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Synergistic effect of water temperature and dissolved oxygen concentration on rates of fertilization, hatching and deformity of hybrid yellow catfish (*Tachysurus fulvidraco*♀ × *Pseudobagrus vachellii*♂)

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ABSTRACT

In the process of selecting and developing freshwater aquaculture species, yellow catfish (*Tachysurus fulvidraco*) have received widespread attention from Chinese farmers, fishery scientists and technologists. Achieving full artificial breeding of yellow catfish would help improve the quantity and quality of fingerlings supplied for large-scale production of this species. Temperature (*T*) and dissolved oxygen (*DO*) are the most important abiotic factors affecting the breeding efficiency of aquatic organisms. In this study, the synergistic effects of *T* and *DO* on fertilization rate (*FR*, %), hatching rate (*HR*, %) and deformity rate (*DR*, %) of hybrid yellow catfish (*T. fulvidraco*♀ × *Pseudobagrus vachellii*♂) were studied by central composite design (CCD) and response surface methodology. A quadratic regression model for the effects of *T* and *DO* on *FR*, *HR* and *DR* was established, and the combination of *T* and *DO* was optimized. The first and second order effects of *T* and *DO* on *FR* and *HR* were significant under the conditions of this experiment ($P < 0.05$). The first and second order effects of *T* on *DR* were significant ($P < 0.05$) but there was no significant effect of *DO* on *DR* ($P > 0.05$). *T* and *DO* had significant interaction effects on *FR* ($P < 0.05$). High *T* and high *DO* environments reduced *FR* and *HR* of yellow catfish eggs and increased *DR* of the newly hatched larvae. The optimal combination of *T* and *DO* was 26.0 °C and 8.3 mgL^{-1}, respectively. Maximum *FR* and *HR* coincided with minimal *DR* whose predicted values were 87.2%, 89.1% and 2.7%, respectively, with reliability of 0.979. Maintaining *T* and *DO* in the best combination will help to improve breeding efficiency and ensure production of the highest quantity and quality of fingerlings.

1. Introduction

Yellow catfish (*Tachysurus fulvidraco*) are typical omnivorous fish that inhabit slow-flowing rivers and lake bottoms. Its flesh is delicate in texture and has a delicious taste, with a high protein content, low fat content and high nutritional value. After several years of demonstration and promotion of its culture potential, this fish has gradually been accepted by farmers and consumers. As a result, the demand for fingerlings continues to increase. However, the production capacity for fingerlings lags behind the requirements for aquaculture production, which restricts further development of the yellow catfish breeding industry. Artificial breeding of yellow catfish could help remove this bottleneck in supply of the quantity and quality of fingerlings, and could provide large-scale production of fingerlings that meets the needs of the majority of farmers.

The early developmental stages of organisms are often the most sensitive phases of their complex life cycles (Cinti et al., 2004). Temperature (*T*) and dissolved oxygen levels (*DO*) are generally important determinants of growth and reproduction in aquatic organisms, and are the most important abiotic factors affecting their fertilization and hatching rates (Wexler et al., 2011; Xie et al., 2018). Hatching of fish eggs involves digestion of the egg shell by a hatching enzyme, and muscular movements of the embryo. *T* is an important factor affecting the secretion of the hatching enzyme by the hatching gland cells in the embryo (Wang et al., 2014). Low *T* has been shown to inhibit hatching enzyme secretion, delay incubation, and reduce survival of the embryos

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to as low as 6.5%–47% (Fan and Shi, 2002). Conversely, *T* exceeding the acclimation range of fish embryos and larvae may also compromise growth and development, resulting in higher mortality and deformity rates (Fan and Shi, 2002). In yellow catfish *T. fulvidraco*, the optimum hatching *T* for embryonic development is 25 °C (Yuan et al., 2005). In channel catfish *Ictalurus punctatus*, the best *T* range for embryonic development is 26 °C; lowering *T* significantly extended the incubation period and increased mortality (Small and Bates, 2001). A suitable *T* for fertilized egg development and larval growth of silver catfish *Rhamdia quelen* is 24 °C (Perelre et al., 2006), while the optimum *T* range for fertilization and hatching of African catfish *Heterobranchus longifilis* was reported to be 25–29 °C (Nguenga et al., 2004).

The optimal hatching *T* for fertilized eggs varies among different fish species and is also dependent on salinity and *DO* level. In the salinity range 20–36‰, the optimum water *T* for hatching of fertilized eggs of silver sea bream *Sparus sarba* was reported to be 18.5 °C, but was 22 °C in the salinity range 28–32‰ (Mihelakakis and Kitajima, 1994). At 20 °C, the hatching rates of Amur sturgeon *Acipenser schrenckii* embryos were 79% and 82% at *DO* concentrations of 6.71 mgL^{-1} and 7.28 mgL^{-1}, respectively, and significantly higher than the hatching rate of 19.9% in a hypoxic group (*DO*, 4.10 mgL^{-1}). Deformity rates in the two normoxic groups were 10.9% and 9.2%, respectively, and were elevated to 25.3% in the hypoxic group (Xie et al., 2018). *DO* is an important ecological factor that directly affects the growth and development of fish (Buentello et al., 2000). However, fish are exposed to water of constantly varying *DO* because of daily changes of *T*, light levels, photosynthesis and respiration, and fluctuations of water flow and dissolved nutrients in the natural environment (Pollock et al., 2007). Increase in water *T* accelerates the breakdown of fish embryo hatching enzymes and increases oxygen consumption of developing embryos (Pollock et al., 2007). At higher *T*, the solubility of gases is decreased and the *DO* of water is reduced (Wexler et al., 2011). Therefore, interactions, and possible synergy, between water *T* and *DO* should be considered. In large-scale aquaculture production it is necessary to pay attention to and understand physical properties such as *DO* and *T*, as well as the requirements and adaptability of species to these factors.

In recent years, we have used hybrids of 3-year-old wild strains of two species of yellow catfish *Pseudobagrus vachellii*♂ and *T. fulvidraco*♀ from the Pearl River as broodstock. In high-density pond culture, the mortality is greatly increased at high water *T* and high dissolved oxygen levels in the water is required for yellow catfish *P. vachellii* (Qiang et al., 2018), but its growth rate is fast. Yellow catfish *T. fulvidraco* is distributed in all major water systems in China. It is more tolerant of hypoxia than *P. vachellii* and is also suitable for pond culture. Progeny of the hybrids have good growth and stress resistance (submitted to another journal), emphasizing the advantages of hybridization. The response surface method (RSM) is a multivariate modeling method that can comprehensively analyze quantitative relationships among two or more test factors and their effects, by combining the analysis with appropriate experimental design. This statistical method, which seeks to find the optimal combination of experimental factors, is now widely used in the biological field (Qiang et al., 2012, 2017; Yao et al., 2017). Using this method, this study explores possible synergistic effects between water *T* and *DO* on fertilization rate, hatching rate and deformity rate of hybrid yellow catfish (*T. fulvidraco*♀ × *P. vachellii*♂), to construct the best combination of these factors for culture and artificial breeding of yellow catfish.

2. Materials and methods

2.1. Experimental parent fish

Experiments were conducted using fertilized eggs of hybrid yellow catfish. The body shape and color of the 3-year-old yellow catfish *P. vachellii*♂ and *T. fulvidraco*♀(Pearl River wild strain) used as broodstock were normal with no apparent disease or disability.

2.2. Hormone injection and dosage

We injected a combination of luteinizing hormone releasing hormone analog No. 2 (LHRH-A 2), domperidone maleate (DOM) and human chorionic gonadotropin (HCG) at doses of 10 μgkg^{-1}, 5 $mgkg^{-1}$, and 4000 $IUkg^{-1}$, respectively. After dilution with sterile 0.65% physiological saline, we injected a total of 0.3 mL per 100 g of fish body weight into females. The needle was inserted obliquely to a depth of about 1 cm into the muscle between the dorsal fin and the lateral line on both sides of the fish body, avoiding the body cavity. The injection occurred in two stages. The first injection consisted of 20%–33% of the full dose in females and the remainder was injected after 8–12 h. At the second injection, males and females were injected simultaneously. The total dose for males was half that for females.

2.3. Artificial fertilization and hatching

Approximately 10–12 h after the second injection, we collected the eggs in a stainless steel basin by gently squeezing the body. Approximately 1000 eggs were randomly selected from each group, 0.2 mL of semen was added, and then 10 mL of water was added for activation. The mixture was stirred for about 2 min until the sperm and eggs were well mixed. After artificial fertilization, the eggs were deadhered with an 8% suspension of yellow mud for 5 min. A sieve was placed over a large plastic pot and the separated fertilized eggs were poured into the sieve, rinsed in a sink and then poured into a hatching tub. Six hatching tubs were included in each experimental group.

2.4. Experimental design

A central composite experimental design (CCD) was used. The response variables were the fertilization rate (*FR*), hatching rate (*HR*) and deformity rate (*DR*). Based on production practices and a preliminary trial indicating significant promotion or inhibition of the response values by experimental factors, the ranges of factors in this experiment were set at: *T* (19°C–33 °C) and *DO* (2.2 $mgL^{-1}$to 12 mgL^{-1}). Each factor was assigned five coded levels by CCD: −a, −1, 0, 1 and a, with 'a' being the star arm. The numbers of factorial and axial points were four and four, respectively. The number of center points was set equal to five; thus the star arm a = ± 1.41421 in this case. The total number of experimental runs was 13. The coded values of *T*, −a, −1, 0, 1, and a corresponded to actual values of 19.0, 21.1, 26.0, 30.0 and 33.0 °C, respectively. The coded values of *DO*, −a, −1, 0, 1, and a corresponded to the actual values of 2.2, 3.6, 7.1, 10.6 and 12.0 mgL^{-1}, respectively. See Table 1 for coded and actual combinations of *T* and *DO* levels; each experimental combination was repeated six times. The *T* of the experimental group was set (± 0.3 °C) by an electronic temperature controller (range 18–36 °C). *DO* was controlled by adjusting the rate of bubbling of nitrogen or oxygen into the water. Real-time readings of *DO* were made using an optical probe (LDO101, measuring range 0.1–20.0 mgL^{-1}; HACH Co., Loveland, CO). The experimental *T* and *DO* values are shown in Table 1.

2.5. Measurement of response values

Ten eggs were removed every hour and examined under a dissecting microscope. When they had reached the mid-intestine stage (9–18 h after fertilization) (Poleo et al., 2005), 100 eggs were randomly collected from each test combination and observed for *FR*. The experiment was repeated six times for each group.

$$FR(\%) = (\text{number of eggs at the mid} - \text{intestine stage} / \text{total number of eggs}) \times 100\%$$

Using the artificial fertilization method described above, the sperm and an egg were simultaneously inserted into a hatching tub having a *T*

Table 1
Experimental design of temperature(*T*) and dissolved oxygen (*DO*) combinations.

Run	Coded *T*	Coded *DO*	Actual *T* (°C)	Actual *DO* (mgL^{-1})
1	a	0	33.0	7.1
2	0	0	26.0	7.1
3	0	−a	26.0	2.2
4	0	0	26.0	7.1
5	−1	−1	21.1	3.6
6	−a	0	19.0	7.1
7	0	0	26.0	7.1
8	0	a	26.0	12.0
9	0	0	26.0	7.1
10	1	1	30.9	10.6
11	0	0	26.0	7.1
12	−1	1	21.1	10.6
13	1	−1	30.9	3.6

Note: Each factor has five levels coded by CCD as: −a, −1, 0, 1 and a, with a being the star arm. The numbers of factorial and axial points are four and four, respectively. The number of center points was set equal to five. A is the star arm and |a| = 1.41421 for this experimental design. The coded values of temperature, −a, −1, 0, 1 and a, correspond to the actual values of 19.0, 21.1 26.0, 30.0 and 33.0 °C. The coded values of dissolved oxygen, −a, −1, 0, 1 and a, correspond to the actual values of 2.2, 3.6, 7.1, 10.6 and 12.0 mgL^{-1}.

of 27 °C and a *DO* of 7 mgL^{-1} to obtain a fertilized egg. Fertilized eggs were selected and placed in a 2000-mL hatching tub at the preset experimental *T* and *DO*. The fertilized eggs (500 eggs ± 20) in each group were micro-inflated to fully roll the fertilized eggs. After hatching from the egg membrane (the incubation time varied with the experimental conditions but was completed within after 5 days), *HR* was calculated. The experiment was repeated six times for each group.

HR(%)= (number of hatched fry/number of fertilized eggs) × 100%

All newly hatched larvae were fixed with 3% formalin solution, and the *DR* was calculated based on the anterior position of the oil droplet, heartbeat irregularity, spinal curvature, or fin damage of the newly hatched larvae.

DR(%)= (number of deformed larvae/total number of hatching larvae) × 100%

2.6. Statistical analysis

Data are expressed as mean values ± standard deviation (SD). A quadratic polynomial regression equation between the factors and the response values was fitted by the least squares method:

$$\hat{Y} = b_0 + b_1T + b_2DO + b_3T \times DO + b_4T^2 + b_5DO^2$$

where $\hat{Y}$ is the predicted response (*FR*, *HR* or *DR*); b_0, b_1, b_2, b_3, b_4, and b_5 are the regression coefficients for the linear effects of *T* and *DO*, the interaction between *T* and *DO*, and the quadratic effects of *T* and *DO*, respectively.

Statistical analysis of experimental data was performed using SAS (V8.2). We established multiple regression equations between the influencing factors and *FR*, *HR* and *DR* of hybrid yellow catfish, and calculated the corresponding fits. The regression equations established for *FR*, *HR* and *DR* were then analyzed to obtain the optimal combination of *T* and *DO* (maximum *FR* and *HR*, minimum *DR*). Values of $P < 0.05$ were considered significant, and $P < 0.01$ highly significant.

3. Results

3.1. Effects of T and DO on FR of hybrid yellow catfish

The experimental data shown in Table 2 were used to perform multiple regression analysis. The following quadratic multiple regression equation of *FR* versus *T* and *DO* was obtained:

$$FR(\%) = -527.78 + 41.34\,T + 12.16DO - 0.83T^2 - 1.41DO2 + 0.46\,T \times DO$$

Table 2
Response observations (mean ± SD) for temperature (*T*)–dissolved oxygen (*DO*) combinations.

Run	Actual *T* (°C)	Actual *DO* (mgL^{-1})	FR (%)	HR (%)	DR (%)
1	33.0	7.1	30.3 ± 2.7	19.3 ± 2.6	24.6 ± 3.2
2	26.0	7.1	82.3 ± 6.4	88.4 ± 6.5	4.1 ± 0.5
3	26.0	2.2	27.9 ± 3.2	34.6 ± 4.5	7.2 ± 0.7
4	26.0	7.1	84.7 ± 5.9	89.3 ± 7.9	3.5 ± 0.4
5	21.1	3.6	34.2 ± 4.1	37.8 ± 4.2	9.7 ± 0.6
6	19.0	7.1	38.4 ± 3.1	21.3 ± 3.4	10.2 ± 1.2
7	26.0	7.1	85.6 ± 6.8	85.7 ± 6.3	2.4 ± 0.3
8	26.0	12.0	78.9 ± 5.7	73.9 ± 5.8	5.6 ± 0.2
9	26.0	7.1	81.5 ± 7.2	84.9 ± 7.6	2.6 ± 0.3
10	30.9	10.6	54.7 ± 4.3	51.1 ± 4.1	16.3 ± 0.7
11	26.0	7.1	87.2 ± 6.3	91.2 ± 7.3	1.7 ± 0.1
12	21.1	10.6	39.6 ± 2.8	45.7 ± 5.1	5.2 ± 0.4
13	30.9	3.6	47.8 ± 4.9	40.6 ± 3.8	19.4 ± 0.8

The model was analyzed for variance (Table 3). The significant test for the regression model coefficients is shown in Table 4. The regression model for *FR* was highly significant ($P < 0.0001$, Table 2). The values of $F = 11.06$, $P > 0.05$, determination coefficient of the equation ($R^2 = 0.97$), and the correlation coefficient ($R^2 = 0.95$), indicate that the fitted quadratic equation is suitable. From Table 3, it can be seen that *T* and *DO* had significant linear effects on *FR* ($P < 0.05$), and that the quadratic effect of *T* and *DO* on *FR* was highly significant ($P < 0.01$); the interaction of $T \times DO$ also had a significant effect on *FR* ($P < 0.05$).

The response surface plot between *T* and *DO* and *FR* is shown in Fig. 1. These dynamics can be used to evaluate the interaction of two factors on the *FR* of hybrid yellow catfish. Under the experimental conditions, *FR* first increased and then decreased as *T* increased from 19.0 °C to 33.0 °C. When the *DO* in water was 7.1 mgL^{-1}, the fertilized eggs had the highest *FR* (> 75%) at *T* between 25.0 and 28.0 °C. At *T* = 26.0 °C and *DO* between 2.2 and 8.5 mgL^{-1}, *FR* increased with increasing *DO* level; at *DO* > 8.5 mgL^{-1}, *FR* decreased significantly ($P < 0.05$). The maximum *FR* (87.2%) of the yellow catfish was obtained at the optimum values of *T* = 27.0 °C and *DO* = 7.8 mgL^{-1}. *T* and *DO* had significant interaction effects on *FR* ($P < 0.05$). The *FR* of the yellow catfish in the high-*T*–high-*DO* environment was significantly higher than that at high-*T*–low-*DO*.

Table 3
Analysis of variance for effects of temperature (*T*) and dissolved oxygen (*DO*) on fertilization rate of hybrid yellow catfish (*Tachysurus fulvidraco*♀ × *Pseudobagrus vachellii*♂).

Source	Sum of Squares	df	Mean Square	F-value	*P*-value
Model	6539.35	5	1307.87	44.57	< 0.0001
T	327.50	1	327.50	11.16	0.0124
DO	1636.63	1	1636.63	55.78	0.0001
$T \times DO$	248.06	1	248.06	8.45	0.0227
T^2	2896.45	1	2896.45	98.72	< 0.0001
DO^2	1982.15	1	1982.15	67.55	< 0.0001
Residual	205.39	7	29.34		
Lack of Fit	183.30	3	61.10	11.06	0.0709
Pure Error	22.09	4	5.52		
Cor Total	6744.74	12			

Table 4
Significance, standard error and 95% confidence interval (CI) regression coefficients for the fertilization rate model by analysis of variance.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High
Intercept	84.26	2.42	78.53	89.99
T	6.40	1.92	1.87	10.93
DO	14.30	1.92	9.77	18.83
T × *DO*	7.88	2.71	1.47	14.28
T^2	−20.41	2.05	−25.26	−15.55
DO^2	−16.88	2.05	−21.74	−12.02

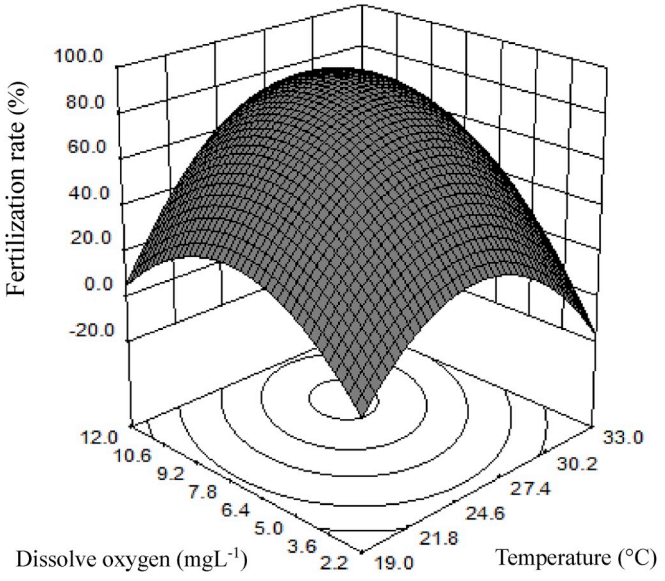

Fig. 1. Response surface plot for the effects of temperature and dissolve oxygen level on the fertilization rate of hybrid yellow catfish.

3.2. Effects of T and DO on HR of fertilized eggs of hybrid yellow catfish

HR values of fertilized eggs of hybrid yellow catfish at different values of *T* and *DO* are shown in Table 2. The regression equation fitted to the data by the least squares method was highly significant ($P < 0.0001$) and the value of the fitting term indicates that the model is valid ($P = 0.1231 > 0.05$) (Table 5). Significance tests for the regression coefficient are listed in Table 6. The linear and quadratic effects of *T* and *DO* on *HR* were significant ($P < 0.05$); the interaction effect of *T* × *DO* was not significant ($P > 0.05$). The actual quadratic regression equation for the effects of *T* and *DO* on *HR* was:

Table 5
Analysis of variance for effects of temperature (*T*) and dissolved oxygen (*DO*) on hatching rate of hybrid yellow catfish (*Tachysurus fulvidraco*♀ × *Pseudobagrus vachellii*♂).

Source	Sum of Squares	df	Mean Square	F-value	*P*-value
Model	7647.17	5	1529.43	52.45	< 0.0001
T	281.84	1	281.84	9.67	0.0171
DO	702.72	1	702.72	24.10	0.0017
T × *DO*	46.24	1	46.24	1.59	0.2483
T^2	5446.61	1	5446.61	186.79	< 0.0001
DO^2	1895.35	1	1895.35	65.00	< 0.0001
Residual	204.12	7	29.16		
Lack of Fit	177.18	3	59.06	8.77	0.1231
Pure Error	26.94	4	6.74		
Cor Total	7851.28	12			

Table 6
Significance, standard error and 95% confidence interval (CI) regression coefficients for the hatching rate model by analysis of variance.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High
Intercept	87.90	2.41	82.19	93.61
T	5.94	1.91	1.42	10.45
DO	9.37	1.91	4.86	13.89
T × *DO*	3.40	2.70	−2.98	9.78
T^2	−27.98	2.05	−32.82	−23.14
DO^2	−16.51	2.05	−21.35	−11.66

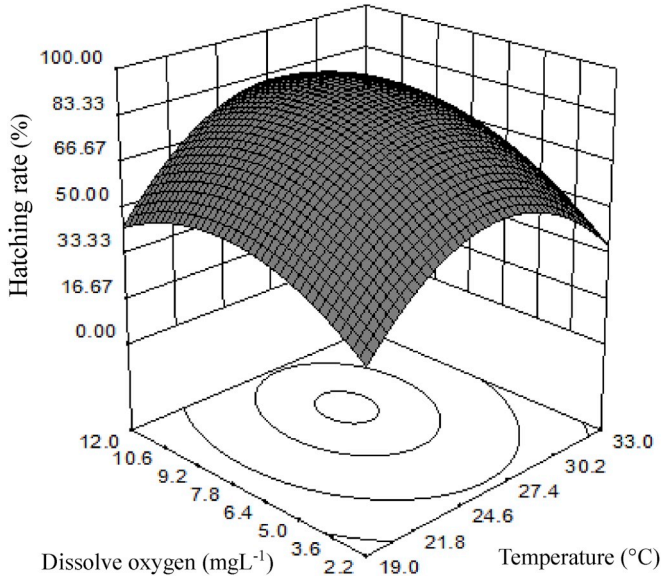

Fig. 2. Response surface plot for the effects of temperature and dissolve oxygen level on the hatching rate of hybrid yellow catfish.

$$HR = -767.25 + 59.18\,T + 17.17DO + 0.20\,T \times DO - 1.14T^2 - 1.37DO^2 (R^2 = 0.97)$$

The response surface for effects of *T* and *DO* on *HR* is shown in Fig. 2. Under the conditions of this experiment, the *DO* in the water environment was 7.1 mgL^{-1}, and *T* was 19.0–26.5 °C; in this range *HR* showed an upward trend with the increase of *T*. *T* had a significant effect on *HR* ($P < 0.05$). As *T* increased above 26.5 °C, *HR* decreased significantly. At *T* = 26.0 °C, *HR* gradually increased as *DO* increased between 2.2 and 8.0 mgL^{-1}, and then declined above *DO* = 8.0 mgL^{-1}. The interaction between *T* and *DO* had no significant effect on *HR* ($P > 0.05$). Therefore, at different water *T*, low or high *DO* environments can reduce the *HR* of yellow catfish fertilized eggs.

3.3. Effects of T and DO on DR of newly hatched larvae

DR values of newly hatched larvae at different *T* and *DO* are shown in Table 2. A regression equation was fitted to the data using the least squares method (Table 7), and the significance tests of the regression coefficients are listed in Table 8. The established regression model was highly significant ($P < 0.0001$); the value of the fitting term indicates that the fitted model is valid ($P = 0.0697 > 0.05$). The linear and quadratic effects of *T* on *DR* were significant ($P < 0.01$) (Table 5). The linear and quadratic effects of *DO* on *DR* were not significant ($P > 0.05$). The regression coefficients indicate that the effect of *T* on *DR* was significantly greater than that of *DO*. The actual quadratic regression equation describing the effects of *T* and *DO* on *DR* was:

Table 7
Analysis of variance for effects of temperature (*T*) and dissolved oxygen (*DO*) on deformity rate of hybrid yellow catfish (*Tachysurus fulvidraco*♀ × *Pseudobagrus vachellii*♂).

Source	Sum of Squares	df	Mean Square	F-value	*P*-value
Model	837.96	5	167.59	65.65	< 0.0001
T	308.14	1	308.14	120.70	< 0.0001
DO	8.92	1	8.92	3.49	0.1038
T × *DO*	0.49	1	0.49	0.19	0.6745
T^2	519.90	1	519.90	203.66	< 0.0001
DO^2	13.54	1	13.54	5.30	0.0548
Residual	17.87	7	2.55		
Lack of Fit	14.30	3	4.77	5.34	0.0697
Pure Error	3.57	4	0.89		
Cor Total	855.83	12			

Table 8
Significance, standard error and 95% confidence interval (CI) regression coefficients for the deformity rate model by analysis of variance.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High
Intercept	2.86	0.71	1.17	4.55
T	6.21	0.56	4.87	7.54
DO	−1.06	0.56	−2.39	0.28
T × *DO*	0.35	0.80	−1.54	2.24
T^2	8.65	0.61	7.21	10.08
DO^2	1.40	0.61	−0.04	2.83

$$DR = 220.58 - 17.24\ T - 2.49DO + 0.02\ T \times DO + 0.35T^2 + 0.12DO^2 (R^2 = 0.98)$$

Fig. 3 shows that, under the conditions of this experiment, as *T* increased (> 24 °C) or decreased (< 24 °C), *DR* of the newly hatched larvae increased significantly ($P < 0.01$). When *DO* in the water was 2.2–12.0 mgL^{-1}, *DR* of the newly hatched larvae was not significantly different at *T* = 19.0 or 33.0 °C ($P > 0.05$). When the *T* was 25.8 °C and the *DO* was 8.5 mgL^{-1}, *DR* of the newly hatched larvae was the lowest (2.4%).

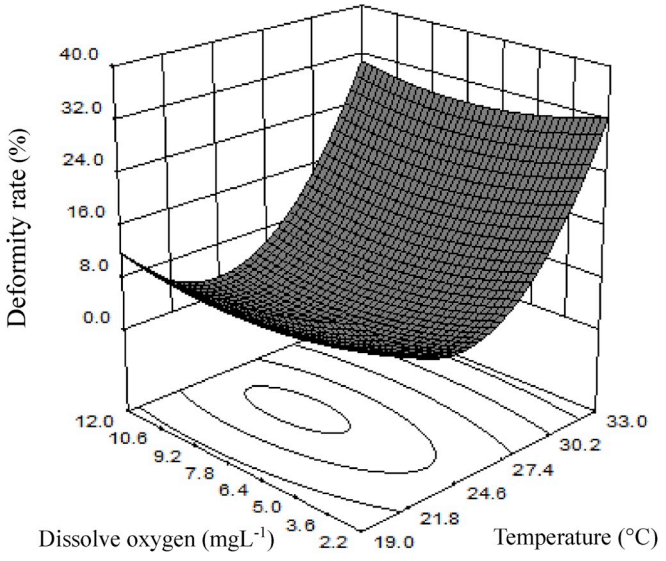

Fig. 3. Response surface plot for the effects of temperature and dissolve oxygen level on the deformity rate of hybrid yellow catfish.

3.4. Optimization of response values

FR, *HR* and *DR* were simultaneously optimized according to the method of Montgomery (2005). The optimal combination of *T* and *DO* was 26.0 °C and 8.3 mgL^{-1}, respectively. Under this combination of conditions, the *FR* and *HR* were simultaneously highest, and *DR* was lowest, with predicted values of 87.2%. 89.1% and 2.7%, respectively, and a reliability value of 0.979.

4. Discussion

Fish gonadal development is a complex and systematic process. Artificial culture employs a restricted ecological environment and for some fish it is difficult to achieve large-scale natural reproduction. Artificial reproduction by means of induction and stimulation by exogenous hormones and environmental factors is therefore required. *T* is an important environmental factor in fish breeding that directly affects fish gonads and embryonic development (Arenzon et al., 2002). *T* had a significant effect on *FR* and *HR* in our experiments. Because we observed that unfertilized eggs were still able to undergo normal division and to develop to the blastocyst stage, we determined *FR* at the mid-intestine stage; embryos that did not divide and died after the blastocyst stage were considered unfertilized embryos. At water *T* of 19.0 °C and 33.0 °C, 65% and 50%, respectively, of the embryos died at the blastocyst stage. Fertilization in fish involves an interaction between the sperm and egg. Low *T* or high *T* may affect sperm activity and structure in hybrid yellow catfish, reducing the binding efficiency of sperm and egg, which needs further study.

Hatching *T* of fertilized eggs is one of the key environmental factors affecting the early survival, feeding and growth of fish, and is also an important environmental condition for reproduction in freshwater fish (Wexler et al., 2011; Imsland et al., 2019). Many biochemical reactions in living organisms are catalyzed by biological enzymes, which can exert their strongest catalytic effect only at the optimum *T*. At low *T*, embryonic development is slow, possibly because the secretion and catalytic activity of hatching enzymes are inhibited (Choi and Kim, 2016). Fan and Shi (2002) found that reduced *T* significantly delayed hatching and that embryo survival was also decreased to 6.5%–47%. In addition, the hatching time of fertilized eggs was significantly negatively correlated with *T*, and the hatching time at 19.0 °C was more than twice that at 28.0 °C in the present study. Qiang et al. (2008) reported that at a *T* of 22 °C, the hatching time of the fertilized eggs of hybrid tilapia *Oreochromis niloticus* × *O.aureus* was 132.7 h and *HR* was 22.3%; at 28 °C hatching time was 83.7 h and *HR* was 90.3%. However, at 30 °C, fertilized eggs of hybrid yellow catfish stopped dividing during embryo formation. Long-term high *T* also inhibits secretion of, or causes premature consumption of, the hatching enzyme, which eventually causes most embryos to be unable to break through the membrane. At *T* = 25–28 °C, *HR* of the fertilized eggs of the hybrid yellow catfish was highest, and *DR* of the newly hatched larvae was lowest. High or low *T* may affect organ differentiation and the initiation of enzyme activity in the late stage of embryonic development, leading to disordered organogenesis or diapause, which would greatly hinder normal development of the embryo (Wang and Tsai, 2000). Liu et al. (2010) and Wei et al. (1997) also found that high *T* during incubation led to uneven embryonic development in striped beakfish *Oplegnathus fasciatus* and roughskin sculpin *Trachidermus fasciatus*, associated with early release of larvae, low *HR*, high *DR* and high mortality.

Insufficient oxygen in the water could cause impaired embryonic development, decreased *HR*, and increased *DR* (Peng et al., 2013). Fertilized eggs of the hybrid yellow catfish sink and are sticky. When the embryos develop to the pre-membrane stage, the hatching enzyme thins the egg membrane, which may increase the surface viscosity of the eggs (Fan and Shi, 2002). In this study, when *DO* in the water was < 3 mgL^{-1}, *FR* and *HR* were significantly lower, and *DR* higher, than in the experimental group with *DO* of 8 mgL^{-1}. Similar

observations were reported in a study of the effects of *DO* on embryonic development of Amur sturgeon (Xie et al., 2018). When the eggs adhere to one another, they are more likely to experience hypoxia, which affects embryonic development and the normal filming of the larvae; in addition, hypoxia might also disturb free radical metabolism of the fish, leading to lipid peroxidation damage of the body. Cronin and Seymour (2000) observed that as embryonic development progressed in the Australian giant squid *Sepia apama*, the need for O_2 increased exponentially, requiring O_2 concentrations in the water greater than 5.5 mgL^{-1}. However, when *DO* in water was > 10 mgL^{-1}, *HR* of hybrid yellow catfish decreased significantly. From the cleavage stage to the primitive gut stage, development was essentially normal but when the heartbeat appeared, the embryos began to die. Excessive oxygen free radicals in a high oxygen environment might attack cellular components, such as proteins, fats and nucleic acids, leading to reduced *HR* and higher *DR* (Fang, 2002).

We constructed a quadratic regression model and report for the first time that the quadratic effects of *T* and *DO* have significant effects on *FR* and *HR* of hybrid yellow catfish. The quadratic effect of *T* also had a significant effect on *DR*. Changes in *T* and *DO* generated maximum values for *FR* and *HR* of hybrid yellow catfish embryos, which could be of great significance for actual production. When each factor deviated from the optimal value, *FR* and *HR* decreased in a curvilinear manner. Therefore, it is necessary to pay attention to maintenance of these factors at the optimal combination of values to ensure maximum *FR* and *HR*, and low *DR* of embryos. We observed a synergistic effect between *T* and *DO* on the *FR* of hybrid yellow catfish, indicating that *T* modulates the effect of *DO*. When the *DO* in the water changes, the effect of *T* on the binding of the egg to the sperm also changes. At high *DO* (> 10 mgL^{-1}), the maximum *FR* of hybrid yellow catfish occurred 28 °C but, when *DO* was < 10 mgL^{-1}, maximum *FR* occurred at 25 °C. The *FR* in the high-*T*–high-*DO* combination was significantly higher than in the combination of high-*T*–low-*DO*, which may be related to the requirements for *T* and *DO* in water during embryonic development of hybrid yellow catfish.

In the production of hybrid yellow catfish fingerlings, higher *FR* and *HR* are important prerequisites for improving the yield of fingerlings. In this study, the effects of *T* and *DO* on the *FR*, *HR* and *DR* of newly hatched larvae were studied by CCD and RSM. The surface model of *T* and *DO* on *FR*, *HR* and *DR* was fitted by a quadratic regression model. The corrected R^2 values were 0.95, 0.96 and 0.96, respectively. When the optimal *T* and *DO* were combined (26.0 °C and 8.3 mgL^{-1}), the *FR* and *HR* of hybrid yellow catfish were highest and the *DR* was lowest. Therefore, *T* and *DO* should be maintained as close as possible to these values in production. In the hot season, increasing the *DO* of the water, or reducing the *T* by adding deep well water or new water would ensure a high reproduction efficiency of hybrid yellow catfish. Other environmental factors, such as pH and fertilized egg density, may also be important factors affecting the *FR* and *HR* of hybrid yellow catfish, and deserve further study.

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Authors'contributions

XP and QJ conceived and designed the experiment, LM and LC cultured hybrid yellow catfish, QJ and ZCY analyzed the database and built models. HJ, TYF and LHX measured FR, HR and DR. QJ and ZCY wrote the paper with contributions from all other authors. All authors read and approved the final version of the manuscript.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.05.003.

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