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Short Communication

Effects of Iodine Treatment on the Development of Eggs and Larvae of Rabbitfish (*Siganus guttatus* Bloch, 1787)

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Abstract

Iodine is proven to be an effective disinfectant for fish eggs and is commonly used in a hatchery to improve hatching and survival rates. However, tolerance to iodine can vary in different fish species, and it is essential to determine the optimal concentration to ensure successful hatchery production. This study investigated the optimum concentration and exposure time to iodine during incubation to improve rabbitfish larvae's hatching and fertilization rate from 4/2022 – 8/2022 in Thua Thien Hue, Vietnam. The study consisted of two experiments: Experiment 1 focused on different iodine concentrations: 0 ppm (C1), 50 ppm (C2), 100 ppm (C3), and 150 ppm (C4). Experiment 2 explored three exposure times: 10 minutes (T1), 20 minutes (T2), and 30 minutes (T3) to increase the fertilization, hatching, and survival rates at five days post-hatching. Data were compiled, analyzed, and compared by ANOVA using SPSS ver. 22.0. The results showed that 100 ppm iodine concentration at 20 minutes of exposure resulted in the optimum hatching, fertilization, and survival rates of *S. guttatus* during artificial reproduction. In the future, integrated solutions in the use of iodine solution for egg treatment and natural food supplementation at the larval stage should be recommended to improve the efficiency in artificial hatchery production of Rabbitfish.

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1. Introduction

Iodine is an essential trace element of great importance for human nutrition (Triggiani *et al*., 2009; Sorrenti *et al*., 2021) and aquatic animal development (Cabanilla-Legaspi *et al*., 2021a). This mineral plays an important role in the synthesis of thyroid hormones (THs) (Sorrenti *et al*., 2021; Cabanilla-Legaspi *et al*., 2021a) involved in normal metamorphosis and development (Yamano, 2005; Ansari, 2010); and protects from oxidation of lipid membranes, proteins, and DNA (Venturi, 2011). The deficiency of iodine content in the diet of marine fish larvae affects their normal growth and development (Srivastava *et al*., 2011; Solbakken *et al*., 2002). Supplementation of an external iodine source can improve larval stage development (Pham and Le, 2016; Cabanilla-Legaspi *et al*., 2021a).

In Vietnam, *Siganus guttatus* is popular among consumers and has a high market value (Nguyen *et al*., 2015). Mass production of fry is still constrained by high mortality rates and severe larval metamorphosis (Juario *et al*., 1985). They can be caused by broodstock nutrition, environmental conditions during broodstock rearing, and nutrition during the larval stage (Ayson, 1989; Tran *et al*., 2023; Kieu *at el*., 2023; Nguyen *et al*., 2015; Phuong *et al*., 2022). Reports in *Siganus guttatus* showed that the addition of iodide to the feed was related to changes in thyroid hormones, thyroxine (T4), and triiodothyronine (T3) during early larval development. Changes in endogenous thyroid hormone levels related to their metamorphosis, growth, and survival (Leatherland, 2010; Cabanilla-Legaspi *et al*., 2021a, 2021b; Pham and Le, 2016). In addition, the use of the iodized solution for sterilization of fish eggs or aquaculture, and water treatment to improve larval quality, egg hatching rate, survival rate, and metamorphosis ability of larvae has also been reported (Ayson and Lam, 1993; Aydin *et al*., 2011; Lai *et al*., 2020; Zawada *et al*., 2014). In this study, for the first time, we used iodine at different concentrations and times in the washing of *S. guttatus* eggs to improve the fertilization rate, hatching rate, the survival rate; and reduce the rate of deformation of larvae in Thua Thien Hue, Vietnam. The study aims to provide a solution on the concentration and time of soaking the iodine solution in the process of handling the fish eggs before being put into the incubation tank. The results will contribute to improved efficiency at an early stage in artificial seed production (5 days after hatching).

2. Materials and Methods

2.1 Materials

Rabbitfish (*S. guttatus*) is one of the food species with economic potential, has a natural distribution, and is being widely cultured in the Tam Giang - Cau Hai lagoon area, Thua Thien Hue, Vietnam (Nguyen *et al*., 2015). Thirty broodstock were selected and collected from the wild before being put into the rearing system at the Center for Research and Application of Aquaculture Technology, Faculty of Fisheries, University of Agriculture and Forestry, University of Science and Technology study in Thua Thien Hue, Viet Nam during two years from 2020 to 2022. Eggs of these broodstock were collected for studies to examine the effect of iodine concentration on fertility, hatching rate, and deformation rate of larva after incubation in two batches (2021 and 2022, from April to July). Chemicals: Iodine used in seafood trading trade name of IODINE 9000, iodine (min): 1.2 % and solvent (glycerin, sodium linear alkyl benzenesulfonate) ingredients just enough 100 % with different concentration levels of iodine for egg sterilization in experimental designs.

2.1.1 Ethical approval

Certificate Reference Number: HUVNO020 Date 10th April 2022. Project title: Effects of iodine treatment on the fertilization, hatching, deformation, and survival rates of rabbitfish. Principal Researcher: Nguyen Quang Linh.

2.2 Experimental Design

Based on data from research by Nguyen and Vu (2020), Forneris *et al*. (2003), El-Gawad *et al*. (2015), Aydin *et al*. (2011), Lai *et al*. (2020), Zawada *et al*. (2014), and biological characteristics of Rabbitfish (Hara, 1987), we used two independent randomized experimental designs to test the effect of iodine solution concentration (with levels of iodine concentrations: 0; 50, 100, and 150 ppm, respectively), and immersion time (10, 20, and 30 minutes, respectively) on the fertilization rate, hatching rate, malformation rate, and survival rate of fish larvae.

Experiment 1. Eggs collected from broodstock were incubated in 20 L plastic buckets with an aeration system. Four different concentrations of iodine were added to the incubation water, including 0 ppm (C1); 50 ppm (C2); 100 ppm (C3), and 150 ppm (C4). Nutrient washing time in each treatment was 20 minutes, incubation density was 100 eggs/L. The experiment was repeated 3 times for each treatment.

Experiment 2. After determining the optimal concentration of iodine for the larval fertility, hatching rate, and deformation rate. The next experiment was

designed to evaluate the effect of iodization time on fertilization ability, hatching rate, deformation rate, and survival rate of larvae. The times of iodine baths used were T1(10 minutes), T2 (20 minutes), and T3 (30 minutes) with three times repeated in each treatment.

2.3. Environmental Conditions

The environmental conditions in the incubation tanks in all tanks in both experiments were maintained within the appropriate range. Water samples werecollected for the measurement of temperature, salinity (S), pH, dissolved oxygen (DO), and NH_4^+/NH_3, using a specialized gauge, electronic thermometer, refractometer ATAGO Master S/MillM, Extech DO600, Hanna HI98017, and Hanna HI715, respectively. Temperature and pH were determined twice a day at 7 a.m. and 3 p.m. Salinity and DO were determined once a day at 7 a.m. The environmental factors in the aquarium are maintained: pH: 7.6 - 8.4, the temperature: 28 - 31°C; DO: 4.5 - 5.1 mg/L, NH_4^+/NH_3: 0 mg/L, salinity: 30 ppt (Table 1).

2.4. The Fertilization Rate (FR)

The egg fertilization rate was determined by examining 200 - 300 eggs for signs of development in each flask. The eggs were collected soon after the detection of spawning and incubated in tanks for seven to nine hours before examination. Developing eggs were easy to distinguish during the early stages of cell division based on the image described by Hara (1987) (Figure 1). Fertilization rate (FR) was calculated as the percentage transformation of micro-injected oocytes into two pronuclei. Eggs that did not develop after the one-hour waiting period were assumed to be unfertilized. The fertilization rate (FR) was determined according to the following formula:

FR (%) = (Number of fertilized eggs/Number of hatched eggs) * 100% Eq (1)

2.5. Hatching Rate (HR), Deformation Rate (DR), And Survival Rate (SR)

For each iodine concentration and washing time, the hatching rate, deformation rates (%), and

Table 1. Environmental factors for two experiments

Environmental factors	Time (h)	Expt. 1 (concentration)		Expt. 2 (time)	
		M ± SE	Min - Max	M ± SE	Min - Max
T (^{0}C)	7:00	28.50 ± 0.50	28.00 - 29.00	28.58± 0.47	28.00 - 29.00
	15:00	30.56 ± 0.53	30.00 - 31.00	0.58 ± 0.51	30.00 - 31.00
pH	7:00	7.93 ± 0.18	7.60 - 8.20	7.97 ± 0.11	7.80 - 8.10
	15:00	8.23 ± 0.11	8.00 - 8.40	8.23 ± 0.11	8.00 - 8.40
DO (mg/L)	7:00	4.59 ± 0.33	4.60 - 5.00	4.50 ± 0.29	4.50 - 5.10
S (ppt)	7:00	30.00 ± 0.00	-	30.00 ± 0.00	-
NH_4^+/NH_3 (mg/L)	7:00	0.00	-	0.00	-

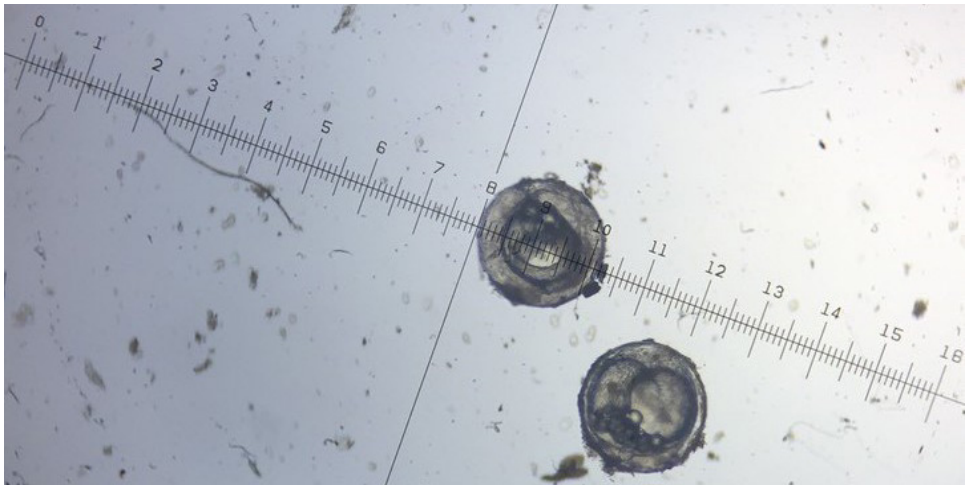

Figure 1. Eggs 11-12 hours after fertilization (570 µm) in normal

survival rate were determined at five days post-hatching. Three hundred eggs contained in a Petri dish were placed into each of the incubation tanks. Hatched larvae were removed and counted each day for five days. The removed larvae were observed with an optical microscope and the number of deformed individuals was recorded. The total hatching rate was defined as the percentage of stocked embryos that hatched by the formula:

Hatching rates (%) = (Amount of larva/Number of fertilized eggs) *100% Eq (2)

The deformation larvae were observed and counted directly on a Nikon microscope. Images of the metamorphosis of larvae as described by Hara (1987) were used as a control image in observing and determining the rate of anomalies. The malformed larva is individual with unusual shapes: curved bodies, scoliosis, short body, and mouth (Figure 3 and Figure 4). The deformation rate was the percent of deformed larvae among the hatched larvae according to the formula:

DR (%) = (Number of larva deformation/Number of observed fries) * 100% Eq (3)

Fish survival was determined by the percentage of fish that survived the incubation period (Figure 2). The number of dead larvae was determined and removed for five consecutive days from the time the fish started hatching to the end of the experiment. The survival rate is calculated as follows:

SR (%) = (Final numbers of fish/ Initial number of fish) * 100% Eq (4)

2.6. Data Analysis

The experimental values are expressed as the means of the measurements over the three experimental replications plus the standard deviation based on Excel 2016. The mean was compared using Duncan's test and the ANOVA analysis of variance. The analytical values were statistically significant, $p < 0.05$ by IBM SPSS Statistics 20.0 software.

Figure 2. Five days larvae in normal

3. Results and Discussion

3.1. Results

3.1.1 Environmental variables

Environmental factors during incubation in both experiments were maintained under stable conditions and the difference was not significant between treatments ($p > 0.05$). The temperature and pH values ranged from 28.50 - 31.08°C and 7.6 - 8.4 respectively. Dissolved oxygen content ranged from 4.00-5.00 mg/L; the salinity was maintained at 28 ppt; and total NH_4^+/ NH_3 was 0 mg/L in all tanks (Table 1).

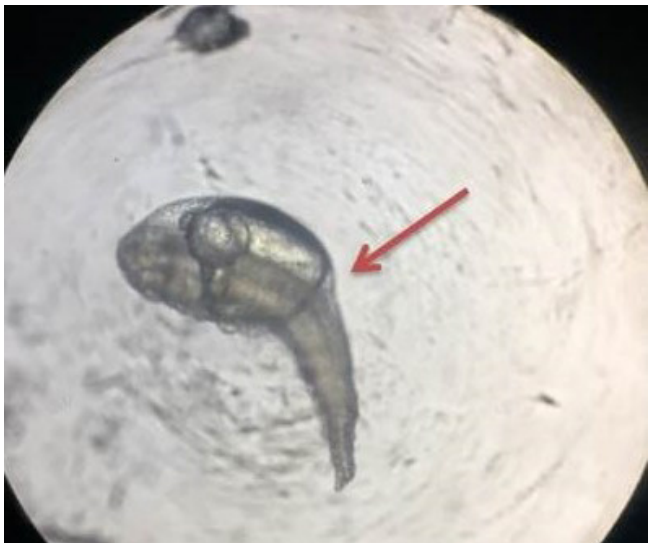

Figure 3. Deformation larva (2 hours after hatchery)

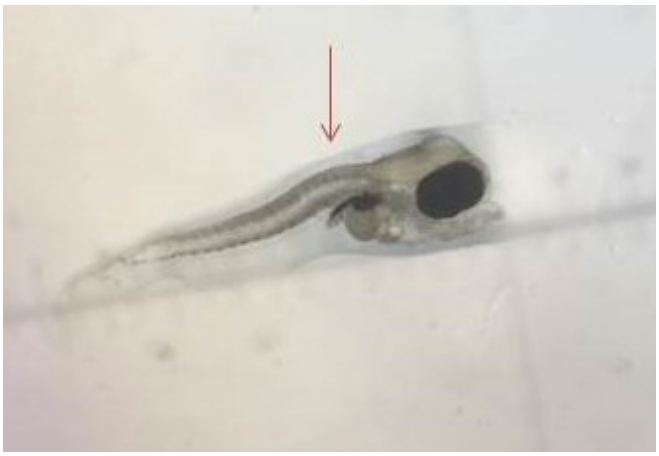

Figure 4. Deformation larva (3 days after hatchery)

Table 2. The egg development and larva survival rates exposed to different concentrations of iodine

Variables	Fertilization rate (%)	Hatchery rate (%)	Deformation rate (%)	Survival rate (%)
C1	82.22^{a} ± 0.51	84.72^{a} ± 1.48	7.67^{b} ± 0.57	36.91^{a} ± 3.27
C2	85.56^{b} ± 1.02	94.87^{b}± 1.74	3.33^{a} ± 0.57	46.31^{b} ± 5.55
C3	86.44^{b} ± 0.84	96.14^{c} ± 1.05	2.67^{a} ± 1.15	58.65^{c} ± 7.60
C4	85.00^{b} ± 1.76	86.77^{a} ± 1.97	3.67^{a} ± 0.57	56.91^{c}± 11.61

Note: $^{a, b, c}$ letters are differences in the same column, with $P < 0.05$.

Table 3. Fertilization, hatching, deformation, and survival rates in different washing times

Variables	Fertilization rate (%)	Hatchery rate (%)	Deformation rate (%)	Survival rate (%)
T1	83.67^{b} ± 1.86	92.42^{b} ± 2.27	2.67^{b} ± 0.58	57.03 ^{b} ± 6.02
T2	85.56^{b} ± 1.17	92.98^{b} ± 0.49	2.33^{b} ± 0.58	59.53^{c} ± 5.25
T3	77.44^{a} ± 2.14	85.92^{a} ± 2.49	4.33^{a} ± 0.58	52.45^{a} ± 7.89

Note: $^{a, b, c}$ letters are the same on the same column, and there is no statistical significance ($p > 0.05$).

3.1.2. Effects of iodine concentration on fertilization rate, hatchery rate, deformation rate, and survival rate

The results of the study on the effect of iodine concentration when washing the eggs before incubation of the scorpion fish (Table 2) showed that the addition of iodine had a significant effect ($p < 0.05$) on the growth of fish eggs development of embryos and larvae of *S. guttatus* up to five days of age. The fertilization rate, hatchery rate (%), deformation rate (%), and survival rate (%) of caviar not using iodine ranged from 82.22 ± 0.51%, 84.72 ± 1.48%, 36.91 ± 3.27 lower than the rate fertilization rate in tanks with iodine. Meanwhile, the đeformation rate (%) of larva in T1 (control) was not using iodine 7.67 ± 0.57 % higher than the rate fertilization rate in tanks with iodine ($p < 0.05$).

At different concentrations of iodine, the fertilization rate of eggs, hatching rate, and survival rate of larva after five days of hatching when washing at T3 concentration (100 ppm) was the highest (86.44 ± 0.84%, 96.14 ± 1.05%, 58.65 ± 7.60% respectively), while the deformation rate of larvae in this treatment was the lowest (2.67 ± 1.15%). Therefore, we have confirmed that a solution of 100 ppm concentration is the best practice for the pretreatment of fish eggs prior to introduction into the incubation system. This concentration was selected for experiment 2.

3.1.3. Effects of washing time of iodin on fertilization, hatching, deformation and survival rates

Different iodine treatment time affects the growth parameters of *S. guttatus* (Table 3). With an iodine concentration of 100 ppm at different soaking times, it showed that the fertilization rate, hatching rate, and mean survival rate at T1 (83.67 ± 1.86 %, 92.42 ± 2.27 %, 57.03 ± 6.02%), T2 (85.56 ± 1.17%, 92.98 ± 0.49%, 59.53 ± 5.25%) were similar and higher than T3 (77.44 ± 2.14%, 85.92 ± 2.49%, 52.45 ± 7.89%) with $p < 0.05$. The larva was influenced when using iodine batching for a long time, and the morphological deformities, body curvature, malformed, and extensive head amputation (Figure 3 and Figure 4) rate of larval in T1 (2.67 ± 0.58%), T2 (2.33 ± 0.58%) was significantly lower than in T3 (4.33 ± 0.58) ($p<0.05$).

3.2. Discussion

The rabbitfish is a species that is naturally distributed in the lagoons of Tam Giang, Cau Hai, Thua Thien Hue, Vietnam (Nguyen *et al.*, 2015), they are able to adapt to the pH conditions in the water of the area. the lagoon system fluctuates strongly from 6.71 to 8.64; DO: 4.5-5.9 mg/L; and Salinity range from 4 to 21 (Truong, 2022). Tests on rearing conditions for brood fish of *S. gustatus* show that environmental conditions affect their

growth. Broodstock fish raised in the cages in the Tam Giang - Cau Hai lagoon area, Thua Thien Hue, Vietnam have significantly higher growth rates and maturation rates ($p < 0.05$) than fish that were raised in cages placed in shrimp ponds and lined ponds (Kieu *et al*., 2023). At various stages of the reproductive process, Rabbit fish can withstand many different salinities (Stattin, 2012). Eggs of *S. guttatus* laid in the wild are more tolerant to changes in salinity than eggs laid in hatcheries (Duray and Kohno, 1988), which can tolerate salinity from 3 to 71 ppt with a hatching rate of 90% (Young and Dueñas, 1993). There was not any significant difference observed in the survival rate of larvae reared at 20-32‰ salinity (Iwamoto *et al.,* 2012**)**. The survival rate of *S. guttatus* larvae was improved when kept at 22-26°C (Kohno *et al*., 1988; Iwamoto *et al*., 2012) with corresponding salinity conditions. In the salinity range from 14 to 37 ‰, rabbitfish eggs have a survival rate of over 50 %, eggs and larvae have the highest survival rate at a salinity of 16 ‰ (Stattin, 2012). According to Rachman Syah *et al*. (2020), most of the parameters for water quality were still at the viable state for the life and growth of rabbitfish, *S. guttatus* were temperature: 25.2-33.7°C; salinity: 26.9-37.2 ppt; pH: 7.5-9.2; DO: 3.3-6.9 mg/L. So, the environmental factors of carrying out studies on the influence of iodine concentration and washing time on the efficiency of broodstock (*S. gustatus*) including temperature, pH, DO, salinity, NH_4^+/NH_3 (Table 1) were maintained stably and within the threshold for embryo and larval development of rabbitfish.

The morphological deformations that commonly occur in larvae can be caused by contaminated water, heavy metals, and chemical influences (Béguer *et al*., 2008; Cabanilla-Legaspi *et al*., 2021a). To improve the efficiency of artificial reproduction, antiseptic bath solutions for eggs and morula stages have been proposed (Hirazawa *et al*., 1999). Iodine solution is recommended as a suitable disinfectant in the treatment of eggs in marine fish through peripheral (water quality control, pathogen killing, etc.) and intracellular (increased production and activity of intracellular hormones). The results shown by Nguyen and Vu (2020) that when crab eggs (*Scylla paramamosain*) were bathed in an iodine solution, the deformity rate of crab larvae was 0.65%, it was lower than that when treated with ozone was 2.10% by studied of Forneris *et al.* (2003). El-Gawad *et al*. (2015) showed that when bathing yellow perch (*Perca flavescens*) at a concentration of 50 ppm for 30 minutes, the hatching rate reached 29.43% and 30.94%, the survival rate of the first-day larvae were 41.97% and 36.7% respectively. Yellow perch eggs (*Perca flavescens* Linnaeus, 1758) without iodine had the highest average hatching rate of 97.60%. When eggs were soaked with iodine (10 minutes) at a concentration of 100 ppm, the hatching rate was 91.3%, while at a concentration of 3,000 ppm, it was only 91.30% (Aydin *et al*., 2011). Abalone eggs (*Haliotis diversicolor* Reeve, 1846) were treated with 100 ppm iodophor solution for 1 minute to limit the infection of harmful bacteria; The hatching rate of eggs was 77.3% and the survival rate to the larval stage was 78.5% (Lai *et al*., 2020). Another study showed that a solution of 100 mL iodophor per 10 dm^{-3} (13.5 mg of active iodine dm^{-3}) for 10 min could be used for routine sterilization of salmon roe (*Salmo trutta* Linnaeus, 1758) after the collection phase (Zawada *et al*., 2014).

Klaren *et al.* (2008) study analyzed the correlation between thyroid hormone concentrations throughout the body during early development and metamorphosis in the Senegal sole fish (*Solea senegalensis*). Under their rearing conditions, most larvae enter stage 1 metamorphosis at 15 days post-hatch (DAH) and metamorphosis is completed in stage 4 at 25 DAH. Pre-metamorphic larvae at 5 DAH thyroid cysts are first observed in the hypopharyngeal region, around the abdominal aorta. By the 12 DAH stage, the thyroid follicles increase, causing the epithelial cell height of the follicle to increase by about 40% compared to the 5 DAH stage larvae. In 15 DAH larvae, whole-body thyroid hormone concentrations increased significantly with a 2.5-fold increase in total thyroid colloid area. Through an increase in the whole body T3/T4 ratio during the metamorphosis stages, an increase in deiodination in the outer ring was shown. For *S. guttatus*, Quinitio and Siladan (2013) showed that the control and treatment treatments supplemented with CEWAF (Chemically Enhanced Water Accommodated Fraction) to disinfect eggs at concentrations of 0.5, 1.0 and 1.5% for fertilization rates ranging from 96.89 to 99.68% ($p > 0.05$). Cabanilla-Legaspi *et al.* (2021a) analyzed the pattern of increase and decrease of iodide and thyroid hormone levels during larval development and its role in the metamorphosis of the larva after 50 DAH under normal rearing conditions. The concentrations of iodide and thyroid hormone (T4, T3) detected from newly hatched larvae of maternal origin were 11.98 ± 4.3 nmol/g; 0.09 ± 0.01 nmol/g; 0.01 ± 8.5E-05 nmol/g respectively. It peaked in fish 10 days after hatching DAH (1416.43 ± 149.6 nmol/g). THs gradually increased as larvae developed and peaked at 20 and 30 DAH, coinciding with the onset of metamorphosis. The addition of iodine at different concentrations to the fresh feed of juvenile (Artemia) at 20 days after hatching (DAH) was carried out and demonstrated that feeding Artemia larvae supplemented with sodium iodide enhances endogenous TH levels,

thereby accelerating metamorphosis and improving fish muscle fiber growth and stress tolerance (Cabanilla-Legaspi *et al*. 2021b). In this study, for the first time, we determine that iodine concentration of 100 ppm (C3) in the treatment of eggs and disinfected water increased the fertilization of rabbitfish up to 86.44%, hatching rate 96.14%, and deformation decreased to 2.67% (Table 2), and increasing of survival larva rate up to 58.65%, with the batching time of 20 minutes to increase 85.56%, 92.98%, and 2.33% respectively (Table 3). Therefore, studies on the combination of the use of iodine solution in egg treatment and the addition of natural food at the larval stage should be carried out as an integrated solution to improve agricultural productivity effectively in artificial seed production in the future.

4. Conclusion

Using iodine in batching eggs and disinfecting for nursing water positively impacted the development of eggs and fries of rabbitfish. For *S. guttatus* eggs, it is recommended to bathe with iodine at a concentration of 100 ppm for 20 minutes for optimal results on some egg development indicators to improve the fertilization rate and reduce the rate of deformation.

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Authors' Contributions

The contribution of authors is as follows, Kieu Thi Huyen; worked on the conceptualization, methodology, investigation, revision, and submission. Nguyen Quang Linh, worked on the conceptualization, methodology, investigation, and project administration. Tran Vinh Phuong; analyzed data and wrote original draft. Tran Nguyen Ngoc; worked on the investigation and analyzed data; Ho Ngoc Tram Anh; worked on the investigation and analyzed data". All authors discussed and contributed to the final manuscript.

Conflicts of interest

All authors declare that they have no conflict of interest.

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