

Research Article

Laboratory Cultivation of *Coregonus ussuriensis* Berg: A Comprehensive Study on Rearing from Egg to Adult Stage

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Conservation aquaculture aids in restoring and rebuilding populations of endangered species through artificial breeding. The Ussuri cisco (*Coregonus ussuriensis* Berg) is a valuable fish species in China, known for its high nutritional value. However, its current yield heavily relies on fishing, leading to a rapid decline in wild populations. This study documents the successful artificial breeding of *C. ussuriensis* and provides a comprehensive description of its life cycle. We performed in situ gamete stripping followed by manual fertilization. Fertilized eggs were incubated at the Bohai cold-water fish experimental station in Mudanjiang, China, where seed breeding and parental fish farming were conducted. Under controlled conditions, we obtained around 110,000 fertilized eggs. After a 2-month incubation period, we obtained around 50,000 oviparous eggs with an eyed rate of 45.5%. Additionally, we observed over 30,000 larvae with a hatching rate of 60%. We produced 20,000 juvenile fish weighing 30 g after 1 year. This procedure facilitates the successful completion of the *C. ussuriensis* life cycle in aquaculture, aiding in the species' recovery.

1. Introduction

Freshwater fish are vital for human sustenance. Freshwater fish species are facing extinction due to climate change, water pollution, alien species invasion, and habitat destruction [1–3]. Conservation aquaculture, proposed by Anders [4], utilizes aquaculture technology to safeguard and rehabilitate at-risk fish populations. Conservation aquaculture has been redefined as the use of human-cultivated aquatic organisms for managing and protecting natural resources [5]. Researchers are prioritizing the development of a sustainable aquaculture environment alongside species conservation efforts. Conservation and protection of species diversity involve establishing indigenous fish nature reserves, restoring habitats, improving fish crossing measures, and implementing artificial proliferation and release [6, 7].

Several artificial breeding successes have been documented, such as *Squalius lucumonis* [8], *Thunnus orientalis* [9], and *Pseudobagrus nitidus* [10]. Advancements in freshwater fish artificial proliferation and release technology have

enhanced the number of indigenous fish reintroduced to their natural habitats, aiding in the restoration of wild fish populations [11, 12]. Artificial proliferation and release involve introducing artificially propagated seedlings or cultivated natural seedlings into designated water bodies. Replenishing native species in specific waters can restore natural populations [13]. Careful planning is necessary, informed by a comprehensive understanding of the species' life history. Understanding the fundamental life history traits and reproductive strategies of endangered species is crucial for population restoration [14, 15].

Coregonus ussuriensis, also known as Ussuri cisco, is a cold-water fish belonging to the Salmoniformes order and Coregoninae subfamily. It is highly valued for its nutritional and economic benefits. The species is found in the middle and lower reaches of the Amur River and its tributaries, as well as in several rivers near the mouth of the Amur River and Sakhalin Island [16]. The fecundity study revealed that *C. ussuriensis* typically reaches sexual maturity at the age of

5+, with a few females at 4+ also capable of reproduction [17, 18]. The embryo development indicated an effective accumulated temperature of 7,753.80°C/hr [19]. The survey data show a decreasing trend in the fishing volume of *C. ussuriensis* over time [18]. The species' sensitivity to rising temperatures suggests that it may face extinction in certain watersheds due to ongoing global warming [20]. *C. ussuriensis* was listed as endangered in the "Red Data Book of China's Endangered Animals" in 1998 [21]. *C. ussuriensis* shows promise for aquaculture in China due to its high economic value. The experimental rearing protocol for artificial reproduction is currently lacking research. Our objective was to identify an experimental feeding regimen to gain valuable insights for the conservation aquaculture of *C. ussuriensis*.

2. Materials and Methods

2.1. Ethics Statement. All experiments were performed according to the European Communities Council Directive (86/609/EEC). Fishes were bred following the guidelines of the Animal Husbandry Department of Heilongjiang, China. All efforts were made to minimize suffering.

2.2. Broodstock Collection and Maturity Identification. Mature brooders of *C. ussuriensis* were collected from the Fuyuan section in Heilongjiang, China. A total of 206 fish were collected during the breeding period. The breeding workshop's temporary pond temperature ranged from 4 to 6°C. After a 2-day acclimation period, the wild parents were anesthetized using a 2-phenoxyethanol solution (100 mg/mL) to begin the identification of the mature stage. We measured the length, weight, egg length, egg diameter, and number of eggs for 30 randomly selected individuals. Additionally, we weighed the testes and ovaries after dissection. The formula for calculating the sexual maturity factor is as follows:

$$\text{Sexual maturity factor (GSI\%)} = \frac{\text{gonadal weight}}{\text{net weight}} \times 100\%. \quad (1)$$

2.3. Breeder Induction and Artificial Insemination. Fifty healthy wild parents (1:1 sex ratio) were selected after identification, and offspring production was induced using exogenous hormones. The hormone ratio used in the study consisted of human chorionic gonadotropin (HCG) at a dose of 1,000 IU/kg, luteinizing hormone (LH) at a dose of 10 µg/kg, and domperidone (DOM) at a dose of 2 mg/kg. These hormones were obtained from Ningbo Sansheng Biological Technology Co., Ltd. in China, with the following catalog numbers: HCG (110911771), LH (110912655), and DOM (110912944). The anesthetized male and female brooders were intramuscularly injected with 1 mL using a 40-U insulin syringe between the anterior region of the dorsal fin and lateral line. After 48 hr, male fish (90% mature) and female fish (40% mature) had fully developed gonads. Semen and eggs were obtained by manually squeezing their abdomen. Dry fecundation was utilized [22].

2.4. Egg Incubation, Domestication of Juveniles, and Individual Development of Juveniles. Fertilized eggs were incubated at the Bohai cold-water fish experimental station, which is affiliated with the Heilongjiang River Fishery Research Institute of the Chinese Academy of Fishery Sciences and located adjacent to Jingbo Lake (44.02°N, 128.74°E), in the Heilongjiang province, Mudanjiang, China. In order to hatch eggs of *C. ussuriensis*, we build a mesh frame (0.45 m in length × 2.5 m in breadth × 0.4 m in height) with a steel bar as the skeleton in a rectangular hatching tank. The eggs were evenly distributed in a single layer in each tank. The water source during incubation was a natural cold spring water with 7 mg/L of dissolved oxygen. During the incubation period, the water temperature was 3–4°C. The developmental stages of the embryos were determined morphologically using a light microscope (Olympus CH2, Japan).

The juveniles were transferred to a rectangular hatching tank (3.0 m in length × 0.5 m in breadth × 0.45 m in height). In an open-flow system, each hatching tank breeds 20,000 seedlings. The source of water was a natural cold spring, and the water flow rate was 5 L/min. From the second day after floating, the fish were fed zooplankton bait (fungi, daphnia, and large branch horns) in the cultivation tank every day, and the opening acclimation time was approximately 30 days, with feeding twice a day. From days 31 to 50, the artificial feed (161, Beijing Hanye Science & Technology Co., Ltd, China) was integrated into the diet of the juvenile fish once a day while they were still fed zooplankton bait (Particle size 500–700 µm). After 51 days, artificial feed was used for cultivation, and the fish were fed six times a day to satiation. Water exchange rate was 2–3 times/day.

Between 07:00 and 10:00 each morning, five individuals were randomly sampled and photographed with a digital camera (Nikon D90) and measured. We used the following formula to calculate the immediate (special) growth rate (*G*) every fortnight:

$$G = \frac{\log_e Y_T - \log_e Y_t}{T - t} \times 100, \quad (2)$$

where *Y_T* is the final size at time *T*, *Y_t* is the size at time *t*, and *e* is the base of natural logarithms.

2.5. Water Quality. Chemical and physical indicators were monitored with a multiparameter probe (Eutech Instruments, mod. PCD650) and maintained at a temperature of 8–10°C, pH 6.8, and dissolved oxygen was 6.0–8.5 mg/L. Additionally, the indices for total phosphorus, nitrogen, and permanganate were 0.062, 0.40, and 1.50 mg/L, respectively.

3. Results

3.1. *C. ussuriensis* Breeder Traits. The growth performance of the breeder of *C. ussuriensis* is shown in Table 1. The female fish had weight that ranged from 554.2 to 1,976.4 g (with an average of 1,190 ± 306.9 g) and length that ranged from 37.4 to 54.1 cm (with an average of 45.8 ± 3.59 cm). The male fish had a range of 270.9–909.8 g weight (with an average of 507.7 ± 187.8 g) and 29.9–44.3 cm length (with an average

TABLE 1: Individual reproductive characteristics of *C. ussuriensis* in the Fuyuan section of Heilongjiang (means \pm SD, $n = 30$).

	Weight (g)	Length (cm)	Ovarian weight (g)	Testis weight (g)	Fecundity (grain)	GSI (%)	Egg diameter (cm)
♀							
Range	554.2–1,976.4	37.4–54.1	62.5–474.2		17,878–778	13.36–34.6	0.18–0.25
Average	1,190 \pm 306.9	45.8 \pm 3.59	214.3 \pm 86.66		9,960 \pm 844	22.62 \pm 0.71	0.20 \pm 0.03
♂							
Range	270.9–909.8	29.9–44.3		4.38–16.9		0.885–2.15	
Average	507.7 \pm 187.8	35.4 \pm 3.99		5.66 \pm 1.62		1.455 \pm 0.28	

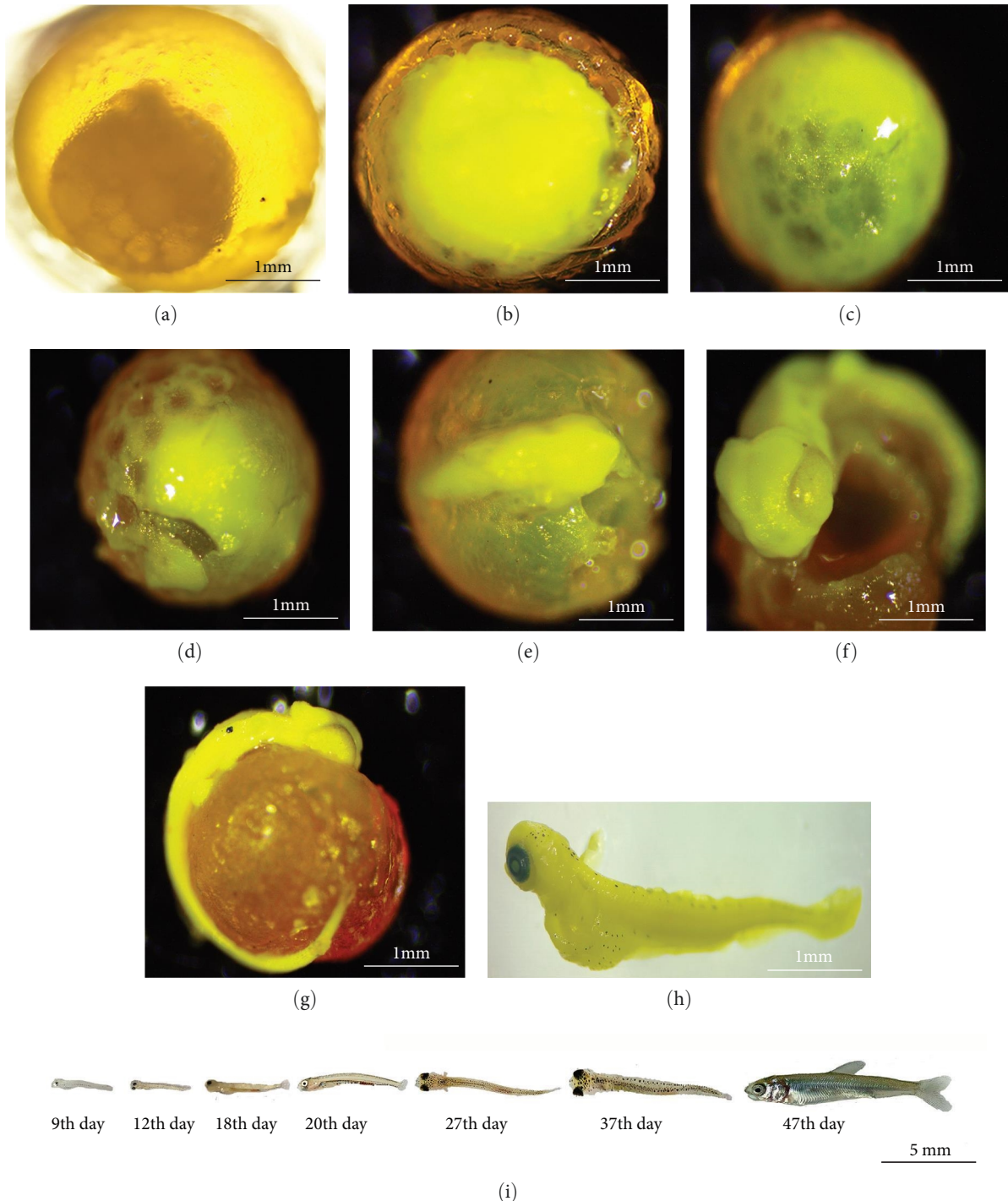


FIGURE 1: The embryo development and morphogens change during the larval stage of *C. ussuriensis*: (a) morula stage, 24 hr after fertilization; (b) early blastocyst stage, 135 hr after fertilization; (c) blastocyst stage, 182 hr after fertilization; (d) gastrulation stage, 216 hr after fertilization; (e) brain differentiation, 292 hr after fertilization; (f) eye sac formation, 341 hr after fertilization; (g) the caudal fin appears, 480 hr after fertilization; (h) larva formation, 1,791 hr after fertilization; (i) development of *C. ussuriensis* larvae (9–18 day was the time after rupture of membranes, 20–47 day was the time after float up).

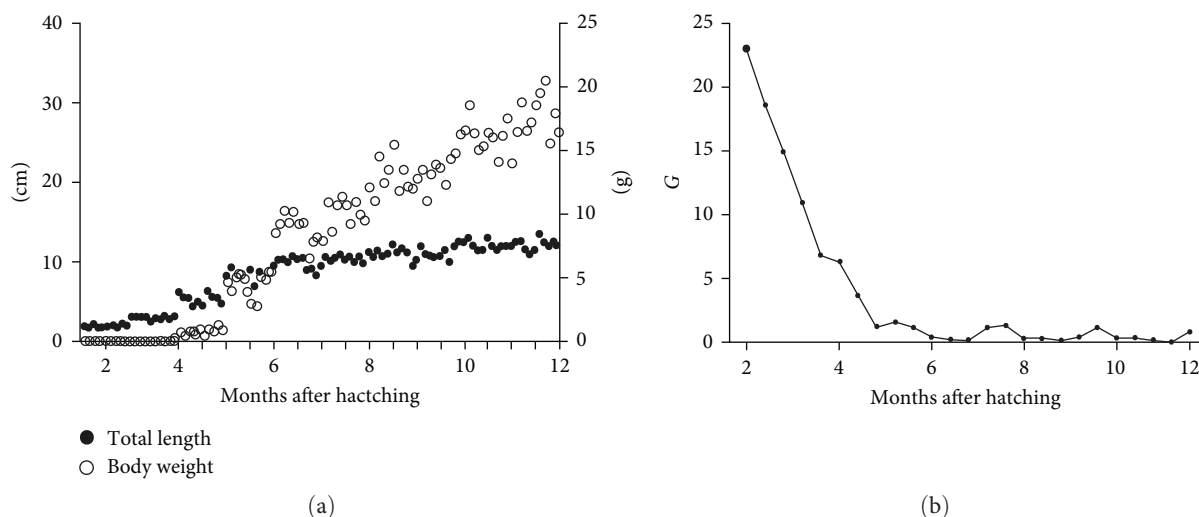


FIGURE 2: Growth and G values of the artificially hatched *C. ussuriensis*: (a) growth curve of larva *C. ussuriensis*; (b) instantaneous (specific) growth rate (G) values calculated every month during the experimental rearing period, $n = 10$.

of 35.4 ± 3.99 cm). The body size of males was significantly smaller than that of females. The average GSI for females was $22.62 \pm 0.71\%$ and $1.455 \pm 0.28\%$ for males.

3.2. Fertile Egg Hatching. The mature eggs of *C. ussuriensis* are spherical in shape, with an egg size of 0.18–0.25 cm and an average diameter of 0.2 ± 0.03 cm (Table 1). Following fertilization, the embryo development of *C. ussuriensis* was completed under 3–4°C. During the morula stage, 24 hr after fertilization, the cytoplasm moved toward the animal pole, and the perivitelline space was clearly visible (Figure 1(a)). The blastocyst stages were completed, with the blastoderm undergoing development and positioned on the surface of the yolk (Figures 1(b) and 1(c)). Visible germ layer migration had occurred along the yolk circumference during the early stages of gastrulation (Figure 1(d)). The brain differentiation was visible at 292 hr after fertilization, and eye development was first visible at 341 hr after fertilization (Figure 1(f)). At 480 hr after fertilization, the caudal fin is gradually formed (Figure 1(g)). The embryonic body has full characteristics of fry characteristics (Figure 1(h)).

In this study, We obtained a total of 50,000 oviparous eggs, with an eyed rate (number of eye eggs/total eggs) of 45.5%. Approximately 30,000 fries hatched, with a hatching rate (number of seedlings/eye eggs) of 60%.

3.3. Larvae Hatching. The larvae from the freshly ruptured membranes had a total length of 0.9234 ± 0.007 cm and a weight of 0.009 ± 0.001 g (Figure 2(a)). The hatchlings were entirely transparent, with oval yolk sacs, and they stayed near the bottom of the pond. After 9 days of membrane rupture, their bodies were 1.5 cm long, and their yolk sacs started to thin (Figure 1(i)). In the third week, the yolk sac had almost disappeared.

On the 18th day of incubation, the larvae began to float up, the yolk sacs were absorbed, the blood vessel distribution was evident, and melanin was deposited in the abdomen. After the larvae floated for 3 weeks, daphnia, large

zooplankton, and waterworms were detected in their digestive tracts, and they were then cultivated with an artificial diet.

3.4. Juvenile Ontogeny. The larvae continued to develop into juveniles after 37 days of floating; the pectoral, ventral, and caudal fins had differentiated at this point. The larvae were 1.9 ± 0.15 cm in length and 0.089 ± 0.003 g in weight (Figure 2(a)). The G values (22.84) were highest in the first 2 months and then drastically decreased (Figure 2(b)).

3.5. Breeding F1 of *C. ussuriensis*. The juvenile fish were lighter in body color than the larvae, and their bodies were slender (Figure 3(a)). Through 4 years of cultivation, the *C. ussuriensis* can achieve natural sexual maturity (Figure 3(b)–3(e)). The growth performance of the *C. ussuriensis* F1 was presented in Tables 2 and 3. The subsequent artificial propagation, fertilized egg hatching, seed breeding, and broodstock cultivation were equivalent to the above steps (Figures 3(e) and 3(f)).

4. Discussion

Artificial proliferation and release are important means to conserve fishery resources, restore freshwater ecological environments, and promote fishery production [23]. Replenishing natural populations by releasing juvenile fish has become a common practice in *Coregoninae* conservation. For example, Wanke et al. [24] systematically studied the proliferation and release plan of *Coregonus albula*. To replenish populations, the primary task is to obtain wild broodstock for artificial reproduction and cultivate healthy primitive groups. In this study, the artificial reproduction of *C. ussuriensis* was successfully achieved with egg collection and dry fecundation, *ex situ* eggs incubation, and larval rearing. Research on the reproductive population of *C. ussuriensis* found that the youngest sexually mature individuals were more than 4 years old in males and 5 years old in females [25]. In this study, *C. ussuriensis* males were sexually mature

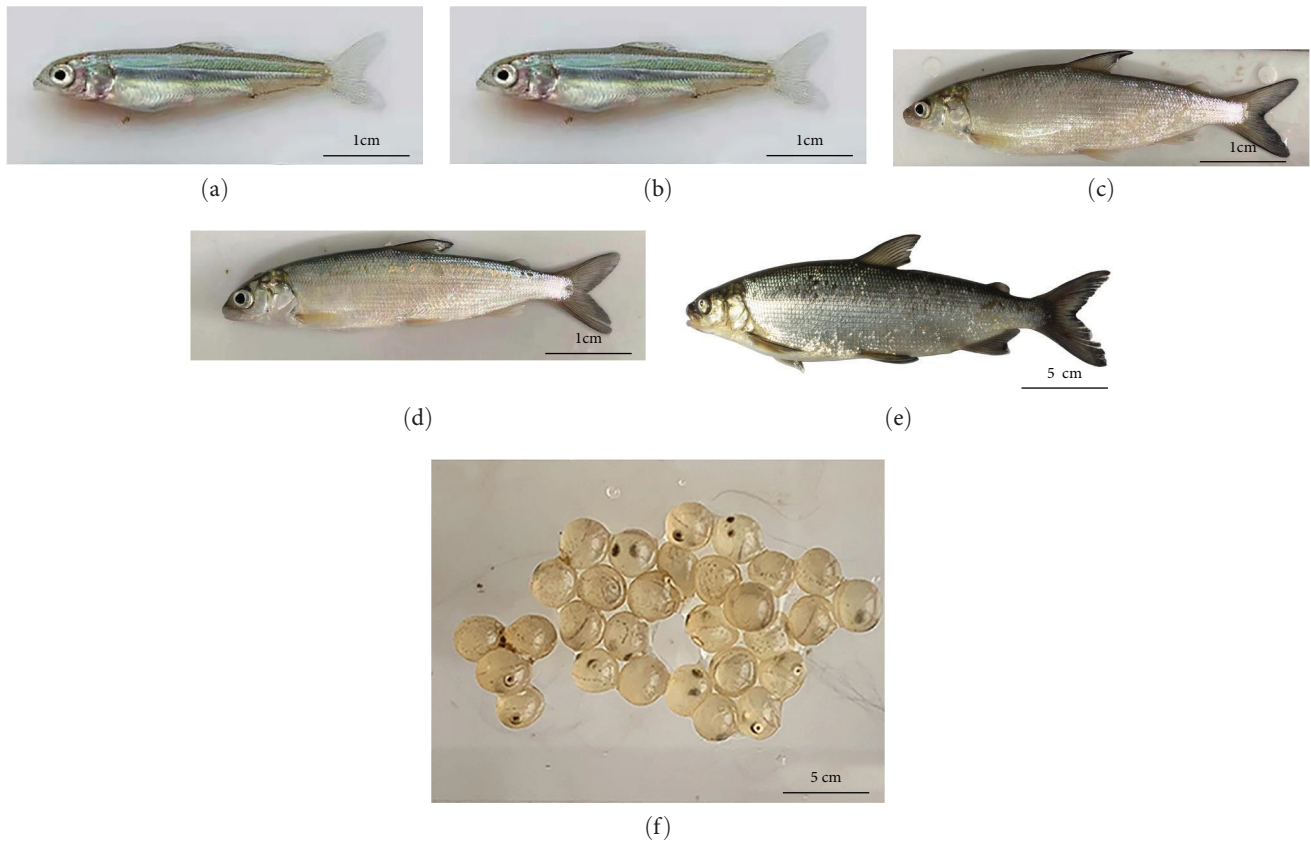


FIGURE 3: The *C. ussuriensis* of different age: (a) larvae; (b) juvenile (1 year old); (c) juvenile (2 years old); (d) juvenile (3 years old); (e) broodstock of F1 (4 years old); (f) eggs.

TABLE 2: Identification of the main reproductive traits of F1 female *C. ussuriensis* (means \pm SD, $n = 10$).

	Average	Standard deviation	Maximum value	Minimum value
Length (cm)	39.30	1.540	41.00	35.00
Weight (g)	800.50	68.29	920.00	680.00
Egg weight (g)	70.04	21.35	113.74	41.54
Egg diameter (cm)	0.22	0.13	0.25	0.20
Egg weight (g)	0.007	0.0009	0.0084	0.005
Egg diameter after water absorption (cm)	0.24	0.018	0.30	0.21
Egg weight after water absorption (g)	0.011	0.001	0.135	0.0875
Fecundity	9,890.03	1,144.77	11,832	8,051.47

TABLE 3: Identification of the main reproductive traits of F1 male *C. ussuriensis* (means \pm SD, $n = 10$).

	Average	Standard deviation	Maximum value	Minimum value
Length (cm)	38.92	1.41	41.00	36.00
Prenatal weight (g)	629.20	68.17	770.00	500.00

at 4⁺ years. Approximately 60% of females were sexually mature at 4⁺ years, but a small number of individuals were sexually mature at 5⁺ years. Compared with the results of a survey on the fertility of *C. ussuriensis*, which showed that mature individuals from the F1 generation that had been cultivated for 4 years reached reproductive capacity [17],

this further shows that the artificial breeding of *C. ussuriensis* is feasible.

Similar to previous studies of other Salmonidae fishes [26], the body size of males of *C. ussuriensis* was significantly smaller than that of females. However, fitting the FL growth of *C. ussuriensis* with the von Bertalanffy equation did not

show a significant difference between the sexes [27]. This difference may be due to the small number of samples size in this study. The reproduction was induced with exogenous hormones, which were composed of HCG, LH, and DOM. About 40% females and 90% males of *C. ussuriensis* broodstocks were induced maturity, which was similar with the study of Kucharczyk et al. [28]. HCG and LH promote the production of sperm and oocytes by regulating the level of steroid hormones [29–31], which are commonly used in aquaculture. DOM acts as a dopamine inhibitor to eliminate its negative effect on gonadotropin secretion and induce the process of ovulation [32–34]. Usually, a combination of these reagents has better effects than a single reagent. The combination of the exogenous hormones HCG, LH, and DOM successfully induced ovulation and maturity of *C. ussuriensis*.

This study determined the embryo development of *C. ussuriensis* under a culture temperature of 3–4°C by providing images and descriptions. The developmental stages of *C. ussuriensis* followed the same sequence described for the *Coregonus clupeaformis* by Sreetharan et al. [35]. Early developmental stages, such as brain differentiation, eye sac formation, and fins differentiation, were reached earlier in *C. ussuriensis* than in *C. clupeaformis*. This variability in the development period may be related to differences in incubation temperatures, in addition to species differences. The results of this study can be generalized to other *Coregoninae* species, as they have similar developmental time, optimal incubation temperature, and size during fertilization and hatching.

The appropriate water area and release time should be selected based on the biological characteristics of the released species, natural habitat, and breeding habitat. In terms of water quality and temperature, *C. ussuriensis* requires unpolluted water, an annual water temperature variation range of 1–20°C, an optimal water temperature of 8–12°C, and high oxygen content (>5 mg/L dissolved oxygen) [16]. We found that during the F1 generation of *C. ussuriensis*, the fish could reach natural maturity and spawn in ponds with micro-flow water, and the floating larvae fed on zooplankton. The survival of fry larvae feeding with zooplanktons was 90%, and feeding with artificial feed was 40%. Therefore, native seedlings would be suitable for stocking in slow tributaries and relatively still water, such as large lakes and reservoirs. The growth inflexion point occurred around the 98th day. So, release time could be selected from April to June every year, and they could be released as soon as the endogenous nutrient consumption of larvae has been completely converted to exogenous nutrients. To ensure that the stress resistance and survival rate of individuals increases, the time of exogenous nutrition feeding could be appropriately increased. The best time for release would be in spring because of the appropriate climate to ensure that the individual's stress resistance and survival rate increase. Moreover, genetic and ethical factors should also be considered. Currently, the genetic problems caused by stocking are not clear. It is believed that artificial proliferation increases the natural population abundance of Chinook salmon (*Oncorhynchus tshawytscha*) and has a small negative impact on genetics [36]. Regarding the

investigation of the genetic diversity of *C. ussuriensis* after stocking, follow-up work still needs to be supplemented.

Data Availability

Data supporting this article are available from the authors upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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