Broodstock cultivation of the yellow seahorse, *Hippocampus kuda* Bleeker, in the aquarium culture system

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Abstract. To improve the yield and survival rate of the offspring, the broodstock cultivation methods of the yellow seahorse, *Hippocampus kuda* Bleeker, were assessed, including the temperature conditions, diets, culture density and mono- or mixed-sex culture. Feeding parent seahorses with live food increased fecundity, and culture density of more than two pairs of parents during mating improved the survival rate of both sexes and decreased the production cycle. Additionally, separate culture of males and females prior to mating improved fecundity as well as the survival rate of juveniles. A higher number of larvae per batch was produced at 26 °C than at 30°C and 22°C, whereas seahorse production occurred at a higher frequency at 30 °C than in the lower temperature treatments. These results suggest that the optimal culture conditions for *H. kuda* are as follows: provide live food when cultivating seahorse broodstock, use equal proportions of males and females during mating, use monosex culture at other times, and maintain seawater temperature of 26 °C.

1. Introduction
The yellow seahorse, *Hippocampus kuda* Bleeker, is a marine species used in traditional Chinese medicine (TCM)[1], which has effects on aphrodisiac, detumescence, tranquilization[2]. The global demand for seahorses is increasing, whereas their output is in sharp decline[3]. In the 1990s, researchers in Europe and the Americas began conducting related research on seahorses[4]. Although there has been some progress, many issues remain poorly understood, such as why mortality is so high during the first weeks of life and details about seahorse reproduction[5]. Today, many seahorse studies are focused on pharmaceutical analysis[6] and disease, but studies of culture technology, especially broodstock culturing, are seldom reported.

In the culture of seahorse broodstock, reproductive development is very important. Water temperature is an important factor in optimizing feeding and gonad development of aquatic animals. For example, Liu[7] reported that a moderate increase in temperature may increase the feeding rate of broodstock of the fish *Oplegnathus fasciatus* while also improve the fecundity and fertility rate. Heating was found to promote maturation and early mating of the Japanese shrimp *Penaeus japonicus*[8]. It is
found that dietary supplementation with vitamin E promoted sexual maturity in the croaker Larimichthys polyactis[9].

Stocking density is another important parameter that affects mating success. Optimal stocking density varies among species, and it can be affected by exogenous factors such as water temperature, water quality, and feed quality/quantity. The cultivated method of broodstocks also plays an important role in the mating process.

To evaluate H. kuda broodstock cultivation techniques and identify optimal culture conditions, we tested the effects of temperatures, different foods, stocking densities, and other culture conditions on the seahorse’s reproductive situation, production cycle, and juvenile survival rate to optimize broodstock rearing conditions and ultimately improve production.

2. Materials and methods

2.1. Materials
Healthy adult seahorses were provided by the Key Lab of Aquaculture and Utilization for Marine TCM Organisms (Ningbo University, China) for propagation and development. Before the experiment, broodstock seahorses were cultured in blue seawater circulation aquaculture tanks with diameter of 80 cm and height of 100 cm. Seawater temperature in the tanks was maintained at 24–25 °C, and salinity was kept at 24–25. The broodstock seahorses were fed with live P. vannamei juveniles (5–10 mm) and frozen Mysis (10–15 mm). The feces and residual food were siphoned off every evening. The P. vannamei juveniles were reared in the laboratory and fed with shrimp flakes (Hailin Corp., Xiamen, China); the temperature in the rearing tanks was controlled by heating rods.

2.2. Temperature experiment
Based on the relevant literature, we selected 22 °C, 26 °C, and 30 °C as the test temperatures, which were controlled by a heating rod with error ± 1 °C. Two-year-old seahorses were used in the experiment. Each treatment contained six replicate pairs. Experimental seahorses were randomly selected from the broodstock tank.

2.3. Food experiment
To explore the effects of food on production of the broodstock seahorses, two experimental treatments (live P. vannamei juveniles and frozen Mysis) were tested in this experiment. Each treatment contained six pairs of seahorses sampled randomly selected from the broodstock tank. The experiment was conducted in 12 tanks with diameter of 80 cm and height of 100 cm; each tank contained one male-female pair of seahorses, and the environmental factors were as follows: DO, 5 ml/L; temperature, 24–25 °C; light intensity, 2500–3000 Lx; and salinity, 24–25 ppt. Broodstock seahorses were fed two times a day (8:00–9:00 and 16:00–17:00). Feces and uneaten food were siphoned from each tank 2 h after each feeding. The experiment was conducted from May to July. The product date, fecundity, bearing date, and 30-day survival rate of juveniles were measured.

2.4. Density experiment
Three density treatments (1 female + 1 male (AA), 2 females + 2 males (BB), and 3 females + 3 males (CC)) were tested. The AA treatment included six tanks, each containing one male-female pair; the BB treatment consisted of three tanks, each containing two male-female pairs; and the CC treatment included two tanks, each containing three male-female, and the experiment was replicated three times per stocking density. The two-year-old seahorses used had previously produced offspring. Environmental factors were the same as those used before. The experiment ran for 3 months. The product date, fecundity, and 30-day survival rate of juveniles were measured.
2.5. Male and female culture method experiment
One-year-old sexually mature individuals from the same brood were used in this experiment. The treatments included separate cultures of male and female seahorses as well as a mixed sex culture. The monosex treatment consisted of six males cultured in one tank and six females cultured in another tank. The mixed culture treatment contained six males and six females cultured together in a tank. Each treatment was replicated three times. When the seahorses in the mixed group began to change color and exhibit mating behavior, the female seahorses in the monosex culture treatment were moved to the male tank. All parent seahorses in each tank were fed with live food during the experiment. The experiment lasted from May to July. The product date, fecundity, bearing date, and 30-day survival rate of juveniles were measured.

2.6. Data analysis
Statistical analyses were conducted using the SPSS13.0. P < 0.05 was considered to be statistically significant.

3. Results

3.1. Temperature effect
At 22°C, the six male-female pairs spawned only once during the 90 days. All pairs spawned twice at 26 °C, with an average production period of 27.83 ± 0.75 days. At 30 °C, the seahorses spawned three times, with average production period of 21.57 ± 1.54 days, which was significantly shorter than that at 26 °C (Table 1).

| Temp. (°C) | Frequency (times) | Production cycle | Product pairs | Fecundity (ind./brood) | Survival after 30 days | Survival rate (%) |
|-----------|-------------------|------------------|---------------|------------------------|-----------------------|-------------------|
| 22        | I                 | 6                | 223.3±17.85c  | 103.5±29.44            | 46.00±9.79A           |
| 26        | I                 | 6                | 221.0±31.18e  | 59.0±5.25              | 27.17±4.58BC          |
|           | II                | 6                | 293.7±15.54a  | 142.8±18.93            | 48.55±5.09A           |
| 30        | I                 | 6                | 182.2±6.37d   | 45.8±3.31              | 25.34±1.16C           |
|           | II                | 6                | 231.2±6.31bc  | 68.7±11.21             | 30.29±5.13BC          |
|           | III               | 6                | 263.8±56.95ab | 86.2±23.09             | 33.53±5.53B           |

The fecundity and 30-day survival rate also differed significantly among the three treatments (Table 1). At the first production of offspring, survival in the 22 °C group was nearly 50%, whereas it was nearly 30% at 26 °C and 30 °C. There was no distinct difference between the 26 °C and 30 °C groups. In the second production of offspring, survivorship of the 26 °C group (48.55 ± 5.09%) was significantly higher than that of the 30 °C group (30.29 ± 5.13%) (p < 0.05). Survivorship at the 30°C group was 33.53 ± 5.53% in the third production.

3.2. Food effect

Table 2. Effects of food type on production of H. kuda

| Food          | Frequency (times) | Production cycle | Product pairs | Fecundity (ind./brood) | Survival after 30 days | Survival rate (%) |
|---------------|-------------------|------------------|---------------|------------------------|-----------------------|-------------------|
| Live food     | I                 | 6                | 224.6±33.44d  | 57.6±4.45              | 26.04±3.92B           |
|               | II                | 6                | 329.2±21.48a  | 86.6±16.26             | 26.21±3.74B           |
|               | III               | 6                | 296.6±8.44ab  | 90.0±15.75             | 30.29±4.96AB          |
|               | 16.8±1.93        | 100%             | 283.5±50.18   | 78.1±19.45             | 27.5±4.42             |
| I             | 6                 | 210.2±24.15d     | 58.5±18.46    | 27.69±8.45B            |                       |
The seahorses fed with live food produced offspring 5–7 days earlier than those fed with frozen food (Table 2). The fecundity and 30-day survival rate of juveniles also differed significantly between the two treatments, and they were slightly significantly different among the three production times within the same treatment (Table 2). The fecundity of the seahorses fed with live food was 224.6 ± 33.44 individuals per brood at the first production, and it increased to more than 300 per brood at the second and third productions. The seahorses fed frozen food had lower fecundity and longer production intervals. Only two of the six pairs produced three times. The survival rate of 30-day-old juveniles was similar between the two treatments at the first production, but average survival was significantly higher in the treatment fed with frozen food than in that fed with live food at the second and third productions (Table 2).

### 3.3. Density effect

| Density | Culture method | Frequency (times) | Product pairs | Product date | Fecundity (ind./brood) | Survival after 30 days | Survival rate (%) |
|---------|----------------|------------------|---------------|--------------|------------------------|------------------------|-------------------|
| AA      | I              | 22.1±29.49       | 4             | 221.3±29.49 | 61.8±7.02              | 28.63±3.76             | 86.8±15.35        |
|         | II             | 295.2±36.86      | 5             | 295.2±36.86 | 86.8±15.35             | 29.57±4.56             | 85.4±30.76        |
| BB      | I              | 264.1±41.53      | 6             | 264.1±41.53 | 72.0±14.37             | 27.77±3.43             | 77.3±17.76        |
|         | II             | 337.3±43.63      | 6             | 337.3±43.63 | 109.7±19.49            | 32.67±6.97             | 30.1±4.33         |
| CC      | I              | 251.4±44.35      | 6             | 251.4±44.35 | 68.0±15.05             | 27.34±3.33             | 80.4±24.33        |
|         | II             | 312.7±36.78      | 6             | 312.7±36.78 | 94.3±13.94             | 30.43±4.99             | 28.7±4.83         |

In the first and second production cycles, mating frequencies of the BB and CC groups were less than 25 days, whereas that of the AA groups was about 28 days (p < 0.05) (Table 3). In all three experimental groups, average fecundity and juvenile survival rate of the second production were significantly higher than those of the first production, and average fecundity was significantly higher in the BB treatment than in the AA and CC groups (p < 0.05). However, the survival rate of juveniles within all the treatments and all levels was similar. LSD multiple comparison tests showed that the BB group was best at producing juveniles.

### 3.4. Different methods of broodstock seahorse production

Separate culture of male and female seahorses before and after experiment is consistent with body color, black brown as the main. The seahorses in the mixed culture started to gradually change color from dark brown to yellow after a week. Table 4 shows the fecundity and 30-day juvenile survival rate for each treatment. The production date of the first production was later in the separate culture group than in the mixed culture group, but production was more concentrated and synchronized (it lasted only 4 days for the six parent pairs compared to about 10 days in the mixed culture group). Production in the second production also was more concentrated in the monosex culture group (6 days) compared with the mixed culture group (13 days).

| Culture method | Frequency (times) | Product pairs | Product date | Fecundity (ind./brood) | Survival after 30 days | Survival rate (%) |
|----------------|------------------|---------------|--------------|------------------------|------------------------|-------------------|

In the first and second production cycles, mating frequencies of the BB and CC groups were less than 25 days, whereas that of the AA groups was about 28 days (p < 0.05) (Table 3). In all three experimental groups, average fecundity and juvenile survival rate of the second production were significantly higher than those of the first production, and average fecundity was significantly higher in the BB treatment than in the AA and CC groups (p < 0.05). However, the survival rate of juveniles within all the treatments and all levels was similar. LSD multiple comparison tests showed that the BB group was best at producing juveniles.
The different culture methods had little effect on mating. In both treatments, the broodstock seahorses all successfully mated. One-way analysis of variance showed that the two groups had similar fecundity and juvenile survival rate in the second production. However, in the first production fecundity and juvenile survival rate were significantly higher in the separate culture group compared to the mixed culture group (Table 4).

4. Conclusion
This study explored the propagation characteristics of broodstock seahorses cultured at different temperatures and with different foods, stocking densities, and breeding methods. Live food compared to frozen food improved the fecundity and prolonged the production period of H. kuda (frequency: more than three times). The culture density of two females and two males improved mating frequency, thus improving fecundity. Separate culture of male and female seahorses prior to mating improved fecundity and juvenile survival rate. Temperature of 30 °C improved production frequency, whereas fecundity was higher at 26 °C.

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