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Survival and Growth of Two Consecutive Broods of Captive-Bred Lined Seahorses (Hippocampus erectus)

Tianyu Ren

Shanghai Starriver Bilingual School, Shanghai, China
er123ethan@163.com

Abstract. Survival and growth pattern of two consecutive broods of captive bred lined seahorse (Hippocampus erectus) in a recirculating aquaculture system was studied. Brood 1 (n = 90) was fed with frozen, decapsulated Artemia (A.) eggs while brood 2 (n = 200) was fed with live rotifers and Chlorella. To describe juvenile performance for the two feeding regimes, survival, and timing of death (mortality rate) and body length were monitored. The estimated survival of brood 1 decreased to 18.9% by day 94 while that of brood 2 decreased to 46.2% by day 64; the estimated survival curves of the two broods differed significantly. For both broods mortality was focussed in the first few days post release. Results from measurements of body length suggested that both cohorts had grown, but due to the fact that only one individual was measured at each time point, it was not possible to compare growth between the two cohorts. There may be feeding and husbandry differences related to the different survival rates, but it is not possible to make causal conclusions given that this was an observational design and other brood-level factors differ. These observations reminding the critical husbandry requirements at an early stage, and further controlled research with respect to feeding regime and environment conditions would lead to a significant improvement in the survival of the juveniles of H. erectus.

Keywords: Hippocampus erectus, juvenile survival, feeding regimes, mortality, recirculating aquaculture system

1. Introduction

Since the market demand of seahorses is still continuing, aquaculture could play a role to feed the market and decrease the dependence on seahorses from wild resources. Previous studies have investigated the reproduction biology of the lined seahorse (Hippocampus erectus) along with its juvenile growth and survival rate when reared at various temperatures [1, 2] with the view of using seahorse for aquaculture. Good production of juveniles however continues to be difficult and feeding practices and husbandry can have a significant impact on survival and growth at early stages of development. Knowledge of the factors involved in the early survival of the juveniles will, therefore, be useful in the improvement of rearing practices, and help produce consistent numbers in the nursery. It will be important in this study to pay attention to early feeding as there are different size, nutrient qualities and mobility and accessibility of prey items. Previous studies on H. erectus have shown that in combination with copepods Artemia nauplii led to improved rate of juvenile

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survival as compared to the offering of Artemia alone [3]. For other seahorse species, research has also shown the importance of the initial feeding regime impacting on the performance of the juveniles. A comparison of various initial feeding regimes was performed in Hippocampus hippocampus by Otero-Ferrer et al. [4] and a study on newly released Hippocampus reidi looking at the prey intake, survival and growth employed various live feeding/rearing protocols [5]. Comparative results from these studies will be used to evaluate the feeding conditions used in the present research and inter-species differences will be compared to.

The interpretation of juvenile survival and growth, in addition to feeding practices, will be inferred based on other husbandry and environmental conditions such as: temperature, and stocking density. Temperature has been found to be an important factor in the culture of H. erectus as the comparatively high survival and growth of H. erectus in 28-29°C under the conditions tested by Lin et al. [2]. Other research has also included studying the effects of stocking density on the performance of juveniles in recirculating aquaculture systems (RAS). Also, analysing the time of death will help point out times of high early rearing mortality. It is important to note that there might be confounders between broods, so that comparisons based on observations present some difficulties in interpretation, and should not be used to find the (independent) effects of a particular factor.

The objective of this study will be to describe and contrast survival to and mortality of two two consecutive broods of captive bred H. erectus in the same recirculating aquaculture system, as well as changes in body length. During the early rearing phase the first brood will be reared under a feeding regime with freeze dried, decapsulated Artemia eggs and the second brood will be reared using live rotifers with Chlorella. The study will provide description of mortality over time, comparison of mortality among broods reared under the same conditions, and obtain body-length measurements to document body length changes. Findings can then be analyzed and compared to previously published work to possibly discover any relationship between feeding practices, rearing conditions and juvenile performance. Since both broods are going to be different in more than one condition, it will not explain differences in survival due to single cause of the diet. The results will lead to a realistic description of the results of early rearing of juveniles and aid in prioritizing future controlled feeding and husbandry experiments in H. erectus.

2. Methods

2.1. Study animals and origin

Two consecutive broods of the lined seahorse, Hippocampus erectus, were produced by a single captive-bred parental group maintained in a private indoor system. All animals were captive-bred and no wild-collected individuals were involved. The genus Hippocampus has been listed in Appendix II of CITES since 2004 [1], only captive-bred stock was used. Brood 1 was released on 18 March 2026 (n = 90) and brood 2 on 29 June 2026 (n = 200).

2.2. Rearing system

Fish were reared in a single recirculating system comprising a main tank (0.7 x 0.7 x 0.7 m) and a sump (0.55 x 0.55 x 0.55 m). The working water volume was approximately 468 L, against a combined geometric capacity of 509 L, as the system was not filled to the brim. Temperature was maintained with an aquarium heater, supplemented by room air-conditioning in summer; 24 measurements ranged from 24.8 to 25.5 C (brood 1: 25.13 +/- 0.19 C, n = 15; brood 2: 25.30 +/-

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0.12 C, n = 9). Stocking density was 0.192 individuals/L in brood 1 and 0.427 individuals/L in brood 2. Salinity, ammonia, nitrite and photoperiod were not recorded.

2.3. Feeding

From release to day 10, brood 1 was offered freeze-dried, decapsulated Artemia eggs, a non-viable diet with no autonomous movement. Brood 2 was offered live rotifers together with Chlorella from day 1 onwards. Rations were given by experience and were not weighed. Housing and husbandry conditions are summarised in Table 1.

Table 1. Rearing conditions for the two broods (dates, initial numbers, water volume, temperature, stocking density, diet and censoring)

Item	Brood 1	Brood 2
Release date	2026/3/18	2026/6/29
Parental origin	Captive-bred parental group (first brood)	Same captive-bred parental group (second brood)
Initial number of juveniles	90	200
Initial total body length (mm)	7	10
Main tank dimensions (m)	0.7 x 0.7 x 0.7	0.7 x 0.7 x 0.7
Sump dimensions (m)	0.55 x 0.55 x 0.55	0.55 x 0.55 x 0.55
Working water volume (L)	468	468
Combined geometric capacity (L)	509	509
Water temperature (deg C)	25.13 +/- 0.19 (24.8-25.5; n = 15)	25.30 +/- 0.12 (25.2-25.5; n = 9)
Temperature control	Aquarium heater; room air-conditioning in summer	Aquarium heater; room air-conditioning in summer
Stocking density (individuals/L)	0.192	0.427
First feed	Freeze-dried, decapsulated Artemia eggs (non-viable); days 0-10	Live rotifers with Chlorella; from day 1
Feed ration	Not weighed (offered by experience)	Not weighed (offered by experience)
Salinity	Not recorded	Not recorded
Ammonia and nitrite	Not recorded	Not recorded
Photoperiod	Not recorded	Not recorded

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Table 1. (continued)

Survivor counts	500 mL scoop, extrapolated proportionally (approximate)	500 mL scoop, extrapolated proportionally (approximate)
Body length	1 individual per time point, snout to tail tip	1 individual per time point, snout to tail tip
Last observation (day)	94	64
Censored individuals	0	108 (54% of the initial number)
Censoring events	None	75 gifted on day 9; 33 sold on day 42

2.4. Monitoring and statistical analysis

Survivors were counted by scooping 500 mL of water with a 1-L bucket, counting the individuals in that sample and extrapolating proportionally to the whole system. Total body length, measured from the snout to the tip of the tail, was recorded once per time point on a single individual chosen as visually typical. Individuals removed from the system by gifting or sale were treated as right-censored from the date of removal. Because the expected number of individuals in a single 500 mL sample was well below one, counts are approximate estimates rather than exact censuses. Survival was estimated by the Kaplan-Meier method [6], 95% confidence intervals were obtained with Greenwood's formula, and the two broods were compared using a log-rank test [7].

3. Results

3.1. Survival

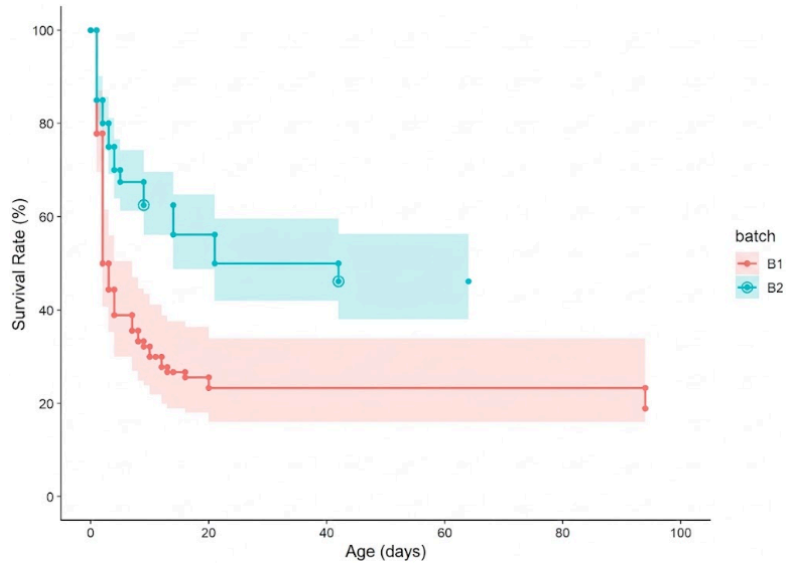


Figure 1. Kaplan-Meier survival curves for the two broods

Kaplan-Meier cumulative survival was 18.9% to day 94 in brood 1 (95% CI 12.3-29.0%; 90 individuals at the start, 21 at risk at day 94) and 46.2% to day 64 in brood 2 (95% CI 38.0-56.3%;

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200 at the start, 4 at risk at day 64) (Fig. 1; Table 2). Median survival was 2 days and 21 days, respectively. The difference between broods was significant (log-rank chi-square = 25.05, df = 1, p = 5.6 x 10^-7; 73 deaths observed in brood 1 against 46.6 expected). In brood 2, 108 individuals, or 54% of the initial cohort, were right-censored, 75 being gifted on day 9 and 33 sold on day 42.

Table 2. Kaplan-Meier survival at selected time points, with 95% confidence intervals and numbers at risk

Age (days)	Brood 1 survival (%)	Brood 1 95% CI	Brood 1 at risk	Brood 2 survival (%)	Brood 2 95% CI	Brood 2 at risk
0	100	100.0-100.0	90	100	100.0-100.0	200
1	77.8	69.6-86.9	90	85	80.2-90.1	200
2	50	40.7-61.5	70	80	74.6-85.7	170
5	38.9	30.0-50.4	35	67.5	61.3-74.3	140
10	30	21.9-41.1	29	62.5	56.1-69.6	50
21	23.3	16.0-33.9	21	50	42.0-59.6	45
42	23.3	16.0-33.9	21	46.2	38.0-56.3	40
64	23.3	16.0-33.9	21	46.2	38.0-56.3	4
94	18.9	12.3-29.0	21	--	--	--

Shaded bands, 95% confidence intervals (Greenwood); open circles, right-censored observations (day 9 and day 42 in brood 2). Each time point is represented twice to generate the step function. B1, brood 1 (n = 90); B2, brood 2 (n = 200).

3.2. Timing of mortality

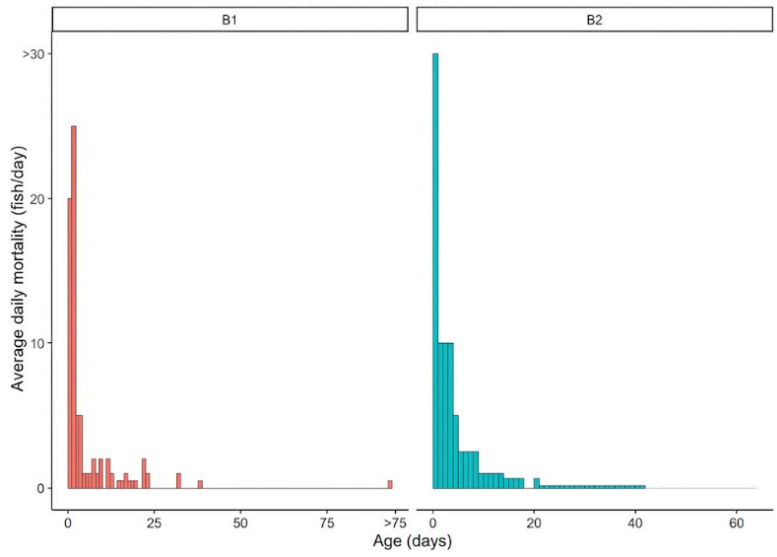


Figure 2. Interval-specific daily mortality

Mortality was concentrated in the first days after release. The highest interval-specific rate was 25.0 individuals/day in brood 1 (days 1-2) and 30.0 individuals/day in brood 2 (days 0-1). It then fell sharply: in brood 1 only 4 deaths were recorded between day 20 and day 94, equivalent to 0.054

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individuals/day, roughly 254 times lower than the 13.75 individuals/day observed over the first four days. The divergence between the two broods was already established within the first two days and remained approximately constant thereafter (Fig. 2).

Bar height, deaths divided by interval length; bar width, interval length in days, so that bar area equals total deaths in that interval. The 74-day interval (days 20-94) in brood 1 should be noted. Zero mortality in brood 2 between days 42 and 64 is a recorded zero, not a missing value.

3.3. Growth

Body length increased from 7 to 79 mm in brood 1 (0.77 mm/day) and from 10 to 72 mm in brood 2 (0.97 mm/day). Because a single individual was measured per time point, these values are presented descriptively and were not compared statistically. Published figures for this species are generally reported as standard length, whereas total length was measured here; the two are not directly interchangeable (Fig. 3; Table 3).

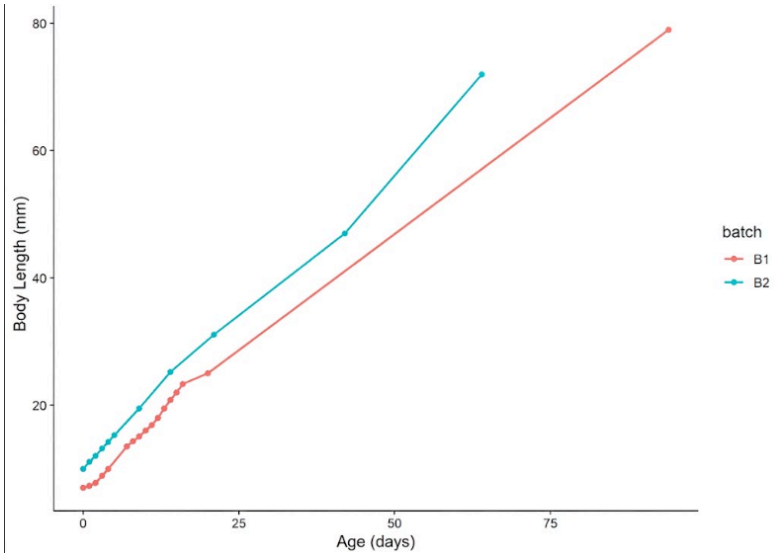


Figure 3. Descriptive changes in total body length (snout to tail tip)

One individual was measured per time point. Lines serve only to guide reading order and do not imply linear interpolation across unsampled periods.

Table 3. Body length (snout to tail tip) by age; one individual per time point

Age (days)	Brood 1 body length (mm)	Brood 2 body length (mm)
0	7	10
1	7.3	11.1
2	7.8	12
3	8.9	13.2
4	10	14.2
5		15.3
7	13.5	

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Table 3. (continued)

8	14.3	
9	15.1	19.5
10	16	
11	16.9	
12	18	
13	19.5	
14	20.8	25.2
15	22	
16	23.3	
20	25	
21		31.1
42		47
64		72
94	79	

3.4. Comparison with published data

At a comparable age of 63-64 days, survival was 23.3% in brood 1 and 46.2% in brood 2, whereas Lin et al. [2] reported 71.11 +/- 10.18% for the same species reared at 28-29 C. Both broods were therefore well below the published value. Because temperature was virtually identical between broods (difference 0.17 C) while being about 3 C below the reported optimum, temperature cannot account for the difference between broods, although it may have contributed to the low survival seen in both.

3.5. Diet in relation to published data

For the same species, Meng et al. [3] reported 62.96% survival in juveniles fed Artemia nauplii alone and 82.41% on a 1:2 Artemia-to-copepod mixture, whereas brood 1 here, fed a non-viable decapsulated Artemia preparation, reached only 18.9%. Otero-Ferrer et al. [4] obtained about 60% survival in H. hippocampus fed Artemia, but high mortality when rotifers were supplied as the sole first feed. The 46.2% achieved here with live rotifers plus Chlorella therefore lies between these published values. Because diet, brood order and initial body length all changed between the two broods, the present data cannot separate their effects.

4. Discussion

The data show that survival was significantly different between the two brood years (brood 2 had estimated survival higher than brood 1 and a longer median survival time than brood 1). In both broods, mortality occurred in the first few days after release, showing the importance of early stage feeding and husbandry in the culture of juveniles. Prey type and feeding regimes have been previously shown to potentially impact juvenile seahorse survival and growth [3-5]. Some difference

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in feeding condition especially in the use of live rotifer in the broods may therefore be responsible for the higher survival observed in brood 2. Because there were some differences between the feeding regime, the initial body length and other brood level factors, however, it is not possible to draw independent conclusions as to the effects of these factors. The differences in overall survival may also have been related to temperature since the temperatures recorded were colder than those seen in other studies of H. erectus [2] which showed relatively high overall survival. Growth was shown in both broods on the basis of body-length measurements, indicating that the results should be regarded descriptively owing to small number of individuals measured. This study, overall, gives information of the survival figures for early juvenile rearing, and indicates that the initial days after release should be given particular attention. More controlled trials comparing feeding regimes under similar environmental conditions are needed in the future in order to determine the successful feeding regimes that can help eliminate high juvenile mortality survival.T.

5. Conclusion

In conclusion, this study compared survival and growth patterns in two consecutive broods of captive-bred Hippocampus erectus. The estimated survival was higher in brood 2 compared to brood 1, and most of the mortality was within the first few days of the release in both broods. The results show the need to conduct feeding and husbandry practices early in the life of juvenile seahorses. However, the design of observations, variations in feeding practices and other brood level factors, limited measures of body length and incomplete records of water quality limited interpretation of the results. However, caution should also be used in interpreting the estimated survival outcomes as there are limitations in the sampling/counting method. In the future, controlled feeding experiments and individual survival records along with repeated growth measurements and systematic water quality monitoring might be able to more reliably pinpoint factors associated with juvenile survival, and improve rearing practices for H. erectus.

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