ISSN : 0974 - 7435 Volume 10 Issue 16

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(16), 2014 [9224-9230]

Effects of different temperature on the growth, enzyme activity and hydrochemical state of pseudosciaena crocea

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ABSTRACT

This experiment researched effects of different temperature on the growth, enzyme activity and hydrochemical state of pseudosciaena crocea through seven temperature groups 20℃, 22℃, 24℃, 26℃, 28℃, 30℃ and 32℃. Water was not changed in the experiment. Research result shows that different temperature obviously ($P<0.05$) affects feeding amount, specific growing rate, feed efficiency and condition factor of pseudosciaena crocea. There was not much difference on growing rate and feeding efficiency at 28℃, 30℃ and 32℃($P>0.05$). When temperature was above 26℃, pepsin and hind intestine protease activities of pseudosciaena crocea increased obviously ($P>0.05$). Amylase and lipase activities increased as temperature rose; but there was no big difference among groups ($P>0.05$). Content of NH_4^+-N and NO_2^--N of farming water increased as a whole while PH value of water decreased.

KEYWORDS

Pseudosciaena crocea; Growth; Enzyme activity; Hydrochemical state.

INTRODUCTION

Fish are poikilothermal animals and temperature is one of the most effective ecological factors to their lives. Farming water temperature can not only affect eating amount, growing rate, feed efficiency and standard metabolizing of fish; but also can it affect immunologic function, digestive enzyme activity and sex determination of fish. Many experts and scholars have researched factors mentioned above[1-4] from home and abroad. At present, *pseudosciaena croceas* are farmed in net cages along the coast where water temperature changes obviously weather. Usually there is a wide range of comfortable temperature for fish. However, in this range growing rate and feed efficiency do not proportionate; at a particular temperature growing rate and feed efficiency are the best[5-7]. Therefore, if this temperature can be found and constant temperature can be maintained in farming factory, pseudosciaena crocea will grow up in the shortest period, farming risk will be decreased and feed efficiency will be improved.

Growing rate of fish and feed efficiency change with water temperature. It is possible that water temperature affects activities of all kinds of enzymes inside fish; especially, digestive enzyme determines digesting, assimilating nutriment abilities, growing rate and feed efficiency. There are many researches home and abroad about effect of enzyme reacting temperature on digestive enzyme[8-10], but not enough reports about effect of water temperature on digestive enzyme activity of fish[3-4]. This experiment searched theoretical basis of the most proper water temperature for pseudosciaena crocea through researching effect of water temperature on digestive enzyme activity.

MATERIAL AND METHOD

Fish in experiment

Fish used in the experiment was Min-Ao Dongzu pseudosciaena crocea of Guanjing Sea Zone and bought from Kengyuan Nursery Ground of Ningde City, Fujian Province. Strong fish which grew well and ate normally was chosen for the experiment. Their average weight was 45.41±1.31g and average length was 14.53±1.61cm.

Feed in experiment

Puffed combinative feed of Xialin Brand was used in experiment. Ingredients of the feed are shown in TABLE 1.

Crude protein	Crude fat	Crude fiber	Crude ash	Water
≥47.0	≥3.0	≤2.0	≤18.0	≤10.0

Experiment method

Set seven temperature groups: 20℃, 22℃, 24℃, 26℃, 28℃, 30℃ and 32℃. Prepared two 200cm-long and 60cm-deep aquariums for each group. Combined the sea water in which *pseudosciaena croceas* ever lived with new sea water and made sure salinity was 25; inlet oxygen 24 hours. Put *pseudosciaena croceas* and let them adjust to the new environment first. One day later, got rid of the dead ones and saved 30 fish for the experiment. Ecological farming mode was chose and water was not changed once in the whole process[11]. Sea water was added up for evaporation to keep ecological balance between microbes and fish. Sediment at the bottom was sucked out by siphonage method every 5 days. Fish were fed full exactly at 7:00 and 17:00 each day. Feeding amount was adjusted according to actual eating status and data was counted after 60 days.

Testing method of digestive enzyme

(a) Prepare sample

Dissected pseudosciaena crocea on an ice plate. Got stomach, fore intestine and hind intestine (front part from the first intestine turning was defined as fore inside contents and fat; washed with 4℃ double distilled water; dried with filter paper and weighted. Cut sample into pieces and homogenized them in glass homogenizer with 4℃ double distilled water at the ratio of 1:5. Use centrifugal machine to centrifuge liquid at 4℃ (8000r/min, 30min) and put liquid in 4℃ refrigerator waiting for test.

(b) Test protease activity

Used Follin phenol reagent method[12]. When PH value was 7.5 (PH value was 2.2 when testing stomach), bathed in 37℃ water, a enzyme activity (U/g) unit was that casein hydro-lysate formed 1μg tyrosine each minute in 1g alive structure.

(c) Test amylase activity

Used iodine-starch color comparison method[13]. When PH value was 7.0, bathed in 25℃ water, a enzyme activity unit (U/g) was that hydrolyzed starch formed 1μg malt sugar each minute in 1g alive structure.

(d) Test lipase activity

Used poly alcohol olive oil emulsion water dissolving method[14]. When PH value was 7.5, bathed in 37℃ water, a enzyme activity unit (U/g) was that hydrolyzed fat formed 1μg fatty acid each minute in 1g alive structure.

Hydrochemical state testing method

Used Nessler's Reagent Colorimetry[15] to test the content of ammoniacal nitrogen (NH_4^+-N) in water; used Naphthalene ethylene-diamine spectrophotometric method[16] to test content of nitrite nitrogen (NO_2^--N) and used PH meter (mine magnetic PHS-2F) to test PH value.

Count data

$$\text{Quality relative increasing rate (\%)} = \frac{m_2 - m_1}{m_1} \times 100$$

$$\text{Specific growing rate (\%·d-1)} = \frac{\ln m_2 - \ln m_1}{t_2 - t_1} \times 100$$

$$\text{Feed efficiency (\%)} = \frac{netweightfishincreased}{eatingamount} \times 100$$

$$\text{Condition factor (\%)} = \frac{W}{L^3} \times 100$$

m1 is average original weight (g); m_2 is average final weight; $t_2 - t_1$ is time of the experiment (d); W is average weight (g); L is average length.

Experiment data was One - Way ANOVA analyzed with SPSS19.0. If the differences were obvious ($P<0.05$), then used Duncan's Multu-Comparison to analyze.

EXPERIMENT RESULT

Effects of different water temperature on growing quality of pseudosciaena croceas

In TABLE 2 it shows that different water temperatures obviously affected feeding quantity, specific growing rate, feed efficiency and condition factor ($P<0.05$).

As water temperature rose, eating positivity of *pseudosciaena croceas* increased and feeding quantity was increased obviously. Feeding quantities of experiment group 30℃ and 32℃ were the biggest.

TABLE 2: Effects of different water temperature on growth, feed efficiency, and condition factor of pseudosciaena crocea

Temperature (℃)	Original weight (g)	Final weight	Feeding quantity (g)	Quantity relatively increasing rate (%)	Specific growing rate (%·d^{-1})	Feed Efficiency (%)	Condition Factor (%)	Survival rate (%)
20	45.77±1.05	57.36±1.67	32.10±1.67^{a}	25.32±2.31^{a}	0.38±0.03^{a}	36.13±3.12^{a}	1.63±0.03^{a}	81.67±6.01^{a}
22	44.83±2.36	62.11±3.25	42.31±1.52^{b}	39.05±4.05^{a}	0.54±0.07^{a}	41.12±4.11^{a}	1.64±0.04^{a}	77.78±12.62^{a}
24	45.55±1.53	72.96±2.45	58.62±2.80^{c}	60.28±7.28^{b}	0.79±0.07^{b}	46.75±3.99^{ab}	1.68±0.03^{a}	81.11±1.92^{a}
26	46.26±1.10	86.19±2.17	70.52±2.02^{d}	86.35±5.15^{c}	1.04±0.05^{c}	56.66±3.76^{bc}	1.67±0.02^{ab}	80.00±6.67^{a}
28	44.76±1.29	98.92±3.66	88.27±3.97^{e}	121.10±9.34^{d}	1.32±0.07^{d}	61.48±5.57^{c}	1.72±0.03^{b}	72.22±6.94^{a}
30	44.63±1.52	100.03±3.62	93.14±2.26^{f}	124.15±3.53^{d}	1.35±0.03^{d}	59.48±2.14^{bc}	1.71±0.04^{b}	69.45±4.81^{a}
32	46.10±1.09	98.53±3.33	94.68±3.12^{f}	113.90±12.17^{d}	1.27±0.09^{d}	55.31±2.77^{bc}	1.70±0.04^{b}	73.89±10.05^{a}

Note: Values in the same column with different superscript letters are significantly different (P<0.05).

Quantity relatively increasing rate and specific growing rate increased as water temperature rose. They are the highest at 30℃. There was not much difference among experiment groups 28℃, 30℃ and 32℃ ($P>0.05$).

Feeding efficiency increased as water temperature rose. It was the highest at 28℃ and decreased at 30℃. There was not much difference among experiment groups 26℃, 28℃ and 30℃ ($P>0.05$).

Condition factor in higher water temperature experiment groups was stronger than lower ones. There was not much difference among experiment groups 20℃, 22℃, 24℃ and 26℃ (P>0.05). There was not much difference among experiment groups 26℃, 28℃, 30℃ and 30℃ (P>0.05) either.

Water temperature did not affect surviving rating obviously (P>0.05).

Effect of different water temperature on digestive enzyme

In TABLE 3 it shows that protease activities were not same in different parts of digestive tract (P<0.05), hind intestine > fore intestine > stomach. When water temperature was above 26℃, activity of stomach protease and hind intestine protease increased obviously (P<0.05). Water temperature did not affect protease activity in fore intestine obviously (P>0.05).

TABLE 3: Effects of different water temperature on protease activity of pseudosciaena crocea (U/g)

Water Temperature Digestiveorgan	20℃	22℃	24℃	26℃	28℃	30℃	32℃
Stomach	625.57±62.7^{a}	671.3±82.6^{a}	735.9±68.4^{ab}	783.8±89.9^{ab}	835.7±69.5^{b}	834.9±125.9^{b}	866.37±90.6^{b}
Fore intestine^{B}	1212.5±115.9^{a}	1191.9±128.6^{a}	1215.5±145.9^{a}	1258.2±124.3^{a}	1253.1±125.4^{a}	1288.4±115.7^{a}	1269.5±128.5^{a}
Hind intestine^{B}	1334.1±63.46^{a}	1358.9±63.4^{a}	1429.8±93.5^{ab}	1458.4±135.2^{ab}	1585.3±89.4^{b}	1577.0±112.5^{b}	1594.6±93.8^{b}

Note: Values in the same row with different superscript lowercase letters are significantly different (P<0.05); Values in the same column with different superscript uppercase letters are significantly different (P<0.05)

In TABLE 4 and 5 it shows that amylase activity in fore intestine was obviously weaker than in stomach and hind intestine (P<0.05). Lipase activity in intestine was stronger than in stomach (P<0.05). As water temperature rose, amylase and lipase activities in digestive tract increased as a whole. But there was not much differences among groups (P>0.05).

TABLE 4: Effects of different temperature on amylase activity of pseudosciaena crocea (U/g)

Water temperature digestive organ	20℃	22℃	24℃	26℃	28℃	30℃	32℃
Stomach^{A}	729.6±89.4^{a}	749.5±83.7^{a}	703.8±134.8^{a}	702.9±82.6^{a}	753.6±63.4^{a}	780.6±73.5^{a}	736.8±88.5^{a}
Fore intestine^{B}	458.7±77.3^{a}	462.3±86.4^{a}	446.3±76.2^{a}	483.4±82.6^{a}	491.3.0±101.6^{a}	476.5±96.4^{a}	439.4±76.4^{a}
Hind intestine^{A}	768.4±89.2^{a}	763.4±59.3^{a}	774.6±59.3^{a}	769.7±92.8^{a}	776.8±63.8^{a}	756.9±68.2^{a}	782.4±59.1^{a}

Note: Values in the same row with different superscript lowercase letters are significantly different (P<0.05); Values in the same column with different superscript uppercase letters are significantly different (P<0.05)

TABLE 5: Effects of different water temperature on lipase activity of pseudosciaena crocea (U/g)

Water temperature digestive organ	20℃	22℃	24℃	26℃	28℃	30℃	32℃
Stomach^{A}	16.3±2.9^{a}	17.2±3.1^{a}	16.5±2.3^{a}	15.9±3.4^{a}	16.9±2.5^{a}	18.2±3.1^{a}	17.6±3.1^{a}
Fore intestine^{B}	40.6±5.9^{a}	43.2±5.9^{a}	39.5±2.8^{a}	42.9±8.9^{a}	47.0±9.2^{a}	46.8±5.3^{a}	43.4±4.9^{a}
Hind intestine^{A}	43.6±3.9^{a}	46.8±6.5^{a}	48.2±8.3^{a}	55.9±6.9^{a}	49.8±5.9^{a}	55.3±9.6^{a}	49.5±10.3^{a}

Note: Values in the same row with different superscript lowercase letters are significantly different (P<0.05); Values in the same column with different superscript uppercase letters are significantly different (P<0.05)

Effect of different water temperature on hydrochemical state

In the following form it shows that the content of $NH_4^+ - N$ and $NO_2^- - N$ in farming water increased while PH value decreased as a whole. The contents of $NH_4^+ - N$ and $NO_2^- - N$ were high in higher temperature groups 30℃ and 32℃ during later period of the experiment; PH values of these two groups were lower than other groups.

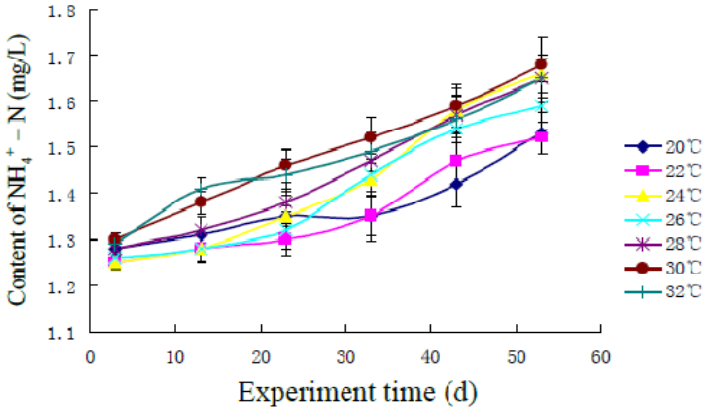

Figure 1: Effects of different temperature on $NH_4^+ - N$

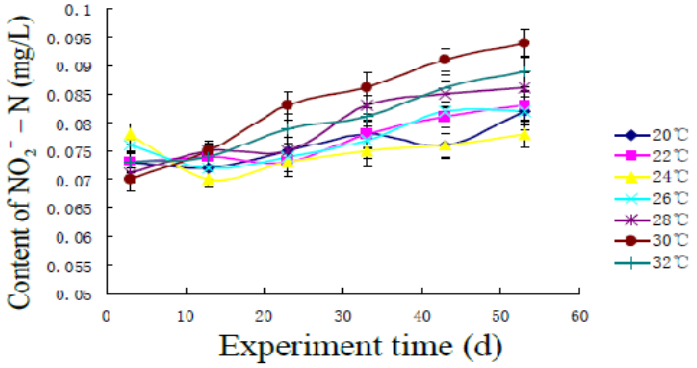

Figure 2: Effects of different temperature on $NO_2^- - N$

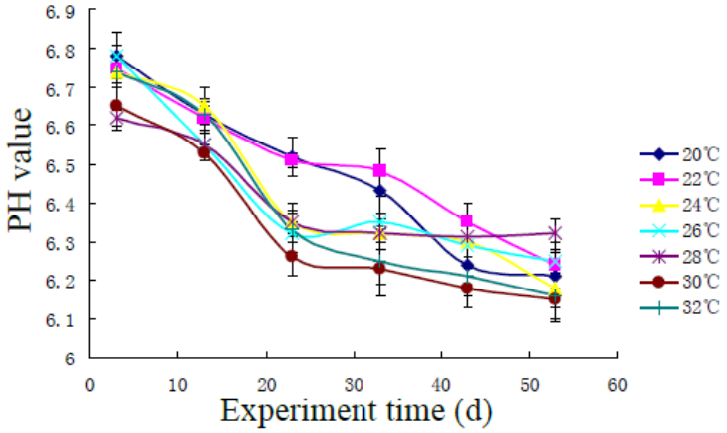

Figure 3: Effects of different temperature on pH

DISCUSSION

Effects of different water temperature on growing quality of pseudosciaena crocea

Usually, eating rate, growing rate and feed efficiency increase as water temperature rises in curtain range. If water temperature rises beyond the comfortable temperature, growing rate will decrease[17-19]. In the research, feeding quantity and specific growing rate increased when water temperature rose. But specific growing rates were not much different among groups 28℃, 30℃ and 32℃ ($P>0.05$). Although feed efficiency difference was not much in group 28℃, 30℃ and 32℃($P>0.05$), it decreased though. Researches show that when water temperature is higher than the temperature which is best for fish to grow, although fish eat more, the energy request for basic metabolizing increases relatively, so growing rate and feeding efficiency will decrease[20]. Therefore, considering feed efficiency and cost of increasing temperature, it is believed that in controllable ecological farming mode like in the research, the best water temperature for growing of pseudosciaena crocea is around 28℃, even though pseudosciaena crocea can eat and grow in 32℃ as well.

Condition factor is another factor to reflect growing status of fish. In the research it was found that condition factor increased as water temperature rose. It means the higher water temperature is, the better pseudosciaena crocea grows.

There was not much difference of survival rate among 7 groups (P>0.05). Pseudosciaena crocea died in each group and it was only because they were shocked by new environment.

Effect of different water temperature on intestine enzyme activity of pseudosciaena crocea

Some researches[21] believe that intestine enzymes are not same in different sections of digestive tract. According to Zhu Aiyi[10] and other people's researches of pseudosciaena crocea, protease activity order in digestive tract is stomach > hind intestine > fore intestine. This is different from our research result. Maybe because sizes of experiment fish and feed are different, the effects on protease activity are different either. In the research, protease activity increased as water temperature rose. It was possible that when water temperature rose pseudosciaena crocea ate more than usual; protease was secreted more relatively in digestive tract and its activity was increased. Our research result is familiar with the results of Tian Hongjie[3] and other people's research on Acipenser schrenckii and of Liuyang[4] and other people's research on Brachymystax lenok.

Although pseudosciaena crocea is a kind of carnivorous fish, amylase activity was proved high by the research. This might because experiment *pseudosciaena croceas* were long term artificial selecting fish fries and artificial combinative feed consisted of many plant materials, so protease was stimulated to form and secret.

In the research, it was found that lipase activity in digestive tract of *pseudosciaena croceas* was low. This result is familiar with the results of Yetty[22] and other people's researches on carnivorous fish-scleropages tab. osus. Low lipase activity may also caused by the content of the feed and the size of the fish in the experiment.

Effects of different water temperature on hydrochemical state

Metabolic waste contains nitrogen is defecated into water directly. Farming water in factories are mainly polluted by organic matter and nitrogen. Too much ammonia in water will stop fish from defecating ammonia, hurt gills of fish, affect growth of fish and even cause death. *Pseudosciaena croceas* are mainly bred in net cages in sea areas. In big environment of flowing sea water, ammonia is not the key effect *pseudosciaena croceas* breeding has to face, eutrophication caused by organic matter pollution is the main concern. In the research, ecological breeding mode was used and water was not changed but was only added up for evaporation. Ecological system which suited fish would form naturally in this mode and microbes digested and metabolized ammonia defecated by fish. As breeding term extended, certain amount of NH_4^+-N and NO_2^--N will piled up in water. Therefore, research on effects of different water temperature on hydrochemical state is very important. Because sea water in which *pseudosciaena croceas* lived before was used, *pseudosciaena croceas* got used to new environment fast. There were certain contents of $NH_4^+ - N$ and NO_2^--N at the beginning of the experiment. After 60 days, contents of $NH_4^+ - N$ and NO_2^--N in higher temperature groups were high. It might because feeding quantity was big in higher temperature groups and the content of ammonia defecated by fish was relatively higher than other groups. PH values in groups 30℃ and 32℃ were the lowest. It might because high temperature caused exuberant metabolizing and fish defecated more organic matter and CO_2.

Among three groups with higher growing rate 28℃, 30℃ and 32℃, hydrochemical state in group 28℃ was the best. Combined with feed coefficient and digestive enzyme activity the experiment analyzed and concluded that, although *pseudosciaena croceas* ate a lot at 28℃, digestive enzyme activity and feed efficiency were high, they defecated less organic. Besides, 28℃ is a proper temperature for microbes to grow in water. Therefore, if 28℃ were maintained as the constant temperature in farming, *pseudosciaena croceas* will grow fast, feed efficiency will be high and adjusting hydrochemical state will be easier. The research believes that 28℃ is the best water temperature for breeding pseudosciaena croceas.

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