Aquaculture 482 (2018) 57–64

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Optimizing packing of live seahorses for shipping

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ARTICLE INFO

Keywords:
Physiology
Metabolism
Welfare
Trade
Production chain

ABSTRACT

The packing and shipping of live marine organisms always poses a potential risk to their survival and well-being, with the costs associated with these practices being paramount for marine ornamental species value chains. The present study describes two experiments employing the longsnout seahorse *Hippocampus reidi* (~80 mm) as a model seahorse species to optimize its packing methods for live shipping. The first experiment evaluated the combined effect of seahorse density (1 ind. per 300 mL, 1 ind. per 450 mL and 1 ind. per 600 mL), transit time (24 vs. 48 h), and use of an oxygen saturated atmosphere for packing (pure oxygen vs. compressed air). The second one evaluated the combined effect of water salinity (15, 25, and 35) and use of a substrate for packed specimens to hold onto it, at a density of 1 ind. per 300 mL. Survival was 100% in all treatments in both experiments up to 48 h after shipping, with ~90% of seahorses displaying a normal feeding behaviour immediately post-transportation. In the first experiment, no significant differences were found ($P > 0.05$) in weight-specific total ammonia nitrogen (TAN) excreted in all treatments within the same transit time. At the end of the transit time, treatments with an oxygen saturated atmosphere displayed an oversaturation in dissolved oxygen (DO) concentrations, whereas those employing compressed air for 48 h ended the experimental trial with a DO concentration above 80%. Water with a salinity of 15 promoted a significantly ($P < 0.05$) lower decrease in pH, followed by water at a salinity of 25 and 35. The lower salinity employed may have reduced breathing frequency of seahorses during transport. The presence of a substrate significantly ($P < 0.05$) decreased weight-specific TAN excreted, possibly due to stress reduction. Overall, *H. reidi* can be packed at a density as low as 1 ind. per 300 mL for up to 48 h, with the use of pure oxygen not being mandatory. Lower salinities and the use of substrate can enhance seahorse welfare when these are shipped over longer transit times without representing additional significant costs. Overall, the findings of the present study may allow traders to ship 3 times more live seahorses than they currently do without negatively impacting their welfare neither increasing associated shipping costs.

1. Introduction

Thousands of seahorses are traded live every year as marine ornamental species to supply the aquarium industry (Foster et al., 2016). As most marine ornamental species, seahorse trade involves transporting live specimens for long-distances by airplanes. Therefore, packing and shipping represent a major cost for traders and pose high mortality risks for the animal (Lim et al., 2007; Wabnitz et al., 2003; Wood, 2001), making the transport a key step for value and production chains. While remarkable improvements have been reported in seahorse aquaculture (Olivotto et al., 2011), researchers and enterprises have mostly overlooked the development of innovative shipping strategies when compared with other culture-related topics. To our best knowledge, there is no multifactor experimental-based study available on packing methods for live seahorses. Thus, improving transport methods for these highly priced organisms may bring economic benefits for producers and secure animal welfare (Cohen et al., 2017).

Most aquatic ornamental species are shipped in closed plastic bags filled with one third of water and two-thirds of pure oxygen inside Styrofoam boxes (reviewed in Correia and Rodrigues, 2017). The size of the bag and the volume of water being used depends on fish species, size and density. Marine ornamental species are pricey products and

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http://dx.doi.org/10.1016/j.aquaculture.2017.09.024
Received 27 June 2017; Received in revised form 12 September 2017; Accepted 14 September 2017
Available online 18 September 2017
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thus, it is a common practice to pack them at a low density (Cole et al., 1999; Lim et al., 2007), commonly a single specimen per bag (mono-packing). Nonetheless, one should consider that the weight of the water being shipped always surpasses that of the fish itself and thus, transportation cost is highly dependent on the amount of water being used per specimen during shipping.

Presently, there is no consistency on packing methods for live seahorses in the industry. Major traders of seahorses employ from mono-packing of *H. reidi*, at densities as low as 1 specimen per L, up to 5 specimens per 2 L (1 ind. per 400 mL) (Miquel Planas and Maik S.C. Hora, person. comm., 2017). Nonetheless, the unique morphology, biology and physiology of seahorses suggests that they can be packed in lower water volumes than other reef fish and without requiring the use of pure oxygen. In other words, it appears to exist room to optimize and standardize packing methods. For example, seahorse upright body position may allow traders to use a narrower bag for packing, thus decreasing the water volume used per specimen. Seahorses are ambush predators, have a low swimming capacity and remain attached to a substrate for long periods of time (Foster and Vincent, 2004). These features suggest a low metabolism and possible resilience to stress caused by transportation. Indeed, an experiment with *Hippocampus abdominalis* showed that this species has a fast recovery after transportation stress (Wright et al., 2007), corroborating the hypotheses above.

To ship live fish successfully, one must ensure suitable oxygen concentrations and water quality. Transit time may vary from < 24 h in domestic transportation up to 72 h on an international shipping (commonly averaging 48 h) (Cole et al., 1999). During shipping, fish consume oxygen, release CO_2 and excrete ammonia to the holding water. Air is more pressurized in closed bags than in the atmosphere and hence oxygen present in the shipping bag rapidly dissolves into the water as packed fish consume it, often not being a limiting factor (Berka, 1986). Nonetheless, the accumulation of CO_2 in closed shipping bags decreases water pH and may affect oxygen-carrying capacity of hemoglobin, regardless of oxygen availability (Berka, 1986; Lim et al., 2007). Notably, under low pH conditions (e.g., < 7.5), most ammonia nitrogen present in marine water is in the form of the ammonium ion (NH_4^+), which is less toxic to fish than un-ionized (NH_3) (Boyd, 2015). Yet, excreted ammonia can reach toxic levels depending on fish species, size, density, stress condition and transit time. A common practice employed to reduce fish excretion and oxygen demand during transportation is fasting the fish for a few days prior to packing.

Water quality on shipping is also closely related to fish stress (Portz et al., 2006). Stressed fish excrete more ammonia, are more sensitive to ammonia toxicity, may display osmoregulatory dysfunction and are more likely to consume higher levels of oxygen (Carneiro and Urbinati, 2001; Randall and Tsui, 2002). Thus, by ensuring optimal packing conditions that minimize fish stress during shipping, one may significantly improve the chances of successfully transporting live specimens. The salinity of shipping water may play an important role in controlling stress and oxygen consumption during transport. Adding salts to freshwater is a common practice to control osmoregulation dysfunctions, or other physiologic disorder, caused by stress when transporting live freshwater fish (Carneiro and Urbinati, 2001; Lim et al., 2007; Long et al., 1977; McDonald and Milligan, 1997). Therefore, a similar rationale may also be valid for saltwater fish, if one considers a decrease in salinity of shipping water. Another issue that remains unaddressed for seahorses is the relevance of adding a substrate to which they may hold. By adding a substrate during transport, it is likely that live seahorses may present lower energy expenditure to stabilize their position in the shipping water, consequently being less prone to stress.

This work evaluated the effect of packing density, transit time, use of an oxygen saturated atmosphere, water salinity and the presence of a substrate to which seahorses may hold on the shipping of *Hippocampus reidi*, one of the most heavily traded seahorse species in the marine aquarium industry (Foster et al., 2016; Cohen et al., 2017). We considered both animal welfare and possible benefits for diminishing shipping costs of traded specimens to evaluate the optimal packing procedure for live seahorses.

2. Material and methods

Two experiments were performed to test the effect of five factors relevant for the shipping of live seahorses. In the first one, it was tested the effect of density, transit time (the period from packing to unpacking) and the use of an oxygen saturated atmosphere. In the second experiment, it was tested the effect of salinity and the use of substrate where seahorse could hold during transportation.

In both experiments, longsnout seahorses (*Hippocampus reidi*) with ~80 mm of total length (TL) were used as a representative model for live trade of seahorses for marine aquariums; all specimens were bred in captivity in the facilities of Instituto de Investigaciones Marinas (CSIC) in Vigo (Spain) (for detailed description of cultured methods for this species, please refer to Planas et al., 2017). This study complied with the bioethical requirements from the Regional Government (Consellería do Medio Rural de Pontevedra, Spain #ES360570202001/16/EDU-FOR07/MPO0) and CSIC bioethics committee.

Seahorses were kept for 24 h without food before packing. After packing, bags with seahorses were placed inside closed Styrofoam boxes. These boxes were kept in the laboratory under controlled room temperature (~21 °C) and gently agitated for approximately 5 min every hour, except during 12 consecutive hours at night. This movement aimed to mimic real shipping conditions experienced by live seahorses.

2.1. Experiment 1: density, transit time and oxygen saturated atmosphere

The effect of density, transit time and oxygen saturated atmosphere were tested through a factorial experiment in a randomized block design with five replicates per treatment. Density was tested as the volume of water employed to pack a single specimen (mono-packing): 300 mL, 450 mL and 600 mL. Transit times tested were 24 h and 48 h. To evaluate the necessity of an oxygen saturated atmosphere inside shipping bags, it was tested the use of pure oxygen contrasted with compressed air. The ratio water:air inside the shipping bags was standardized at 1:2. Thus, to respect a minimum water column of ~85 mm to cover the seahorse, it were used three different shaped bags for this experiment (Fig. 1).

Overall, 60 seahorses were divided in five blocks with 12 treatments each – one Styrofoam box was employed per block. The water used for packing seahorses was 1 μm filtered UV-irradiated seawater retrieved from the grow-out system where all seahorses were stocked prior to the experiment.

2.2. Experiment 2: salinity and substrate

The effect of salinity and substrate were tested through a factorial experiment in a completely randomized design with five replicates per treatment. Based on preliminary results from experiment 1, each specimen employed was packed in a bag with 300 mL of water and 600 mL of pure oxygen for 48 h. Salinities tested were 15, 25 and 35. These salinities were selected considering that *H. reidi* has an isosmotic point of ~12 (Hora et al., 2016) and that it has been commonly cultured in salinities between 24 and 35 (reviewed in Planas et al., 2017).

As seahorses were initially stocked in a system at a salinity of 35 before the experiment, specimens were acclimated to the salinities of 15 and 25 one day before packing. For this acclimation seahorses were randomly separated in three aquariums (~30 L each) in a static system with aeration and controlled temperature (25 °C) and freshwater purified through reverse osmosis was slowly dripped until the aquarium water matched the target salinities. This process took approximately 8 h

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