temperature can exceed 30˚C, and Hallmann (2012) reports that *Parosphromenus* spp. can die at “a little above 30˚C”, though in captive environments this genus has been kept and bred with water temperatures as high as 32˚C without observed detrimental effects (pers. obs.). At the very north of the range, air temperatures can get as low as 22.9˚C in January as an extreme minimum, but even for this region at this time of year the average temperature is usually 25.1˚C.

Figure 10: *Parosphromenus* habitats (A) *P. paludicola* habitat (Photo credit: C. Hinz), (B) *P. rubrimontis* biotope (Photo credit: C. Hinz), (C) Biotope of *P. harveyi,* (Photo credit: C. Hinz), (D) *P. spec. aff. allani* (Photo credit: C. Hinz).

Carbonates and bicarbonates are usually less than 1dKH, with low conductivity, measured at a maximum of 125±52µScm^{-1} even at roadside locations where detritus and pollutants may be an issue, and measuring 8 – 28µScm^{-1} in more favourable conditions (Goldstein, 2015; Schindler and Linke, 2012; Beamish *et al.,* 2003).

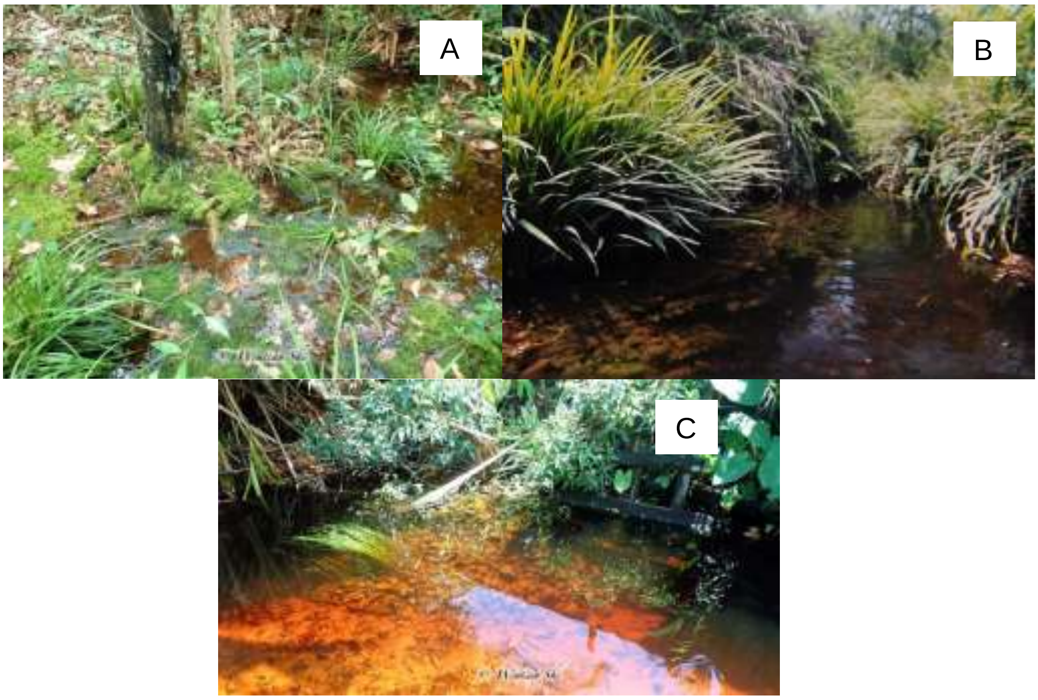

Figure 11: *Parosphromenus* habitats (A) *P. parvulus* habitat (Photo credit: W. Shi), (B) *P. bintan* biotope (Photo credit: H. Kishi), (C) *P. filamentosus* habitat (Photo credit: W. Shi).

1.5.3 Population

Of the 23 currently described species, population trend studies have so far only been carried out on *P. alfredi, P. ornaticauda,* and *P. tweediei,* but all of these populations appear to be decreasing (Low, 2019a, 2019b, 2019c; Shi *et al.*, 2021).

1.5.4 Conservation status

Parosphromenus are highly endemic, for example *P. kishii* is now only known in a single river flowing through an oil palm plantation, and this endemism leaves them very open to threat (Shi *et al.*, 2021).

Species	IUCN Red listing	Population trend
Parosphromenus alfredi	CR, *proposed EW*	Decreasing
Parosphromenus allani	VU	Unknown
Parosphromenus anjunganensis	EN	Unknown
Parosphromenus barbarae	Not listed, *proposed EW*	Unknown
Parosphromenus bintan	VU, *proposed EN*	Unknown
Parosphromenus deissneri	EN, *proposed CR*	Unknown
Parosphromenus filamentosus	EN, *proposed NT*	Unknown
Parosphromenus gunawani	CR	Unknown
Parosphromenus harveyi	EN	Unknown
Parosphromenus juelinae	Proposed CR	Unknown
Parosphromenus kishii	Proposed CR	Unknown
Parosphromenus linkei	EN, *proposed VU*	Unknown
Parosphromenus nagyi	VU	Unknown
Parosphromenus opallios	EN, *proposed VU*	Unknown
Parosphromenus ornaticauda	CR	Decreasing
Parosphromenus pahuensis	EN, *proposed NT*	Unknown
Parosphromenus paludicola	EN, *proposed VU*	Unknown
Parosphromenus parvulus	VU, *proposed LC*	Unknown
Parosphromenus phoenicurus	CR, *proposed EN*	Unknown
Parosphromenus quindecim	CR	Unknown
Parosphromenus rubrimontis	EN, *proposed CR*	Unknown
Parosphromenus sumatranus	NT, *proposed EN*	Unknown
Parosphromenus tweediei	EN, *proposed CR*	Decreasing

Table 10: Conservation listings of *Parosphromenus* spp. Text in italic indicates changes proposed by Shi (pers. comm., 2023) not yet in formal literature.
Information from Shi, pers. comm., 2023; Low, 2019a, 2019b, 2019c; Shi *et al*., 2021; Tan and Jongkar, 2020.

While overharvesting for the aquarium trade has been a perceived threat in the past, particularly for *P. tweediei* (Kottelat and Ng, 2005; Giam *et al*., 2012), it has been noted that sites where this species was recorded in the 1980's have since been reclaimed for agricultural, industrial and urban uses (Kottelat and Ng, 2005), and it is thought that the only extant populations are in the Pontian and Batu Pahat areas in the southern part of its range within oil palm plantations.

On Bangka Island, the island where *P. juelinae* is found, natural lowland habitats have been seriously affected by mining activities, oil palm plantations, and human settlement. During surveys between 2016 and 2019 dramatic habitat loss and degradation was noted (Shi *et al.,* 2021). This echoes other comments about habitat clearance and the destruction of swamp forest by Kottelat and Ng (2005), as well as oil palm plantations and construction of the Pan Borneo Highway rendering the habitat of *P. barbarae* unable to support species composition levels previously recorded.

Figure 12: (A) Destruction of *P. allani* habitat (Photo credit: L. Kent), (B) previous *P. harveyi* habitat (Photo credit: K. Weissenberg).

1.6 Diet and feeding behaviour

Studies are limited on the wild diets of *Parosphromenus* spp*.,* Linke (1991) notes a number of insects and invertebrate larvae as a vital food source in a description of *P. nagyi* habitat, while Hallmann (2012) recorded *Parosphromenus* spp*.* as feeding almost exclusively on juvenile shrimp.

There is also a 2017 study by Zainordin and Ab Hamid (2017) on trophic levels in rice fields which records *Parosphromenus deissneri* as top predators in the area feeding on *Chironomidae* larvae. While it seems likely that *Parosphromenus* spp*.* would feed on chironomid larvae, the validity of this study must be questioned as *P. deissneri* is not found in the studied region, and *Parosphromenus* spp*.* are not often found in rice paddies.

1.7 Reproduction

1.7.1 Age of sexual maturity

The age of sexual maturity in wild specimens is not known, but in captivity can be achieved around 9 – 12 months of age (Kopic, 2012).

1.7.2 Seasonality of cycling

Ng *et al.* (1994) found more male *P. harveyi* in full breeding colouration in August and September, theorising a higher incidence of this species, and others, breeding during periods of low water. Linke (1991) notes November monsoon rains inducing development of invertebrate life and the importance of these for growing young fish, and recording half-grown fish amongst vegetation in January.

1.7.3 Incubation

Parosphromenus spp. are bubble nesters that usually exhibit male parental care (Hubert *et al.,* 2015; Kopic, 2012; Rüber *et al*., 2006; Vierke, 1991; Pinter, 1986). Please see **Section 2.4** on captive management for further reproduction information.

1.7.4 Clutch size

The eggs of *Parosphromenus* spp. are similar to that of *Betta* spp., and differ from some other anabantoids as they have reduced oil globules and larval oil vesicles, a wrinkled egg surface, and wartlike attachment cells (Britz, 2001; Britz and Cambray, 2001; Gilch, 1957; Vierke, 1991; Vierke, 1975, all cited in Rüber *et al*., 2006). Information on wild clutch sizes is not known at this time.

1.7.5 Hatching details and season

From captive observations, eggs hatch within days (pers. obs.; Linke, 1991; Richter, 1988; Pinter, 1986), and it would seem likely that the highest rate of hatching is also likely to occur during low water periods, matching the breeding seasonality theorised by Ng *et al.* (1994).

1.8 Behaviour

1.8.1 Activity

Wild *Parosphromenus* spp. are found almost exclusively in the vegetation at the edges of the blackwaters and peat forest streams in which they reside (Shi *et al.,* 2021; Tan and Jongkar, 2020; Linke, 2012).

1.8.2 Locomotion

Parosphromenus spp. predominantly use paired fin propulsion but are also capable of carangiform swimming when disturbed, the use of posterior body and caudal fin to provide propulsion (pers. obs.).

1.8.3 Predation

While formal records for predation of *Parosphromenus* spp. are difficult to come across, there are a number of piscivorous species that are recorded in the same or similar habitats and it would not be unreasonable to expect *Parosphromenus* to form part of their diets. These include *Channa* spp., *Luciocephalus aura, L. pulcher,* and *Monopterus albus* (Shi *et al.,* 2021; Tan and Jongkar, 2020; Goldstein, 2015; Beamish *et al.,* 2003) as well as unidentified spiny eels (Linke, 1991). Kingfishers and other fish-eating birds are also present in the swamp environment, though it is theorised that the high colouration of the blackwater habitat may serve as protection (Beamish *et al*., 2003). It is also worth noting that the carnivorous plant, *Utricularia sp.,* has also been noted in the same habitat as *Parosphromenus* spp. (Shi *et al.,* 2021) which may be capable of feeding on young fry (Miranda *et al.,* 2021).

1.8.4 Social behaviour

No detailed field observations have been recorded of social behaviour of these species in the wild, though Hallmann (2012) notes that they are not found together *en masse*, though may be found in larger numbers where food and potential spawning sites are plentiful.

1.8.5 Sexual behaviour

Males will display to females through fin-spreading and the maximum expression of colouration, and may make passes above and below the female. Courtship may be expressed through head-up ‘standing’ in *P. ornaticauda, P. parvulus* and *P. sumatranus,* head-down courting in the *P. harveyi* group*,* and horizontal courtship position in other *Parosphromenus* species (Hallmann, 2012). Please see **Section 2.4** for further detailed breeding behaviours.

Section 2: Management in zoos and aquariums

The following section discusses the management of *Parosphromenus* spp. in captivity in more detail. For ease, a suggested equipment list is provided for the reader in **Appendix 3**.

2.1 Enclosure

2.1.1 Substrate

Linke (1991) and Richter (1988) advise to use bare-bottomed tanks to aid tank cleanliness; however, if a substrate is used, it must be one that is not going to negatively affect the water parameters of soft water with low pH that are needed. Although peat and peat pellets are sometimes used (Noga, 2010; Linke, 1991), this is not something that we recommend due to the negative environmental impacts. A dark tank base can sometimes seem to give the fish more sense of security, this could be by a vinyl or dark layer below the glass tank.

Although leaves can be suitable as substrate and hides, care should be taken as they can on occasions negatively affect water parameters. This is not always easy to anticipate as it can vary by species and also by the growing conditions of the plant. Leaves to purchase that are suitable include Indian almond (*Terminalia catappa*), cocoa (*Theobroma cacao*) and jackfruit (*Artocarpus heterophyllus*). Several suitable species can also be collected locally such as several oak species (*Quercus* spp.), beech (*Fagus* spp.) and European alder (*Alnus glutinosa*) of which the dried catkins can also be suitable.

2.1.2 Furnishings and maintenance

Tanks do not need to be heavily decorated or furnished, depending on the number of fish in a tank. Some hiding opportunities are recommended – but can be provided by just leaves on the bottom, plants such as Java moss (*Taxiphyllum barbieri)*, Java fern (*Leptochilus pteropus*) or artificial hides. In addition to the sense of security offered to dominant pairs, in group settings these provide some visual barriers for subordinate animals.

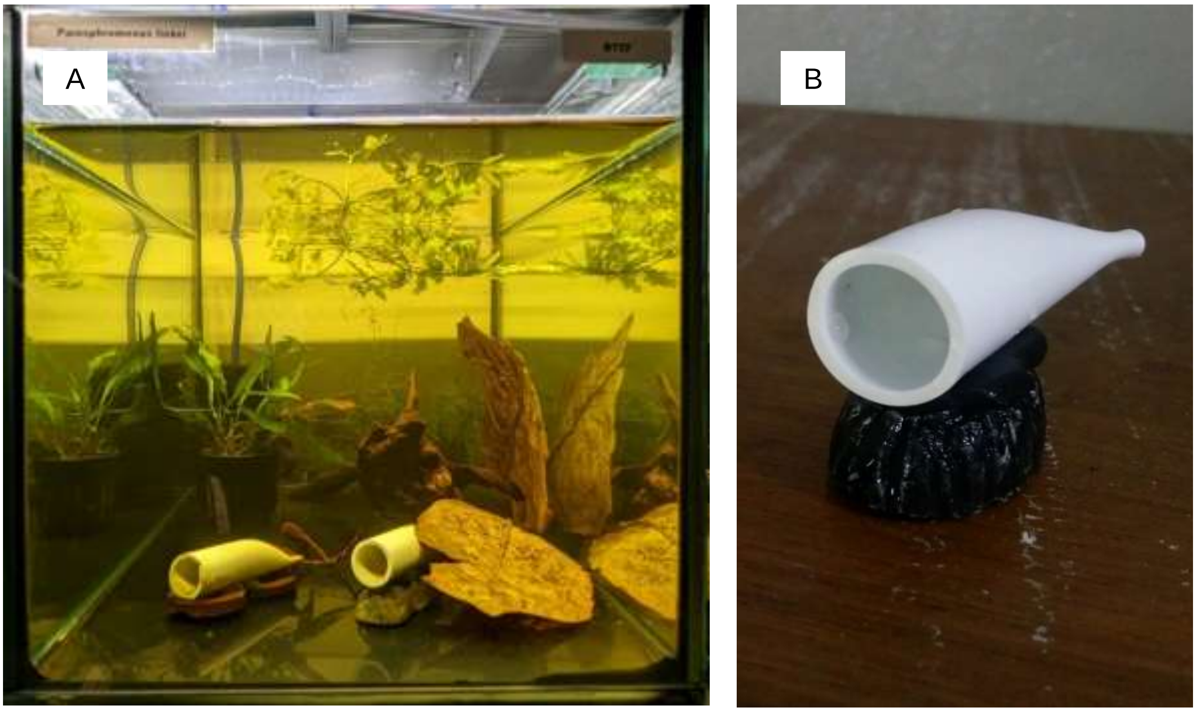

Figure 13: (A) A simple sparse breeding tank (Photo credit: J. Hutchins). (B) Example of a suitable spawning cave (Photo credit: B. Goodwin).

Figure 13 A shows an example of a simple, sparse breeding tank at Chester Zoo. Note the bare tank base as recommended by Linke (1991), artificial spawning caves, wood and planting for visual barriers and both Indian almond (*Terminalia catappa*) leaves and European alder (*Alnus glutinosa*) cones for provision of organic acids. There is an air driven filter in the back corner and the tank is usually fitted with a light obscuring lid – which was removed for the purposes of photography.

Caves or other suitable cover are important to males and necessary to induce spawning behaviour. They are not usually picky over the choice, so if a cave is placed where it is easy to see in and monitor, it will usually be used. The male will spend most of the time in or around the cave and will guard the territory, though defence of this is not very intense.

Suitable caves can be provided by small clay tubes, or any structure made of clay, wood, slate, plastic etc. The only stipulation is, as with substrate, it must be either inert or made of a material that will not negatively affect water parameters. **Figure 13 B** shows an example of a spawning tube used at Chester Zoo, this is PVC pipe glued with adhesive sealant, specifically Sikaflex, to a plastic plant weight, they are easy to clean and see inside, and from personal observation the lighter-coloured tubes are often favoured by *Parosphromenus* for spawning. Occasionally, though not often in captivity, the male will simply occupy a small space under a leaf if suitable.

Some plants which can survive in very low pH and soft water include Java moss (*Vesicularia/Taxiphyllum* spp.), Java fern (*Microsorum/ Leptochilus* spp.), and floating fern (*Salvinia* spp.).

Care with avoiding *Hydra* growth is necessary as they can cause irritation to adults and can catch and kill fry that are unable to move away (Mulla and Tsai, 1978; Clady and Ulrikson, 1968). The regular wiping of surfaces and sparse feeding of *Artemia nauplii* can assist in this.

2.1.3 Environment

The most important environmental aspect is correct water parameters. The tank water needs to be very low in dissolved compounds to replicate natural conditions with some organic acids from wood and leaves. Linke (2012) suggests that information on their natural habitat is indispensable in order to provide them with optimal living conditions in the aquarium.

The conductivity should be low; while values of 50 – 75µS/cm are reported by Linke (1991, p. 100-115), some of these are noted to be in periods of drought, and in the same report recordings for most species range between 6 – 39µS/cm, averaging 18µS/cm. Even including these higher readings, the average conductivity is 29µS/cm. This is best achieved by using good quality (<5µS/cm) reverse osmosis (RO) or de-ionised (DI) water, then adding leaves and wood which may raise conductivity slightly. Success can also be had with conductivity in the tank as low as 3µS/cm. Carbonate hardness (KH) and general hardness (GH) should be 0 on commercially available test kits. Low levels of both hardness and pH are very important, especially for successful reproduction as water hardness affects adhesion capacity of the eggs (Pinter, 1986, p. 106). This should be achieved by creating the correct overall conditions rather than the active addition of a strong acid. Conditions of pH suitable to species have been shown to prevent metabolic stress, aid utilisation of nutrients and digestion thereby increasing growth (Rebouças *et al*., 2015; Bolner and Baldisserto, 2007). The degree of sensitivity to pH varies between species. Species such as *P. parvulus* can be more specialist and require a low pH of around 4.5, while others, such as *P. linkei* and *P. paludicola*, can be less demanding surviving and breeding in water with a pH of up to 6. However, some institutions, e.g., Zoo Ostrava, have reported breeding of *P. linkei* at a pH above 7 and a conductivity of 220µS/cm (Rejlkova, pers. comm., 2023), though this differs significantly from the 9µS/cm reported in their native range (Linke, 1991, p. 115). It is noteworthy that that even though Zulkifle *et al.* (2021) found wild *P. tweediei* in waters with a pH of up to 5.9, their captive experiments reported significant increases in both growth rate and survival rate of *P. tweediei* fry when tested at lower pH values, between 4.5 – 6.0. This corresponds with the low pH of around 4 found by Kottelat and Ng (2005) in the natural range of *P. tweediei*.

DI, RO and rainwater have all been used in the past; however, rainwater should be used with caution as the chemical composition can change and is not consistent between areas (Whalley, 2018; Gould, 1985).

The necessity of using extra water filtration beyond just reverse osmosis will also depend on the location and the parameters of mains water; there will be many locations where this will be mandatory. The extent of this need will depend on the water supply. In some areas RO filtration alone will produce completely suitable water, some areas will need the use of de-ioniser after the RO unit. Areas with harder mains water may benefit from the use of a commercially available water softener prior to RO/DI filtration. The use of RO/DI water gives more consistent results but does not fully eliminate potential fluctuations caused by municipal supply.

Water quality is also important. Levels of phosphates and nitrogenous wastes should be kept as close to zero as possible. Large water changes can be useful to maintain correct water quality and chemistry because the high rate of feeding can quickly increase organic loading and change water quality.

Temperature should replicate the natural conditions of approximately 25 – 30˚C (Norhisyam *et al.,* 2012; Schindler & Linke, 2012; Beamish *et al.,* 2003), although opinion varies with some aquarists keeping water temperatures below 25˚C, especially for species such as *P. harveyi* (Parosphromenus Project, no date f). Wilden (pers. comm., 2023) reports extended lifespan with animals kept as low as 22˚C over the winter period, returning to 26˚C during summer months. It is, however, worth nothing that according to the site ClimateData.org (2023), air temperatures in Kuala Lumpur, near the range of *P. harveyi*, still average 25.1˚C in December, even though they can be as low as 22.3˚C or as high as 28.8˚C at this time. Temperature may also have an effect on sex determination, see **Section 2.4**.

Table 11 shows general recommended water parameters for *Parosphromenus* spp., however, any species-specific information mentioned in text should be considered to overrule this.

Parameter	Minimum Value	Optimum Value	Maximum Value
Temperature (˚C)	25*	28	30
Conductivity (µS/cm)	3	<39	75*
pH	4	4.5 – 6	6*
Ammonia (ppm)	0	0	0
Nitrite (ppm)	0	0	0
Nitrate (ppm)	0	0	10
Phosphate (ppm)	0	0	0.25
Carbonate hardness (dKH)	0	0	0
General hardness (dGH)	0	0	0

Table 11: Recommended water parameters for *Parosphromenus* species (*See notes in text) (Norhisyam et al., 2012; Schindler & Linke, 2012; Beamish et al., 2003; Linke, 1991).

It is suspected from personal observation of animals at Chester Zoo that, along with the rest of the *Osphronemidae*, *Parosphromenus* spp. can exhibit pale colouration with irregular pigmented patches when any conditions are not suitable or there are stressors present.

Figure 14: *Betta patoti* showing pigmented patches (Photo credit: B. Goodwin).

2.1.4 Dimensions

Aquariums of smaller sizes are most suitable for breeding purposes, housing one pair in one tank. A suitable volume is around 20 – 30 litres per pair. Fry should not be reared in tanks that are too big either, as this will make it difficult to maintain a suitable feeding density. The same sized tank is a good size for raising up to around 30 fry for several weeks. It is often easier to manage them by moving them carefully into larger tanks or splitting them into smaller groups as they grow rather than starting in a larger tank.

Larger groups of adults or older juveniles can be housed together in larger tanks providing there are enough available hides. They do not appear to show any aggression in groups, but it can become increasingly more difficult to monitor the population. Floor space is more important than height.

2.1.5 Environmental enrichment

If environmental conditions are met and a suitable selection of live food is offered, no further enrichment is needed.

2.2 Feeding

2.2.1 Basic diet

Parosphromenus species are well known for being difficult, or almost impossible to get to take any food except live food (Kopic, 2012). It can be possible to habituate young *Parosphromenus* to take some varieties of non-live food, but this is not very reliable and usually depends on the presence of other species which take the food. The ability to provide a range of suitable live foods should be considered essential. Caution must be used if feeding with non-live food: even if they take a little it is very unlikely that they will eat all available food; therefore, monitor the water quality carefully.

2.2.2 Special dietary requirements

Some suitable live food options, culture and harvest methods are found in **Appendix 1**. A staple diet used at Chester Zoo is a combination of grindal worm (*Enchytraeus buchholzi*) and microworm (*Panagrellus* spp.) that is suitable in size for the fish palatability, presence at the correct level of water column, ease of cultivation, protein level (see below), and longevity of the food item in an acidic environment and has little effect on the water parameters. Other invertebrate food items are offered on a supplementary basis including, but not limited to, young *Daphnia*, *Artemia* spp. (newly hatched nauplii to adult, depending on fish size), vinegar eel (*Anguillula aceti*), copepods including *Cyclops*, bloodworm and mosquito larvae. Live food will be fed and reared on a good diet (see **Appendix 1**) and, where possible, should be gut loaded prior to being fed out. Kopic (2012) states the preference of newly hatched *Artemia* for raising fry but states that other food organisms of suitable size should be added to the diet as soon as possible. Variety was shown to result in better growth and survival of *Betta splendens* fry where it is speculated that the availability of different sized food items allows fry to preferentially choose food items (Herath and Atapaththu, 2012).

The optimal dietary requirements are an area that would benefit from further research, especially for protein intake of *Parosphromenus* species and particularly for wild diet. In several species of *Osphromenidae* there were trends seen with growth rate, age at maturity and fecundity affected by dietary protein levels with a peak around the 35% mark, decreasing at higher levels and more steeply decreasing at lower levels. It was found that optimal levels of protein were 35% for *Betta splendens* (James and Sampeth, 2003), for *Trichopodus leerii* 26 – 36% (Degani & Gur 1992, then known as *Trichogaster leerii*), for *Trichogaster lalius* 35% (Shim *et al.,* 1989, then *Colisa lalia*) and 36.62% (Zuanon *et al.,* 2013) for *Trichopodus trichopterus* (Mohanta *et al.,* 2013, then *Trichogaster trichopterus*).

Table 12 shows protein levels of some commonly used food species. This may be worth noting when choosing the diet.

Species	Protein Content
***Panagrellus* spp.**	~40%
***Daphnia* sp.**	~50%
***Artemia* sp. – newly hatched to adult**	42.5% - 62.8%
***Moina* sp.**	<70%
Cyclops abyssorum divergens	36.6 - 40%

Table 12: Protein levels from dry weight in some of our commonly used food species.
Information from El-khodary *et al.,* 2020; Hoff and Snell, 1987; Helfrich, 1973; Ivleva, 1969.

2.2.3 Method of feeding

Where appropriate, live foods should be rinsed thoroughly, ideally with RO/DI water, with small feeds multiple times per day. Quantity needs to be judged on size and number of individuals; but, unlike many other species, having some food available at all times benefits the feeding behaviour of *Parosphromenus* sp. As with most fish, be wary of overfeeding as left over foods can spoil the water quality.

2.2.4 Water

Although the provision of water is paramount to aquatic species, it constitutes too much of their environment to be excluded from **Section 2.1.3.** Therefore, please refer back to that section for more details of water parameters.

2.3 Social structure

Parosphromenus species live in small congregations but are not shoaling fish. Hallmann (2012) states that they are never found in large groups in their natural environment but may occur in larger numbers in suitable conditions. The social structure centres on the males who will create small territories around their chosen spawning site. The male will stay in this area and attempt to attract females to join the site. Once a female has taken part in the spawning, she may stay around the cave, protecting it against other fish. Some females can act quite defensively, or aggressively towards other females or younger males. However, not all females will engage in this behaviour. Aggression between *Parosphromenus* is rarely fatal, the less dominant fish will retreat and often hide but will likely continue to feed. For this reason, however, it is best to remove conspecifics once a pair has formed.

2.3.1 Sharing enclosure with other species

Parosphromenus species can do well in larger tanks together with other species which are native to their natural biotopes. Larger, more voracious species can outcompete for resources, particularly food. Suitable species are *Boraras* spp., small wild bettas such as *Betta livida, B. uberis, B. hendra* and *B. persephone.* These *Betta* can live well together with *Parosphromenus* when the tank surroundings are spacious enough and well furnished with suitable hiding places for both species.
However, when actively attempting to breed *Parosphromenus*, it is easier to maintain them in species-specific tanks, allowing pairs to form naturally, and moving established pairs into their own small breeding tank. This prevents any intraspecific aggression or disturbance of the breeding pair, and makes managing the breeding process easier, as it can be much harder to monitor numbers and demographics in the population if a larger number is kept in a larger space.

2.3.2 Population management

While freshwater fish may differ from many conservation biology paradigms based on birds and mammals, as with any species kept for conservation value, we must take care not to actively select for or against any trait. Any individual with obvious deformity should not be used to breed, but otherwise keeping as much genetic variety as possible is beneficial (Coe *et al*., 2009).

This can apply to our choosing of pairs, for example letting them pair up naturally from a group or making an active effort to pair them randomly. When forming a group to move to another collection this is also important to bear in mind. Ideally, they should be randomly selected from within your group and care taken not to choose to retain any that you feel are aesthetically pleasing. Both of these can be useful tools to avoid line breeding your group and affecting the genetic variation and heterozygosity within your population (Coe *et al*., 2009). Preservation of the genetics should take precedence over preservation of phenotypes, as there is mounting evidence that adaptive phenotypes can recurrently evolve in freshwater fish, sometimes rapidly (Moritz *et al*., 2002), while heterozygosity and genetic diversity has been correlated with other measures of fitness (Kempenaers, 2007; Reed and Frankham, 2003). In particular, low heterozygosity has been linked with low resistance to parasites in the endangered rainbowfish *Melanotaenia eachamensis* (Moritz *et al*., 2002; Zhu *et al.,* 1998).

Considerations such as these are compounded due to the fact that often relatively small population sizes are held for each species at each location. For similar reasons, if new stock is brought in, especially if wild caught, try to get as many offspring from this founder population as possible before moving to reproduce the F1 or subsequent generations to aid the preservation of genetic diversity (Schönhuth *et al.,* 2003).

It is unlikely that numbers of *Parosphromenus* spp. held would reach excessive levels as the aims for the genus generally are to increase numbers and the locations that hold them. As such, euthanasia for population management is not currently a recommended course of action, instead following EAZA's disposition protocol for transferring animals to other collections. Should euthanasia prove necessary for health reasons, consult with your veterinary surgeon. However, an often-recommended method is the treatment with 2-phenoxyethanol (brand name AquaSed, www.vetark.co.uk/products/fish/aqua-sed) following the bottled instructions at 4x sedation rate.

If the population held was getting too high, the simplest method of population management is reproductive management. This is best achieved by disrupting eggs whilst in the bubble nest stage, or simply removing the eggs and destroying them via freezing (Lopez, pers. comm., 2023).

2.4 Breeding

For the initial selection of pairs, it is often easiest to keep animals in slightly larger groups of around 8 – 20 individuals, even before they can be sexed and allow pairs to form naturally.

There appears to be at least a degree of temperature-based sex determination (pers. obs.), with, it is believed, higher temperatures tending towards producing males. Although this has not been studied in *Parosphromenus*, Francis (1992) states that teleosts have sexual development that is more plastic than other taxa and Lowe and Larkin (1975) found temperature to affect sex differentiation in the related osphromenid *Betta splendens*.

Parosphromenus species are bubble nest breeders, though with less substantial nests than most other known bubble nest builders. The bubble nest will be built in a cave or overhang, which in the wild can be anything which provides a hidden, uninterrupted space. The male will choose and guard this area and is the one to build the nest. The bubble nests will be stuck to the upper surface of the cave and are relatively small, usually totalling only a few cm^2.

The number of eggs can vary significantly depending on individuals and species, from only a few eggs to around 150 in *P. nagyi* (Kopic, 2012), although the highest of these numbers is not frequent. Jaroensutasinee and Jaroensutasinee (2021) found in wild *Betta splendens* that bubble nest size was correlated to male body size but not to number of eggs, it is not clear if this is also true for *Parosphromenus*. They can consist of anything from a few small bubbles stuck to the surface to many bubbles stuck together and sometimes only a single larger bubble.

The male will build the nest and protect the cave and will provide the majority of parental care to the developing larvae. The eggs are kept clean and attached to the nest and the buoyancy of the nest is maintained. The male will guard the nest until the larvae have developed and the free-swimming fry have left the cave, there is no subsequent parental care after the fry have left the cave.

In an aquarium, breeding *Parosphromenus* species depends significantly on the water parameters (**Section 2.1.3**) as well as a suitable and varied diet (discussed above in **Section 2.2**). Once a pair has begun breeding, they will likely continue with high frequency and without many pauses, unless anything occurs that changes conditions.

Young and unexperienced *Parosphromenus* pairings can often be seen having trouble creating suitable bubble-nest, the eggs not being fertilised or viable eggs with poor adhesion that fall.

Figure 15: *P. linkei* with a nest under a leaf (Photo credit: R. Eggli).

2.4.1 Mating

Mature males will actively display to females by what is often called flashing behaviour. The male pursues a female, with courtship including contact to the body and displaying fins and colouration in an attempt to encourage her into the cave and the bubble nest. In displaying the fins to the female, a head down courtship position is often shown (**Figure 16**) predominantly by the males, but females can also show this stance to some extent. In three species, *P. sumatranus, P. ornaticauda* and *P. parvulus*, the stance is opposite, and the male will show a head up courtship position (Hallmann, 2012) (**Figure 17**). Fins are extended with the whole body visible and colouration exaggerated.

Figure 16: *P. linkei* male showing head down courtship position (Photo credit: H. Schoubye Johansen).

Figure 17: *P. parvulus* male showing head up courtship position (Photo credit: H. Schoubye Johansen).

When ready for spawning, the female will react to the male, slowly changing colours from normal beige-black stripes to a light brownish yellow uniform body colour. Some females will show a characteristic expression of the eyes with a broad black band running vertically through the pupil (**Figure 18**) which indicates imminent spawning. This is colloquially referred to as 'sexy eyes' and in most species some, but not all, males will express this. However, in *P. parvulus* and *P. ornaticauda*, neither sex ever exhibits 'sexy eyes', and unlike other species, males of both species will approach the female from above (Hallmann, 2012).

Figure 18: *P. nagyi* exhibiting eye expression indicating spawning (Photo credit: H. Schoubye Johansen).

2.4.2 Egg laying

After initial courtship the female will enter the cave and the pair will begin mating. The mating behaviour itself is fairly typical of other bubble nest spawning osphromenids. The male will surround the female when releasing the egg and the male will fertilise it. The egg sinks to the bottom of the cave then both male and female will pick the eggs up and attempt to place them in the bubble nest. The bubbles are coated in a mucous which allows the bubbles to remain and the eggs to stick onto the nest, possibly due to being glycoprotein rich as in *Betta splendens* (Kang & Lee, 2010).

Figure 19: *P. nagyi* showing egg laying (Photo credit: D. Jones).

2.4.3 Hatching

Figure 20: *P.* sp*.* newly hatched larvae hanging from cave ceiling (Photo credit: S. Kechek).

Development of eggs and larvae (**Figure 20**) will vary with temperature, from personal observation the eggs will hatch into larvae usually in under 24 hours post fertilisation but definitely with 48 hours (at 27 – 29˚C). Others report longer hatching periods and this could be just due to temperature differences. They will stay hanging from the bubble nest for several more days, from personal observation this is approximately 5 – 6 days post hatch. Development time will also vary depending on temperature, but the time frame is generally quite reliable for a known species and temperature (pers. obs.). The fry will then drop from the ceiling and begin to leave the cave, which may happen over some time. They are initially not strong swimmers and largely remain on the bottom of the tank. The free-swimming fry are small and fairly cryptic making them difficult to view in the aquarium, for this reason it is often more practical to remove the larvae prior to them becoming free swimming (see further details below).

2.4.4 Development and care of young

The male will stay in, or close to, the cave for the whole development of the larvae. In some cases, he will have initiated another spawn with the female and continue the occupation of the cave after the fry from the first spawn have developed to the point of dropping from the bubble nest.

The fry initially have a yolk sac and will not feed for around 1 – 2 days after becoming free swimming; however, it is a good idea to make sure there is food available from the time they leave the nest so it is available immediately when they are able to eat. If they are in a well-furnished tank with a lot of leaf litter, there is likely to be food available for the early days. Supplemental feeding of items such as *Paramaecium* spp. or microworm (*Panagrellus* spp.) is beneficial and required if in a less furnished tank. Microworm makes a good staple until they are able to take larger items, this is usually until they are around 6 – 8mm in length. Larger items such as grindal worm (*Enchytraeus buchholzi*) can then begin to be mixed in, with smaller items still offered. See **Section 2.2** for further feeding details.

Figure 21: *Parosphromenus harveyi* larvae at day (A) day 4 (B) day 24 (C) day 32 and (D) day 75 (Photo credit: Z. Zaccharia).

For *P. paludicola and P. linkei*, they reach approximately 12 – 15mm length including tail in around 4 months (pers. obs.), this is something that further details would be useful to assess growth rate as factors including pH, temperature and different diets can affect growth rate and final size such as in Zulkifle *et al.,* (2021).

Whilst the exact age to sexual maturity for most species is not currently recorded in wild populations, in captivity it has been observed to be reached in around 9 – 12 moths (Kopic, 2012).

Even within current limits of knowledge we have observed that it takes a relatively long time for *Parosphromenus* sp. to reach maturity when compared to similar species. For example, *Betta splendens* is reported to take 60 – 75 days (James & Sampath 2003) to around 140 days (Balasubramani & Pandian, 2008) post hatch to reach maturity. Diet and environmental factors are almost certain to affect growth rate as has been shown in many species (Balasubramani & Pandian 2008; James & Sampath 2003; Degani & Gur 1992). It is also likely there will be variations between species that affect growth rate, but there is currently not enough information on this.

As mentioned briefly in **Section 2.2**, optimal dietary requirements are not fully known, however, in Zulkifle *et al*., (2021) greatest TL of *P. tweediei* fry was recorded with a rearing diet of *Artemia* nauplii from days 6 – 27, *Moina* sp. from days 28 – 48, and grindal worm (*Enchytraeus buchholzi*) from days 49 onwards compared to diets of *Artemia* and *Moina* sp. alone, and the same diet with grindal worm instead introduced on day 44.

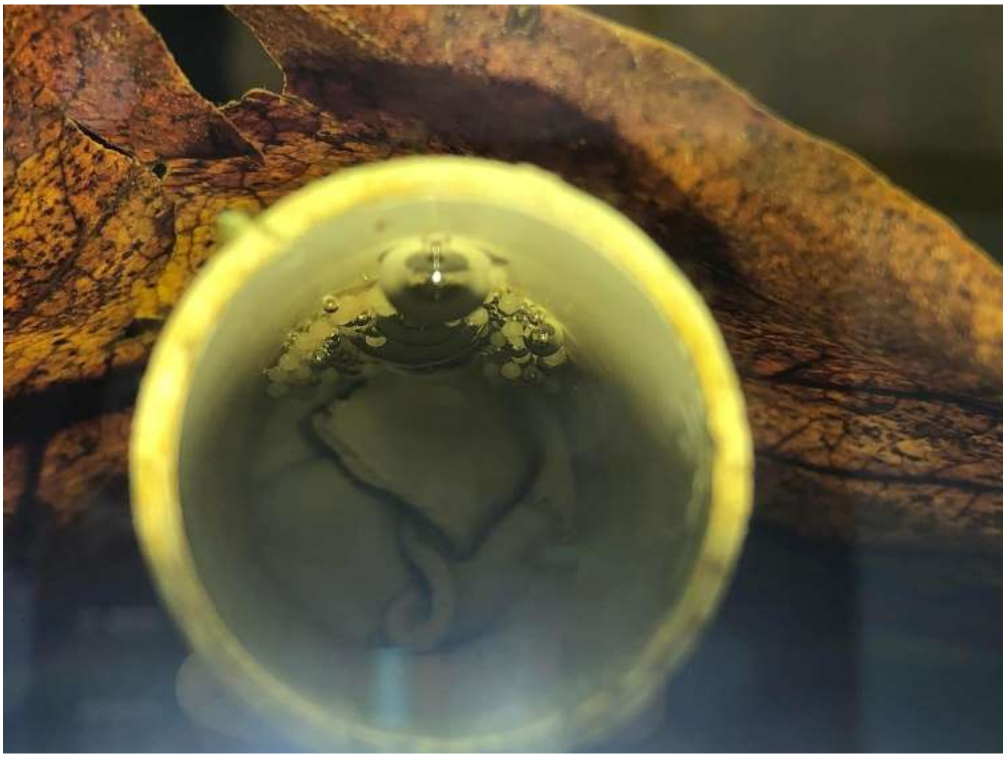

Figure 22: *P. paludicola* cave spawn (Photo credit: J. Crabtree).

Figure 23: *P. harveyi* larvae (Photo credit: Z. Zaccharia).

2.4.5 Artificial incubation and raising fry

For more intensive breeding it is possible to transfer the cave at the time just before the first larvae are dropping out of the nest. This provides the benefits of paternal care whilst in the egg, whilst still being able to move all larvae together relatively easily and before the risk of maternal predation increases (pers. obs.). The larvae can also be moved after this point but are then harder to keep confined.

The simplest way to achieve removing the cave is by lowering a small container into the breeding tank and transferring the cave into it underwater, and then placing it into a separate rearing tank. This tank can be filled with water from the original tank, but the larvae seem to be extremely tolerant to small fluctuations at this point and can do well with minimal acclimatisation if parameters are similar (pers. obs.). At 27 – 29°C the larvae will hatch within 24 hours. Approximately the fifth day post laying is the optimal time to move the cave, with larvae detaching around the seventh day; they are not quite swimming at this point but begin to leave the cave area (pers. obs.).

Instead of moving the nest cave to a new tank, the parents can be relocated instead for the same effect. Leaving fry with the adults is another viable option with no observed impact on fry survival but it can be harder to manage (Pinter, 1986).

2.5 Handling

2.5.1 Individual identification and sexing

Whilst juveniles are often difficult to sex, determining sex in adults are usually easier as females are duller in colouration. Markings may make the identification of different individuals possible but is not often practicable.

2.5.2 General handling

Handling is not typically required outside of reasons where catching would be occurring, and *Parosphromenus* will usually move away during tank maintenance, however, if desired it is also possible to first encourage the fish to one end of the tank with live foods rather than startling them from the other.

2.5.3 Catching and restraining

Due to their small size, *Parosphromenus* should be caught using very fine nets, or ideally, herded into a jug or other small container (**Figure 24 A**)

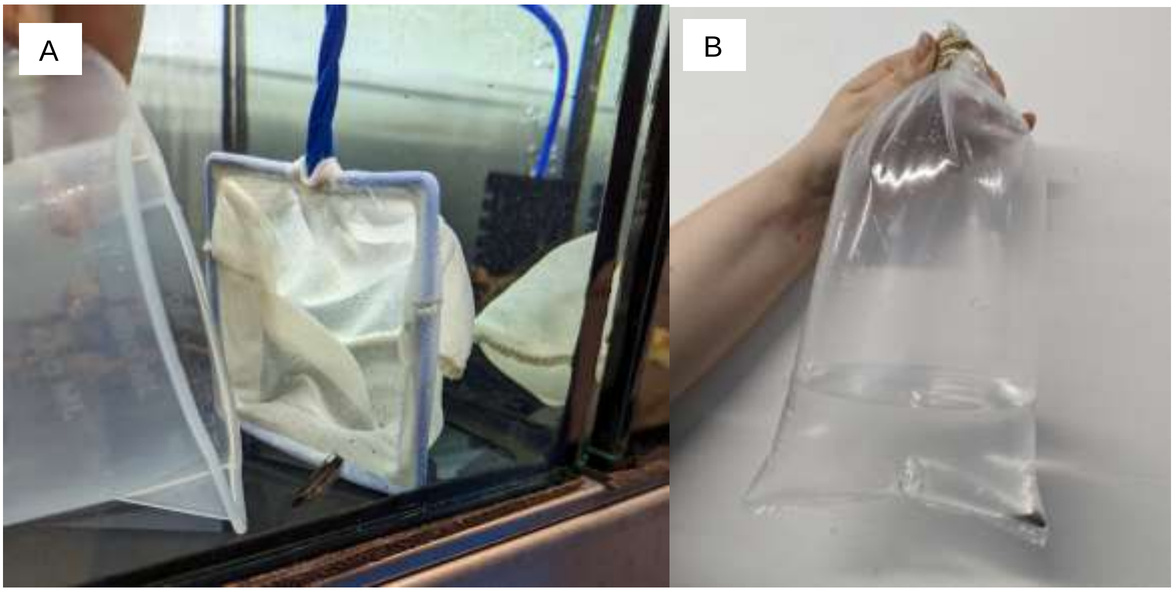

Figure 24: (A) Example of catching *P. opallios* (Photo credit: J. Chattell). (B) Example of suitable travelling bag (Photo credit: J. Chattell).

2.5.4 Transportation

The standard practice of securely bagging fish in a bag or container with one-quarter water and three-quarters atmospheric air is appropriate for the transport of this genus, with the atmospheric air being replaced with oxygen-rich air mixes for journeys approaching 24 hours, though this figure is a guide and depends on water volume and stocking density. Swann (1993) suggests 75% of the bag pure oxygen and 25% of the bag water, but this was giving the ability to transport far higher densities of fish than proposed here, i.e., up to 230g of fish per litre for fish the size of *Parosphromenus*. Using 100% oxygen allows the water to stay at or around saturation. The fish can be contained individually if the container is small, but if multiples are housed together, they can be transported in the same container if suitably sized. A suggested size for the container is a 2L bag, holding 500ml water for one pair of *Parosphromenus* (see **Figure 24 B**).

As with many tropical fish, care should be taken to keep the water temperature stable for the duration of the journey. This can be achieved by transporting the fish bags or containers in an insulated box, tightly packed with additional bags filled with warm water, and heat packs where appropriate, though care must be taken when using heat packs to avoid overheating. Care must be taken to avoid shocking the fish with too much light after transporting them in sealed, dark containers. Although unlikely due to the small size of the fish and relative body size to water volume, ammonia levels may have increased during transit. Testing this upon arrival is recommended and respond accordingly.

If there are concerns over ammonia rising during transport, ammonia-neutralising agents, such as Ammo-lock (https://apifishcare.com/product/ammo-lock), can be added to the transport water prior to shipping. However, this should not be necessary unless transporting for very long journeys, and it may be more appropriate managing this through suitable packing of fish in relatively sparsely stocked containers, as well as moderate underfeeding prior to transport, to both reduce and dilute any waste.

2.5.5 Safety

Care must be taken when handling *Parosphromenus* spp*.* as due to their small size it would be incredibly easy to do serious harm. The use of nets that are too coarse can cause damage to the fins or risk the fish getting stuck in the holes themselves.

Parosphromenus spp*.* do not directly pose a unique threat to human health, however, as with other fish species there is the risk of zoonoses, such as mycobacteriosis, (Gauthier, 2015) and good hygiene practices should be observed.

2.6 Specific problems: Considerations for health and welfare

Parosphromenus can be shy animals and appropriate hiding places and cover should be available in their tank to avoid unnecessary stress. To prevent animals jumping out of the tank when startled, the tanks should be provided with a well-fitting lid or a large gap between the water surface and the edge of the tank.

As previously mentioned in **Section 2.1.3**, *Parosphromenus* spp*.* are largely intolerant of dissolved minerals in their environment, and both water chemistry and water quality parameters should be strictly adhered to in order to maintain conditions conducive to animal health.

The general signs that may indicate poor fish health are as applicable to *Parosphromenus* spp*.* as they are to any other fish, including, but not limited to the symptoms described by Noga (2010) and Untergasser (1989);

- Excessive 'flashing' or scraping the body on substrate or tank décor
- Dorsal or lateral recumbence
- Increased respiration rate
- 'Clamped' fins held close to the body
- Abnormal swimming such as rapid spinning
- Variation in level of water column occupied – *Parosphromenus* spp*.* are usually occupying lower levels of the tank so animals near the surface should be monitored.
- Colour change not associated with breeding behaviour or acute stressors (e.g. transport)
    - Particularly darkening, or dark patches as with other Osphronemidae
    - Unusual reddening of the skin caused by inflammation
    - Bluish or whitish skin colour due to a thickening of the skin
- Visible lesions
- Loss of fin tissue
- Trauma to eyes or mouth, particularly exophthalmus
- Abdominal swelling (not associated with maturity in female fish)
    - Scale protrusion may also be noted in individuals with abdominal swelling
- Emaciation, evident through lack of dorsal muscle and concave abdomen
- Skeletal deformities, particularly scoliosis and lordosis

Some conditions that affect labyrinth fish include the parasites *Piscinoodinium pillulare* (Richter, 1988, then known as *Oodinium pillularis*) and *Ichthyobodo necator* (Wickins *et al.,* 2011) and *Mycobacterium* spp. and it is important to investigate and diagnose disease so that they can be responded to appropriately (Wickins *et al.,* 2011).

New acquisitions at Chester Zoo are subjected to a 30-day quarantine period with tight biosecurity procedures, using designated tanks and tools treated with Safe4 disinfectant (1:50) (www.safe4disinfectant.com) as appropriate. We do not, however, use any prophylactic treatments due to the sensitivity of these fish. Discuss this with your veterinary surgeon. An example of a post-mortem form for submission for necropsy or histology is included in **Appendix 2**.

2.7 Recommended research

Ecology

One area for further research is wild water parameters at different sites, and the same site across different seasons, which could aid aquarists in closer matching the wild environment and potentially allow manipulation of breeding seasons through the introduction of seasonality. However, the complexity of this task given the difficulty of site access during certain seasons, variation caused by human activity and the potential for environmentally induced shifts in behaviour (Linke, 2012) (e.g., the temperature of the water can vary with depth and it may be that the fish move to deeper water depths during periods of heat) cannot be understated.

Studies to confirm or refute temperature-related sex determination in *Parosphromenus* spp. may also prove informative for both management of populations in captivity, and awareness with regard to any threats that may be posed by temperature changes in their wild habitats.

Ex-situ conservation

As demonstrated by the Parosphromenus Project, hobby aquarists are a vital resource of experience in the maintenance and management of this genus, as well as being presumed to hold a larger reservoir of genetics than European zoos and aquariums. With limited genetic diversity currently in captivity, it would be beneficial to work with the Project and such hobbyists if a well–organised clear strategy can be agreed upon (Finke, 2012).

In-situ conservation

As habitat destruction and degradation are significant threats, habitat quality should be assessed in degraded areas. Though Kottelat and Ng (2005) found *P. tweediei* within oil palm plantations, it was also noted that areas of *P. barbarae* range that had been affected were unable to sustain the same species composition as prior to human activity, so methods of establishing how much degradation affects the carrying capacity of the habitat could aid in the management of these areas. This is important as discussion with Shi (pers. comm., 2023) suggests that *P. barbarae* may now be extinct in the wild.

Optimal Diet

While Zulkifle *et al*. (2021) trialled different feeding regimes on the growth of *P. tweediei*, it would be useful to know if optimal protein levels for *Parosphromenus* differed from the levels found in other osphromenids in the studies mentioned in **Section 2.2.2**. It may also be beneficial to have further information on wild diet preference and the nutritional value of these items.

Section 3: References

Balasubramani, A., and Pandian, T. J. (2008). Norethindrone ensures masculinization, normal growth and secondary sexual characteristics in the fighting fish, *Betta splendens*. *Current Science* [Internet] Vol. 95(10): 1446–1453. Available at: http://www.jstor.org/stable/24103095 [Accessed 15 February 2023].

Beamish, F. W. H., Beamish, R. B. and Lim, S. L.H. (2003). Fish assemblages and habitat in a Malaysian blackwater peat swamp. *Environmental Biology of Fishes.* Vol. 68: 1-13. Available at: https://link.springer.com/article/10.1023/A:1026004315978 [Accessed 12 April 2022.

Bolner, K.C.S. and Baldisserto, B. (2007). Water pH and urinary excretion in silver catfish, *Rhamdia quelen*. *Journal of Fish Biology*. [Internet] Vol. 70: 50-64. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1095-8649.2006.01253.x [Accessed 15 February 2023].

Brook, B. W., Sodhi, N. S. and Ng, P. K. L. (2003). Catastrophic extinctions follow deforestation in Singapore. *Nature.* [Internet] Vol. 424: 420-423. Available at: https://www.nature.com/articles/nature01795 [Accessed 12 April 2022].

Clady, M. D. and Ulrikson, G. U. (1968). Mortality of recently hatched Bluegill fry as a result of hydra. *The Progressive Fish Culturist.* Vol. 30(1): 39-40. Available at: https://www.tandfonline.com/doi/abs/10.1577/1548-8640%281968%2930%5B39%3AMORHBF%5D2.0.CO%3B2?journalCode=uzpf20 [Accessed 15 February 2023].

ClimateData.org. (2023). *Climate Data for Cities Worldwide.* [Internet] Available at: https://en.climate-data.org/ [Accessed 07 March 2023].

Coe, T. S., Hamilton, P. B., Griffiths, A. M., Hodgson, D. J., Wahab, M. A. and Tyler, C. R. (2009). Genetic variation in strains of zebrafish (Danio rerio) and the implications for ecotoxicology studies. *Ecotoxicology*. [Internet] Vol. 18: 144-150. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=d9b0be193af7c3985f3609e344f35b86a148d7bb [Accessed 15 April 2023].

Degaini, G., and Gur, N. (1992). Growth of juvenile *Trichogaster leerii* (Bleeker, 1852) on diets with various protein levels. *Aquaculture research* [Internet] Vol. 23(2): 161-166. Available at: https://doi.org/10.1111/j.1365-2109.1992.tb00606.x [Accessed 15 February 2023].

Dennis, C. and Aldhous, P. (2004). Biodiversity: A tragedy with many players. *Nature.* Vol. 430: 396-398.

El-khodary, G. M., Mona, M. M., El-sayed, H. S. and Ghoneim, A. Z. (2020). Phylogenetic identification and assessment of the nutritional value of different diets for a copepod species isolated from Eastern Harbor coastal region. *The Egyptian Journal of Aquatic Research.* [Internet]. Vol. 46(2): 173-180. Available at: https://www.sciencedirect.com/science/article/pii/S1687428520300315 [Accessed 06 April 2023].

Finke, P. (2012). The *Parosphromenus* Project: A global conservation network. *Amazonas*. [Internet] Vol 40: 44-49. Available at: https://www.parosphromenus-project.org/attachments/article/192/AMAZONAS%20Licorice%20Gouramis.pdf [Accessed 09 April 2022].

Fishbase. (2013). *Fish Identification: Find Species.* [Internet] Available at: https://www.fishbase.se/identification/SpeciesList.php?genus=Parosphromenus#result [Accessed 12 April 2022].

Francis, R. C. (1992). Sexual Lability in Teleosts: Developmental Factors. *The Quarterly Review of Biology*. [Internet] Vol. 67(1): 1–18. Available at: http://www.jstor.org/stable/2830890 [Accessed 15 February 2023].

Gauthier, D. T. (2015). Bacterial zoonoses of fishes: A review and appraisal of evidence for linkages between fish and human infections. *The Veterinary Journal.* [Internet] Vol. 203(1): 27-35. Available at: www.sciencedirect.com/science/article/abs/pii/S1090023314004419 [Accessed 17 February 2023].

Giam, X., Scheffers, B. R., Sodhi, N. S., Wilcove, D. S., Ceballos, G. and Ehrlich, P. R. (2012). Reservoirs of richness: least disturbed tropical forests are centres of undescribed species diversity. *Proceedings of the Royal Society B.* [Internet] Available at: https://royalsocietypublishing.org/doi/abs/10.1098/rspb.2011.0433 [Accessed 12 April 2022].

Goldstein, R. (2015). *The Betta Handbook.* New York: Barron's Educational Series, Inc.

Gould, R. (1985). *Going Sour: Science and Politics of Acid Rain*. Boston: Birkhäuser.

Hallmann, M. (2012). Why are Licorice Gouramis the way they are? *Amazonas*. [Internet] Vol 40: 36-42. Available at: https://www.parosphromenus-project.org/attachments/article/192/AMAZONAS%20Licorice%20Gouramis.pdf [Accessed 12 April 2022].

Heisig, G. (1977). Mass cultivation of *Daphnia pulex* in ponds: The effects of fertilization, aeration and harvest on the population development. In: Styczynska-Jurewicz, E. Backiel, T., Jaspers, E. and Persoone, G. *Cultivation of Fish Fry and Its Live Food.* European Mariculture Society Special Publication 4. pp. 335-359.

Helfrich, P. (1973). The feasibility of brine shrimp production on Christmas Island. *Sea Grant Technical Report UNIHI – Sea grant TR-73-02*: 1-173. Available at: https://repository.library.noaa.gov/view/noaa/40234 [Accessed 15 February 2023].

Herath, S. S. and Atapaththu, K. S. S. (2012). Effects of different live feeds on growth performance of fighter fish (*Betta splendens*) larvae. *The 2nd International Symposium.* [pdf] Available at: http://ir.lib.seu.ac.lk/bitstream/123456789/2844/1/2nd%20int%20sym%202012%20pp129-131.pdf [Accessed 15 April 2023].

Hoff, F. H and Snell, T. W. (1987). *Plankton culture manual*. Florida Aqua Farms, Inc., Florida.

Hubert, N., Kadarusman, Wibowo, A., Busson, F., Caruso, D., Sulandari, S., Nafiqoh, N., Pouyaud, L., Rüber, L., Avarre, J-C., Herder, F., Hanner, R., Keith, P. and Hadiaty, R. (2015). DNA Barcoding Indonesian freshwater fishes: challenges and prospects. *DNA Barcodes.* [Internet] Vol 3(1): 144-169. Available at: https://hal.archives-ouvertes.fr/hal-01958706/document [Accessed 09 April 2022].

Ivleva, I.V. (1973). Mass cultivation of invertebrates: biology and methods. *Israel program for scientific translations*. Vol. 601078.

James, R., and Sampath, K. (2003). Effect Of Animal And Plant Protein Diets On Growth And Fecundity In Ornamental Fish, Betta Splendens (Regan). *The Israeli Journal of Aquaculture – Bamidgeh*. Vol. 55(1): 39-52.

Jaroensutasinee, M. and Jaroensutasinee, K. (2001). Bubble nest habitat characteristics of wild Siamese fighting fish. *Journal of Fish Biology* Vol. 58: 1311–1319.

Kang, C.K. and Lee, T. H. (2010). The Pharyngeal Organ in the Buccal Cavity of the Male Siamese Fighting Fish, Betta splendens, Supplies Mucus for Building Bubble Nests. *Zoological Science*. [Internet] Vol. 27(11): 861-866. Available at: https://doi.org/10.2108/zsj.27.861 [Accessed 15 February 2023].

Kempanaers, B. (2007). Mate Choice and Genetic Quality: A Review of the Heterozygosity Theory. Advances in the Study of Behavior. [Internet] Vol. 37: 189-278. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0065345407370058 [Accessed 15 April 2023].

Kopic, G. (2012). Breeding licorice gouramis. *Amazonas*. [Internet] Vol 40: 36-42. Available at: https://www.parosphromenus-project.org/attachments/article/192/AMAZONAS%20Licorice%20Gouramis.pdf [Accessed 09 April 2022].

Kottelat, M. and Ng. P. K. L. (2005). Diagnoses of six new species of *Parosphromenus* (Teleostei: Osphronemidae) from Malay Peninsula and Borneo, with notes on other species. *Raffles Bulletin of Zoology.* [Internet] Vol. 13: 101-113. Available at: https://lkcnhm.nus.edu.sg/wp-content/uploads/sites/10/2021/01/S13_101-113.pdf [Accessed 12 April 2022].

Leger, P., Vanhaecke, P. and Sorgeloos, P. (1983). International study on Artemia. XXIV. Cold storage of *Artemia nauplii* from various geographical sources: Potentials and limits in aquaculture. *Aquacultural Engineering* [pdf] Vol. 2: 69-78. Available at: https://core.ac.uk/download/pdf/35103209.pdf [Accessed 15 February 2023].

Liem, K. (1963). *The Comparative Osteology and Phylogeny of the Anabantoidei (Telostei, Pisces).* Urbana: The University of Illinois Press.

Linke, H. (1991). *Labyrinth Fish. The Bubble-Nest-Builders.* Tetra-Press: Melle.

Linke, H. (2012). Traveling in Licorice Gourami biotopes. *Amazonas*. [Internet] Vol 40: 28-35. Available at: https://www.parosphromenus-project.org/attachments/article/192/AMAZONAS%20Licorice%20Gouramis.pdf [Accessed 09 April 2022].

Low, B.W. (2019a). *Parosphromenus alfredi*. *The IUCN Red List of Threatened Species* 2019: e.T89824307A89824311. [Internet] Available at: https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T89824307A89824311.en. [Accessed 10 November 2023].

Low, B.W. (2019b). *Parosphromenus ornaticauda*. *The IUCN Red List of Threatened Species* 2019: e.T91315657A91315674. [Internet] Available at: https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T91315657A91315674.en. [Accessed 10 November 2023].

Low, B.W. (2019c). *Parosphromenus tweediei*. *The IUCN Red List of Threatened Species* 2019: e.T91315953A91315962. [Internet] Available at: https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T91315953A91315962.en. [Accessed 10 November 2023].

Lowe, T. P. and Larkin, J. R. (1975). Sex reversal in *Betta splendens* Regan with emphasis on the problem of sex determination. *Reproductive Biology.* [Internet] Vol. 191 (1): 25-31. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/jez.1401910104 [Accessed 23 May 2022].

Lopez, J. (2023). Email to Joe Hutchins, 20 April.
Miranda, V. F. O., Silva, S. R., Reut, M. S., Dolsan, H., Stolarczyk, P., Rutishauser, R. and Płachno, B. J. (2021). A Historical Perspective of Bladderworts (*Ulticularia*): Traps, Carnivory and Body Architecture. *Plants.* [Internet] Vol. 10 (12): 2656. Available at: https://www.mdpi.com/2223-7747/10/12/2656/htm [Accessed 12 April 2022].

Mohanta, K.N., Subramanian, S. and Korikanthimath, V.S. (2013). Effect of dietary protein and lipid levels on growth, nutrient utilization and whole-body composition of blue gourami, *Trichogaster trichopterus* fingerlings. *Animal Physiology and Animal Nutrition*. [Internet] Vol. 97(1): 126-136. Available at: https://doi.org/10.1111/j.1439-0396.2011.01258.x [Accessed 15 February 2023].

Moritz, C., McGuigan, K. and Bernatchez, L. (2002). Conservation of freshwater fishes: integrating evolution and genetics with ecology. In: Collares-Pereira, M. J., Coelho, M. M. and Cowx, I. G. *Conservation of Freshwater Fishes: Options for the Future*. Oxford: Fishing News Books. pp. 293-310.

Mulla, M. S. and Tsai, S. C. (1978). Fish fry kill by hydra and planaria. *Mosquito News.* Vol. 38 (1): 43-47.

Ng, P. K. L. and Kottelat, M. (1998). *Osphronemus deissneri* Bleeker, 1859 (currently *Parosphromenus deissneri;* Osteichthyes, Perciformes): proposed replacement of holotype by a neotype. *Bulletin of Zoological Nomenclature.* [Internet] Vol. 55 (3): 155-158. Available at: https://ia802909.us.archive.org/6/items/biostor-2918/biostor-2918.pdf [Accessed 12 April 2022].

Ng. P. K. L., Tay, J. B. and Lim, K. K. P. (1994). Diversity and conservation of blackwater fishes in Peninsular Malaysia, particularly in the North Selangor peat swamp forest. *Hydrobiologia*. [Internet] Vol. 285: 203–218. Available at: https://link.springer.com/article/10.1007/BF00005667 [Accessed 12 April 2022].

Noga, E. J. (2010). *Fish Disease: Diagnosis and Treatment.* 2nd Edition*.* Wiley-Blackwell: Iowa.

Norhisyam, M. S., Roshani, O., Zuhairi, A. N. M., Akmal, S. N., Mohd Syahril, M. Z. Ainil Hawa, S., Wahidah, M. N. and Harmin, S. A. (2012). Water quality, diversity and distribution of blackwater fishes in selected locations of Raja Musa Peat Swamp Forest Reserve. *Conference: Malaysian Sciences and Technology Congress 2012.* [pdf] Available at: https://www.researchgate.net/profile/Roshani-Othman/publication/233918845_Water_Quality_Diversity_and_Distribution_of_Blackwater_Fishes_in_Selected_Locations_of_Raja_Musa_Peat_Swamp_Forest_Reserve/links/0912f50ceed151cafb000000/Water-Quality-Diversity-and-Distribution-of-Blackwater-Fishes-in-Selected-Locations-of-Raja-Musa-Peat-Swamp-Forest-Reserve.pdf [Accessed 15 February 2023].

Parosphromenus Project. (No Date a). *Other Forms.* [Internet] Available at: https://www.parosphromenus-project.org/en/species/other-forms#close [Accessed 12 April 2022].

Parosphromenus Project. (No Date b). *parvulus.* [Internet] Available at: https://www.parosphromenus-project.org/en/parvulus [Accessed 12 April 2022].

Parosphromenus Project. (No Date c). *Description of the 23 known species.* [Internet] Available at: https://www.parosphromenus-project.org/en/species/describtion-of-the-20-known-species [Accessed 28 February 2023].

Parosphromenus Project. (No Date d). *Genus.* [Internet] Available at: https://www.parosphromenus-project.org/en/the-genus-parosphromenus/genus [Accessed 12 April 2022].

Parosphromenus Project. (No Date e). *Species Map.* [Internet] Available at: https://www.parosphromenus-project.org/en/species/species-map [Accessed 12 April 2022].

Parosphromenus Project. (No Date f). *harveyi*. [Internet] Available at: https://www.parosphromenus-project.org/en/p-harveyi [Accessed 28 February 2023].

Pinter, H. (1986). *Labyrinth Fish*. Barron's Educational Series, Inc.: New York.

Rebouças, V. T., Lima, F. R. D. S., Cavalcante, D.D. H. and Sá, M. V. C. (2015). Tolerance of Nile tilapia juveniles to highly acidic rearing water. *Acta Scientiarum Animal Sciences.* Vol. 37 (3): 227-233. Available at: https://www.scielo.br/j/asas/a/VjKkQFRgYvD4wZKmzMZzSvQ/?format=pdf&lang=en [Accessed 15 February 2023].

Reed, D. H. and Frankham, R. (2003). Correlation between Fitness and Genetic Diversity. *Conservation Biology*. [Internet] Vol. 17(1): 230-237. Available at: https://www.academia.edu/download/45496104/Correlation_between_Fitness_and_Genetic_20160509-22272-u1khdr.pdf [Accessed 15 April 2023].

Rejlkova, M. (2023). Email to Hannah Thomas, 02 April.

Richter, H.-J. (1988). *Gouramis and other anabantoids.* T.F.H. Publications Limited: Maidenhead.

Rüber, L., Britz, R. and Zardoya, R. (2006). Molecular Phylogenetics and Evolutionary Diversification of Labyrinth Fishes (Perciformes: Anabantoidei). *Systematic Biology.* [Internet] Vol. 55 (3): 374-397. Available at: https://academic.oup.com/sysbio/article/55/3/374/1667762?login=true [Accessed 09 April 2022].

Schindler, I. and Linke, H. (2012). Two new species of the genus *Parosphromenus* (Teleostei: Osphronemidae) from Sumatra. *Vertebrate Zoology.* [Internet] Vol. 62 (3): 399-406. Available at: https://www.senckenberg.de/wp-content/uploads/2019/08/06_vertebrate_zoology_62-3_schindler_399-406.pdf [Accessed 12 April 2022].

Schönhuth, S., Luikart, G. and Doadrio, I. (2003). Effects of a founder event and supplementary introductions on genetic variation in a captive population of the endangered Spanish killifish. *Journal of Fish Biology.* [Internet]. Vol. 63: 1538-1551. Available at: https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1095-8649.2003.00265.x [Accessed 01 June 2022].

Shi, W. (2023). Zoom call with Chester Zoo Aquarium team, 14 March.

Shi, W., Guo, S., Haryono, H., Hong, Y. and Zhang, W. (2021). Diagnoses of two new species of *Parosphromenus* (Teleostei: Osphronemidae) from Bangka Island and Kalimantan, Indonesia. *Zootaxa.* [Internet] Vol 5060 (1): 71-92. Available at: https://www.biotaxa.org/Zootaxa/article/download/zootaxa.5060.1.3/69034 [Accessed 09 April 2022].

Shim K. F., Landesman L. and Lam, T. J. (1989). Effect of dietary protein on growth, ovarian development and fecundity in the dwarf gourami *Colisa lalia* (Hamilton). *Journal of Aquaculture in the Tropics.* Vol. 4: 111-123.

Sorgeloos, P., Lavens, P., Leger, P. and Tackaert, W. (1993). The use of Artemia in marine fish larviculture. *TML Conference Proceedings*. [pdf] Vol 3: 73-86. Available at: https://core.ac.uk/download/pdf/35102839.pdf [Accessed 15 February 2023].

Swann, L. (1992). *Transport of fish in bags*. Aquaculture Extension, Illinois-Indiana Sea Grant Program.

Tan, H.H. and Jongkar, G. (2020) *Parosphromenus barbarae*, a new species of Licorice Gourami from Sarawak, Borneo (Teleostei: Osphronemidae). *Vertebrate Zoology.* [Internet] Vol. 70 (3): 349–356. Available at: https://www.senckenberg.de/wp-content/uploads/2020/08/07_vertebrate_zoology_70_3_2020_Tan.pdf [Accessed 09 April 2022].

Untergasser, D. (1989). *Handbook of fish diseases.* T.F.H. Publications Limited: Maidenhead.

Ventura, R. F., and Enderez, E. M. (1980) Preliminary studies on *Moina* sp. Production in freshwater tanks. *Aquaculture.* Vol. 21: 93-96.

Vierke, J. (1991). Brutpflegestrategien bei Belontiiden (Pisces, Anabantoidei). *Bonner Zoologische Beiträge.* Vol. 42: 299-324. Available at: https://zoologicalbulletin.de/BzB_Volumes/Volume_42_3_4/299_324_BZB42_3_4_Vierke_J%C3%B6rg.pdf [Accessed 17 February 2023].

Whalley, C. (2018). Chemicals in European waters: Knowledge developments. *European Environment Agency report*. No. 18/2018. [pdf] Available at: https://www.eea.europa.eu/publications/chemicals-in-european-waters [Accessed 15 February 2023].

Wickins, S. C., Dennis, M. M., Landos, M., Šlapetam J. and Whittington, R. J. (2011). Histopathological survey of lesions and infections affecting sick ornamental fish in pet shops in New South Wales, Australia. *Diseases of Aquatic Organisms.* [pdf] Vol. 94: 143-152. Available at: https://www.int-res.com/articles/dao2011/94/d094p143.pdf [Accessed 30 March 2023].

Wilden, B. (2023). Zoom call with Chester Zoo Aquarium team, 14 March.

Zainordin, A. F. and Ab Hamid, S. (2017). Determination of trophic structure in selected freshwater ecosystems by using stable isotope analysis. *Tropical Life Sciences Research.* [Internet] Vol 28 (2): 9-29. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5584840/ [Accessed 10 April 2022].

Zhu, D., Degnan, S. and Moritz, C. (1998). Evolutionary Distinctiveness and Status of the Endangered Lake Eacham Rainbowfish (Melanotaenia eachamensis). Conservation Biology. [Internet] Available at: https://conbio.onlinelibrary.wiley.com/doi/abs/10.1111/j.1523-1739.1998.96330.x [Accessed 15 April 2023].

Zuanon, J.A., Carneiro, A.P., Nascimento, L.D., Silva, D.A., Pontes, M.D., Kanashiro, M.Y. and Salaro, A.L. (2013). Protein requirement for *Trichogaster lalius*, blue variety. *Revista Brasileira De Zootecnia*. [Internet] Vol. 42, 144-147. Available at: https://www.scielo.br/j/rbz/a/jN87TPHtX4QyLHBJd4bkM3K/abstract/?lang=en [Accessed 15 February 2023].

Zulkifle M, Christianus A, and Zulperi Z. (2021). Effects of pH, feeding regime and thyroxine on growth and survival of *Parosphromenus tweediei* (Kottelat and Ng, 2005) fry. *Journal of Survey in Fisheries Sciences*. [Internet] Vol. 8 (1): 89-105 Available at: http://sifisheriessciences.com/index.php/journal/article/view/71 [Accessed 15 February 2023].

Appendix 1 Live food culture, maintenance and harvesting

Below is a guide to the management of a selection of live foods suitable for feeding to *Parosphromenus* spp. This is by no means a fully exclusive list of live foods, nor is it the only way to culture such species.

Microworm (*Panagrellus* spp.)

A small terrestrial nematode is a good staple food for very young fry and a suitable supplemental food for juveniles and even for adults. Several species of slightly differing sizes are easily available. Common ones include microworm (*P. redivivus*), walter worm (*P. silusioides*) and banana worm (*P. nepenthicola*). Banana worms are the smallest species and are therefore slightly more suitable for newly hatched fry. Otherwise, there is little difference in their care or in their suitability as food.

Set-up

Small containers of almost any manageable size can be used. Clear plastic containers of around 1 litre with sides around 4-5cm in height are ideal. There needs to be adequate ventilation which can be achieved through multiple small holes drilled in the lid.

Make up a mix of porridge oats into a fairly thick porridge by adding hot water. It should be thick enough to need spreading out a little when added to the containers. The addition of spirulina powder can be beneficial both to the nematodes and as a gut loading: by allowing the nematodes to feed on the spirulina before being fed out themselves, it makes the nematodes a vehicle for getting this nutritious supplement into fish who may not consume it in its raw state. The porridge does not have to be boiled or cooked, heating to 60˚C or above is fine. Put into containers a layer 1-2cm deep; importantly there should be plenty of space left between the porridge and the lid. Allow the porridge to cool fully before adding the microworm. Scrape a thin layer from an old culture and smear over the top of the new porridge. Do not mix it in as they will stay near the surface to breathe. This can then be left for several days for the culture to mature or until the surface seems to ripple when looked at closely and there are plenty of worms moving up the sides.

The worms will reproduce faster at warmer temperatures of around 22-27˚C, basically any room temperature is fine. They are tolerant to a very wide range of temperatures which, however, might decrease growth rates, Ivleva (1969) records live worms at temperatures as low as 1.5˚C and personal experience has witnessed their survival up to around 32˚C.

Collection

Do not collect the worms directly from the porridge mix as this will foul the aquarium water. The worms will move up the sides of the tub and can be wiped off using a finger or a spoon and can then be added to water to feed out without transferring any porridge material.

Maintenance

The length of time a culture lasts varies with temperature, the amount used, the amount of food at set up etc. Therefore, it is best to time the setting-up of a new culture when the old culture begins to slow its production; this should be about every 2 weeks. I.e., for continual use there should be cultures in two stages kept, some which are growing on and some which are in use for harvesting, each week replacing one and switching them over. The older culture can have a little new porridge mix added and checked that it is not too wet or dry; it can be sprayed lightly or have some dry oats added if needed.

Grindal Worm (*Enchytraeus buchholzi*)

A small oligochaete that reaches a maximum length of around 10mm and makes a very good food for adults and juveniles once they are able to take it.

Setup

A tub of almost any size can be used. Tubs of 1 – 5 litres capacity are the most suitable and easiest to handle. Fill to a depth of about 2 – 3cm with coconut fibre that should be kept damp but not wet. Add the culture to the fibre and place a small piece of plastic mesh (with holes around 1mm is good) on top. This plastic mesh is where food for the worms, such as a pinch of porridge oats, can be placed, allowing easy removal should the food spoil, and can also aid in harvesting the culture, as the worms naturally separate themselves from the fibre to come and feed. Do not harvest until the culture looks stable and has increased to a good size.

They do best at temperatures around 22 – 27˚C but can tolerate a little wider temperature range for short periods of time. They can do well in similar locations as microworm but are not tolerant to quite as wide a temperature range.

Collection

They are best harvested just before feeding. Remove the mesh and rinse a corner of it into a pot of RO water. Be careful to assess how much can be harvested based on how the culture is doing. If necessary, gently dry the mesh with tissue before replacing it. The harvested worms may need rinsing again with RO water as grindal worms can affect the tank parameters (pers. obs.), especially if contaminated with any fibre material. Allow the container of RO water and harvested worms to settle, as this allows the worms to sink and be collected with tweezers or a pipette more easily. Try to minimise the amount of this RO water added to the *Parosphromenus* tank to further reduce contamination.

Maintenance

Check and feed daily. Feed a small amount of porridge oats and occasionally good quality fish pellets. Feed an amount which can be fully consumed within one to two days. Having two or more pieces of mesh can be helpful so that fresh food can be placed on each in rotation, allowing all food on one piece to be fully eaten to prevent spoilage whilst not leaving the culture without food. Monitor that the substrate stays damp but not wet and does not get compacted, but this very rarely needs replacing.

Brine shrimp nauplii (*Artemia* spp.)

Newly hatched brine shrimp can be a suitable food for individuals around 1 centimetre in length and above. It is best used as a supplementary food item as it does not survive for long in tanks, particularly encourages hydroid growth and can affect water parameters if not extremely carefully rinsed with RO water before being fed out.

Set-up

Clear containers are preferable; choose a size based on needs, a volume of around 2 litres is often ideal. An airline should be added at a moderate speed to mix the water well. It is better for the bottom of this airline to be as close to the base of the container as possible or cysts will gather below; introducing air from beneath or using rigid airline from above can help accomplish this. A container with a tap at the bottom can make collection of nauplii easier but is not strictly necessary. Water temperature should be maintained at approximately 25˚C, depending on the ambient room temperature a water heater may be needed.

Fill the culturing container with water with a salinity of around 30 – 35‰ to a few centimetres below the top. *Artemia* are tolerant to wide ranges of salinity (Hoff and Snell, 1987), but 30 – 35‰ is a good level for aquarium use. Any aquarium salt is fine to use. The cysts take around 24 hours to hatch at 25˚C but this varies with temperature.

Collection

The nauplii should be collected as soon as possible after hatching as they will begin to use up nutrition from their yolk reserves immediately; if allowed to reach second instar, they lose 22-39% of their energy (Sorgeloos *et al*., 1993) and will require feeding to stop further losses.

Once hatched the airline should be removed, and the culture allowed to settle, so that the shells of the cysts float to the surface, dead nauplii and sheds sink to the bottom and the live nauplii should be near to but not directly on the bottom. *Artemia* exhibit phototaxis, moving towards the light, so a light source can be used to gather them towards one location.

If polarised cysts are used, magnets (as large as practical) can be placed on one or both sides of the collecting vessel. This increases the efficiency of cyst removal and reduces waste. After around 5-10 minutes most of the culture can be slowly drained away, leaving the surface layer that contains the floating cysts on the first drain. Rinse the vessel and return the culture with the magnets, repeat this 2-3 times or until there are very few remaining cysts.

If non-polarised cysts are used or large magnets are not available, leave to settle a little longer, approximately 10-20 minutes. Open the tap at the bottom of the culture container part way and discard the very first small amount that will contain dead and unhatched cysts. Collect most of the contents into a collection container and stop when the majority of the nauplii are collected, leaving the top section with floating cysts. Alternatively, this same section can be siphoned out if no tap is available.

Sieve this though a brine shrimp sieve with mesh of 120µm. Wash this thoroughly with fresh water (RO water is beneficial before feeding out to *Parosphromenus* tanks) then rinse the nauplii into a small container to be fed out directly. Any remaining nauplii can be stored in saltwater in the fridge for up to 24 hours without issue (Leger *et al*., 1983), or in freshwater for just a few hours.

Maintenance

Discard the remaining culture once harvested, clean the container out well and disinfect it periodically. Set up again as described.

Daphnia (*Daphnia* spp. and *Moina* spp.)

Small freshwater crustaceans of the genus *Daphnia* and *Moina* are often known as daphnia or more colloquially 'water fleas'. While their nutritional content varies considerably due to age and food type, the protein content of *Daphnia* spp. usually averages ~50% of the dry weight, with *Moina* spp. averaging ~70% dry weight, and as such may not be suitable as a routine staple of the diet, see **Section 2.2.2**.

Set-up
While open ponds can provide good cultures of daphnia, production can be highly variable, with closed cultures better suited for controlling temperature and light levels, as well as having a reduced risk of contamination. Containers as small as 2 litres can sustain continuous cultures of daphnia; however, larger shallow containers with a large surface area are preferable. Ventura and Enderez (1980) suggest a water depth of 40-50cm, with 1m being the maximum advised (Ivleva, 1973).
A third to half of the culture should be shaded, though the shaded area can be increased if not relying on natural algae growth. While daphnia are largely tolerant of temperature extremes, *Moina* reproduction and survival reduces above 32˚C, the optimal range being 24-31˚C. Larger species such as *Daphnia magna* and *D. pulex* have lower optimal ranges of 18-22˚C.
Dechlorinated municipal water can be used, though it is worth noting that *Daphnia* are extremely sensitive to metal ions such as copper and zinc. Despite this, Hoff and Snell (1987) advise against the use of distilled water.

Collection
Daphnia cultures can easily be harvested by catching the required number with a net, though care should be taken that initial harvests from continuous cultures are not excessive (not more than ¼ of the population daily).

Maintenance
Inoculating microalgae into the daphnia culture can help to provide nutrition, as well as aiding water quality. Yeast and yeast-based products can also be used as a feed, though these should be avoided as the sole food: extended shortages of microalgae have been shown to adversely influence population (Heisig, 1977), and yeast-based foods can be easily overfed. Roti-rich, available from Florida Aqua Farms - Aquaculture Products, can also be used as a feed, as can rehydrated spirulina algae.

Water changes should occur when, or before, water quality begins to deteriorate. While temperature changes associated with water changes can be tolerated, care should be taken not to change the pH too much to avoid crashing the culture.

Paramecium (*Paramecium* spp.)

Paramecia are ciliated protozoans. Due to their small size, they can be a good first food for hatchling fish/fry; however, they do not survive well in the water conditions most suitable for *Parosphromenus*. Additionally, any culture water being introduced can disrupt the parameters of the *Parosphromenus* tank, so caution is suggested, and it is not particularly suitable as a main food item for fry.

Set-up
A glass or preferably at least transparent jar of almost any size can be used. A lid is not necessary, but a ventilated one can be used. Water that has been passed through a HMA (Heavy Metal Axe) filtration system can be added to approximately double the culture volume. Feed lightly until the water is slightly cloudy with brewer's yeast or a purchased product. Rotirich is a good option. Pararich is also very good, but difficult to access in small quantities in Europe. Both are available from the company Florida Aqua Farms - Aquaculture Products.

When starting a new culture or increasing the size of a culture it generally works better to not increase the volume too quickly as it can become difficult to regulate the feeding density. Increasing it by around 100-200% per day is usually appropriate. Monitor the density and adjust accordingly; there should be plenty of paramecia visible in at least the top third of the water column. Alternatively, they can be raised by causing a bacterial bloom from some sort of organic matter and allowing the paramecia to feed on it. We have found this to be a less reliable method and it has a high chance of polluting the tank water.

Collection
The paramecia can be fed directly from the culture. Pour or pipette what is required from the culture, but take care with the volume added to limit the effects on the tank's water quality. Try not to feed out soon after the paramecium culture has been freshly fed, the culture should be relatively clear before doing so. In the case when paramecia are needed regularly it is best to have multiple cultures of *Paramecium* which have staggered feeding so that they can be continuously fed out.

Maintenance
Paramecium do best with continuous harvesting and replacing of water, otherwise they can reach excessive densities and cause a crash. Feeding should be daily or every few days, feeding just enough that the culture will easily clear in this period. Frequent smaller feeds are preferable. Periodically remove the culture, clean the vessel and put the culture back. Some detritus in the container is not a problem.

Vinegar eels (*Anguillula aceti*)

A nematode similar in size and characteristics to microworm and they survive for prolonged periods in tank water. However, they often dwell close to the surface which can make them less suitable for *Parosphromenus* spp.

Set-up
Fill a jar with cider vinegar or white wine vinegar and a chunk of apple; homemade vinegars including an active culture ('mother of vinegar') are best. The apple is a source of food for the vinegar bacteria and will break down over time. It is best to avoid diluting the vinegar with water as the vinegar forms the basis of the vinegar eel's food, and dilution of the vinegar reduces their food supply, likely reducing the productivity of the culture. The cultures can be covered with a fine mesh; this is only to keep debris and dead flies etc. out as fruit flies can become a problem. Add the starter culture and leave this alone for around a month to mature or until there is a dense culture in the top section.

Collection
Pour the culture into a collection bottle with a thin neck, to the base of the neck of the bottle. Put a piece of sponge on a string into the bottle, up to the vinegar level. Carefully pour water over the sponge filling near to the top of the neck. Leave this for several hours and the nematodes will move up through the sponge to get to the oxygenated water. This can then be pipetted out and fed. It can be left like this overnight; however, you then need to be extremely careful not to remove too much as most of the culture will be in the small volume at the top. Only a very small amount is needed as the density is usually high. Return any unused vinegar eel to the culture vessel, rinse the sponge (this can be done into a pot and fed out for a last feed or put back in the culture) and pour the contents of the bottle back into the culture container.

Alternatively, you can carefully pipette a small amount of worms from the very surface of the culture and feed directly.

Maintenance

If any vinegar is lost through use or by evaporation, it can be topped up over time. The accumulated detritus at the bottom is not a problem at all, it is from the broken-down apple and is a part of the mother of vinegar that is the food for the vinegar eels. A little can be removed if too much of it collects. As with the paramecium, if the container gets particularly dirty, the whole culture should be removed and the container cleaned out. A little more apple can also be added again when it has been depleted. As mentioned in collection, be careful not to over-collect from each culture.

Appendix 2 Example of a fish-specific post-mortem form

AQUARIUM DEATH AND POST-MORTEM EXAMINATION SUBMISSION FORM

ANY DECEASED ANIMAL SUBMITTED TO AHC

OR ANIMAL FOR HD MUST HAVE THIS FORM COMPLETED

(Vets use only PM case number: ______________________

PERSON COMPLETING FORM: AQUARIUM	
SPECIES:	LOCAL ID: OTHER I.D:
DATE SUBMITTED:	DATE OF DEATH: DATE DISCOVERED:
DOB or Approx. age: SEX: M F U	TANK: WEIGHT:
Number of this species remaining in enclosure: (record as estimate if necessary)	Any other species in enclosure: If yes, what are they?
Was the specimen euthanized: Y N Is the body: Fresh or mildly / moderately / severely autolysed?	
Recent History and Observations: Please circle: Prev. healthy / Under treatment / Long term health problems / Disease or deaths in group <u>PLEASE REPORT DETAILS OF RECENT HISTORY OR REASON FOR HD:</u> (e.g.: Why do you think it is dead. Recent changes/problems/new introductions/moved etc) PTO if required. Date of last water test: Results: Temp: pH: Nitrite: Nitrate: Ammo: SG:	
VET TEAM TO COMPLETE Pathology Case Number: Vet: Dead on ZIMS? Y N PM STANDARD TEXT: Necropsy/Priority: U H M L ("include in recent history") + / - OR TBC HISTO Carcass: Return to Chester Zoo / incineration / cosmetic pm? PDF TITLE FOR LEAHURST = SPECIES/CZ PATH NUMBER/LOCAL ID	

Appendix 3 Suggested equipment list

- **Tank** (see **Section 2.1** for details)
  - 20 – 30 litres per pair, larger for groups
  - Floor space is more important than height
  - Tight fitting lid
  - Aquarium heater
  - Air driven filtration
- **Water** (see **Section 2.1.3** for details)
  - Reverse osmosis filtration advised
  - Commercial water softener prior to reverse osmosis and de-ionising media after reverse osmosis may be necessary depending on local conditions
- **Tank furniture** (see **Section 2.1** for details)
  - Planting
    - Java moss (*Taxiphyllum barbieri*)
    - Java fern (*Leptochilus pteropus*)
    - Floating fern (*Salvinia* sp.)
  - Hides
    - Wood
    - Inert stone
    - Inert ceramics
    - Inert plastics
  - Leaves
    - Indian almond (*Terminalia catappa*)
    - Cocoa (*Theobroma cacao*)
    - Jackfruit (*Artocarpus heterophyllus*)
    - Oak (*Quercus* spp.)
    - European beech (*Fagus* spp.)
    - European alder (*Alnus glutinosa*) – catkins of this species are also appropriate
- **Management** (see **Section 2.5.3** for details)
  - Very fine nets
  - Small jug or similar handled container
- **Live food** (see **Appendix 1** for details)
  - Microworm
    - Starter culture
    - 1 litre tubs
    - Porridge oats
    - Spirulina
  - Grindal worm
    - Starter culture
    - 5 litre tubs
    - Coconut fibre
    - Plastic mesh
    - Porridge oats
  - Brine shrimp
    - *Artemia c*ysts
    - Hatching cone
    - Salt water
    - Air line
    - Small aquarium heater
    - 120µm sieve

    - Magnets (depending on what cysts purchased)
  - Daphnia
    - Starter culture
    - Desired container (2 litre minimum)
    - Heavy Metal Axed water (HMA)
    - Microalgae
    - Dried spirulina
  - Paramecium
    - Starter culture
    - Transparent jar
    - Heavy Metal Axed water (HMA)
    - Yeast/RotiRich
  - Vinegar eels
    - Starter culture
    - Jar or bottle for culture
    - Wine bottle for collection
    - Vinegar (apple cider, white wine or homemade)
    - Apple
    - Filter floss
    - Pipette