THESIS FOR THE DEGREE OF MASTERS OF SCIENCE

*Haematological Parameters and Respiratory Function in Diploid and Triploid Far Eastern Catfish, **Silurus asotus***

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Department of Marine Bioscience and Environment

The Graduate School

Korea Maritime University

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Advisor: Prof. In-Seok Park

by

Dong-Won SEOL

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*Haematological Parameters and Respiratory Function in Diploid and Triploid Far Eastern Catfish, **Silurus asotus***

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Abstract

Haematological features pertaining to aerobic capability were compared between diploid and triploid specimens of the far eastern catfish, *Silurus asotus*.

No significant differences between diploids and triploids were found for the haematocrit value, total haemoglobin, and mean corpuscular haemoglobin concentration, while the mean corpuscular volume, mean corpuscular haemoglobin, and plasma glucose concentration were significantly greater in triploids than in diploids, and the number of red blood cells was significantly lower in triploids than in diploids.

The oxygen consumption rate did not differ significantly between diploid and triploid fish ($P > 0.05$). Nevertheless, the respiratory frequency was higher in triploids than in diploids ($P < 0.05$). Triploids were characterized by a lower concentration of circulating blood cells, and aquaculture practice should consider the need for a lower surface/situation.

Key words: Diploid, Triploid, Haematological parameter, Respiratory function, *Silurus asotus*

Thesis Advisor: Prof. In-Seok PARK, Ph. D.

INTRODUCTION

The induction of triploid has been achieved in a number of different freshwater and marine fish species (Thorgaard, 1983; Benfey, 1989; Ihssen *et al.*, 1990; Felip *et al.*, 2001). The main benefit of triploidy is sterility condition. Sterility allows and organism to avoid the metabolic costs of sexual maturation, resulting in continued somatic growth in triploid fish, with maintenance of flesh quality during the period when diploids sexually mature. In addition, sterility prevents fish mortality related to spawning (Utter *et al.*, 1983; Ihssen *et al.*, 1990; Mair, 1993; Benfey, 1999). Because of these advantages, the induction and rearing of triploid fish is practiced in the aquaculture of several economically relevant species (Hulata, 2001). Furthermore, sterile triploid fish are unable to breed and contribute to the local gene pool if they escape from the confinement. By conferring in the desired introduction of exotic fish species for a limited purpose, triploidy can serve as an effective method by which to reduce or eliminate the environmental risks of genetically modified organisms (Kim *et al.*, 1994; Dunham and Devlin, 1999).

Numerous studies have demonstrated that erythrocyte cellular and nuclear dimensions are increased and number of erythrocytes are decreased in triploids (Benfey, 1999). Therefore, it is easy to distinguish between diploid and triploid fish by assessing the size and number of erythrocytes, which are reduced in triploidy in

proportion to the erythrocyte size (Benfey & Sutterlin 1984; Benfey 1999). In sweetfish, *Plecoglossus altivelis*, triploid specimens had larger erythrocytes and lower erythrocytes number than diploid specimens, and also showed higher hematological parameters (mean corpuscular volume and mean content of haemoglobin) and oxygen consumption were higher triploid than diploid (Aliah *et al*., 1991).

An important consequence of increased nuclear and/or cellular volume in triploid fish is the resulting decrease in the ratio of surface area to volume. This could affect processes limited by surface area, such as nutrient and metabolite exchange, passive and active ion exchange, and membrane binding of hormones and other messengers. Due to decreased cell number in the ration of surface too volume also applies to whole tissues and organs as well (Benfey, 1999). A second important consequence of increased nuclear and/or cellular volume is that, depending on the shape of the cell and its nucleus, the internal transport and diffusion distance may be increased. This could affect processes such as signal transduction from the cell surface to the nucleus, and resultant production and movement of RNA and protein within and outside of the nucleus and cell (Benfey, 1999). Some of these potential disadvantages of triploid cell may be offset by the energetic advanntages arising from reduced production and maintenace of cellular membranes and from the smaller relative surface area across which ionic and osmotic gradients must be maintained (Szarski, 1976; Benfey, 1999).

The Far Eastern catfish, *Silurus asotus* (Linnaeus) (order Siluriformes, family Siluridae), is distributed widely throughout the Northeast Asia and is an important species that is used as food in Korean freshwater aquaculture (Kim *et al.*, 2001b). However, there are two major limitations in culturing of this species. Firstly, there is a sex-related dimorphism in the growth rate, i.e. the females grow much faster than males (Kim *et al.*, 2001a). The sex-related size difference leads to difficultly in effective stock management and also frequently results in severe cannibalism in farms during the early stages of life. Secondly, the precocious maturation prior to the fish reaching marketable size necessitates an extended cultivation period beyond sexual maturity. Upon attaining sexual maturity, these fish begin to experience reduced growth and decreased feed efficiency (Choi *et al.*, 1992). Therefore, the induction of triploidy offers fast-growth and an added value due to the increased production of large-sized Far Eastern catfish.

Therefore, the purpose of the present study was to investigate the haematological characteristics in relation to the efficiency of metabolism-related growth and respiratory function in the transport of oxygen by erythrocytes to tissue in triploid and diploid Far Eastern catfish.

MATERIALS AND METHODS

I. Animals

Triploid induction of Far Eastern catfish, *Silurus asotus*, was carried out according to the method of Kim *et al.* (2001). Mature females were induced to spawn using a single intraperitoneal (IP) injection of 1,000 IU of human chorionic gonadotropin (Sigma, USA) per kg body weight (BW) of the catfish. Sperm were also obtained by scissoring the surgically removed testes of males that had been given an IP injection of hCG at 500 IU · kg/BW. Eggs were fertilized with sperm diluted in saline using the wet method. Five minutes after fertilization, they were rapidly rinsed to remove excess sperm and were immediately submitted to a cold-shock treatment (4℃) for 60 min to prevent the extrusion of the second polar body. Untreated fertilized eggs were used as diploid controls.

Diploid and triploid the Far Eastern catfish rearing was carried out as described Choi & Kim (1996). Diploid and triploid anmals were reared in 45 L tanks, under the same hydrological conditions. Water temperature was maintained at 24 ± 1.5℃ and the mean water oxygen concentration was kept close to saturation level (mean: 9.4 ± 0.3 mg/L). Animals were periodically sampled and their ploidy was determined by flow-cytometric assessment of the nuclear DNA content in erythrocytes or fin cells (Colombo *et al.*, 1995; Francescon *et al.*, 2004). Specimens were used at 100 days post-hatching, and had an

average body mass of 102.3 ± 9.71 g (length 12.7 ± 2.31 cm).

II. Erythrocyte and erythrocyte nuclear size

For determination of erythrocytes and their nuclear sizes, blood was collected from the caudal vein of 20 diploid and 20 triploid animals with 3 mL sterile syringes (23 G × 11/4 needle) and kept at 4℃ in polyethylene vials to which heparin (70 IU/mL blood) had been added. Erythrocytes and erythrocyte nuclei for ploidy were determined from dry blood smears. Air-dried blood smears were prepared from each fish using the conventional method. The smears were then fixed in methyl alcohol and stained with May-Grünwald-Giemsa.

For observation of live red blood cells, whole was diluted 1:10 with phosphate-buffered saline (PBS: 0.8% NaCl, 0.02% KCl, 0.02% KH_2PO_4, 0.115% Na_2HPO_4) and a drop of cell suspension was placed in the centre of a slide glass, which was then covered with a coverslip.

For scanning electron microscopy (SEM) analysis, 100 ㎕ of blood, diluted 1:100 with PBS was placed in the centre of a coverslip that had previously been coated with 50 ㎍/mL poly-L-lysine. Cells were left to adhere for 30 min and fixed with a solution of 1% glutaraldehyde and 1% sucrose in PBS; they were then dehydrated in ethanol, subjected to critical point and sputtered with gold.

Erythrocytes and their nuclear major and minor axes were

determined in both live and fixed cells using a light microscope (Carl, Zeiss, Germany) equipped with a Axioskop 4.1 image analysis system and SEM erythrocyte major and minor axes were deciphered from SEM images. One-hundred and twenty cells were measured for each specimen.

Erythrocyte and nuclear surface areas were calculated as $S = \pi \cdot a \cdot b/4$, where a and b are the major and the minor axis of the cell and of the nucleus, respectively. The cell and nuclear major and minor axis and surface for the cell and nucleus of specimens with each ploidy were compared.

III. Measurement of haematological indices

Ten diploid and ten triploid fish were separated by flow-cytometry, and haematological parameters were subsequently analyzed. Total red blood cell count (RBC), haematocrit (Ht), and haemoglobin (Hb) were determined by an auto-haematology analyzer (Sysmex XE-2100D, Sysmex Corporation, Japan).

From the previous parameters, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were obtained using the following formulas (Sezaki *et al.*, 1977).

Plasma glucose levels were analyzed using the by Chemistry System (Hitachi 7180, Hitachi, Japan).

IV. Oxygen consumption rate and respiratory frequency

The oxygen consumption rate was measured according to the method of Jo and Kim (1999). The respirometer chamber utilized a simple circulating system. The flow of water was circulated from the reservoir (170 L) to the head tank by a circulating pump, passed by a respirometer chamber, and then flowed back into the reservoir (Fig. 1). The head tank was equipped with a temperature controller and 10 μm and 3 μm cartridge filters equipped for the exclusion of particles before they were circulated from the reservoir to the head tank, and a flow-through UV lamp was utilized for the reduction of oxygen consumption by microbes. Water flowing from the respirometer chamber passed by an oxygen measurement chamber. During the period of experiment, the average water flow was 59.6 ± 0.5 L/h.

As described by Jo & Kim (1999), the respirometer chamber was comprised of an acrylic resin box with a thickness of 8 mm; the overall dimensions of the box were 10 cm (width) × 25 cm (length) × 10 cm (height). A rubber pad was used as a cover for the respirometer chamber to prevent the inflow of air; a hole was made in the cover and a small valve was attached for the removal of air in the respirometer chamber. Inflow water in the respirometer chamber was diffused through a 10 mm pipe, which was capped at the end, where a few holes were made. Water flowing from the

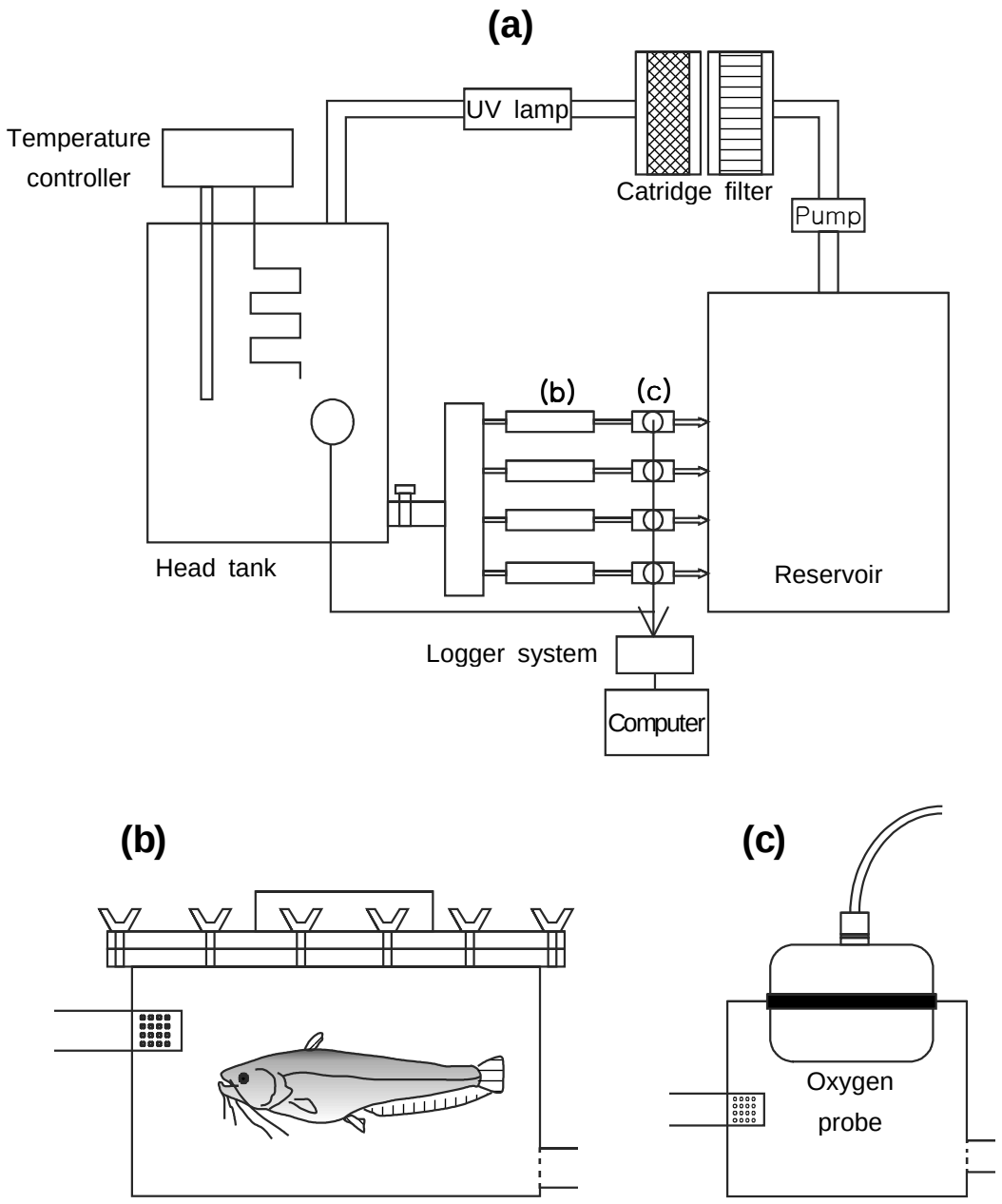

Fig. 1. Schematic diagrams of (a) the respirometer system, (b) the respirometer chamber and (c) the dissolved oxygen measurement chamber used in this study.

respirometer chamber was flowed into the dissolved oxygen measurement chamber, the dimensions of which were 10 cm (width) × 10 cm (length) × 6 cm (height). The respirometer chamber could use three chambers at once, and each chamber was connected to a dissolved oxygen measurement chamber.

Dissolved oxygen measurement chambers were equipped with an oxygen probe and air in this chamber was removed by same method used for the respirometer chamber. Dissolved oxygen was measured using an oxygen measurement electrode and a multi-data logger system (Oxyguard, Denmark). Inflow and outflow dissolved oxygen of the respirometer chamber was measured using by μLog VL 100 Software at five minute intervals over 24 hours at 20, 25, and 30 ℃. Measurements of oxygen and oxygen consumption rates at each temperature were saved by the multi-data logger, as described Jobling (1982).

Oxygen consumption rate (mg O_2/kg/h)=(C_i-C_o)×Q/W

C_i = Dissolved oxygen concentration of inflow, mg/L

C_o=Dissolved oxygen concentration of outflow, mg/L

Q = Inflow water volume, L/min

B = Weight of specimen, kg

Fish were starved for 1 day; over a 45 hour period, the respiratory frequency of observed gill cover movements was recorded for 1 minute every 5 hours.

V. Statistical analysis

The differences among groups were analyzed using Student's *t*-test of the SPSS statistics package (SPSS 9.0, SPSS Inc., USA).

RESULTS

I. Ploidy in the examined animals

All of the putative triploid Far Eastern catfish, *Silurus asotus*, were characterized by 1.5-fold increase (3.3 pg/cell) in the amount of nuclear DNA compared to the diploid (2.2 pg/cell) fish (data not shown), thus confirming their triploid status and the success of triploidization (Fig. 2).

II. Erythorcyte and erythrocyte nuclear size

Far Eastern catfish were elliptical with a central condensed nucleus (Fig. 3). Living cells were slightly smaller than air-dried cells and larger than those observed by SEM.

Light and SEM morphological analysis indicated that triploid red blood cells had significantly ($P<0.05$) larger major and minor axes and cell surface when compared with cells from diploid fish. The same was true for the major and minor axes and surfaces of nuclei of erythrocytes (Table 1).

III. Haematological indices

The haematological indices obtained with the auto-haematology analyzer (Sysmex XE-2100D) are presented in Table 2. The haematocrit

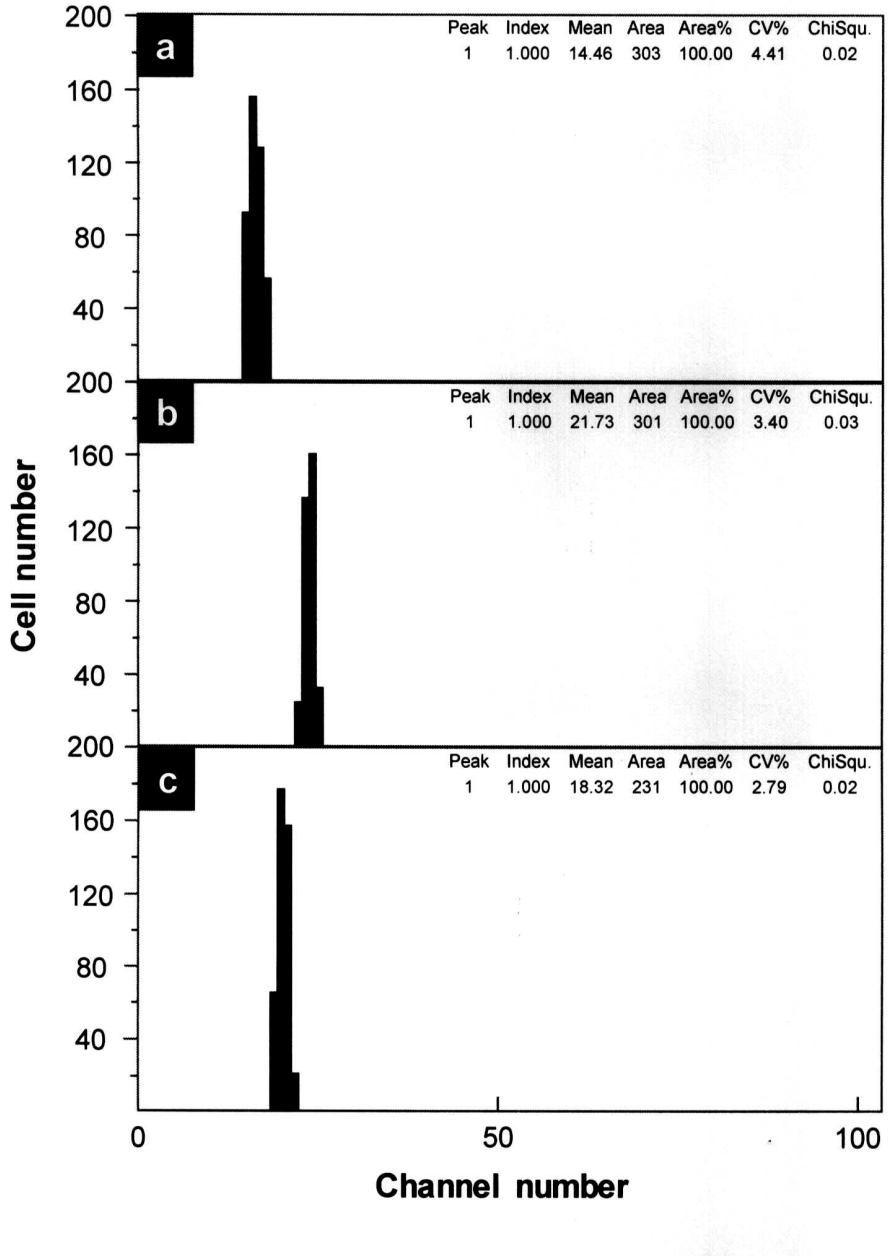

Fig 2. Representative histogram for flowcytometric analysis of diploid (a) and triploid (b) *Silurus asotus*. DNA content of Mud loach, *Misgurnus mizolepis* (c) red blood cells is also shown as internal control.

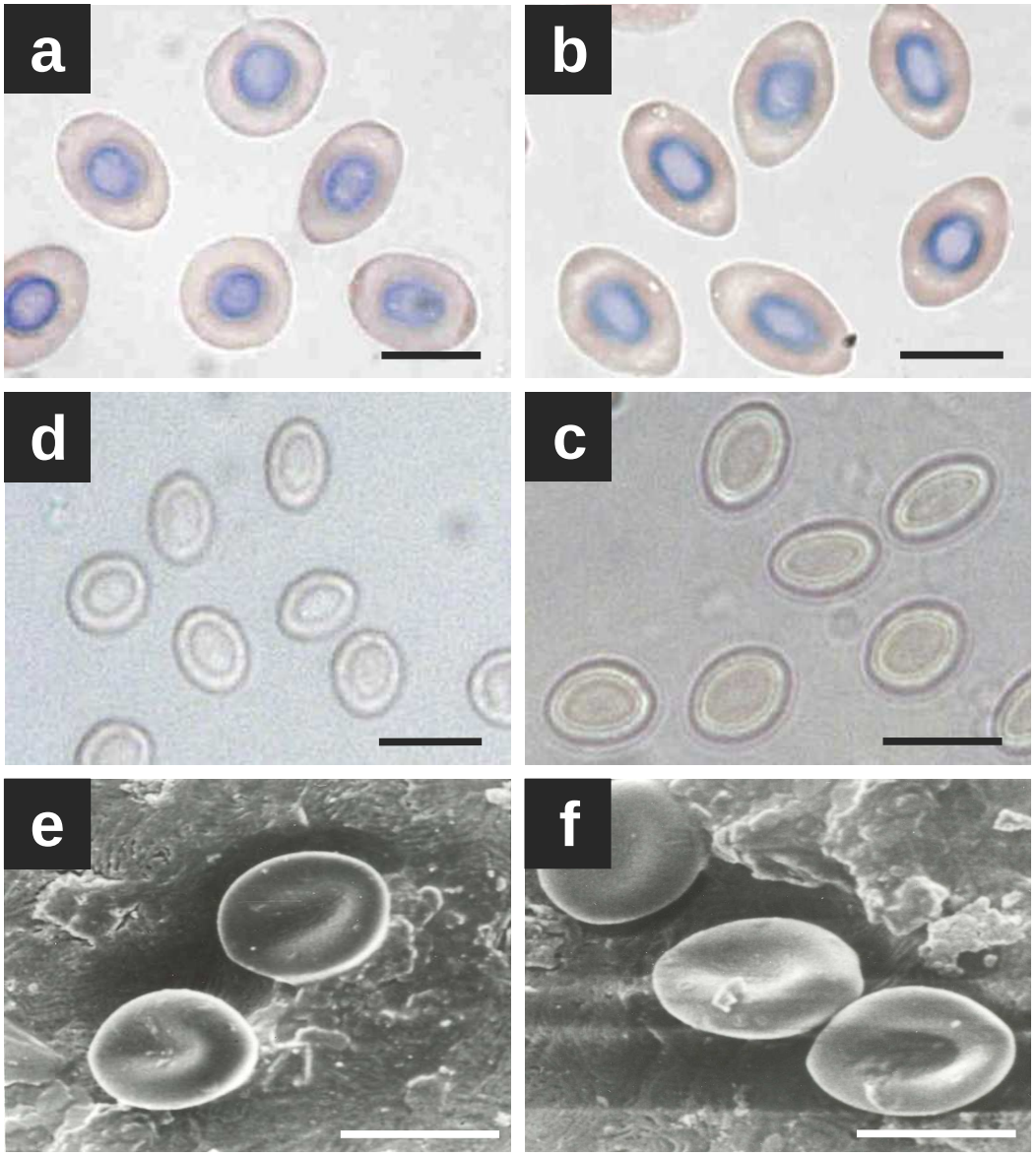

Fig 3. Diploid (a, c and e) and triploid (b, d and f) erythrocytes from far eastern catfish *Silurus asotus*. a, b : air-dried blood smears stanined May-Grünwald-Giemsa; c, d : living cells; e, f : SEM micrographs. Scale bars : 10 ㎛.

Table 1. Differences in erythrocyte size under different conditions in diploid and triploid far eastern catfish, *Silurus asotus*

Parameter	Diploids (2n)	Triploids (3n)	Significance*
Linear length (㎛)			
Major axis, living cells	12.10 ± 1.215	16.24 ± 1.053	$P < 0.001$
Major axis, blood smears	12.39 ± 0.741	16.40 ± 0.514	$P < 0.001$
Major axis, SEM	10.99 ± 0.890	14.52 ± 1.007	$P < 0.001$
Minor axis, living cells	8.13 ± 1.097	10.26 ± 1.052	$P < 0.001$
Minor axis, blood cells	8.71 ± 0.271	10.52 ± 1.502	$P < 0.001$
Minor axis, SEM	6.68 ± 0.520	7.96 ± 0.528	$P < 0.001$
Major nuclear axis, living cells	5.35 ± 1.301	6.45 ± 1.319	$P < 0.05$
Major nuclear axis, blood smears	4.48 ± 0.633	5.52 ± 0.552	$P < 0.05$
Minor nuclear axis, living cells	3.39 ± 0.760	4.09 ± 0.970	$P < 0.05$
Minor nuclear axis, blood smears	3.01 ± 0.158	3.79 ± 0.247	$P < 0.05$
Area (μm^2)			
Cells area, living cells	82.57 ± 5.342	112.18 ± 9.550	$P < 0.001$
Cells area, blood smears	89.64 ± 3.427	136.56 ± 6.553	$P < 0.001$
Cells area, SEM	77.36 ± 6.113	108.21 ± 6.324	$P < 0.001$
Nucleus area, living cells	9.27 ± 1.125	12.21 ± 0.892	$P < 0.05$
Nucleus area, blood smears	8.58 ± 0.603	10.93 ± 3.096	$P < 0.05$
Volume (μm^3)			
Cell volume, living cells	391.22 ± 22.195	552.70 ± 40.181	$P < 0.05$
Cell volume, blood smears	377.38 ± 30.028	518.89 ± 31.347	$P < 0.05$
Cell volume, SEM	367.56 ± 31.412	511.01 ± 39.743	$P < 0.05$
Nucleus volume, living cells	13.66 ± 0.970	19.83 ± 1.774	$P < 0.05$
Nucleus volume, blood smears	13.27 ± 1.054	18.24 ± 0.726	$P < 0.05$

*Difference between diploid and triploid is significant at this level.

Twenty individuals for each ploidy were used, mean ± S.D.

Table 2. Comparison of haematological parameters between diploid and triploid far estern catfish, *Silurus asotus*

Parameter*	Diploids (2n)	Triploids (3n)	3N/2N	Significance**
Erythrocyte count (RBC count, 10^6 cells/mm^3)	2.56 ± 0.247	1.16 ± 0.057	0.45	$P < 0.001$
Haematocrit value (Ht, %)	35.73 ± 3.176	33.65 ± 2.636	0.95	NS
Mean corpuscular volume (MCV, μm^3)	139.25 ± 5.419	203.95 ± 4.455	1.46	$P < 0.001$
Total haemoglobin content (Hb, g/100 mL)	9.33 ± 0.826	9.35 ± 0.354	1.00	NS
Mean corpuscular haemoglobin (MCH, pg)	36.50 ± 2.977	54.75 ± 0.354	1.50	$P < 0.001$
Mean corpuscular haemoglobin concentration (MCHC, %)	26.25 ± 1.930	26.85 ± 2.778	1.02	NS
Plasma glucose concentration (mg/L)	28.51 ± 1.707	44.50 ± 3.109	1.56	$P < 0.001$

*Abbreviations : RBC, red bood cell; Hb, haemoglobin concentration; Ht, haematocrit value; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentraton.

*Difference between diploid and triploid is significant at this level.

Difference between diploid and triploid is not significant ($P > 0.05$).

value (Ht), total haemoglobin content (Hb) and mean corpuscular haemoglobin concentration (MCHC) did not differ when triploid and diploid Far Eastern catfish were compared. However, the mean corpuscular volume (MCV) was 46 % higher and the mean corpuscular haemoglobin (MCH) was 50 % higher, and the plasma glucose concentration was 56 % higher ($P<0.05$) in triploid specimens, whereas the total red blood cell count was increased 2.2-fold in diploid specimens. The total surface of erythrocytes per blood volume (surface of erythrocyte × erythrocyte counter) was 229.5 × 10^4 μm/μL in diploids and 158.4 × 10^4 μm/μL in triploids, with diploid specimens being 45% larger than triploid specimens.

IV. Oxygen consumption rate and respiratory frequency

Table 3 shows the comparison of oxygen consumption and respiratory frequency (gill cover movement) between diploids and triploids at water temperatures of 20, 25 and 30 ℃. The routine metabolism, as indicated by oxygen consumption exhibited a large difference among individuals, resulting in no significant difference between diploid and triploid specimens. The respiratory frequencies of triploids at 20, 25, and 30 ℃ were significantly higher than those of diploids. Triploids showed a respiratory frequency 1.81 times higher at 20 ℃ and 1.74 times higher at 25 ℃ and 1.5 times at 30 ℃ compared to diploids. This was more clearly demonstrated by plotting both values for individual specimens (Fig. 4). Therefore, it was concluded that oxygen uptake per unit of respiratory movement was lower for triploids than for diploids.

Table 3. Oxygen consumption rate and respiratory (gill cover movement) in diploid and triploid Far Eastern catfish, *Silurus asotus*

	20 ℃		25 ℃		30 ℃	
Ploidy	Oxygen consumption (O_2/mg/kg/hr)	Respiratory frequency (/min)	Oxygen consumption (O_2/mg/kg/hr)	Respiratory frequency (/min)	Oxygen consumption (O_2/mg/kg/hr)	Respiratory frequency (/min)
Diploid	129.3 ± 12.03	43.5 ± 11.75	242.9 ± 21.77	52.5 ± 12.04	347.4 ± 36.87	65.0 ± 13.38
Triploid	121.6 ± 7.87	78.7 ± 15.28	233.9 ± 23.01	91.2 ± 14.58	345.3 ± 34.63	97.4 ± 9.43
Significance	NS[1]	$P < 0.05$[2]	NS	$P < 0.05$	NS	$P < 0.05$

[1]Difference between diploidy and triploidy is not significant ($P>0.05$).

[2]Difference between diploidy and triploidy is significant at this level.

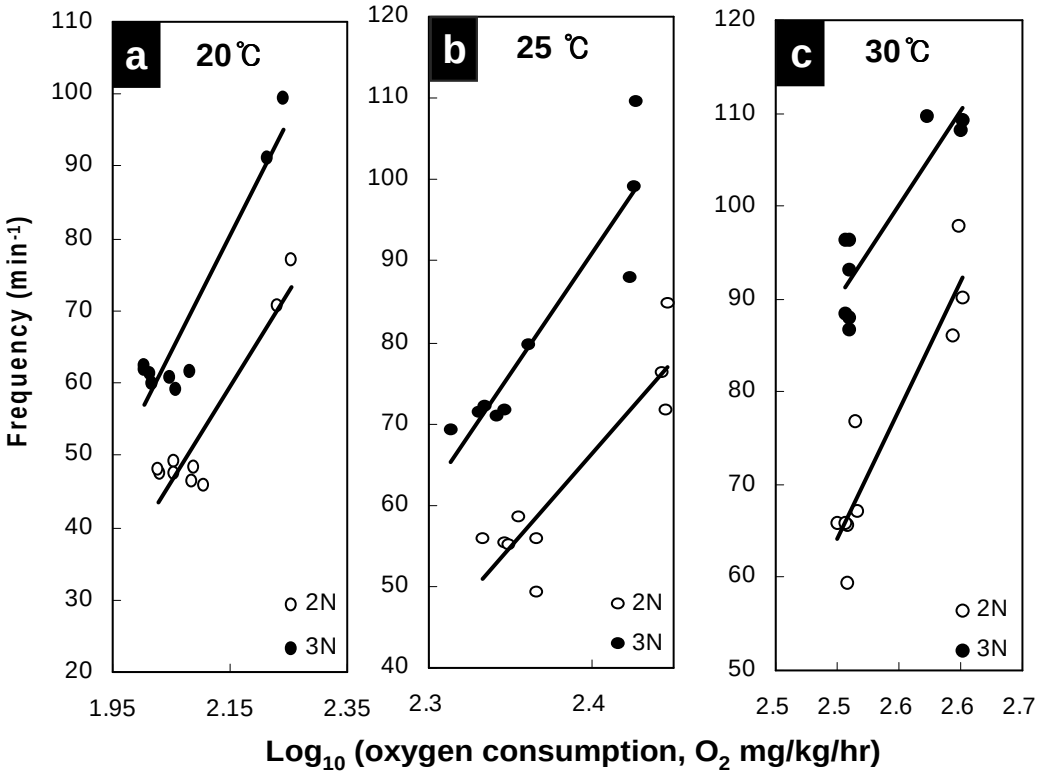

Fig. 4. Relationship between oxygen consumption and respiratory frequency (gill cover movement) for diplod (○) and triploid (●) far eastern catfish, *Silurus asotus*. a : 20 ℃, diploid: y = 131.08x - 222.27 (r^2 = 0.863) triploid : y = 159.8x - 263.03 (r^2 = 0.895); b : 25 ℃, diploid : y = 229.56x - 484.6 (r^2 = 0.800), triploid : y = 296.58x - 620.72 (r^2 = 0.856); c: 30 ℃, diploid : y = 278.1x - 631.06 (r^2 = 0.864), triploid : y = 202.95x - 417.37 (r^2 = 0.821).

DISCUSSION

The induction of a triploid condition in fish has usually been reported to be accompanied by modification in physiology that may reduce the resistance to acute stress (Ojolick *et al.*, 1995a; Cotter *et al.*, 2002; Ballarin *et al.*, 2004). This lower resistance can influence the productivity of triploids during artificial rearing, which is characterized by crowding and/or occasional reductions in oxygen concentration.

In this study, some haematological parameters were compared in diploid and triploid specimens of the Far Eastern catfish, *S.asotus*. The results showed an increase in erythrocyte size in triploids, in agreement with the previously reported increase in the cell volumes of polyploidy animals (Purdom, 1993; Gue *et al.*, 1996; Benfey, 1999). In teleost fish, the increase in erythrocyte size associated with triploidy has already been reported and the measurement of red blood cell dimensions was proposed as a rapid and in expensive assay for triploidy (Krasznai *et al.*, 1984; Sezaki *et al.*, 1988; Sezaki *et al.*, 1991; Yamamoto & Iida 1994; Libertini *et al.*, 1996; Benfey, 1999). Data have usually been obtained from blood cells subjected to air-drying, but this method may lead to alteration in cell morphology. In order to prove that the observed differences were not a consequences of different responses of diploid and triploid cells to air-drying, erythrocyte dimensions were compared under different

conditions (i.e. air-dried cells, fixed and dehydrated cells for SEM observations, and living cells). In all cases, the differences were confirmed. Air-dried cells were somewhat bigger than living cells, likely due to their flattening on the slide surface. The opposite situation was shown by cells treated for SEM, probably due to shrinkage associated with dehydration. The observed increase in erythrocyte nuclear size in triploids is a consequence of their higher DNA content, is in agreement with data reported in (Sezaki *et al.*, 1988; Libertini *e t al.*. 1996; Benfey, 1999). Shrinkage of nuclei was also observed after air-drying.

In this study, the haematocrit value (Ht), total haemoglobin content (Hb) and mean corpuscular haemoglobin concentration (MCHC) were not significantly different between diploid and triploid catfish, but the erythrocyte size, erythrocyte count (RBC count), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) were increased in triploid catfish. This increase in cellular size was offset by a decrease in cell number, which explains the lack of a difference in haematocrit observed between diploid and triploid catfish, as reported in other fish species (Benfey 1999).

Among the haematological parameters, the haemoglobin concentration and haematocrit value are regarded as being directly related to the respiratory function and activity of fish, while the number of and size of erythrocytes are not (Ikeda *et al.*, 1986). Therefore, if the haemoglobin concentration and haematocrit value are

constant, a smaller size with an increased number of erythrocytes of smaller size in a unit blood volume is advantageous in terms of both respiratory function and activity since these erythrocyte conditions lead to an increase in the surface area of erythrocytes for the exchange of oxygen. It is well- known that polyploidization of fish results in an increase of erythrocyte size (Sezaki et al., 1983; Sezaki *et al.*, 1988). In this study, the decrease of the erythrocyte surface in triploid catfish was 45% and the increase of mean corpuscular volume was 46% compared to that of diploid catfish. Therefore, triploid catfish had a lower capacity of oxygen exchange than diploid catfish, due to a decrease of the erythrocyte surface by polyploidization.

Total blood haemoglobin was not siginificantly different in fish of different ploidy, although the mean corpuscular haemoglobin was higher in triploid erythrocytes. These findings are in agreement with data on freshwater (Baker *et al.*, 1983; Sezaki *et al.*, 1991; Parsons, 1993; Benfey & Biron, 2000) and marine teleosts (Felip *et al.*, 2001; Ballarin *et al.*, 2004). A similar total haemoglobin content implies a similar capability of oxygen transport. This hypothesis is supported by the observation that oxygen consumption is similar in diploid and triploid fish under various experimental conditions (Sezaki *et al.*, 1991; Benfey, 1999). In this study, comparison of the total haemoglobin content and oxygen consumption rate between diploidy and triploidy indicated a lower oxygen capability of triploid erythrocytes along with a higher mean corpuscular haemoglobin level in triploid catfish.

A lower aerobic metabolism capacity, consequent to a rapid rise of lactate and an earlier switch to anaerobic metabolism, was reported in triploid rainbow trout, *Oncorhynchusmykiss* (Virtanen *et al.*, 1990). This can explain the observed higher plasma glucose concentration in triploid catfish, which is probably related to an increased depletion of liver glycogen which, according to Ojolick *et al.* (1995b), confers triploid fish a sustained ability to with stand anaerobic metabolism. In addition, a lower capability of triploid cells to internalize glucose, related to a lower density of either glucose carriage or insulin receptors per unit of volume, can also contribute to the increase in plasma glycaemia observed in triploid fish (Ballarin *et al.*, 2004).

The relationship between oxygen consumption and respiratory frequency at 20, 25 and 30 ℃ was higher in triploids than in diploids, although diploid and triploid catfish showed similar oxygen consumption. Therefore, the lower oxygen capability of triploidy than diploidy is in agreement with the haematological characteristics of triploidy. Davison (1959) claimed that an increase in erythrocyte size leads to unpleasant conditions such as the accelerated heart pulsation and decreased transformation capacity of erythrocytes passing through peripheral blood vessels. Thus, it is suggested that triploid catfish compensate for such disadvantages concerning of oxygen transport by increased respiratory movement.

Haematological studies have been performed in many fish species but few studies have been conducted in catfishes. The results

of this study add haematological information for a new species of catfish, which contributes to the alleviation of this situation. Our results show triploidy-associated changes in haematological parameters that might affect Far Eastern catfish physiology, specifically the capacity of this fish to use oxygen in low concentration conditions and thus to react to acute hypoxia. Current farming practices such as in-farm continuous monitoring of water quality and rearing tanks certainly would minimize the impact of triploidy-induced changes in oxygen-use capabilities. Nevertheless, these changes, as they have been determined in the present study, should be taken into account when assessing the feasibility of triploid Far Eastern catfish for intensive aquaculture systems where unfavorable rearing situations may occur.

KOREAN ABSTRACT (국문요약)

이학석사 학위논문

메기, *Silurus asotus* 2배체와 3배체의 혈액성상과 호흡능력 비교

설 동 원

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(지도교수: 수산학박사 박 인 석)

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메기, *Silurus asotus*의 2배체와 유도 3배체의 혈액학적 특징에 기인된 호흡 능력에 관한 비교를 위하여 적혈구와 적혈구 핵의 크기, 표면적 및 부피를 살아있는 세포, Giemsa 염색 및 전자현미경(SEM)을 통하여 측정하였으며, 단위 부피당 적혈구 수, haematocrit 치, 평균 적혈구 용적, 총 haemoglobin 함량, 평균 적혈구 haemoglobin 량, 평균 적혈구내 haemoglobin 농도 및 혈장 glucose 농도를 측정 하였으며, 산소 소비율 및 호흡수를 측정하였다.

3배체의 적혈구 세포 및 핵의 크기, 표면적 및 부피는 2배체 보다 크게 나타났으며 또한, 살아있는 세포, Giemsa 염색 및 전자현미경을 통

한 측정치 모두 2배체 보다 3배체가 크게 나타났다($P < 0.05$).

2배체와 3배체의 혈액성상은 haematocrit 치, 총 haemoglobin 함량 및 평균 적혈구 혈색소내 농도는 서로 유의적인 차이를 보이지 않았으나 ($P > 0.05$), 평균 적혈구 용적, 평균 적혈구 haemoglobin 량 및 혈장 glucose 농도는 3배체가 높게 나타났다. 반면, 단위 부피당 적혈구 수는 2배체가 3배체 보다 많은 수를 보였다($P< 0.001$).

3배체와 2배체의 산소 소비율은 서로 유사하게 나타났으나, 호흡수에 있어서 3배체가 2배체보다 많은 호흡수를 보였다. 이러한 결과는 3배체의 혈액학적 특징과 일치하는 낮은 산소 이용율을 반영하고 있다.

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