JZAR www.jzar.org JOURNAL OF ZOO AND AQUARIUM RESEARCH

EAZA

JZAR Evidence-based Practice

OPEN ACCESS

Research article

Courtship behaviour of the freshwater pipefish *Microphis aculeatus* (Syngnathidae): A case study in captive breeding

Barrett L. Christie

The Maritime Aquarium at Norwalk, 10 N. Water St., Norwalk, CT 06854 USA

Correspondence, Barrett L. Christie, email; bchristie@maritimeaquarium.org

Keywords: courtship, freshwater, *Microphis aculeatus*, pipefish, reproduction, Syngnathidae

Article history:
Received: 6 Jun 2020
Accepted: 21 Jan 2022
Published online: 31 Jan 2022

Abstract

An account of the first documented spawning of the pipefish *Microphis aculeatus* (Syngnathidae) is presented. Pipefishes and seahorses are sex-reversed and males brood ova, displaying a level of parental investment extraordinary among teleost fishes. Pipefishes are known to exhibit complex courtship rituals, described here for *M. aculeatus* from a series of spawning events in a captive population at a public aquarium. Reproductive individuals ranged from 125–145 mm (male) and 136–144 mm (female) in total length giving a first estimate of size at maturity for the species. Spawning events (n=19) occurred in November over a period of 10 days immediately following illumination of the exhibit, suggesting that lighting is a primary cue in reproductive behaviour for this species. The courtship behaviour is initiated by males and can be divided into five distinct phases (described herein) which are characterised by displays of body quivering, rostral pointing, caudal fin fanning and curvature of the body into a distinctive "S" shaped curve while displaying the ventral side (harbouring the brood pouch) towards the female. Respiration rates were markedly increased during copulatory behaviour, peaking at 128 min^{-1} (male) and 120 min^{-1} (female). The observations of breeding and ventilation rates have implications for the nutritional status, age class and general husbandry practices required for spawning these pipefishes in an aquarium.

Background

The Syngnathidae are predominantly a marine taxon, and while the seahorses are all found in seawater, there are a number of pipefishes found in freshwater worldwide. Some marine pipefish species, such as *Syngnathus scovelli*, have been known to become landlocked and establish brackish or freshwater populations (Gasparini and Teixeira 1999), but a few species of the genera *Doryichthys* and *Microphis* have adapted to life entirely in freshwater (Dawson 1979, 1984). *Microphis aculeatus* is commonly collected in west Africa from Senegal to the Congo basin and is often found in the ornamental fish trade under the (invalid) names of *Microphis smithii, Oostethus brachyurus aculeatus, Doryichthys aculeatus* and others.

The syngnathid fishes as a sex-reversed taxon are an interesting model for reproductive studies (Masonjones and Lewis 2000); the parental investment of egg-brooding is arguably among the most significant known among teleost fishes and coupled with a relatively low fecundity distinguishes their reproductive habits from other fishes. The tendency of some species to be monogamous such as *S. scovelli*, *Syngnathus biaculeatus* and most *Hippocampus* spp. (Lourie et al. 1999, Masonjones and Lewis 1996, Miranda-Marure et al. 2004) is unusual for bony fishes. Bizarrely, in some populations of *Syngnathus typhle* and *Nerophis ophidion*, female egg production may even exceed total male brood pouch availability (Berglund et al. 1989) contravening the evolutionary norms that ova are bioenergetically costly to a species, and as such, are the limiting factor in a species' fecundity.

The first observations of syngnathid reproduction (by Aristotle) date to 350 BCE (Frias-Torres 2004), and pipefishes

Table 1. Duration of distinct phases in the courtship behaviour of *Microphis aculeatus* and change in respiration as indicated by opercular ventilation rates (V) for both male and female specimens.

Courtship phase	Duration (min)	Male ventilation (V min^{-1})	Female ventilation (V min^{-1})
Phase 1	6–8	91.6	84.0
Phase 2	2–3	128.6	90.0
Phase 3	2–3	112.5	120.0
Phase 4	1–2	52.0	48.0
Phase 5	8–10	67.8	78.0
None: Baseline	-	78.2	74.0

and seahorses have been spawned in laboratories and aquaria since at least the mid-19th century (Breder and Rosen 1966). Many syngnathiform species are imperilled worldwide (Lourie et al. 1999) and available information on reproduction has been largely anecdotal (Koldewey and Martin-Smith 2010, Masonjones and Lewis 1996) and based on reports from captive specimens (Koldewey 2005). Aquaculture is a promising conservation tool but is dependent on refining breeding techniques (Faleiro et al. 2006, Woods 2003). Therefore, aquaria and zoos can make significant contributions in these areas of inquiry, and to that end, this report is presented.

Actions

Animals and their management

Eight specimens of *M. aculeatus* were acquired from two different fish vendors who had misidentified them as *Microphis smithii* and *Doryichthys martensii*. The country of origin in both cases was Senegal, narrowing the potential species to *Enneacampus kaupi* or a species of *Microphis* (Stiassny et al. 2007). Keys provided by Dawson (1984) were used to confirm the identification as *M. aculeatus* through counts of dorsal fin rays, trunk rings and tail rings (41–51, 19–21 and 22–24 respectively). Size of specimens was determined through analysis of scaled photos using ImageJ v1.52 (National Institutes of Health, Bethesda, Maryland, US). Animals were not handled or manipulated at any point for data collection.

Specimens were housed at the Dallas Aquarium at Fair Park in a 682 L glass tank with conventional life support systems (sump with low-density polyethylene [LDPE] bio-media and 100 µm mechanical filtration), six very high output (VHO) fluorescent lights (6700 and 14,000 K) on a 12 h light/dark cycle (illuminated 0800–2000) and planted with live *Vallisneria spiralis.* Lighting was measured from first light as photosynthetically active radiation in µmol m-2s^{-1} using a Quantum Sensor (Apogee Instruments, Logan, UT) at the level of the substrate. The exhibit was maintained at a salinity of 1–2 g L^{-1}, 26°C, pH 8.4–8.6 and a total alkalinity of 3.0–3.5 mEq L^{-1}, due to the high mineral content of the freshwater drawn from the aquarium's well.

Specimens were fed a mixture of live *Artemia* sp. adults enriched with microalgae (*Isochrysis* sp. and *Nannochloropsis* sp.), arachidic acid (AHA), and docosahexaenoic acid (DHA). The specimens were also fed live *Daphnia* sp. enriched with brewer's yeast (*Saccharomyces* spp.), and *Spirulina* sp., live *Gammarus* sp. amphipods, frozen *Mysis relicta* and frozen calanoid copepods. Feedings were carried out twice daily.

Behavioural observations

Observations of animals were made each day 30 minutes prior to illumination (artificial dawn) and for 60 minutes afterwards for a total of 10 days in November (until courtship ceased to be displayed). Video and still photos were taken during this time period and ventilation rates of the animals as indicated by opercular movement were measured with a stopwatch and recorded in 30-second intervals every other minute throughout courtship. Baseline ventilation rates were measured both mid-day and in the evening when the animals were not engaged in breeding and were displaying normal swimming and feeding behaviours.

Normal behaviours are defined to differentiate the observed spawning behaviours reported below. Normal swimming is characterised by the pipefish swimming horizontally across the substrate, holding a fixed position in the water column facing into the direction of water flow or holding position amid submerged vegetation. The species may aggregate together in a head-down posture using existing vegetation as cover but does not school. Under normal swimming conditions, pipefish are not observed to be in close contact with one another (i.e. within 5–10 cm) for more than 60 seconds.

Consequences

A total of 19 courtship displays were observed over 10 days from three male and three female pipefish. Males had an average total length (TL) of 138 mm (125–145 mm) and females had an average TL of 142 mm (136–144 mm).

Baseline ventilation was determined under normal (non-reproductive) conditions from six measurements each of both males and females. During courtship, ventilation rates (V) rose markedly, peaking at 14 min (V=128 min^{-1}) in male specimens and 16 min (V=120 min^{-1}) in female specimens; these data are summarised for brevity in Table 1 and Figure 1.

Courtship behaviours followed a defined pattern that was repeated each day by each pair of animals. These behaviours can be grouped into distinct phases characterised by the body posture and movements exhibited in each. Courtship displays began 9 to 11 minutes after first light and continued for 19–26 minutes thereafter. Light levels rose to 153–159 µmol m2 s^{-1} within the first 10 minutes of artificial daylight (Figure 2). No courtship behaviour was seen more than 40 minutes after first light, with the single exception of a male who appeared to be attempting to initiate courtship early in the evening just after the exhibit lights turned off. In this instance the female showed no interest and the courtship ritual did not proceed.

https://doi.org/10.19227/jzar.v10i1.545

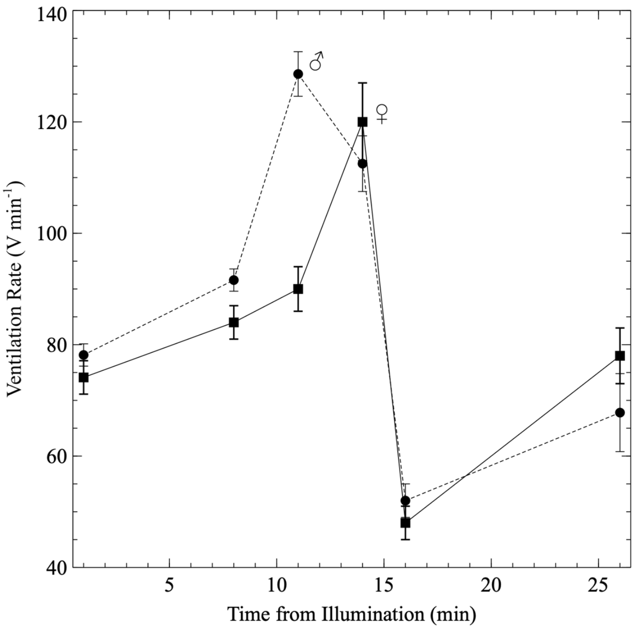

Figure 1. Change in respiration rate of *Microphis aculeatus* during courtship behaviour as indicated by opercular ventilation rate (V) per minute. Dashed line indicates male ventilation, solid line indicates female.

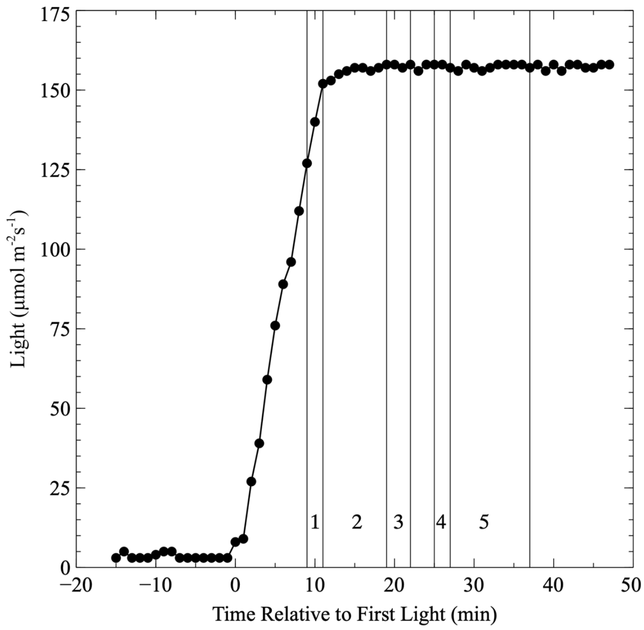

Figure 2. Distinct phases of courtship behaviour in *Microphis aculeatus* as compared to lighting levels (measured as µmol m^{2}s^{-1} photosynthetically active radiation) at artificial dawn. Vertical lines and numerals under the curve of light intensity delineate the distinct behavioural phases defined in this study for the species.

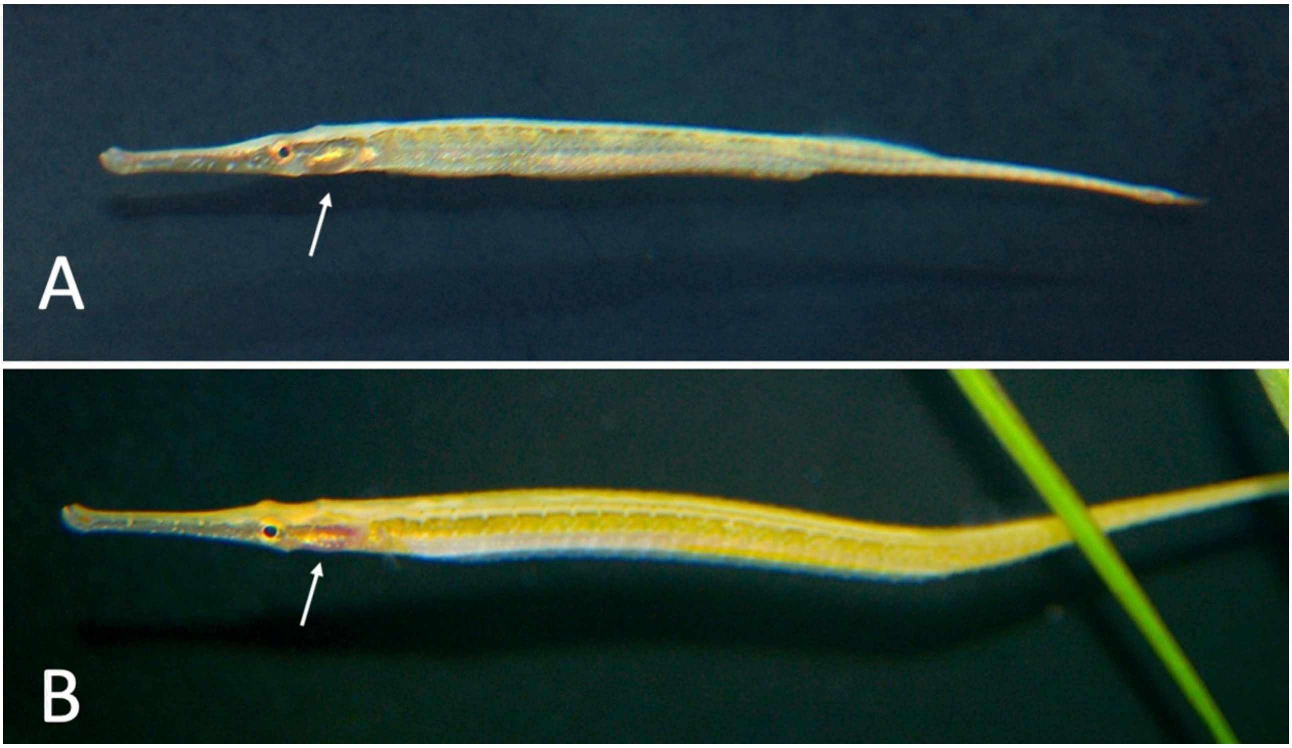

Figure 3. Colour change observed in the gills of *Microphis aculeatus* during spawning. Photo at top (A) shows a normal (female) specimen hours after courtship. Photo at bottom (B) shows a (male) specimen immediately preceding courtship behaviour. Note the marked colour difference of the gills in a reproductive individual which was accompanied by distinct changes in ventilation rate (Figure 1).

Description of courtship behaviours

From the initiation of daylight the precopulatory behaviour includes females darkening in colour, revealing some light spots on the head and tail and vertical banding on the trunk. The gills flushed with blood in both males and females in this time period, creating a noticeably increased red colouration (Figure 3) that grows more prominent as ventilation rates increase. The phases of courtship (Figure 4) in *M. aculeatus* are characterised, following the work of Masonjones and Lewis (1996) with *Hippocampus zosterae*, as follows:

Phase 1: Initiation of courtship. Females positioned among vegetation in a slightly head-down (normal) posture; males

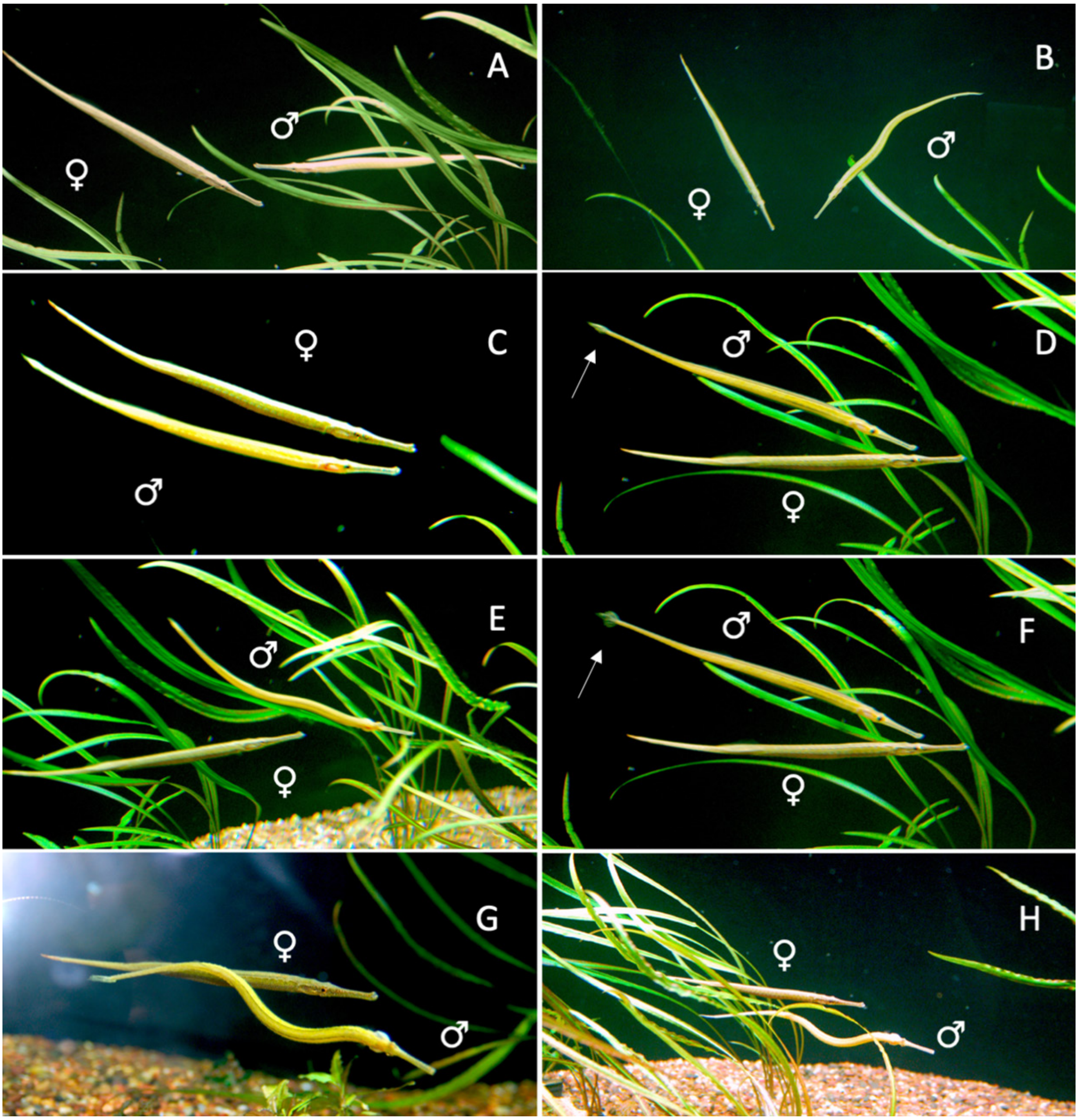

Figure 4. The characteristic behaviour exhibited by *Microphis aculeatus* during the four phases of courtship as defined in this study. Phase 1 (A) the male (right) approaches the female (left) minutes after daylight. Phase 2 (B) is characterised by the two changing orientation to a head-down position and the male quivering. In Phase 3 (C, D, F) the male (top) orients himself parallel to the female (C) then repeatedly displays his caudal fin (D, F) in a fan-like motion. Phase 4 (E, G, H) sees the male repeatedly dart forward (E) in a curved posture then turn 90°, displaying his ventral side to the female and contorting into an "S" shape (G, H). Note the colouration of gills throughout courtship, and the darker colouration of females relative to males.

https://doi.org/10.19227/jzar.v10i1.545

approach head-to-head in a horizontal posture with some quivering motions of the body and tail. Female responds by pointing her rostrum towards male (Figure 4a). Male and female ventilation rates rise to 117% and 113% of baseline respectively. Duration: six to eight minutes.

Phase 2: Male and female remain in a head-down posture; male positions himself nearly perpendicular to female with his trunk and tail slightly curved. The male's body quivers significantly and gills are flushed a bright red (Figure 4b). Male and female ventilation rates rise to 165% and 121% of baseline respectively. Duration: two to three minutes.

Phase 3: Male positions himself parallel with female 5–10 mm apart (Figure 4c). After approximately 30–45 seconds, the female begins pointing her rostrum towards the male at which point the male backs up and elevates his tail 20–30 degrees above the female in a head-down posture and begins flaring his caudal fin then clamping it closed in roughly three-second intervals, which proceeds for a minute or longer (Figure 4d, f). Male ventilation drops to 144% of baseline while female ventilation rate rises to 162% of baseline. Duration: two to three minutes.

Phase 4: The male repeatedly and rapidly swims forward approximately 2–3 cm ahead of the female, contorting his body into a rigid curved shape with his operculum flared. The male holds this position for 8–11 seconds then swims back and repeats the movement. Male and female ventilation rates both drop to 67% and 65% of baseline respectively. Duration: one to two minutes.

Phase 5: After the display of darting forward and contorting the body into a curved posture, the male will position himself approximately 1 cm ahead of the female, bodies nearly parallel, and rotate his body 90 degrees so that the ventral side is facing the female, only 5–10 mm apart. The male then contorts his body into a striking "S" shape and holds this position with opercula flared to display his gills. The male will hold this position for 80–90 seconds before retreating and repeating the behaviour (Figure 4g, h). If the female signals him by turning her ventral side toward the male, the two will rise up 5–10 cm in the water column pointed upwards and egg transfer occurs. If no signal is sent by the female, the male will repeat this behaviour until the two eventually separate (Figure 5) or eggs are transferred. Male and female ventilation rates rise to 87% and 105% of baseline respectively. Duration: eight to ten minutes.

On day 10, egg transfer was completed from female to male in one pair (<10) and there was some egg droppage in the process as has been observed in *Syngnathus abaster* (Silva et al. (2006). The (open) brood pouch of the male was never visibly distended and no eggs were observed to hatch in this breeding season.

The breeding behaviours of *M. aculeatus* were previously unknown. In an exhaustive review, Breder and Rosen (1966) note that *Microphis cuncalus* had produced offspring in captivity and they describe in detail the courtship behaviours of *Syngnathus floridae* as reported by Gudger (1905). Riehl and Baensch (1982) note that *Enneacampus ansorgii*, another African freshwater

Figure 5. The distinctive "S" shape exhibited by males of *Microphis aculeatus* during the final period (Phase 4) of their courtship ritual. This striking behaviour is the penultimate act in their reproductive behaviour immediately preceding egg transfer from gravid female to the brood pouch on the ventral side of the male.

Table 2. Known reproductive characteristics of some pipefishes known to inhabit freshwater. Reproductive season indicated and time refers to the period of day when courtship and spawning behaviours have been observed.

Species	Season	Time	Fecundity (number of ova per animal)	Reference
Syngnathus floridae	Summer	Night	-	Gudger 1905
Syngnathus scovelli	None	Morning	36–165	Gasparini and Teixeira 1999
Microphis aculeatus	November	Morning	-	Present study
Microphis lineatus	May–November	-	-	Frias-Torres 2004
Microphis lineatus	Summer, winter	-	10–943	Miranda-Marure et al. 2004
Microphis leiaspis	June–December	-	75–241	Ishihara and Tachihara 2008

pipefish, had reproduced in captivity but data on the spawning of *M. aculeatus* was absent from the literature.

The courtship ritual observed here should be placed in the context of known reproductive characteristics of closely related species, which are summarised in Table 2 for brevity. The species most closely related to *M. aculeatus* whose breeding habits are better studied is *Microphis lineatus*, which has two reproductive seasons in Brazilian populations but reproduces constantly from May to November in the greater Caribbean with a mean fecundity of 409 ova male^{-1} and 765 ova female^{-1} (Miranda-Marure et al. 2004).

The lack of vertical movement in courtship in *Corythoichthys intestinalis* was hypothesized by Gronell (1984) to be an adaptation to life in shallow intertidal habitats and perhaps the minimal vertical movement observed here in *M. aculeatus* is a result of the species' native habitat being shallow rivers and streams in West Africa. This stands in stark contrast to some other oceanic syngnathids, which have significant vertical migration as part of the courtship ritual (Forsgren et al. 2006). Many pipefishes are known to intertwine their bodies like twisted rope during courtship (Gronell 1984) but the distinct "S" shape curves exhibited here by *M. aculeatus* are similar to courtship behaviours in other species such as *S. floridae* and *S. abaster* (Breder and Rosen 1966, Gudger 1905, Silva et al. 2006).

It is known that the frequency of courtship behaviour may be depressed by predation risk in *Syngnathus typhle* (Fuller and Berglund 1996), though these predation cues should be absent in a captive setting allowing for the greatest potential number of mating occurrences to be observed, assuming all other factors are met (nutritional needs, habitat complexity, water quality, etc.). As researchers have begun to explore the potential of aquaculture techniques for syngnathid conservation (Faleiro et al. 2008, Koldewey and Martin-Smith 2010, Woods 2003) these needs are beginning to be elucidated. In wild populations of syngnathiform fishes, food availability has been a determinative factor in female fecundity (Berglund et al. 1989) and this presumably holds true for captive specimens as well. Gardner (2003) found that addition of copepods to the diet of young seahorses was more likely to ensure higher survivorship of cultured animals and it has been noted by Koldewey (2005) that adult *Artemia* sp. should be enriched with highly unsaturated fatty acids as they are not themselves a highly nutritious diet for syngnathids.

Pipefishes of the genus *Microphis* have an 'open' brood pouch, consisting of a spongy vascularised material that supports ova during development (Miranda-Marure et al. 2004) rather than the traditional membrane-enclosed pouch found in seahorses and other pipefishes. In this investigation, the open brood pouch of male *M. aculeatus* was never visibly enlarged, which may indicate that the males were underdeveloped. Alternatively, the single female who produced (few) eggs could have been too young or underdeveloped, or perhaps the nutritional needs of the animals were not adequately met. The animals in this study were acquired shortly before mating behaviour was observed, so although the diet offered (following industry best practices for marine syngnathid fishes) was assumed to be optimal, they may have previously not had adequate nutrition to support healthy reproduction. In *M. lineatus*, insect larvae were the most common food item in gut content analyses and larval fishes also had a high prevalence (Frias-Torres 2004), so perhaps these freshwater pipefishes need higher protein content or a different composition of fatty acids for optimal nutrition in captivity.

The average sizes of male and female *M. aculeatus* were smaller than reports of average sizes reported anecdotally by other aquarists keeping the species and significantly smaller than the maximum sizes (20 cm) reported by Dawson (1990), so it is also possible these specimens were at the minimum size or age required for sexual maturity and these observations represented their first breeding season. Miranda-Marure et al. (2004) report male fecundity in the smallest *M. lineatus* specimens was as low as 10 ova per brood, so the small number of eggs seen transferred here in *M. aculeatus* (<10) supports the hypothesis that specimens were either underdeveloped or just reaching sexual maturity.

The involvement of light in the courtship behaviours observed is also noteworthy. Lighting cues are known to be important to reproduction in many fishes, and in this case the artificial dawn created when the aquarium was illuminated by fluorescent lighting was the trigger for courtship initiation. When the phases of spawning behaviour observed in this study were overlaid on a plot of lighting intensity (Figure 1) the initiation of courtship (9–11 min after first light) begins just as the exhibit lighting reaches its maximum intensity. Thus, lighting is an important cue for spawning. However, it cannot be determined from these data if there is a latent period from the onset of illumination or a threshold of illumination intensity that is the critical factor in the initiation of courtship behaviour.

In most known vertebrate mating systems, complex male displays ostensibly allow females to select the fittest partners. Given the duration of courtship behaviour observed here (up to 26 minutes) and the marked increase in respiration rates throughout, it can be reasonably assumed this display has a significant energetic cost to the animals. This assessment of endurance could have evolved as a test of fitness and may have been amplified by sexual selection to result in females who prefer males able to display stamina through the courtship ritual.

Journal of Zoo and Aquarium Research 10(1) 2022
https://doi.org/10.19227/jzar.v10i1.545

Conclusions

Continued elucidation of the diversity of breeding systems present in the Syngnathiformes will undoubtedly shed new light on the dynamics of sex-reversed fishes, and some may prove useful as model organisms given their proclivity to breed readily in captivity. In this case the copulatory behaviour of *M. aculeatus* has been documented and lighting shown to be a critical environmental cue to initiate courtship. Ventilation rates and activity levels are markedly raised during courtship, which may persist for 10 days or more, which has implications for nutritional needs and care during breeding seasons. It is hoped these data and observations of distinctive reproductive behaviours will help other aquarists worldwide in breeding freshwater pipefishes.

Acknowledgements

Thanks are due to Eric Julius and Charles Yancey, formerly of the Dallas Aquarium at Fair Park, for the husbandry of the pipefishes in this study and assistance with observations and data collection. Thanks also to Dr. Lori Elliott for assistance preparing the manuscript.

References

Berglund A., Rosenqvist G., Svensson I. (1989) Reproductive success of females limited by males in two pipefish species. *The American Naturalist* 133(4): 506–516. doi:10.1086/284932

Breder C.M., Rosen D.E. (1966) *Modes of Reproduction in Fishes*. New York, New York: Natural History Press.

Dawson C.E. (1979) Review of the polytypic doryrhamphine pipefish *Oostethus brachyurus* (Bleeker). *Bulletin of Marine Science* 29(4): 465–480.

Dawson C.E. (1984) Revision of the genus *Microphis* Kaup (Pisces: Syngnathidae). Bulletin of Marine Science 35(2): 117–181.

Dawson C.E. (1990) Syngnathidae. In: Quéro J.C., Hureau J.C., Karrer C., Post A., Saldanha L. (eds.) *Check-list of the Fishes of the Eastern Tropical Atlantic, Vol. 2.* Lisbon, Portugal: UNESCO.

Faleiro F., Narciso L., Vicente L. (2008) Seahorse behaviour and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? *Aquaculture* 282(1–4): 33–40. doi:10.1016/j.aquaculture.2008.05.038

Fricke R., Eschmeyer W.N., van der Laan R. (eds.) (2020) Eschmeyer's Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [Accessed 19 May 2020].

Forsgren K.L., Lowe C.G. (2006) The life history of weedy seadragons, *Phyllopteryx taeniolatus* (Teleostei: Syngnathidae). Marine and *Freshwater Research* 57(3): 313–322. doi:10.1071/MF05094

Frias-Torres S. (2004) Notes on aquarium brood release and feeding of the opossum pipefish, *Microphis brachyurus lineatus. Gulf and Caribbean Research* 16(1): 73–75. doi:10.18785/gcr.1601.11

Fuller R., Berglund A. (1996) Behavioural responses of a sex-role reversed pipefish to a gradient of perceived predation risk. *Behavioural Ecology* 7(1): 69–75. doi:10.1093/beheco/7.1.69

Gardner T. (2003) The copepod/*Artemia* tradeoff in the captive culture of *Hippocampus erectus*, a vulnerable species in lower New York State. In: Cato J.C., Brown C.L. (eds.) *Marine Ornamental Species: Collection, Culture & Conservation.* Ames, Iowa: Iowa State Press and Blackwell Publishing, 297–303.

Gasparini J.L., Teixeira R.L. (1999) Reproductive aspects of the Gulf pipefish, *Syngnathus scovelli* (Teleostei: Syngnathidae), from Southeastern Brazil. *Revisita Brasileira de Biologia* 59(1): 87–90. doi:10.1590/S0034-71081999000100011

Gronell A.M. (1984) Courtship, spawning and social organization of the pipefish, *Corythoichthys intestinalis* (Pisces: Syngnathidae) with notes on two congeneric species. *Zeitschrift für Tierpsychologie* 65(1): 1–24. doi:10.1111/j.1439-0310.1984.tb00369.x

Gudger E.W. (1905) The breeding habits and segmentation of the egg of the pipe-fish (Siphostoma floridae). *Proceedings of the US National Museum* 29(1431): 447–500.

Ishihara T., Tachihara K. (2008) Reproduction and early development of a freshwater pipefish, *Microphis leiaspis* in Okinawa-Jima Island, Japan. *Ichthyological Research* 55: 349–355. doi:10.1007/s10228-008-0049-y

Koldewey H. (ed.) (2005) *Syngnathid Husbandry in Public Aquariums*. London, UK: Project Seahorse and the Zoological Society of London.

Koldewey H., Martin-Smith K.M. (2010) A global review of seahorse aquaculture. *Aquaculture* 302(3-4): 131–152. doi:10.1016/j.aquaculture.2009.11.010

Lourie S.A., Vincent A.C.J., Hall H.J. (1999) *Seahorses: An Identification Guide to the World's Species and their Conservation*. London, UK: Project Seahorse.

Masonjones H.D., Lewis S.M. (1996) Courtship behavior in the dwarf seahorse, *Hippocampus zosterae. Copeia* 1996(3): 634–640. doi:10.2307/1447527

Masonjones H.D., Lewis S.M. (2000) Differences in potential reproductive rates of male and female seahorses related to courtship roles. *Animal Behaviour* 59(1): 11–20. doi:10.1006/anbe.1999.1269

Miranda-Marure M.E., Martínez-Pérez J.A., Brown-Peterson N.J. (2004) Reproductive biology of the opossum pipefish, *Microphis brachyurus lineatus*, in Tecolutla Estuary, Veracruz, Mexico. *Gulf and Caribbean Research* 16(1): 101–108. doi:10.18785/gcr.1601.17

Riehl R., Baensch H.A. (1982) *Aquarium Atlas.* Melle, Germany: Baensch Publishing.

Silva K., Monteiro N.M., Vieira M.N., Almada V.C. (2006) Reproductive behaviour of the black-striped pipefish *Syngnathus abaster* (Pisces: Syngnathidae). *Journal of Fish Biology* 69(6): 1860–1869. doi:10.1111/j.1095-8649.2006.01229.x

Stiassny M.L.J., Teugels G.G., Hopkins C.D. (eds.) (2007) P*oissons d'Eaux Douces et Saumâtres de Basse Guinée, Ouest de l'Afrique Centrale, Vol. 2.* Paris, France: Muséum National d'Histoire Naturelle.

Woods C.M.C. (2003) Effect of stocking density and gender segregation in the seahorse *Hippocampus abdominalis. Aquaculture* 218(1-4): 167–176. doi:10.1016/S0044-8486(02)00202-8