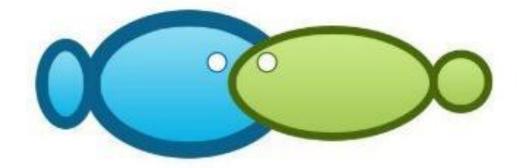
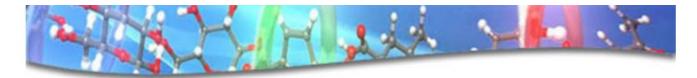
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# **BIOFLUX SRL**

Identity: Research organization, CAEN 7219, CUI: 23753059 from 18.04.2008 Address: 54 Ceahlau street, Cluj-Napoca 400488, Romania, European Union, Europe. Lesmana D., Supriyono E., Widanarni, Nirmala K., Jusadi D., 2022 Physiological response of the puerulus of scalloped spiny lobster, *Panulirus homarus*, raised at different tank color. AACL Bioflux 15(6):3238-3249.

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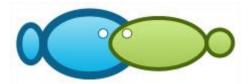
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### Koi fish (*Cyprinus rubrofuscus*) seed production management in koi farm, Sukabumi District, West Java, Indonesia

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**Abstract**. *Cyprinus rubrofuscus* is one of the most popular ornamental fish in the community with a high market demand and a high economic value, which stimulates people's interest to develop it. *C. rubrofuscus* has become a mainstay commodity in several areas such as Sukabumi, Cianjur, and Blitar because it has succeeded in lifting the community's economy. This research was conducted for 3 months at a koi farm, Sukabumi Regency, West Java, on the cultured *C. rubrofuscus* from the broodstock, ponds and nursery ponds. The purpose of this research was to determine the technical aspects of *C. rubrofuscus* fish seed production and the performance of fish farming including fecundity, fertilization rate (FR), hatching rate (HR), and survival rate (SR), in order to increase production yields and the quality of cultivation for an improved regional economy. Data collection was carried out by field observation and measurement, combined with interviewing technicians. The performance of the seedling cultivation of *C. rubrofuscus* consisted of a fecundity ranging from 109,000 to 114,500 grains per brood, an FR ranging from 66 to 72%, a HR ranging from 68 to 77%, a seed growth per day ranging from 4.3 to 15.7% per day, and an SR ranging from 66 to 68% and from 87 to 90% at the first and second harvest, respectively. These figures, although acceptable, did not allow the cultured fish at the third cycle to reach their growth target of an average size of 5 cm within 60 days.

Key Words: hatchery, Koi shiro and showa, fecundity.

**Introduction**. Koi *(Cyprinus rubrofuscus)* is one of the freshwater fishery commodities that is currently the mainstay in the fisheries sub-sector (Dupamana et al 2020), being a freshwater ornamental fish (Domasevich et al 2022; Kottelat & Freyhof 2007; Balon 1995). *C. rubrofuscus* began to be bred in Japan in the 17<sup>th</sup> century (Utomo et al 2007) under the name "Nishikigoi", which means fish of various colors (David et al 2004; Balon 2006). The beauty of *C. rubrofuscus* lies in its back which has unique colors and patterns and has approximately 100 types of colors (Qur'ania & Verananda 2017; Utomo et al 2007). Nishikigoi is a world-famous koi fish (Domasevich et al 2022; Kottelat & Freyhof 2007) that is one of the most important raw materials that is still in demand today. *C. rubrofuscus* have high economic value both on the national and international markets (Putri & Dewi 2019; Kusrini et al 2015).

*C. rubrofuscus* originates from Japan in Niigata Prefecture as an ornamental fish after several generations of selective breeding (Balon 1995) and it is in great demand because it is believed to bring good luck by koi lovers in Indonesia. The color scale and pattern of color distribution can vary greatly between koi varieties, and the pattern is nearly unique and nearly impossible to repeat between any two individuals (De Kock & Gomelsky 2015). In addition, *C. rubrofuscus* are often used as pond decorations and are art consumption for devotees (Lesmana & Dermawan 2007). Koi fish are characterized by attractive colors and different variations. Koi fish comes from the carp or carper which is Japan's national fish (kokugyo), generally classified into 13 categories, namely Kohaku, Sanke, Showa, Bekko, Utsurimono, Asagi, Shusui, Tancho, Hikari, Koromo, Ogon,

Kinginrin and Kawarimono (Triyanti & Yulisti 2012). Sukabumi is one of the most appropriate areas of *C. rubrofuscus* production, according to the criteria for cultivating *C. rubrofuscus* from seeding to rearing.

The aim of this study was to observe the implementation of koi hatchery management which includes broodstock rearing, brood spawning, egg hatching, larval rearing, seed rearing, feeding, water quality management, pest and disease prevention, sorting, seed selection, harvesting, and marketing, so that can assist cultivators in increasing production yields and the quality of *C. rubrofuscus* cultivation.

**Material and Method**. This research was conducted from March 2 to May 23, 2020, at Mizumi Koi Farm, Sukabumi Regency, West Java. Tools and materials used in the research on the *C. rubrofuscus* hatchery techniques include: broodstock ponds, spawning ponds, nursery ponds, scales, aerators, paralon pipes, rulers, oxygen tubes, aeration hoses, pH meters, thermometers, and cameras. The materials: male and female *C. rubrofuscus* broodstock, floating pellets, powder pellets, and DO test kit. The methods used for data collection, were direct observation and measuring directly in the field, and the direct interviews conducted with technicians in the field.

### **Results and Discussion**

**C.** rubrofuscus broodstock maintenance. *C.* rubrofuscus male and female broodstock, originating from Ogata Farm, Japan, were reared separately, in order to avoid mass spawning, in accordance with Arddhiagung et al (2009). A number of 10 males and 10 females of *C.* rubrofuscus broodstock specimens of shiro and showa strains were reared. The total weight of the *C.* rubrofuscus broodstock was in the range of 2.5-4 kg. *C.* rubrofuscus broodstock were fed on floating pellets with a size of 5 mm. Feeding pellets aims to help growth, increase fish weight and maintain the quality of *C.* rubrofuscus broodstock so that the color of the fish does not fade, in accordance with the opinion of Utomo et al (2007), who state that fish's nutritional needs for maintenance and growth can be covered by artificial feeds that meet the digestive and taste requirements of *C.* rubrofuscus. The pellet feed used can be seen in Figure 1 below.

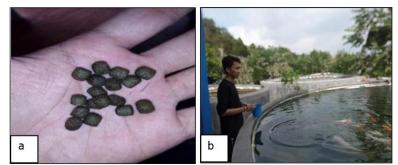


Figure 1. (a) Type of pellet (b) Feeding.

Feeding is done with a frequency of once a day, in the morning at 09.00, by spreading it. This is not in accordance with the opinion of Tiana & Murhananto (2004), which states that feeding should be carried out twice a day, in the morning and evening.

In fish farming, nutrition is the most crucial economic factor to take into account, due to its contribution of 50 to 70% to the production costs (Rana et al 2009; Gebremichael et al 2021). The nutritional content of the used broodstock feed was of 35% protein, according to the product's specifications, in accordance with the opinion of Papilon & Efendi (2017), who state that the minimum composition is of 28-32% protein and 6-8% fatty acids, for a feed dose of 3-5% of the broodstock's weight. According to Bartlett & Bartlett (2007), the content of feed for *C. carpio* fish throughout the year must have a protein content of 30-36%. The content of the broodstock feed used can be seen in Table 1.

Table 1

*Cyprinus rubrofuscus* broodstock feed content

Component	Content (%)
Protein	35
Fat	5
Fiber	3
Ash	12
Water content	12
Calcium	2

The water quality of the main tank was measured as follows: temperature and pH twice a day, at 07:00 am and 05:00 pm, and the dissolved oxygen once a week. The average results of water quality measurements are presented in Table 2.

Table 2

Parameter	<i>Result of measurement</i>	According to SNI*	Description
Temperature (°C)	23-26	20-28	In accordance
pH	7.1-7.5	6.5-8	In accordance
DO (mg L <sup>-1</sup> )	5.5-6.5	>5	In accordance

Water quality measurement

5NI 7734:2011 Indonesian National Standard.

Based on the table above, the value of water quality is in accordance with 7734:2011 Indonesian National Standardization (SNI). This is because during the maintenance period using water sources from direct springs and away from industrial waste and the use of aerator as an oxygen supply.

Selection of C. rubrofuscus broodstock. Selection of C. rubrofuscus broodstock with mature gonads was carried out at 10:00 am, by using a drain and then selecting by visually examining some of their characteristics. Based on the data obtained, the selected female broodstock at the gonadal maturity was characterized by sluggish movement, rounded and reddish sex shape, enlarged abdomen, with an average weight of 3.6 kg. The male selected broodstock was characterized by agile movements, the shape of the genitals was elongated and if the stomach was massaged towards the anus it would emit a white liquid with a weight of about 3.3 kg. The age of the male broodstock is 2 years while the age of the female broodstock is 3 years. This is in accordance with the statements of Sugiarto (2008) and Papilon & Efendi (2017) (Table 3).

Table 3

Parameters	Female	Male	Description
Movement	Slow	Agile	In accordance with Sugiarto (2008)
Age	More than 2 years	More than 1 year	In accordance with Papilon & Efendi (2017)
Genitals	Rounded shape, reddish color	The genitals are elongated, if the stomach is pressed towards the anus, it will release sperm fluid	In accordance with Sugiarto (2008)
Body shape	Body size is relatively	The body shape is	In accordance

Characteristics of the broodstock gonadal maturity

Parameters	Female	Male	Description
	larger than that of male, the abdomen is enlarged to the anus and soft at pressing.	elongated	with Sugiarto (2008)

The selected female *C. rubrofuscus* broodstock which will spawn have slow movements and the male moves with agility, the genitals are mature, the female body shape is larger and the abdomen is enlarged to the anus, while the male has a slender and elongated body shape. The male broodstock is aged of 2 years and the female broodstock of 3 years, in accordance with Sugiarto (2008) and Papilon & Efendi (2017). The male and female broodstock of *C. rubrofuscus* shiro are shown in Figure 2a and 2b and those of *C. rubrofuscus* showa can be seen in Figure 2c and 2d.

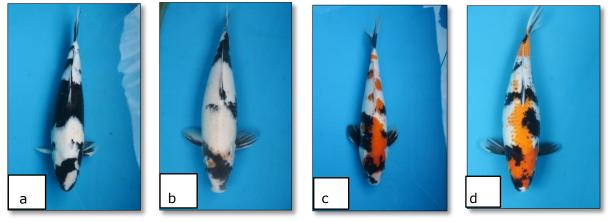


Figure 2. *Cyprinus rubrofuscus* shiro broodstock (a) male, (b) female; *Cyprinus rubrofuscus* showa broodstock (c) male, (d) female.

**Spawning**. Spawning is carried out after the selection process of mature gonads which produces 3 broodstock ready to spawn. Spawning is done with a ratio of males and females 1:1. According to Kusrini et al (2015), the implementation of natural, semi-artificial, and purely artificial hatcheries is done by using 1:1, 1:2, 1:3 broodstock, respectively, depending on the readiness of the existing broodstock. The number of *C. rubrofuscus* broodstock in the spawning tank is 1 male and 1 female. This is believed to improve the quality of *C. rubrofuscus* seeds and make it easier to choose fish that will be used as prospective broodstock. The spawning broodstock data can be seen in Table 4.

Spawning of *Cyprinus rubrofuscus* broodstock

Table 4

	Spawning Broodstock strain		Broodstock weight (g)			
No.	date –	DIOUUSLOCK SLIAIII		Bef	fore	After
ŭ	uale	Male	Female	Male	Female	Female
1	Feb 1, 2020	Shiro	Showa	3,129	3,565	3,274
2	Mar 1, 2020	Shiro	Showa	3,209	3,638	3,282
3	Apr 4, 2020	Showa	Shiro	3,637	3,592	3,261

Spawning is done naturally. This was done to anticipate the risk of physical changes in *C. rubrofuscus* due to the stripping carried out on artificial spawning. Before the broodstock is put into the spawning tank, it is weighed first. Spawning was carried out during the day: male and female *C. rubrofuscus* brooders who were about to spawn were transferred to the spawning tub and left for approximately 22 hours.

According to Ramadhan & Sari (2019), the spawning process occurs at night, being marked by the sound of splashing water produced when the male chases the female. After spawning, the broods are slowly removed and weighed again, then the broods are put into plastic packing and then transferred to a quarantine tank. The purpose of separating the broodstock after spawning is to avoid disturbing the eggs that have been released. The transfer of the broodstock of *C. rubrofuscus* is in accordance with the opinion of Agus & Asmara (2007), which state that the spawning process is better when the broodstock is moved, so that the eggs are not eaten by the mother, and the eggs are hatched in the spawning pond.

**Egg hatching**. The hatching of eggs is carried out in a spawning tank. The fertilized eggs are visually clear, while the unfertilized eggs are white. Eggs attached to the kakaban (medium in a spawning pond, in the form sugar palm fiber) substrate were allowed to hatch in the spawning tub from the first day of spawning until they hatched for approximately 3 days, without any water recirculation. This is in accordance with the opinion of Agus & Asmara (2007).

**Larvae care and harvest**. When the larvae hatch until they are 4 days old, the feed used is egg yolk; when the larvae are more than 4 days to 7 days old, they are given feed in the form of egg yolks that have been boiled and dissolved in water; feeding is done once a day, at 07.30-09.00 am, by spreading evenly. According to Purbomartono & Suwarsito (2016), the protein contained in the egg yolk feed is sufficient for the consumption of koi fish. Chicken egg yolks have a fairly high protein content, of 30.9% (Purbomartono & Suwarsito 2012). The larval rearing process is carried out in a spawning tank. Larvae were harvested in spawning tanks after 7 days of age and then stocked in nursery ponds. At the time of harvesting, larvae grading was carried out to obtain quality *C. rubrofuscus* showa and shiro specimens. The characteristics of larvae that will be sorted and stocked in nursery ponds, according to the company standards, are black color, while larvae that do not pass the sorting are reddish white. Black larvae are counted and will be stocked in nursery ponds and reared up to a size of 5 cm, while white larvae will be freely stocked in ponds (Figure 3).

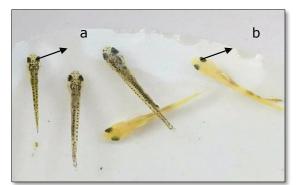


Figure 3. Larvae (a) Qualified; (b) Not qualified.

Harvesting the larvae is done by lifting the kakaban while slowly pouring water so that no larvae are carried away, after the hapa used for spawning is removed slowly, in order to reduce the space for the larvae movements and facilitate the harvest. To make sorting easier, the tools use vaccum pump (a modification of hi-blow, buckets, and aeration hoses), buckets, and basins. Larvae are scooped up and placed in a basin in order to be sorted, then the black larvae are sucked up using a vacuum pump, while the red larvae are placed in another bucket.

Larvae that have passed the sorting are then stocked in nursery ponds to be reared up to a size of 5 cm or approximately 2 months old. Before the larvae are stocked, acclimatization is carried out first by slowly adding water from the nursery pond, so that the larvae can adjust to the temperature in the new environment. The stocking of these larvae is done in the morning to avoid the high temperatures. Temperatures that are too high can kill *Cyprinus carpio* seeds (Kusrini et al 2015) or cause stress to fish and subsequent diseases (Lembang & Kuing 2021).

**Seed maintenance**. The distribution of larvae was carried out two times, namely at the larvae and carp fry stadia, each of which was divided into three time cycles and the number of stockings are described in Table 5 below. Stocking is done in the morning at 10:00 am. Larvae were stocked into nursery pond 1, with a maintenance period of 30 days, until the carp fry stadia. After the sorting, the carp fry that pass the sorting will be stocked into nursery ponds 1 and 2 have an area of  $27 \times 22 \times 1 \text{ m}^3$ , with a water height of 0.2 m. Larvae are released into the basin until they can swim. The number of stocked specimens can be seen in Table 5.

Table 5

Cycle	Stocking date	Stocked individuals
	Larvae stadia	
1	Feb 08	18,928
2	Mar 07	13,986
3	Apr 11	14,883
	Carp fry stadia	
1	Feb 29	3,144
2	Mar 28	2,709
3	May 02	3,063

*Cyprinus rubrofuscus* stocking number

**Feed management**. During the three cycles (Table 5), the seeds received the same treatment, being fed once a day, as it can be seen in Figure 4 below.

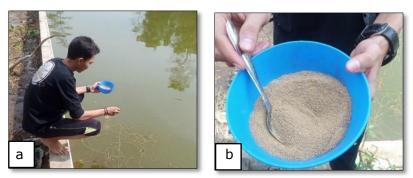


Figure 4. Seed feed: (a) Feeding; (b) Powder pellets.

Powdered feed is used for seed maintenance until day of culture (DOC) 30. After the seeds enter DOC 31-60, the feed will be given in the form of pellets measuring 1 mm. The nutritional content contained in the artificial feed can be seen in Table 6 below.

Table 6

No	Components	Powder pellets (%)	1 mm pellets (%)
1	Protein	35	35
2	Fat	5	5
3	Fiber	3	3
4	Ash	12	12
5	Moisture content	12	12
6	Calcium	2	2

#### Seed feed content

The feed content contained on the label used is acceptable for *C. rubrofuscus* in accordance with the opinion of Bartlett & Bartlett (2007), stating that 25-36% of protein content is good for *C. rubrofuscus*.

**Water quality management**. Water quality management is very important, since water is the main medium in cultivation activities, and in nursery ponds it can be performed by checking DO, pH, and temperature (Shahady & Boniface 2018). This check is carried out twice a day, namely at 07:00 am and at 05:00 pm, except for DO, which is checked once a week, in the morning and evening time.

1. Temperature: The results of temperature measurements for the three cycles can be seen in Figure 5 below.

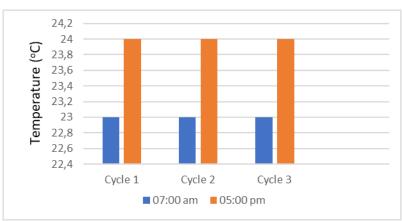


Figure 5. Average nursery temperature for *Cyprinus rubrofuscus*.

Based on the graph above, the value of the temperature measurements in cycles 1 to 3 did not show any difference, because nursery 1 was carried out in an earthen pond and had the same water source and the same treatment. The average temperature value obtained is still in accordance with SNI 7734-2011.

2. pH: The average pH obtained during the 3 cycles maintenance period can be seen in Figure 6 below.



Figure 6. Average pH of *Cyprinus rubrofuscus* nursery.

Based on the graph above, the pH measurements in cycles 1 to 3 were similar, for the reasons previously mentioned. However, the average pH is still in accordance with Bartlett & Bartlett (2007), stating that the pH ranges ideally from 6.8 to 7.2 but *C. rubrofuscus* can survive in a wider range, of 6.5 to 9.0.

3. Dissolved Oxygen (DO): During the maintenance period of the 3 cycles, the average DO value obtained can be seen in Figure 7.

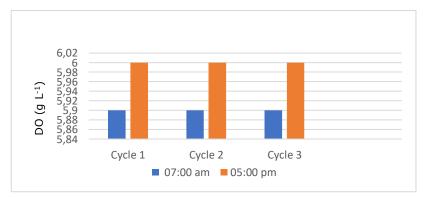


Figure 7. Average DO value of Cyprinus rubrofuscus nurseries.

Based on the graph above, the similar DO values. However, the average temperature is still in accordance with SNI 7734-2011.

4. Sampling: Sampling was carried out once a week by taking 8 samples at one point, using a scoop, and then length measurements were taken. The average seed length can be seen in Figure 8 below.

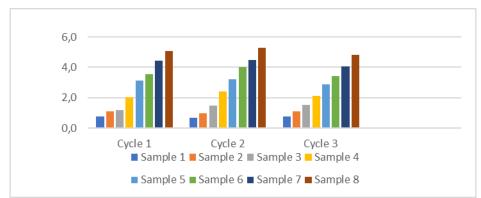


Figure 8. Average length of *Cyprinus rubrofuscus*.

Based on the graph above, it can be seen that the growth of fish seeds in sampling 1 to sampling 8 experienced a slow growth, but the average seed growth had reached the minimum limit for the size of the fry, set by the SNI 01-6136-1999: 1-3 cm at the age of 20 days, 3-5 cm at the age of 40 days and 5-8 cm at the age of 70 days. This is caused by the low temperature of the environment and affects the growth of fish. According to Laila (2018) and Dedi et al (2016), temperature has a very significant effect on the growth of carp fry. The optimal temperature ranges from 28-30°C, but the best temperature to grow the carp fry is 28°C, while the temperature in the nursery ponds ranged from 23-26°C.

**Pest and disease prevention**. Prevention of predators and competitors for oxygen and feed in nursery ponds is not carried out specifically. According to Brönmark & Hansson (2017), their presence can reduce the maximum productivity by losing cultured fish due to the predation. In the presence of predators, the actions taken are to catch them using a drain and immediately throw them away or destroy them. For prevention purposes, biosecurity is applied, vitamin feed is provided and also health checks are performed by sending 30 samples regularly, every 6 months, to the microbiology test laboratory, virology, and fish health.

**Post-harvest**. After a 30-day maintenance period, the seeds were harvested at the carp fry stadium. Harvesting is done by sorting the quality seeds. The fish are scooped up and then put into the basin to be transferred to the sorting tank for selection. After sorting, the quality seeds will be stocked in nursery pond 2, while the seeds that are not qualified will be flowed into the pond. The volume of harvested carp fry can be seen in Table 7.

No.	Harvest of carp fry	SR (%)	Grading of carp fry	Passed sorting (%)
1	12,579	66	3,144	25
2	9,453	68	2,709	29
3	10,168	68	3,063	30

After the carp fry seeds were stocked in nursery pond 2, they were kept until DOC 60. After that, the seeds with grade 8 to 12 cm are harvested. After harvesting, the next step is grading, which is carried out in a sorting tank with a water net (waring) on top by scooping the fish and putting them into the small pail little by little using a scope net to observe the pattern and color. The tank used is also equipped with an aerator to supply oxygen to prevent fish death. This grading aims to separate *C. rubrofuscus* based on good patterns and colors. Sorting activities are carried out to separate seed quality according to strain and color pattern (Kusrini et al 2015). *C. rubrofuscus* that pass the sorting will be distributed to the rearing unit and the fish that do not pass will be released into the pond. Data on quality seeds can be seen in Table 8.

Table 8

No.	Harvest seed	SR (%)	Seed grading	Passed the sorting (%)
1	2,739	87	1095	40
2	2,257	83	847	38
3	2,773	91	918	33

The yield of the seed (size 8 to 12 cm)

*C. rubrofuscus* has been sorted and then packed. Packing is usually done in two systems, open and closed packing (Anggraini et al 2014). The plastic used has a thickness of 0.05 mm, a width of 50 cm and a length of 85 cm. The packing technique was carried out by filling water into a plastic bag one-third the size of the plastic, then by filling it with oxygen at a ratio of 1:2 to  $H_2O:O_2$ ; 200 seeds of *C. rubrofuscus* were placed into the plastic bag, which was tied using a rubber band. *C. rubrofuscus* that has been packed was then distributed to the enlargement unit.

### Cultivation performance

*Fecundity*. Fecundity resulting from three spawning times can be seen in Table 9.

	i ccui	laity	
No	Number of egg samples/ 100 cm² (Grains)	Sample area (cm²)	<i>Number of eggs (Grains/broodstock)</i>
1	229	50,000	114,500
2	218	50,000	109,000
3	221	50,000	110,500

Fecundity

Of the three spawning cycles, the highest number of eggs was found in the first spawning, namely 114,500 eggs and the least results occurred in the second spawning 109,000 eggs per brood. The number of eggs produced was in accordance with the SNI (1999) range, namely from 85,000 to 125,000 eggs.

*Fertilization Rate (FR)*. From the results of three spawning times during the study, the FR value can be seen in the following table.

Table 10

Table 11

Table 12

Fertilization	rate (	(FR)
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Spawning	Number of eggs (Grains)	Number of fertilized eggs (Grains)	FR (%)
1	114,500	82,500	72
2	109,000	72,000	66
3	110,500	77,000	70

In the three spawning cycles, the average FR value is 69%. The highest FR value was obtained during the first spawning and the lowest value was obtained during the second spawning, probably as a result of the quality of the spawning broodstock. According to Setyono (2009), for the fertilization of eggs, the number of spermatozoa released is very large compared to the number of eggs to be fertilized.

**Hatching Rate (HR)**. The number of eggs that hatch and the HR values (compared to the number of fertilized eggs) obtained during the study can be seen in Table 11.

Hatching rate									
Spawning —	Λ								
	Total	Fertilized	Hatch	– HR (%)					
1	114,500	82,500	63,750	77					
2	109,000	72,000	48,750	68					
3	110,500	77,000	56,250	73					

HR values obtained from the three spawning cycles during the final practice ranged from 68-77%. According to Firmantin et al (2015), the degree of hatching of eggs is 60% and according to Kusrini et al (2015), the degree of hatching of the eggs in a good environment, from healthy and good brooders can produce HR values above 50%. This shows that the HR value obtained is quite good.

**Survival Rate (SR)**. The achievement of the production results is strongly influenced by the survival rate. The results of the SR of koi fish seeds during research can be seen in the following table.

Survival rate (SR)

Surviva rate (SK)								
Cycle –	Early spread	Harvest	SR	Spread	Harvest	SR		
	DOC 7	DOC 30	%	DOC 30	DOC 60	%		
1	18,928	12,579	66,46	3,144	2,739	87.12		
2	13,986	9,453	67,59	2,709	2,257	83.31		
3	14,883	10,168	68,32	3,063	2,773	90.53		

DOC-day of culture.

The SR results during the research were in accordance with SNI 01-6137-1999 which stated that the survival rate in carp hatcheries ranged from 60-80% while the resulting SR values ranged from 66-91%. If it is averaged, it gets an SR value of 77%. The SR value obtained is less good where Ramadhan & Sari (2019) states, the mortality rate at goldfish hatchery naturally is 35-75% According to Ridwantara (2019), the size of the fish affects their survival, the risk of death being higher when the size of the goldfish is small because the body's resistance cannot yet face the environmental pressures.

**Conclusions**. From the results of the study it can be concluded that the problem with the *C. rubrofuscus* hatchery at the koi farm is that fish growth is relatively low. This is

caused by several factors, namely the frequency of ineffective and not timely feeding. The performance of *C. rubrofuscus* hatchery cultivation was as follows: a fecundity ranging from 109,000 to 114,500 grains per brood and a FR ranging from 66 to 72%, a HR ranging from 68% to 77%, a seed growth per day around 4.3 up to 15.7% and a SR of 66 to 68% at the first harvest and of 87 to 90% at the second harvest. This was acceptable, but the growth of fish in the third cycle did not reach the target of 5 cm within 60 days. The hatchery technique for *C. rubrofuscus* is quite good but still needs to be improved to increase the growth and SR of fish in the rearing ponds.

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