



Criteria for assessing juvenile quality of the lined seahorse, *Hippocampus erectus*

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ABSTRACT

Juvenile quality has been demonstrated to be the key for commercial fish production. Variations in juvenile quality leading to varying growth and mortality are often encountered and well documented in many marine fish species. It is valuable to discover simple and reliable indicators for assessing juvenile quality in fish aquaculture. In this study, we established criteria for evaluating the juvenile quality of the lined seahorse, *Hippocampus erectus*, a Chinese traditional medicine and popular aquarium species. We raised 18 batches for 45 days from birth to determine the potential criteria for assessing the juvenile quality of the lined seahorse. There was a strong and significant correlation between survival through the fifth day and that on the day 45. By comparing the ratio of trunk width at 1/2 trunk length to trunk length (TW/TL) of dead and live juveniles during the first 5 days, we found that TW/TL is a reliable indicator of the juvenile quality, with the distinct threshold of 0.190.

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1. Introduction

Significant breakthroughs in the commercial culture of seahorses have only occurred in the last 10 years. This has in part been the result of increasing culture efforts since all 33 recognized seahorse species were listed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2004), due to overexploitation of the wild populations to meet growing demand in Chinese medicine and ornamental market (Lourie et al., 1999; Vincent, 1996). More than 10 seahorse species have been reared successfully in captivity, such as *Hippocampus abdominalis* (Woods 2000, 2003), *Hippocampus comes* (Job et al., 2006), *Hippocampus erectus* (Correa et al., 1989; Lin et al., 2008, 2009; Scarratt, 1995; Zhang et al., 2010), *Hippocampus kuda* (Job et al., 2002; Lin et al., 2006; Lin et al., 2007), *Hippocampus reidi* (Olivotto et al., 2008), *Hippocampus subelongatus* (Payne and Rippingale, 2000), and *Hippocampus trimaculatus* (Sheng et al., 2006). Research efforts have focused on the effects of food type and feed regimes, and various environmental factors, including temperature, salinity, light intensity and photoperiods on growth and survival of juvenile seahorses to establish appropriate rearing protocols (e.g., Lin et al., 2006, 2008; Olivotto et al., 2008; Payne and Rippingale, 2000; Woods, 2000, 2005). Although a big progress in technique of rearing seahorses has been made, variation in juvenile survival is still high. For example, survival of some batches of

the lined seahorse (*H. erectus*) could reach >70% at day 60 post hatch, while other batches may all die at day 20 post hatch under the same culture conditions (unpublished data). The variation in juvenile survival may result from intrinsic juvenile quality as has been observed in others in many fish species (Aristizabal et al. 2009; Kjorsvik et al., 2003; Matsuo et al., 2006; Shimma and Tsujigado, 1981).

Egg and larval quality of cultured fish has been recognized as one of the most important limiting factors of seed production and the main obstacle for aquaculture success (Bromage, 1985; Kjorsvik et al., 1990). Egg and larval quality is usually assessed with several parameters, including morphology, behavior, survival from stress test, and biochemistry (Aristizabal et al. 2009; Bromage, 1985; Kjorsvik et al., 2003; Matsuo et al., 2006; Shimma and Tsujigado, 1981). Developing a simple and reliable way to determine whether larvae are worth further rearing at the early stage is essential to avoid wasting resources. Different from other marine fish species, seahorses are released from the male pouch as juveniles. Up to date, there still is no juvenile quality criteria applied to seahorses.

The lined seahorse, *H. erectus*, a highly valued species in both medicinal and aquarium trades (Correa et al., 1989; Foster et al., 2003; Lourie et al., 1999; Scarratt, 1995), has been reared successfully in captivity, and recognized as a good species for commercial aquaculture (Correa et al., 1989; Lin et al., 2008, 2009; Zhang et al., 2010). In this study, we evaluate juvenile quality parameters to establish simple and effective assessment protocols using the lined seahorse as the model species.

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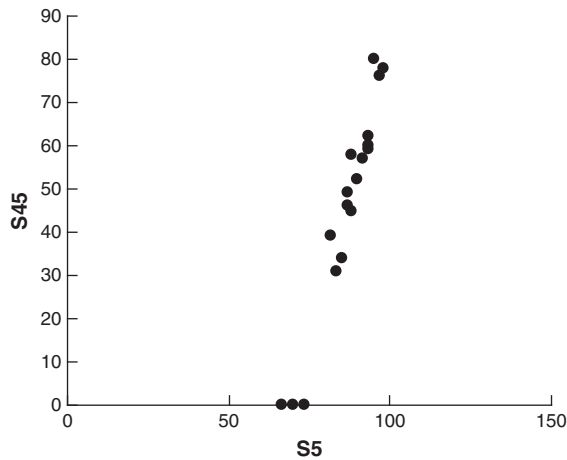


Fig. 1. Correlation ($r = 0.9753$, $P < 0.05$) between the juvenile survival (%) on day 5 (S5) and on day 45 (S45).

2. Materials and methods

2.1. Broodstock seahorse

Twenty pairs of F_2 seahorses (16–20 cm in height (HT): the vertical distance from the tip of the coronet, to the tip of the outstretched tail, with the head held at right angles to the body (Lourie et al. 1999)), were used as broodstock and maintained in flow-through tanks ($90 \times 80 \times 60$ cm) at the Vero Beach Marine Laboratory, Vero Beach, Florida, USA with sand filtered seawater pumped directly from the Atlantic Ocean at a rate of 0.5–0.6 L/min. Salinity, temperature, light intensity, and photoperiod were 35‰, 26.5 ± 0.5 °C (mean \pm S.D., the same format throughout this paper), 1000 lx, 14 L/10 D, respectively. Plastic plants and corallites were used as the substrate and holdfasts for the seahorses. The broodstock seahorses were fed twice a day (0900 h and 1600 h) with frozen *Mysis* sp. (Hikari™) at a daily rate of approximately 15% wet body weight, and feces and uneaten food in the tanks were siphoned out 2 h after each feeding. Hatching occurred in the broodstock tanks, and newly hatched juveniles were moved out with a net for juvenile rearing.

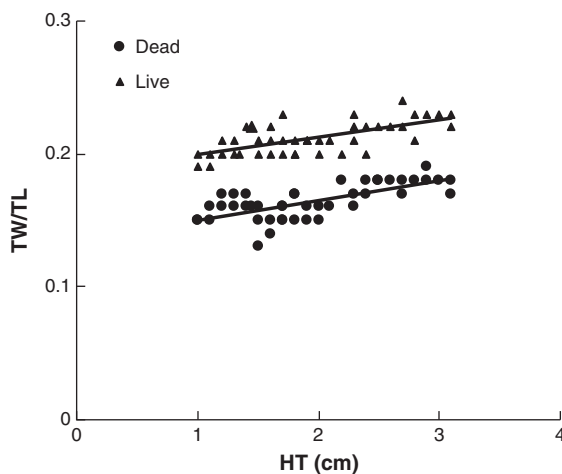


Fig. 2. Relationship between height (HT) and the ratio of trunk width at 1/2 trunk length to trunk length (TW/TL) in dead and live seahorses. TW/TL of dead juveniles was significantly (ANCOVA, $F_{1,83} = 628.106$, $P < 0.0001$) lower than that of live juveniles.

2.2. Juvenile rearing

Juveniles were reared at stocking density of 2 individuals/l with three replicate tanks ($50 \times 25 \times 30$ cm). Each flow-through tank contained 30 l seawater with gentle aeration and flow rate of 0.2 l/min. Newly hatched juveniles from individual males were haphazardly allocated to the three tanks. Plastic plants were used as holdfasts for the seahorses. The juveniles were fed twice at 0900 and 1600 h each day with enriched *Artemia sinica* nauplii (Bohai strain, China) at approximately 15 nauplii/ml. The 8-hour-old nauplii were enriched for 12 h with Selco® HUFA using the producer's recommended protocol. Before each feeding, the bottom of the tanks was siphoned to remove feces and uneaten food. Salinity, temperature, light intensity, and photoperiod were 34–35‰, 26.5 ± 0.5 °C, 1000 lx, 14 L/10 D, respectively, throughout the experimental period (up to 45 days).

2.3. Juvenile quality parameters

Early juvenile survival and the ratio (TW/TL) of trunk width (TW) at 1/2 trunk length to trunk length (TL) were used to evaluate juvenile quality. We assume if early juvenile survival is low, survival of juveniles at late stage would be low as well. TW/TL was used as a potential indicator for assessing juvenile quality because it is similar to the condition factor (ratio of weight to height) of the juvenile, which is for assessing the stoutness of fish. Generally, low condition factor is a symbol of poor quality. In seahorse, the condition factors of dead ones are low and the body is thin. Hence, TW/TL could be a quick indicator of juvenile seahorse quality. Measuring trunk length and width is easier than measuring height (HT) and wet weight (WW). In the experiment, a total of 18 batches from 18 males were cultured for up to 45 days. Ten seahorses were haphazardly selected from each tank on day 5 for measuring TL, HT, TW, and WW to determine growth differentiation. Thereafter, the seahorses were measured on days 15, 25, 35 and 45 post hatch. When the experiment was terminated on day 45 all the seahorses were counted. Specific growth rate [$SGR = 100 \times (\ln \text{final WW} - \ln \text{initial WW}) / \text{time}$], condition factor [$CF = 100 \times WW(g) / HT \text{ (cm}^3\text{)}$], and W/TL of the juveniles were calculated. During the culturing period, if any mortality occurred, dead seahorses were removed upon daily inspection and recorded. HT, TL, and TW of dead individuals were measured. Meanwhile, same number of live juvenile(s) was/were haphazardly selected from the same tank and its/their HT, TL, and TW were measured.

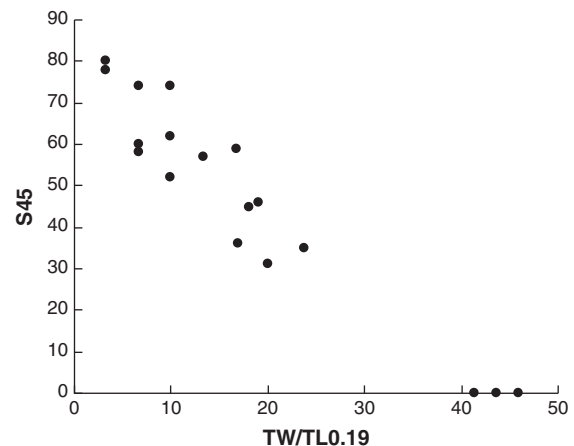


Fig. 3. Correlation ($r = -0.9583$, $P < 0.05$) between percentage of juveniles with TW/TL (the ratio of trunk width at 1/2 trunk length to trunk length) of < 0.190 (TW/TL.0.19) and the juvenile survival (%) on day 45 (S45).

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