Latin American Journal of Aquatic Research, 51(3): 447-451, 2023
DOI: 10.3856/vol51-issue3-fulltext-2996

Short Communication

Transport conditions in the planktonic phase for seahorses *Hippocampus reidi* Ginsburg, 1933 (Teleostei: Syngnathidae)

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ABSTRACT. Thousands of seahorses are traded every year as marine ornamental species. Although packing methods for live adult seahorses have already been addressed, there is no information on the transportation of juvenile seahorses. Thus, this study aimed to evaluate the possibility of packing and transportation for newly-birthed seahorses. Three batches of newborn long-snout seahorses *Hippocampus reidi* were transported in three independent events from Ubatuba, north coast of São Paulo, to the city of São Paulo, covering 248 km in each trip, with an average duration of 3.5 h. A mean of 350 offspring of one day old was distributed in 1 L containers with round borders at equal densities, provided with constant aeration by portable pumps. Aeration was kept so that large bubbles would create an ascending flow keeping juveniles in suspension throughout transportation. Values of survival achieved in this study, even after 10 days post-transportation, were surprisingly high, reaching over 70%. No significant shift in water temperature, pH, or salinity was observed for any transportation events. For oxygen levels, although portable pumps were used, a drop of around 15% in DO level was recorded. In view of the trending ornamental fish market, considering the limited swimming capacity of newly born seahorses and that a great number of marine fish undergo a planktonic phase, this study provides important directions to a better understanding on seahorse transportation and yet, directions for the design of new transport methodology for an efficient shipping protocol for other species.

Keywords: *Hippocampus reidi*; shipping; packing; ornamental fish; transportation; offspring

The sustainability of the exploitation of aquatic resources is an issue that has been considered in recent studies (Kuhnen et al. 2019, Bergamo et al. 2021). The marine aquarium trade often depends on organisms collected from reef environments. However, reefs are declining because of multiple anthropogenic stressors, and sustaining functional diversity is critical for maintaining reef resilience (Carvalho et al. 2022). Several technological advances have been developed to increase the sustainability of fisheries resources. Examples of such technologies are developing larviculture protocols for threatened species or species likely to be traded in the aquarium industry, such as seahorses (Willadino et al. 2012, Cohen et al. 2017). However, aquatic organisms' transport techniques need to evolve for trading, including developing techniques for fingerlings transportation.

The trade-in of live ornamental fish for the aquarium industry is a global activity that allows species from different regions of the world to be acquired and maintained anywhere else (Livengood & Chapman 2007, Correia & Rodrigues 2017). Traditionally, freshwater and marine fish have been transported in recirculating and static systems (Portz et

Corresponding editor: Alejandra Volpedo

al. 2006, Stieglitz et al. 2012). Considering the increasing variety of traded species, although most practices aim to minimize stress and increase survival rates throughout transportation, shipping methodology must be developed for each species or developmental stage (Emata 2000, Paterson et al. 2003, Harmon 2009).

Thousands of seahorses are traded yearly as marine ornamental species (Cohen et al. 2017, Foster et al. 2019). Although packing methods for live adult seahorses have already been addressed (Cohen et al. 2018), there is no information on the transportation of newly-born seahorses. Besides supplying the aquarium industry, implementing efficient shipping protocols in all developmental stages is fundamental for maintaining viable populations in captivity for conservation purposes. Therefore, packing and shipping represent a major step for traders as it poses high mortality risks (Lim et al. 2003, Wabnitz 2003, Cohen et al. 2018).

Considering the unique morphology, biology, and physiology of juvenile seahorses and that there is no information available on the transportation of juveniles, this study aimed to evaluate the possibility of packing and transportation for newly-born offspring.

For testing the proposed method, three spawns of newborn long-snout seahorses *Hippocampus reidi* were transported, each batch in an independent event by land from the Ubatuba Aquarium, north coast of São Paulo, Brazil, to the Biosciences Institute of the University of São Paulo. The total distance covered in each trip was 248 km with an average duration of 3.5 h. Transport and maintenance of individuals were carried out with authorization from the ethics committee of the University of São Paulo (Nº3017/2018) and under authorization from the Ministry of the Environment (License SISBIO/ICMBIO Nº65473/2).

Before each transportation event, a mean of 350 ± 42 offspring of one day old from the same birth event was distributed in 1 L containers with round borders at equal densities (1:10, ind:mL), provided with constant aeration by battery-powered aerators (BOYU D-200). Water was filled to the top of the bottles. As number of offspring per birth differed, the number of bottles used varied within trips, comprising 3 to 4 bottles (Table 1). Animals were shipped in the same water they were kept at the aquarium to avoid rapid changes in water quality. Aeration was kept at a low flow, approximately 180 bubbles min^{-1}, provided by a regulated output of 2 L min^{-1}, with no air stone, and silicon tube going as far as the middle of the bottles in a way that large bubbles would create an ascending flow keeping juveniles in suspension at all times throughout transportation (Fig. 1). Bottles were kept with no lid. However, a soft sponge was used to hold air tubes in place and seal up the top of the containers. Physicochemical parameters of the water were monitored at the beginning and end of each experiment (Table 2). After transportation, growth, and survival were evaluated over 10 days, with measures of growth being taken at arrival (day 1) and at day 10, and survival registered daily.

Upon arrival, animals were kept in three separate plankton-kreisels equipped with physical and biological filters, besides a skimmer and UV filter, keeping parameters close to those prior to transportation (24ºC, pH 8, and salinity 25). Feeding was conducted five times daily with commercial rotifers (*Brachionus plicatilis*). The mean survival rate was 95% up to 48 h post-transportation and stabilized at 75% from the 7th day onwards (Fig. 2). For the third transportation event, survival was kept at 100% until day 3 but showed the lowest rate on day 10 (70%). For the second event, although mortality was high for the first days, survival kept stable from day 4, ending up with 80% of survival. For growth rate, at day 1, referring to the day of transportation, animals measured 0.63 ± 0.17 cm and presented a mean growth of 0.36 ± 0.02 cm within 10 days post-transportation, summing up to 0.99 ± 0.2 cm. Growth curves followed the same pattern for the three independent trips (Fig. 3).

Temperature can change during transport, affecting oxygen consumption and gas solubility in the transport water and metabolic rate. Initially, the average temperature of shipping water was 23.6 ± 0.5°C (Table 2). In this study, no significant change in water temperature was observed for any transportation events (Table 2). Likewise, during these short-term trips, pH and salinity did not change throughout the transport period. However, although aerators were used for oxygen levels, a drop of around 15% in dissolved oxygen (DO) was recorded (Table 2).

The survival of all offspring after transportation and recovery suggests that *H. reidi* is a remarkably resistant species, with most of them feeding immediately after shipping and keeping a steady survival seven days post-transportation, and can be successfully shipped under different conditions, as previously stated by Cohen et al. (2018).

Low-density packing is a common practice for marine ornamental species as animals represent high trade prices (Cole et al. 1999, Lim et al. 2003, Cohen et al. 2018). For seahorses, most traders pack single individuals or pack at very low densities for adult *H. reidi* (Cohen et al. 2018). Most of the data on the transport of seahorses regards adults or sub-adults rather

Table 1. Transport conditions of live newly born seahorse *Hippocampus reidi* in each transport event.

Event	Births number	Offspring's number	Number of bottles	Mean individuals per mL
1	1	356	4	1: 8.9
2	1	392	4	1: 9.8
3	1	308	3	1:1.02

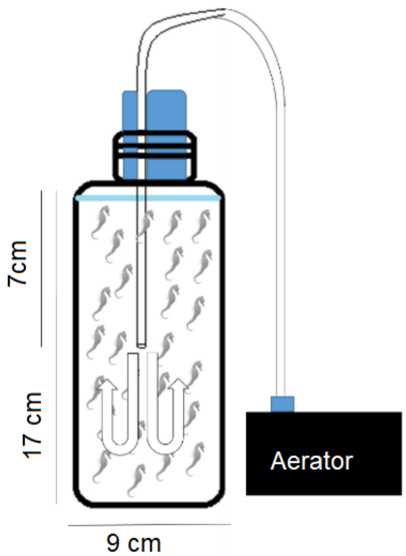

Figure 1. Schematic representation of the system tested in the shipping experiment of live juvenile seahorses *Hippocampus reidi.*

Table 2. Water parameters (means ± standard deviation) before and after transporting live planktonic seahorse *Hippocampus reidi*. DO: dissolved oxygen.

	Start	End
Temperature [°C]	23.6 ± 0.5	24.0 ± 0.3
Salinity	25.7 ± 0.2	25.7 ± 0.3
pH	8.0 ± 0.03	8.0 ± 0.03
DO [%]	76.3 ± 0.04	69.38 ± 1.19
DO [mg L^{-1}]	5.6 ± 0.01	4.6 ± 0.01
Nitrite [NO_2]	0.05 ± 0.05	0.1 ± 0.1
Ammonia [NH_3]	0	0

than newly-born juveniles; thus, further research on the application of this method to different sizes is required. Considering that the weight of the water being shipped will influence the cost of transportation, this study brings the possibility of transporting juvenile seahorses at densities as high as 100 ind L^{-1}, thus, reducing shipping costs. However, it is important to consider that the size of the animals evaluated in this study (0.63 ± 0.17 cm) and the duration of transport are the major factors to allow such high densities for the species.

During shipping, fish consume oxygen, release CO_2 and excrete ammonia into the holding water (Tang et al. 2009). Although increased values for nitrite were not expected, this might reflex the initial upload registered, especially considering stocking densities per bottle. Although the levels reached in this study were low, it is important to consider that excreted ammonia can reach toxic levels depending on fish species, size, density, stress condition, and transit time (Portz et al. 2006). Nevertheless, in static systems, the accumulation of CO_2 will reduce water pH and may affect the oxygen-carrying capacity of hemoglobin (Lim et al. 2003). In this study, the continuous flow of air was enough to compensate for the accumulation of CO_2, maintaining steady pH levels, but still, a drop in values of DO was observed. Thus, the evaluation of water parameters during long-term transportations is also required in order to ascertain if values of DO would stabilize or keep declining to limiting levels.

Seahorses have a low survival rate during early stages, especially in the first 15 days of life, which comprises a period of feeding transition as the offspring grow. Thus, the values of survival achieved in this study, even after 10 days post-transportation, were surprisingly high compared to those observed by Willadino et al. (2012), which were as low as 30% at the first 10 days of life. Moreover, growth rates were equally steady for the three groups evaluated, besides differences in magnitude related to the initial size. The light flow provided by the system, keeping juveniles in suspension at all times throughout transportation, is probably a major factor in survival rates. Although micro-bubbles might induce air ingestion in juvenile seahorses (Sanaye et al. 2013), the absence of a bubble stone provided enough flow while minimizing the risks of ingestion. Although efficient, the system depends on portable air pumps and might need extra equipment in case of failures.

Although more specific studies need to be carried out, this study's first report on the successful packing and transportation of juvenile seahorses provides important data on adequate conditions for the early stages of *H. reidi* transportation. In view of the trending

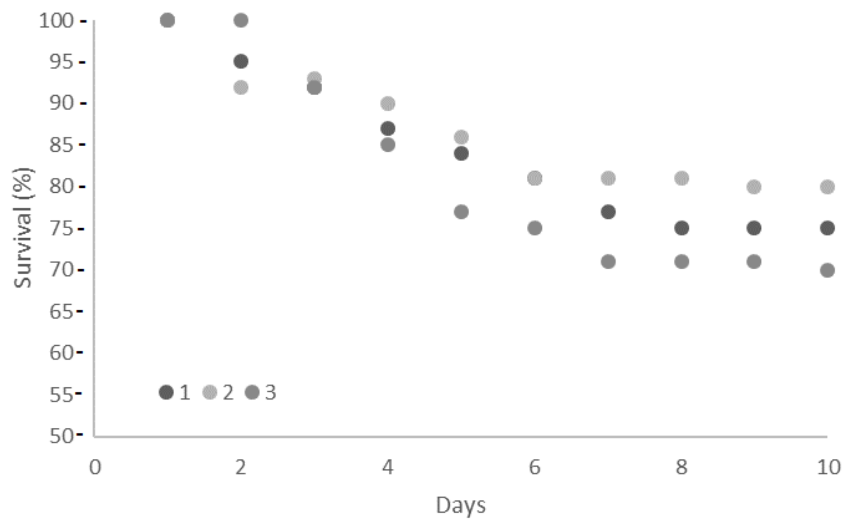

Figure 2. Survival of juvenile seahorses *Hippocampus reidi* from day 1 (transport) through 10 days post-transportation, representing the mean of the three trips. Circles 1, 2, and 3 represent the means of three independent trips.

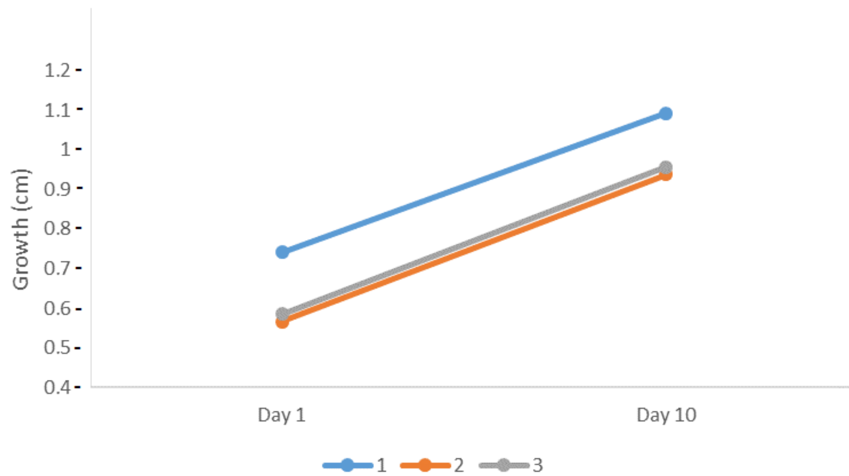

Figure 3. Mean length of juvenile seahorse *Hippocampus reidi* after shipping, in three batches, measured on arrival (day 1) and 10 days post-transportation. Lines 1, 2, and 3 represent the means of three independent trips.

ornamental fish market, and that a great number of marine fish undergo a planktonic phase, this study provides important directions, such as air flow and containers design, to the implementation of efficient shipping protocols in other species. Such protocols are fundamental once will allow the maintenance of viable populations in captivity for conservation purposes and supply for the aquarium trade, besides trades associated to scientific research.

Conflict of interest

The authors declare that they have no competing interests.

Authors' contributions

B.L.M. and J.A.S.N. were responsible for data collection, statistical analyses, interpretation of data, and drafted paper. R.E.K. helped with the analysis, interpretation of data, and drafted paper. H.G.N. helped with analysis and financial support. E.G.S. was responsible for the designed study, interpretation of data, statistical analyses, and study supervision. All authors read and approved the final manuscript.

REFERENCES

Bergamo, G.C.A., Olier, B.S., Sousa, O.M., Kuhnen, V.V., Pessoa, M.F.G. & Sanches, E.G. 2021. Economic feasibility of mussel (*Perna perna*) and cobia

(*Rachycentron canadum*) produced in a multi-trophic system. Aquaculture International, 29: 1909-1924. doi: 10.1007/s10499-021-00762-x

Carvalho, L.M., Mies, M., Inagaki, K.Y., Sanches, E.G., Souza, M.R., Longo, G.O. & Tomás, A.G.R. 2022. The marine ornamental market in Brazil (Southwestern Atlantic) frequently trades prohibited and endangered species and threatens the ecosystem role of cleaning mutualism. Marine Policy, 146: e105305. doi: 10.1016/ j.marpol.2022.105305

Cohen, F.P., Valenti, W.C., Planas, M. & Calado, R. 2017. Seahorse aquaculture, biology and conservation: knowledge gaps and research opportunities. Reviews in Fisheries Science & Aquaculture, 25: 100-111. doi: 10.1080/23308249.2016.1237469

Cohen, F.P., Planas, M., Valenti, W.C., Lillebø, A. & Calado, R. 2018. Optimizing packing of live seahorses for shipping. Aquaculture, 482: 57-64. doi: 10.1016/ j.aquaculture.2017.09.024

Cole, B.E., Clyde, S. & Bailey, R. 1999. Shipping practices in the ornamental fish industry. Center for Tropical and Subtropical Aquaculture, 131. CTSA, Honolulu.

Correia, J.P. & Rodrigues, N.V. 2017. Packing and shipping. Marine ornamental species aquaculture, Wiley, New Jersey, pp. 597- 607.

Emata, A.C. 2000. Live transport of pond-reared milkfish *Chanos chanos* broodstock. Journal of the World Aquaculture Society, 31: 279-282. doi: 10.1111/j. 1749-7345.2000.tb00364.x

Foster, S.J., Kuo, T.C., Wan, A.K.Y. & Vincent, A.C. 2019. Global seahorse trade defies export bans under CITES action and national legislation. Marine Policy, 103: 33-41. doi: 10.1016/j.marpol. 2019.01.014

Harmon, T.S. 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. Reviews in Aquaculture, 1: 58-66. doi: 10.1111/j.1753-5131.2008. 01003.x

Kuhnen, V.V., Costa, L.G., Raiol, K.L., Sousa, O.M. & Sanches, E.G. 2019. Mariculture impacts on the benthonic ichthyofauna of Itaguá Bay, Ubatuba, southeast Brazil. Boletim do Instituto de Pesca, 45: e500. doi: 10.20950/1678-2305.2019.45.4.500

Lim, L.C., Dhert, P. & Sorgeloos, P. 2003. Recent developments and improvements in ornamental fish packaging systems for air transport. Aquaculture Research, 34: 923-935. doi: 10.1046/j.1365-2109.2003. 00946.x

Livengood, E.J. & Chapman, F.A. 2007. The ornamental fish trade: an introduction with perspectives for responsible aquarium fish ownership. IFAS Extension, 24.

Paterson, B.D., Rimmer, M.A., Meikle, G.M. & Semmens, G.L. 2003. Physiological responses of the Asian sea bass, *Lates calcarifer* to water quality deterioration during simulated live transport: acidosis, red-cell swelling, and levels of ions and ammonia in the plasma. Aquaculture, 218: 717-728. doi: 10.1016/ S0044-8486(02)00564-1

Portz, D.E., Woodley, C.M. & Cech, J.J. 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries, 16: 125-170.

Sanaye, S.V., Pawar, H.B., Murugan, A., Sreepada, R.A., Singh, T. & Ansari, Z.A. 2013. Diseases and parasites in cultured yellow seahorse, *Hippocampus kuda* (Bleeker, 1852). Fishing Chimes, 32: 65-67.

Stieglitz, J.D., Benetti, D.D. & Serafy, J.E. 2012. Optimizing transport of live juvenile cobia (*Rachycentron canadum*): effects of salinity and shipping biomass. Aquaculture, 364: 293-297. doi: 10.1016/j.aquaculture. 2012.08.038

Tang, S., Thorarensen, H., Brauner, C.J., Wood, C.M. & Farrell, A.P. 2009. Modeling the accumulation of CO_2 during high density, recirculating transport of adult Atlantic salmon, *Salmo salar*, from observations aboard a sea-going commercial live-haul vessel. Aquaculture, 296: 102-109. doi: 10.1016/j.aquaculture. 2009.07.020

Wabnitz, C. 2003. From ocean to aquarium: the global trade in marine ornamental species. UNEP-WCMC Biodiversity Series, 17.

Willadino, L., Souza-Santos, L.P., Mélo, R.C., Brito, A.P., Barros, N.C., Araújo-Castro, C.M., et al. 2012. Ingestion rate, survival, and growth of newly released seahorse *Hippocampus reidi* fed exclusively on cultured live food items. Aquaculture, 360: 10-16. doi: 10.1016/j.aquaculture.2012.06.025

Received: October 18, 2022; Accepted: February 11, 2023