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Short Communication

Observation on the laterally conjoined juvenile seahorse, *Hippocampus kuda* (Bleeker, 1852) under captivity

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Abstract

Yellow seahorse, *Hippocampus kuda* (Bleeker, 1852), is one of the heavily traded seahorse species for Traditional Chinese Medicines (TCM), aquariums and curios in a global perspective. Experimental trials to standardize breeding, mass-scale rearing, and culture were undertaken under captive conditions at the National Institute of Oceanography, Goa. During the breeding trail among one spawn of 865 juveniles released by male *H. kuda,* it was noticed one juvenile with an abnormality with a presence of two heads (8 mm height, tip of the coronet to tip of the tail). The two-headed juvenile survived for 62 h only after being released. Such observations have been reported previously in other wild and cultured teleost fishes in captivity. In this communication, morphological information about the rare bicephalic seahorse juvenile born during captive breeding trials. The present observation suggests the possibility of captive conditions influencing the deformities in seahorses has been discussed along with earlier observations.

Keywords: Bicephalic, Captive breeding, Husbandry welfare, Syngnathidae, Yellow seahorse

Highlights

- Captive breeding of seahorse under captive conditions
- Reporting of bicephalic juveniles and their behavior
- Welfare requirements for captive breeding programs
- Morphometric details of the conjoined juveniles

The demand for seahorses in traditional Chinese medicine triggered the traditional fishermen community, driven by local traders through financial support spearheaded the fishing activities to target seahorse resources from various fishing practices (Marichamy *et al*., 1993; Murugan and Durgekar, 2008; Vinod *et al*., 2018). Due to the overexploitation of the resources from the Tamil Nadu coast and international regulations like CITES addressing seahorse trade restrictions, to conserve this species, the Government of India placed them under Schedule I of the Wildlife Protection Act, 1972 from 2001 much before the trade restriction amended by CITES in 2002 (Vaidyanathan and Vincent, 2021). On the other hand, scientific institutions, through government-funded projects, have been involved in the closing of their life cycle under *ex-situ* conditions from 2000 onwards. However, further research has been undertaken, but no research findings from the outcome of the aquaculture projects have been implemented for conservation or commercial initiatives to date despite state-of-the-art technology has been perfected for this unique animal (Murugan *et al*., 2009; Pawar *et al*., 2011; Murugan *et al*., 2017).

Behavioral studies linked to welfare measures are not widely undertaken by the aquaculture industry (Chandroo *et al*., 2000); hence certification system is playing a major role in streamlining the welfare needs of captive animals (UNEP-WCMC, 2003). In the present scenario, positive welfare practices in aquaculture produce healthier animals that are less susceptible to disease (Gonzalez, 2023). During the captive breeding initiatives, most of the welfare issues concerned with broodstock management for seahorses are not taken under consideration since the seahorse breeding industry mostly depends on wild-caught brooding males (Vincent and Koldewey, 2006). Hence, deformity and disease-related problems during the seahorse culture period have been reported based on experimental trials, whereas welfare issues faced during pilot studies during the implementation of aquaculture production project during brood stock period, pelagic juvenile and settled juvenile period during the culture period exits, but information pertaining to deformity and clutch dropping of immature eggs are not reported on scientific terms. Observation of

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snout and tail deformity in juveniles has been reported due to the role of temperature and nutrients provided to the brooding seahorses (Woods, 2002; Murugan, 2004; Blanco *et al*., 2012).

Morphological abnormalities in the animal kingdom have been reported from aquaculture and wild-caught species in vertebrates as well in invertebrates from time to time (Owusu-Frimpong and Hargreaves, 2000). Fish deformities are detrimental to aquaculture and conservation initiatives; it is also an ethical problem and causes substantial financial losses to the aquaculture industry (Chandra *et al*., 2023). Artificial environmental provided during captive condition can affect the morphology, physiology, and behavior of fish (Kihslinger and Nevitt, 2006). Environmental stress and oocyte ageing can give rise to decreased developmental stability of individuals reared in controlled conditions (Clarke, 1995; Moller and Swaddle, 1997; Nowosad *et al*., 2018) causing morphological abnormalities or deformities.

Bicephaly refers to the presence of two heads in a single individual (Bárcenas-Ibarra *et al*., 2015). Animals with bicephalic (two heads/Siamese twins) have been recorded and reared successfully to some extent in higher vertebrates like reptiles, amphibians and mammals (Velo-Anton *et al*., 2007; Fernandez-Alvarez *et al*., 2011; Martín-del-Campo *et al*., 2021), whereas it is reversed in the case of lower vertebrates and invertebrates with rare success. Morphological abnormalities are observed commonly in hatchery-reared flatfishes and are assumed to develop during the period of asymmetric change and tissue differentiation associated with metamorphosis (Seikai *et al*., 1987). The occurrence of two headed juvenile seahorse has been reported by Blanco *et al.* (2012), whereas aquarists/hobbyists might have observed these abnormalities but not reported them scientifically. Other deformities in newborn juveniles, like tail and snout deformities, have been observed during captive breeding in seahorses (Murugan, 2004).

There has been growing interest in captive breeding, rearing, and culturing of seahorses in many countries to reduce the pressure on the wild stocks (Job *et al*., 2002; Wong and Benzie, 2003; Murugan *et al*., 2009). Commercial seahorse culture for species like *H. kuda, H. trimaculatus* and *H. erectus* has been carried out for the aquarium industry from many other countries following the CITES guidelines (Vincent and Koldewey, 2006; Koldewey and Martin-Smith, 2010; Koning and Hoeksema, 2021). However, seahorse aquaculture is a relatively new industry with many challenges, such as low reproductive efficiency and juvenile survivorship (Lin *et al*., 2007), and much research is still required to improve the efficacy and commercial viability of seahorse aquaculture. The age and associated size of the parent of syngnathids can influence the embryonic size (Berglund *et al*., 1986; Masonjones and Lewis, 2000), fecundity, growth, and survival of the offspring (Dzyuba *et al*., 2006). Environmental factors such as food and nutrition can also greatly influence brood size by affecting gonad development (e.g. ovary development) and fertilization (Foster and Vincent, 2004).

Captive breeding trials and mass-scale culture of the yellow seahorse *H. kuda* were carried out at the Aquaculture Laboratory of the National Institute of Oceanography, Goa. For all the mating pairs, optimum water quality parameters were maintained (salinity, 26±1 ppt; temperature, 27±2ºC; dissolved oxygen, >6 mg L^{-1}), and the feeding was done *ad libitum* by providing wild-caught mysids (*Mesopodopsis orientalis*) and cultured amphipod (*Grandidierella nioensis*). Out of the observed 7 captive spawning of *H. kuda*, one juvenile released by the last spawning pair was found to be born with a bicephalic head with a laterally conjoined body. Under normal circumstances, the juvenile inflates the swim bladder and exhibits pelagic behavior immediately after the release from the male brood pouch (Woods, 2002).

However, the bicephalic juvenile did not exhibit pelagic behavior and was found to be confined to the bottom to mid water of the juvenile rearing tank. The main reason might be due to the independent movement of the head; through this behavior, the pelagic movement was restricted while the two head juvenile could able to swim and reach out only up to the middle of the water column, whereas the water depth maintained in the juvenile rearing tank was 50 cm. Twinning in fish of the family Syngnathidae has been previously described in the pipefish, *Syngnathus floridae* (Jordan and Gilbert, 1882; Cable, 1940), but not in seahorses, whereas the first reported observations of conjoined twins in newborn seahorses, *Hippocampus guttulatus* was made in 2012 (Blanco *et al*., 2012), in multiple spawning events and such scientific reporting has been not yet reported in recent times. We observed that due to non-coordinated movement of the head, they could start the first feeding behavior, but they exhibited feeding strikes which might not be successful, and they could survive only for 62 h. During the 62 h survival period, the laterally conjoined juveniles attempted 14 feeding strikes. Deformities in fish can hinder their ability to capture prey and forage effectively, which ultimately leads to biological problems (Branson and Turnbull, 2008; Boglione *et al*., 2013). Deformities adversely affect the swimming performance of fish (Le Vay *et al*., 2007), whereas in the case of seahorse juvenile rearing inflating the swimming bladder coupled with pelagic behavior and lack of yolk sac resulting in immediate initial feeding after spawning are the biological threats in the case of deformed seahorses. In aquaculture practices, especially in salmon and sea bream, deformities like conjoined larvae and abnormalities in fins and vertebral column development have been noticed (Nguyen *et al*., 2008). The reason for those abnormalities might be due to high stocking, lack of nutritious diet, chemicals, and temperature of the cultured system, whereas the present study suggests that the bi-cephalic in seahorses might be due to the physiological activities involved during the gestation period. Further studies regarding conjoined twins need to be undertaken by researchers who are working in marine aquariums as well as in seahorse hatcheries to further improve the knowledge to overcome the shortfalls and an achievement in rearing the conjoined twins will be an interesting subject even for the marine ornamental industry.

Laterally conjoined bicephalic twins might have emerged from the unipolar embryonic development with bifurcated heads (Owusu-Frimpong and Hargreaves, 2000), whereas the exact mechanism of deformities is still a mystery (Chandra *et al*., 2023). Morphometric and meristic measurements of the dead conjoined juvenile were made as per the standard protocols described by (Lourie *et al*., 1999). Morphometric measurements carried out using an ocular micrometer under a compound microscope (Olympus BX40) are listed in Table 1. In the observed two headed juvenile, the body was found to be laterally fused from TD4 (between 4th and 5th trunk ring) to TD9 (9th and 10th trunk rings) with a common tail (Fig. 1). At birth, the wet weight of the two headed juvenile was 3.3 mg

Table 1. Morphometric characters and meristic counts of normal and bicephalic juvenile of yellow seahorse (*Hippocampus kuda*)

Morphometric characters	Bicephalic juvenile		Normal juvenile
	First body	Second body	
Height (Ht) (mm)	8		8
Weight (W) (mg)	3.3		1.9
Orbital diameter (OD) (mm)	0.48	0.48	0.49
Snout length (SnL) (mm)	1.1	1.1	1.1
Snout depth (SnD) (mm)	0.6	0.6	0.6
Head length (HL) (mm)	2.4	2.42	2.41
Head depth (HD) (mm)	1.5	1.5	1.5
Trunk length (TrL) (mm)	2.5	2.5	2.7
Trunk width between 4th and 5th trunk ring (TW 4) (mm)	1.6 for joined animal		0.7
Trunk width between 9th and 10th trunk ring (TW 9) (mm)	0.9	-	0.8
Trunk depth between 4th and 5th trunk ring (TD 4) (mm)	0.8	0.8	0.8
Trunk depth between 9th and 10th trunk ring (TD 9) (mm)	0.85	0.85	0.8
Tail length (TaL) (mm)	3.5	-	3.6
Dorsal fin length (DL) (mm)	0.7	-	0.7
Pectoral fin length (PL) (mm)	0.5	0.5	0.5
Anal fin length (AL) (mm)	0.3	0.3	0.3
Dorsal fin rays (DF)	18	-	18
Pectoral fin rays (PF)	16	16	17

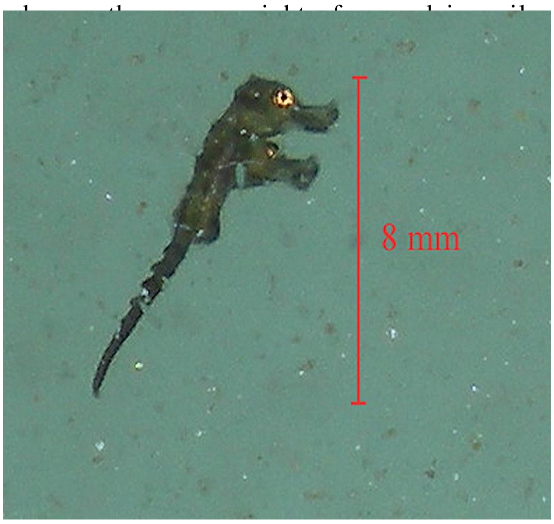

Fig. 1. Laterally conjoined twins of yellow seahorse (*Hippocampus kuda*)

Knowledge about the effects of captivity and environmental stress on fish populations is basic to the culture of any organism. Environmental stress can give rise to decreased developmental stability of individuals, which may result in reduced performance of fitness components. Animal welfare parameters should be given priority in aquaculture systems, which might reduce the profit a little bit but provide optimum conditions for the growing species and for sustainable growth of the industry.

The specific mechanisms of deformities are poorly understood; thus, the exact mechanisms behind the deformities are unclear (Chandra *et al.*, 2023). Behavioral studies can also constitute a useful tool for aquaculture and contribute to the improvement of rearing protocols, by allowing the evaluation of animal response to culture conditions and animal welfare assessment. Behavioral alterations can be indicative of environmental disturbances and animal distress (Mench and Mason, 1997; Huntingford *et al.*, 2006; Scherer *et al.*, 2018), and this can be particularly useful in aquaculture facilities.

Several factors are responsible for the induction of deformities in fish; still, the phenomenon is mysterious among the scientific fraternity (Chandra *et al.*, 2023). Adopting and practicing good aquaculture initiatives such as broodstock husbandry, gamete management, species specific standard breeding and larval rearing protocol, management of water quality parameters, and providing nutritionally balanced feed, especially to the broodstock and developing larvae (Nowosad *et al.*, 2018; Chandra *et al.*, 2023). Hence, the development of a seahorse husbandry manual is of prime importance to address this issue. The international aquarium trade for seahorses has seen a shift from wild to captive-bred sourcing (Foster, 2023), which will promote this industry for sustainable trade. Hence, it is important to develop a seahorse husbandry plan which will help in the captive breeding and rearing process for addressing issues like abnormalities, diseases, and aquaculture advancement techniques.

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REFERENCES

Bárcenas-Ibarra A, de la Cueva H, Rojas-Lleonart I, Abreu-Grobois FA, Lozano-Guzmán RI *et al.*, 2015. First approximation to congenital malformation rates in embryos and hatchlings of sea turtles. Birth Defects Res A Clin Mol Teratol, 103(3): 203-224, doi: 10.1002/bdra.23342

Berglund A, Rosenqvist G and Svensson I, 1986. Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). Behav Ecol Sociobiol, 19: 301-307, doi: 10.1007/BF00300646

Blanco A, Quintas P and Planas M, 2012. First observations of conjoined twins in newborn seahorses, *Hippocampus guttulatus* Cuvier. J Fish Dis, 35(9): 705-708, doi: 10.1111/j.1365-2761.2012.01373.x

Boglione C, Gisbert E, Gavaia P, Witten PE, Moren M *et al.*, 2013. Skeletal anomalies in reared European fish larvae and juveniles. Part 2: main typologies, occurrences and causative factors. Rev Aquac, 5: S121-S167, doi: 10.1111/raq.12016

Branson EJ and Turnbull T, 2008. Welfare and Deformities in Fish. In: Fish Welfare (Branson EJ, edn.), Blackwell Publishing, Oxford, pp 202-216

Cable LE, 1940. A two-headed embryo of the pipefish, *Syngnathus floridae*. J Elisha Mitchell Sci Soc, 56(1): 135-139

Chandra G, Saini VP, Kumar S and Fopp-Bayat D, 2023. Deformities in fish: A barrier for responsible aquaculture and sustainable fisheries. Rev Aquac, 16(2): 872-891, doi: 10.1111/raq.12872

Chandroo KP, Moccia RD and McKinley RS, 2000. Utilization of physiological telemetry to monitor behavioural responses of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to captive culture conditions. Bull Aquac Assoc Can, 4: 34-36

Clarke GM, 1995. Relationships between developmental stability and fitness: application for conservation biology. Conserv Biol, 9: 18-24, 10.1046/j.1523-1739.1995.09010018.x

Dzyuba B, Van Look KJW, Cliffe A, Koldewey HJ and Holt WV, 2006. Effect of parental age and associated size on fecundity, growth, and survival in the yellow seahorse *Hippocampus kuda*. J Exp Biol, 209(16): 3055-3061, doi: 10.1242/jeb.02336

Fernandez-Alvarez FÁ, Recuero E, Martinez-Solano Í and Buckley D, 2011. First Record of Bicephaly in *Lissotriton boscai* (Amphibia, Caudata, Salamandridae). North-west J Zool, 7(1): 161-163

Foster SJ and Vincent ACJ, 2004. Life history and ecology of seahorses: implications for conservation and management. J Fish Biol, 65(1): 1-61, doi: 10.1111/j.0022-1112.2004.00429.x

Foster SJ, 2023. Implementation of CITES Appendix II listing for seahorses in the context of export bans and suspensions. Fish Cent Res Rep, 31(3): 306

Gonzalez TJ, 2023. 'Positive' animal welfare in aquaculture as a cardinal principle for sustainable development. Front Anim Sci, 4: 1206035, doi: 10.3389/fanim.2023.1206035

Huntingford FA, Adams C, Braithwaite VA, Kadri S, Pottinger TG *et al.*, 2006. Current issues in fish welfare. J Fish Biol, 68(2): 332-372, doi: 10.1111/j.0022-1112.2006.001046.x

Job SD, Do HH, Meeuwig JJ and Hall HJ, 2002. Culturing the oceanic seahorse, *Hippocampus kuda*. Aquaculture, 214(1-4): 333-341, doi: 10.1016/S0044-8486(02)00063-7

Jordan DS and Gilbert CH, 1882. Notes on fishes observed about Pensacola, Florida, and Galveston, Texas, with description of new species. Proc US Natl Mus, 5: 241-307

Kihslinger RL and Nevitt GA, 2006. Early rearing environment impacts cerebrellar growth in juvenile salmon. J Exp Biol, 209: 504-509, doi: 10.1242/jeb.02019

Koldewey HJ and Martin-Smith KM, 2010. A global review of seahorse aquaculture. Aquaculture, 302: 131-152, doi: 10.1016/j.aquaculture.2009.11.010

Koning S and Hoeksema BW, 2021. Diversity of seahorse species (*Hippocampus* spp.) in the international aquarium trade. Diversity, 13(5): 187, doi: 10.3390/d13050187

Le Vay L, Carvalho GR, Quinitio ET, Lebata JH, Ut VN *et al*., 2007. Quality of hatchery-reared juveniles for marine fisheries stock enhancement. Aquaculture, 268(1-4): 169-180, doi: 10.1016/j.aquaculture.2007.04.041

Lin Q, Gao Y, Sheng J, Chen Q, Zhang B *et al*., 2007. The effects of food and the sum of effective temperature on the embryonic development of the seahorse, *Hippocampus kuda* Bleeker. Aquaculture, 262(2-4): 481-492, doi: 10.1016/j. Aquaculture.2006.11.011

Lourie SA, Vincent ACJ and Hall HJ, 1999. Seahorses: An Identification Guide to the World's Species and Their Conservation. Project Seahorse, London, pp 214

Marichamy R, Lipton AP, Ganapathy A and Ramalingam JR, 1993. Large scale exploitation of seahorse (*Hippocampus kuda*) along the Palk Bay coast of Tamil Nadu. Mar Fish Infor Serv T & E Ser, 119: 17-20

Martín-del-Campo R, Calderón-Campuzano MF, Rojas-Lleonart I, Briseño-Dueñas R and García-Gasca A, 2021. Congenital malformations in sea turtles: puzzling interplay between genes and environment. Animals (Basel), 11 (2): 444, doi: 10.3390/ani11020444

Masonjones HD and Lewis SM, 2000. Differences in potential reproductive rates of male and female seahorses related to courtship roles. Anim Behav, 59(1): 11-20, doi: 10.1006/anbe.1999.1269

Mench JA and Mason GJ, 1997. Behaviour. In: Animal Welfare (Appleby MC, Hughes B O, edn.), CAB International, Wallingford, pp 127-141

Moller AP and Swaddle JP, 1997. Asymmetry, Developmental Stability, and Evolution. Oxford Series in Ecology and Evolution. Oxford University Press, New York, pp 291

Murugan A and Durgekar R, 2008. Beyond the Tsunami: Status of Fisheries in Tamil Nadu, India: A Snapshot of Present and Long-term Trends. UNDP/UNTRS, Chennai and ATREE, Bangalore, India, pp 75

Murugan A, 2004. Biology and culture of the seahorse *Hippocampus trimaculatus* (Leach, 1814). PhD Thesis, Annamalai University, India, pp 176

Murugan A, Dhanya S, Sreepada RA, Rajagopal S and Balasubramanian T, 2009. Breeding and mass scale rearing of three spotted seahorse, *Hippocampus trimaculatus* Leach under captive conditions. Aquaculture, 290: 87-96, doi: 10.1016/j.aquaculture.2009.01.033

Murugan A, Sreepada RA, Sanaye SV and Pawar HB, 2017. Captive breeding and rearing of the yellow seahorse, *Hippocampus kuda* (Bleeker, 1852) in support of the marine ornamental fish industry and conservation. Indian J Geo-Mar Sci, 46 (10): 1996-2002

Nguyen VT, Satoh S, Haga Y, Fushimi H and Kotani T, 2008. Effect of zinc and manganese supplementation in *Artemia* on growth and vertebral deformity in red sea bream (*Pagrus major*) larvae. Aquaculture, 285: 184-192, doi: 10.1016/j.aquaculture.2008.08.030

Nowosad J, Sikora M and Kucharczyk D, 2018. Survival rates and the occurrence of larval malformations, including Siamese twins, following fertilization of post-ovulatory aged oocytes in ide *Leuciscus idus*. Dis Aquat Organ, 127(3): 237-242

Owusu-Frimpong M and Hargreaves JA, 2000. Incidence of conjoined twins in tilapia after thermal shock induction of polyploidy. Aquac Res, 31: 421-426, doi: 10.1046/j.1365-2109.2000.00440.x

Pawar HB, Sanaye SV, Murugan A, Sreepada RA and Tanu, 2011. Effect of tank background colour on the growth and survival of juvenile yellow seahorse, *Hippocampus kuda* (Pawar, 1852) in the pelagic phase. Isr J Aquac Bamidgeh, 63(4) IIC: 63.2011.621

Scherer L, Tomasik B, Rueda O and Pfister S, 2018. Framework for integrating animal welfare into life cycle sustainability assessment. Int J Life Cycle Assess, 23: 1476-1490, doi: 10.1007/s11367-017-1420-x

Seikai T, Shimozaki M and Watanabe T, 1987. Estimation of larval stage determining the appearance of albinism in hatchery-reared flounder *Paralichthys olivaceus*. Nippon Suisan Gakk, 53: 1107-1114

UNEP-WCMC, 2003. Global Marine Aquarium Database. Cambridge (UK). Available In: https://resources.unep-wcmc.org/products/WCMC_DT004

Vaidyanathan T and Vincent ACJ, 2021. State of seahorse fisheries in India, nearly two decades after they were banned. Biodivers Conserv, 30(5): 2223-2253, doi: 10.1007/s10531-021-02188-6

Velo-Anton G, Buckley D, Daoudi AD and Rivera AC, 2007. Bicephaly in *Salamandra salamandra* larvae. Herpetol Bull, 101: 31-33

Vincent ACJ and Koldewey HJ, 2006. An uncertain future for seahorse aquaculture in conservation and economic contexts. In: JH Primavera, ET Quinitio & MRR Eguia (Edn.), Proceedings of the Regional Technical Consultation on Stock Enhancement for threatened Species of International Concern, Iloilo City, Philippines, 13-15 July 2005, Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center, pp 71-84

Vinod K, Saravanan R, Johnson B, Murugan A, Joshi KK *et al*., 2018. Seahorse resources in India: need for paradigm shift in approach for sustainable fisheries and conservation. Marine Fisheries Policy Series -8, pp 1-106

Wong JM and Benzie JAH, 2003. The effects of temperature, *Artemia* enrichment, stocking density and light on the growth of juvenile seahorses, *Hipppocampus whitei* (Bleeker, 1855), from Australia. Aquaculture, 228: 107-121, doi: 10.1016/S0044-8486(03)00320-X

Woods CMC, 2002. Natural diet of the seahorse *Hippocampus abdominalis*. NZ J Mar Freshw Res, 36: 655-660, doi: 10.1080/00288330.2002.9517121

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