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Characteristics of induced triploid Far Eastern catfish, *Silurus asotus*

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Korea Maritime and Ocean University

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by

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ABSTRACT

Characteristics of induced triploid Far Eastern catfish, *Silurus asotus*

by

Hyun Woo GIL

Submitted to
The Department of Marine Bioscience and Environment
Graduate School of Korea Maritime and Ocean University
Korea
(Supervised by In-Seok PARK, Ph. D.)

The objective of this study is to determine characteristics of induced triploid Far Eastern catfish, *Silurus asotus*: comparative analysis of cell cycle, expression of cell cycle protein, histological characteristics, morphometric characteristics, physiological response, body composition, hematological characteristics, concentrations of sex hormone and growth hormone, occurrence of amitosis were analyzed in diploid and induced triploid Far Eastern catfish.

Cell cycle of gill tissue were significantly different in the percentages of each cell cycle fraction between diploid and induced triploid Far Eastern catfish ($P<0.05$). Cyclin D1 and cyclin E expressions after organized damage were lower than those before organized damage in both tissues of diploid and induced triploid Far eastern catfish, respectively. In all experimental groups, protein expressions of induced triploid were higher than those of diploid. Significant difference of cyclin D1 and cyclin E expressions were not determined between gill tissue and tail fin tissue.

Cell and nuclear size of induced triploid Far Eastern catfish were higher than those of diploid Far Eastern catfish, however cell number were lower than diploid.

Significant variables of morphometric characteristic between diploid and induced triploid Far Eastern catfish were the direct distance between the anterior edge of the lower lip and the anterior insertion of the dorsal fin (DALAD), the horizontal distance between the anterior edge of the lower lip and the anterior insertion of the ventral fin (HALAV), the direct distance between the anterior edge of the upper lip and the first nostril (DAUF), the direct distance between the anterior edge of the upper lip and the second nostril (DAUS), the interorbital width (IW), and the mandible barbel length (ManBL). Therefore, induced triploid had smaller heads and shorter mandible barbels than diploid.

Stress hormone concentrations and reaction velocity of diploid Far Eastern catfish were generally higher and slower than those of induced triploid Far Eastern catfish. In addition, stress hormone concentrations of low water temperature stress was higher than those of high water temperature stress.

The differences body compositions and hormonal parameters between the diploid and induced triploid Far Eastern catfish were investigated in the spawning and non-spawning season. Spawning season in May and non-spawning season in January were determined at 2014, respectively. Estradiol and testosterone of diploid were higher than those of induced triploid in spawning season (P<0.05). Thyroid stimulating hormone and thyroxine of induced triploid were higher than those of diploid in spawning season (P<0.05). However, estradiol, testosterone, thyroid stimulating hormone and thyroxine were not different significantly between diploid and induced triploid in non-spawning season.

Erythrocyte count of diploid Far Eastern catfish was higher than that of induced triploid Far Eastern catfish in spawning season and non-spawning seasons. Mean corpuscular volume and mean corpuscular hemoglobin of induced triploid were significantly higher than those of diploid in both seasons (P<0.05).

Crude fat of induced triploid Far Eastern catfish was higher than diploid Far Eastern catfish in spawning season, but there was no significant difference of crude fat between diploid and induced triploid in non-spawning season.

The types of atypical cell were determined three types in Far Eastern catfish; asymmetric division, irregular-shaped and absence of nucleus. Occurrence frequency of amitosis-like nuclear division in induced triploid was higher than that in diploid.

This study was determined sterility and other characteristics of induced triploid by histology, morphology and physiology. The reason why this condition doesn't cause giantism is proved by histological characteristics. In addition, comparative analysis of sex hormone and growth hormone between diploid and induced triploid were proved to convert the energy used in the reproductive growth of the body. Therefore, the result of this study could use evidence of previous studies about induced triploid for improvement of productivity in a fish aquaculture industry.

Key words: Far Eastern catfish, histological characteristics, induced triploid, morphometric characteristics, physiological response, sterility

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Approved as qualified thesis of **Hyun Woo Gil** for the degree of **Doctor of Philosophy** by the Evaluation Committee of the Graduate School, Korea Maritime and Ocean University, Korea in May 2016.

KOREAN ABSTRACT
(국문요약)

유도 3배체 메기, *Silurus asotus*의 특성

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메기, *Silurus asotus* 유도 3배체의 특징을 구명하기 위해서 본 연구는 메기 3배체를 유도하여 이들의 세포주기, 조직학적 특징, 계측형질, 생리학적 특징, 체조성, 혈액학적 특징, 성호르몬 및 성장호르몬의 농도, 그리고 비정형 적혈구의 출현 여부를 2배체 메기와 비교·조사하였다.

메기 2배체와 유도 3배체를 대상으로 아가미조직의 세포주기 및 단백질 발현을 조사한 결과, cell cycle fraction간 비율에서 2배체와 유도 3배체 간의 유의한 차이가 나타났으며($P<0.05$), 유도 3배체의 세포주기 단백질, cyclin D1과 cyclin E의 발현량이 2배체 보다 높게 나타났다. 유도 3배체의 세포와 핵의 크기가 2배체 보다 크게 나타났으나 반면, 세포수는 2배체 보다 낮게 나타났다($P<0.05$).

계측형질 중 유의한 차이를 보인 항목은 direct distance between the anterior edge of the lower lip and the anterior insertion of the dorsal fin (DALAD), the horizontal distance between the anterior edge of the lower lip and the anterior insertion of the ventral fin (HALAV), the direct distance between the anterior edge of the upper lip and the first nostril (DAUF), the direct distance between the anterior edge of the upper lip and the second

nostril (DAUS), the interorbital width (IW) 및 the mandible barbel length (ManBL)로 나타났다(P<0.05). 즉, 유도 3배체 메기는 2배체에 비해 비교적 작은 머리 및 짧은 하악수염을 가진 것으로 나타났다.

스트레스 반응의 경우, 2배체 메기의 스트레스 호르몬의 농도 및 반응 속도가 전반적으로 유도 3배체 메기에 비해 높고 천천히 증가하였으며, 저온 스트레스가 고온 스트레스에 비해 더 높게 나타났다(P<0.05).

2014년에 산란기(5월)와 비산란 시기(1월)의 2배체 메기와 유도 3배체 메기를 대상으로 체조성과 호르몬 변화를 측정한 결과, 산란기의 estradiol과 testosterone 함량은 2배체가 유도 3배체 보다 높게 나타났으나, thyroid stimulating 호르몬과 thyroxine 함량은 유도 3배체가 2배체에 비해 더 높게 나타났다(P<0.05). 그러나 비산란시기의 estradiol, testosterone, thyroid stimulating 호르몬과 thyroxine 함량은 2배체와 3배체간에서 유의한 차이가 나타나지 않았다(P>0.05).

2배체 메기의 적혈구 수는 측정 시기와 관계없이 유도 3배체 메기에 비해 높게 나타났고, 평균 혈구용적과 평균 혈구 혈색소 함량은 유도 3배체가 2배체에 비해 높게 나타났다(P<0.05).

산란시기에 유도 3배체 메기의 조지질 함량이 2배체 메기에 비해 월등히 높게 나타났으나(P<0.05), 비산란시기가 되면서 2배체간에서 유의한 차이가 나타나지 않았다(P>0.05).

비균형 분열, 불규칙적인 핵의 모양 및 핵의 부재와 같은 비정형 적혈구 유형이 2배체 메기와 유도 3배체 메기에서 관찰되었으며, 유도 3배체의 비정형 적혈구 출현 빈도가 2배체에 비해 높게 나타났다(P<0.05).

본 연구는 유도 3배체의 불임 현상을 조직학, 형태학 및 생리학적 특징 측면에서 구명하였다. 본 연구의 조직학적 특징을 통해 유도 3배체 메기의 거대화(giantism)가 나타나지 않는 이유를 파악하였으며 아울러, 호르몬 변화 측정을 통해 유도 3배체 메기의 불임으로 인한 성숙에너지의 전환을 설명하였다. 본 연구의 결과들은 양식산업에서 생산성 향상을 위한 어류 유도 3배체에 관한 정보제공과 아울러 기존 관련 연구들을 보완할 것으로 사료된다.

주제어: 메기, 불임, 생리학적 특징, 유도 3배체, 외부 계측형질, 조직학적 특징

Characteristics of induced triploid Far Eastern catfish, *Silurus asotus*

1. Introduction

Far Eastern catfish, *Silurus astous*, is a member of the typical freshwater Siluridae, and an important commercial catfish in Korea (Kim *et al.*, 1988). The aquaculture production of Far Eastern catfish in Korea was 4,194 tons at 2010, and increasing gradully until now (Lim *et al.*, 2012). The fish formerly inhabited rivers in Manchuria, Japan, Taiwan, China and Northeast Asia, and elsewhere; and it is widely distributed throughout freshwater systems (Park and Im, 2001). This species is an example of tasty and nutritious food, and has been used as such; it is also widely used in private treatment (Park and Im, 2001). In addition, Far eastern catfish has good taste than the channel catfish, *Ictalurus punctatus*, is that increasingly popluar in the spotlight to consumers (Lim *et al.*, 2012)

In particular, the induction of induced induced triploid, sterile catfish by a chromosome-engineering technique is drawing attention as a way to enhance the productivity of fish farming per unit effort in the short term (Thorgaard, 1986; Cassani *et al.*, 1990). Production of infertile fish, either through direct triploidization induction or by breeding induced tetraploid females with diploid males, is widely practiced in aquaculture and has been

shown to produce fish which exhibit improved survival and extended growth (Hulata, 2001).

Various basic researches of Far Eastern catfish are still required for stably production of induced triploid. First, anesthesia of induced triploid is necessary for surgical incision, handling and sedation of Far Eastern catfish (Appendix 1). Experimental samples were died by removal of gonads (Appendix 2). In addition, suitability of passive integrated transponder (PIT) tag method is required for breeding system of diploid and induced triploid Far Eastern catfish (Appendix 3). Surgical incision of Far Eastern catfish is necessary for prevent mortality of samples.

In the Far Eastern catfish farming industry, chromosome-engineering techniques have been applied using genetic and breeding methods to improve productivity. Preliminary studies on this species have addressed the temperature-dependent somatic cell division cycle (τ_0), nuclear division of the egg, gonadogenesis, and the cytogenetic production of gynogenetically diploid, all-female diploid, and induced triploid strains (Kim *et al.*, 2001; Park *et al.*, 2004). However, comparative analyses of various characteristics between diploid and induced triploid were not proceeded until recently. Comparative analysis of cell cycle, morphometric trait, histological characteristic, stress response, hematological characteristic, endocrine hormone, body composition between diploid and induced triploid Far

Eastern catfish were necessary for breeding system of induced triploid Far Eastern catfish.

Triploidization is a technique used to generate sterile aquatic animals by taking advantage of the incompatibility in pairing the three homologous chromosomes during meiosis I (Don and Avtalion, 1986). This technique has also been used to enhance the productivity of several fish species because of its assumed ability to increase yield by channeling the energy required from gonadal development to somatic growth (Tave, 1993). More importantly, it generates fish that are unable to breed and contribute to the local gene pool if they were to accidentally escape from confinement. By conferring sterility of exotic fish for a limited purpose, induced triploidy can serve as an effective method for reducing or eliminating the environmental risks of genetically modified organisms (Kim *et al.*, 1994; Murray *et al.*, 1999). Induced triploidy was confirmed by the 1.5-fold increase in nuclear volume, cellular DNA content and chromosome number as estimated by erythrocyte counting, respectively (Seol *et al.*, 2008). Fraction of nuclear organizer regions (NORs) of different ploidy levels of Far Eastern catfish, *Silurus asotus*, was also well coincided with the previous study on induced triploid salmonid (Phillips *et al.*, 1986).

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). Induced triploid was not normal gonad development in spawning season (Lincoln and Scott, 1984). Gonad index of induced triploid was also very low, and induced triploid was produced a heteroploid gamete. Therefore induced triploid became functionally sterile (Thorgaard, 1983). Because induced triploid sterile, to convert the energy used in the reproductive growth of the body. Growth is continued as compared to diploid in the spawning season (Ihssen *et al.*, 1990; Kim *et al.*, 1990). Sterility allows organism to avoid the metabolic costs of sexual maturation, result in continued somatic growth in induced triploid fish, with maintenance of flesh quality during the period when diploids sexually mature (Seol *et al.*, 2008). Induced triploid grew significantly faster than diploid from the the same spawns, reared under similar culture conditions (Qin *et al.*, 1998). Induced triploid the cell size is increased, but decreased the number of cell in body. So induced triploid doesn't cause giantism (Beatty and Fischberg, 1951).

Atypical or divided erythrocyte nuclei are commonly distinguished in induced triploid fish such as induced triploid rainbow trout, *Oncorhynchus mykiss* (Han *et al.*, 2007). However, Dorafshan *et al.* (2008) suggested that atypical erythrocytes in induced triploid Caspian salmon, *Salmo trutta*, were behind the mechanism to cope with stress in fish, and induced triploids might be less tolerant to stress than diploids; such a nuclear pattern in induced triploid brook trout, *Salvelinus fontinalis*, reflected some kind of

functional depression or mobilization of granulocytes (Wlasow *et al.*, 2004). The atypical cells are regarded as a cytological marker for induced triploidy (Liu *et al.*, 2003). So far, studies of amitotic erythrocytes are respiratory problem and erythrocyte activity (Ueno, 1984).

There are numerous studies in the literature which have investigated various aspects of induced triploid fish identification methodology including analysis of chromosome sets (Thorgaard, 1986), the microfluorimetry of nuclear DNA content (Komaru *et al.*, 1988), the nuclear DNA content by flowcytometry (Allen and Stanley, 1978), the measurement of erythrocyte and nuclear size (Thorgaard, 1986; Kim *et al.*, 1990; Park and Kim, 1994; Park *et al.*, 1994), the distinction of nucleolar number (Philips *et al.*, 1986), the measurement of cell number (Ueno, 1984; Park and Park, 1994), and the measurement of cell and nuclear size in different tissues (Swarup, 1959; Aliah *et al.*, 1990). Park and Kim (2000) reported that characteristics of the some tissues of retina, optic tectum and trunk kidney in induced triploid and diploid hybrid between female mud loach, *Misgurnus mizolepis*, and male cyprinid loach, *M. andguillicaudatus*.

Flowcytometry has a wide variety of clinical applications in oncology for understanding surface expression, intracellular signaling, cell cycle content analysis, and a number of other interesting parameters (Vanparys *et al.*, 2006). Recent advances in instrument platforms, calibration methods,

and reagent quality have now made flowcytometry a promising tool for DNA content analysis (Estevam *et al.*, 2011). These calibration packages can detect if the parameters are within acceptable ranges and thus allow for consistent sample acquisition over time. One of the advantages of flowcytometry is the rapidity of the measurement, making it possible to measure thousands of cells over a short period of time, and the ability for multi-color immunophenotyping (Estevam *et al.*, 2011).

However, for cell cycle analysis by flowcytometry, care should be taken to collect cells at a proper rate. In order to yield a good signal in gap2 (G_2)/mitosis (M) and to discriminate between singlets and doublets, samples should be analyzed at rates below 1000 cells per second (Nunez, 2001). Samples processed through the cell cycle assay described were analyzed below this cellular threshold rate. Since the data obtained is not a direct measure of the cellular DNA content, reference cells, such as human leukocytes or red blood cells from chicken or trout should be used (Nunez, 2001). Incorporation of these reference standards can be used to determine the position of cells with a normal diploid amount of DNA and thus allows for a more consistent interpretation of the data (Estevam *et al.*, 2011).

Induced triploids generally have similar, if not identical, morphological and meristic characteristics to diploids (e.g., Bonar *et al.*, 1988). However, several morphological differences and abnormalities have been associated

with induced triploidy in fish. A variety of deformities were reported in the induced triploid pejerrey, *Odontesthes bonariensis* (Strüssmann and Takashima, 1993), but it is not clear whether these fish were in fact induced triploid or aneuploid (i.e., having a chromosome number other than a complete multiple of haploid). Changes in the scale pattern and the degree of reduction in the scale cover were observed in the induced triploid common carp, *Cyprinus carpio*, and were attributed to differences in allelic ratios for genes controlling these traits (Gomelsky *et al.*, 1992). Flajshans *et al.* (1993) described differences in pelvic fin shapes and lengths between the induced triploid and diploid tench, *Tinca tinca*, and Tave (1993) observed facial deformities in the induced triploid bighead carp, *Hypophthalmichthys nobilis*, and grass carp, *Ctenopharyngodon idella*. Probably the best and most frequently described gross morphological difference in induced triploid fish is the development of lower jaw deformities in the induced triploid Atlantic salmon, *S. salar* (Lee and King, 1994). Although conclusive data are lacking, this deformity may be linked to rapid growth rates in seawater (Lee and King, 1994).

Morphologic differences between species or populations are understood and compared by general figures or specific anatomical shapes (Strauss and Bond, 1990). Morphometric characteristics of fish, unlike meristic, countable characteristics, are measured characteristics; they can be measured in

millimeters. Although understanding of the morphometric characteristics of fish is limited because they can be modified by the environment, the general figure of fish is mainly determined by genetic factors (Currens *et al.*, 1989; Park *et al.*, 2004). Morphometric characteristics of aquatic animal are used in three major ways: to make distinctions between sex and species and to identify confusing species such as crossbreed hybrids; to study figure modifications in groups and species; and to identify and classify biotypic linkages (Park *et al.*, 2004; Park *et al.*, 2008b).

In intensive culture systems fish are continuously exposed to stress (Gamperl *et al.*, 1994), including increased density, inadequate nutrition, poor sanitation, injury during handling, high water temperature or low water temperature. Stress responses can include physiological changes such as oxygen uptake and transfer, metabolic and hematological changes, mobilization of energy substrates, reallocation of energy away from growth and reproduction, and suppressive effects on immune functions (Pickering and Pottinger, 1989; Barton and Iwama, 1991). These changes can increase disease susceptibility leading toincreased mortality and subsequent economic losses.

The physiological response of fish under stress can be sorted by first, second, and third responses (Barton and Iwama, 1991). The first response is to increase internal secretion activities by promoting the secretion of

catecholamine and glucocorticoid, thus inducing the second response where the fish then undergoes metabolic and hematological changes which subsequently induce the final and third response by which time the fish starts to exhibit obvious signs of stress and discomfort (Thompson *et al.*, 1993).

2. Materials and Methods

2-1. Induction of triploid

On May, 2011, triploid induction of the 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 injection of 1000 IU of human chorionic gonadotropin (hCG, Sigma, St Louis, MO, USA) per kg body weight (BW). For minimize the effects of stress in 600 ppm clove oil (Containing 85% eugenol; Sigma, St Louis, MO, USA) contain anesthesia was performed (Appendix 1). Using surgical incision, sperm were obtained by cutting the surgically removed testes of males that had been given an IP injection of hCG at 500 IU/kg BW (Appendix 2). For prevent death of female sample, unfertilized eggs were obtained by surgical incision (Appendix 2). Eggs were fertilized with sperm diluted in saline using the wet method. Five mins after fertilization, the eggs were rinsed rapidly to remove excess sperm and were immediately subjected to cold-shock treatment (4°C) for 60 mins to prevent the extrusion of the second polar body. Untreated fertilized eggs were used as diploid controls.

Diploid and induced triploid individuals (n=100) were cultivated by the method of Kim *et al.* (2001). All fish were reared in 450-L tanks under the same hydrological conditions. Water temperature was maintained at 24 ± 1.5°C, and the mean water oxygen concentration was kept close to saturation

level (mean: 9.4 ± 0.3 mg/L). Experimental fish were fed twice daily, totaling 2% of the average body weight during the experimental time (3 years). At 3 months after hatching, fishes of each group were periodically sampled and anesthesia of each sample were performed in 300 ppm clove oil for prevent death of each samples (Appendix 1). Their ploidy was determined by flowcytometric assessment of the nuclear DNA content in erythrocytes or fin cells (Francescon *et al.*, 2004). Diploid and induced triploid samples were implanted passive integrated tag (PIT) chips in dorsal muscle (Appendix 3). Specimens were used at 100 days posthatching, and the diploid samples had an average body mass of 100.7 ± 8.92 g and average body length of 11.2 ± 2.54 cm (n=50). The induced triploid samples had an average body mass of 102.3 ± 9.71 g and average body length of 12.7 ± 2.31 cm (n=50).

2-2. Cell cycle and cell cycle protein expression analysis

Specimens were sourced at 3 months after hatching. Flowcytometric analysis was performed to estimate the average celluar DNA content of 20 individuals from diploid and induced triploid Far Eastern catfish, *Silurus asotus*. Experimental samples of each ploidy used 1 year hatchings. Using anesthesia of 300 ppm clove oil (Appendix 1), fin tissues and gill tissues were collected from tail fin and gill arch of experimental animal. Samples

were fixed in 10 mL of cold 70% ethanol and filtered through a 30 μm filter. The cell solution was stored at 4ºC. One million cells were collected and stained using a high-resolution DNA Staining Kit (Partec GmbH, Germany) under dark conditions at room temperature for 15 mins. Stained samples were analyzed on Partec flowcytometer (Ploid Analyzer, PA-II, Partec GmbH, Germany) to determine the relative DNA content. The red blood cells with 2.8 pg DNA/nucleus of mud loach, *Musgurnus mizolepis*, were used as a standard reference (Park *et al.*, 1999). Partec PA-II flowcytometer calculates the percentage of cells in G_1 (gap 1)-, S (synthesis)- and G_2 (gap 2)+M (mitosis)-phase fractions in the diploid cell population and in the induced triploid cell population.

For western blots, tail fin and gill tissue were extracted from diploid and induced triploid Far Eastern catfish, and mixed with lysis buffer (40 mM Tris, 120 nM NaCl, 1mM phenylmethylsulfonyl fluoride, 10 mg/µl leupeptin, 2 mM sodium orthovanadate, 10 μg/ml aprotinin). Samples were homogenized by homogenizer prior to centrifugation (Centrifuge Micro 17R, Hanil Science Industrial Co., Ltd, Incheon, Korea) for 20 mins at 12,000 rpm, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% SDS-PAGE) and electrotransfer. Extracted protein was transferred to nitrocellulose membrane for 2 hrs at 80 V. To prevent a non-specific response, blocking reaction in room temperature was executed using 5%

nonfat milk for one hr, and treated with a HRP-conjugated anti-rabbit, goat IgG (1:10,000; Santa Cruz Biotechnology, USA) and ECL detection Kit (Amersham Pharmacia Biotech, England, UK). After 20 days, for comparing protein expression of cell cycle adjustment protein along organized damage, regeneration site of tail fin and gill tissue were extracted from diploid and induced triploid Far Eastern catfish, and were analyzed with western blot analysis.

2-3. Cellular nucleus analysis

Using passive integrated tag (PIT) method, diploid and triploid Far Eastern catfish, *Silurus asotus*, were sourced from 1 year hatchlings (Appendix 3) which had an average body mass of 302.1 ± 15.22 g (mean ± S.D.) and a standard length of 31.5 ± 4.19 cm (mean ± S.D.). Ten specimens were used for histological observations from each group. Fish were euthanized with an overdose of 600 ppm clove oil (Sigma, St Louis, MO, USA) at 22ºC (Appendix 1) and immediately dissected on an ice-cold cutting board. The retina, kidney, liver and midgut epithelium were removed and extracted tissue samples fixed in 10% neutral formalin solution (100 mL formalin; 6.5 g $Na_2HPO_4 \cdot 12H_2O$; 4.5 g KH_2PO_4; 900 mL distilled water) for 24 hrs. The samples were then refixed in Bouin's solution for a further 24 hrs. All fixed tissues were routinely dehydrated in ethanol, equilibrated in xylene

and embedded in paraffin according to standard histological techniques. Transverse sections were then cut at 6 μm and routinely stained using Mayer's Haematoxlyin and eosin Y-phroxine B before being observed under a high-powered optical microscope (Axioskop, Carl Zeiss, Germany).

The Axioskop 4.1image analysis software (Axiovision, Carl Zeiss, Germany) was employed to measure area and volume of cells and nuclei using the following formulas:

Surface area = $1/4 \times ab\pi$

Volume = $4/3 \times \pi(a/2) \times (b/2)^2$

Where: a = the major axis of a cell or nucleus; b = the minor axis of a cell or nucleus. The differences among groups were analyzed using Student's *t*-test from the SPSS statistics package (SPSS 9.0, SPSS Inc. Chicago, IL, USA).

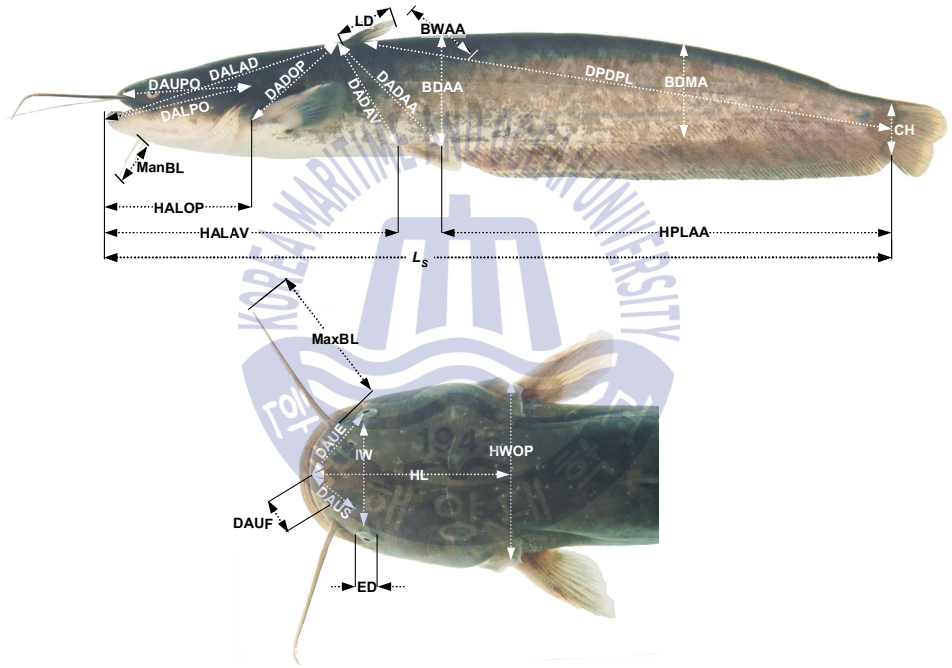
2-4. Morphometric traits analysis

Using PIT tag method, diploid and triploid Far Eastern catfish, *Silurus asotus*, were sourced at 1 year after hatching and 3 years after hatching, respectively (Appendix 3). During the experiment, to avoid sampling fish with guts that were distended by large quantities of food, fish were starved for 24 hrs before sampling (Park *et al.*, 2001). On May, 2012, samples (n=50, 1 year after hatching) from each group were randomly captured and were anesthetized with 600 ppm clove oil for photographing and measuring

(Appendix 1). Standard length measurements of anesthetized individuals were taken to the nearest 0.01 cm using digital vernier calipers (CD-20CP; Mitytoyo, Kawasaki, Japan). As studied by Park *et al.* (2004), body outline measurements were taken for 25 distances between landmarks for both truss and classical dimensions (Fig. 1). *L*s, HALOP, HALAV, and HPLAA indicate horizontal distance, and the other indicates direct distance. On May, 2014, the standard length and body outline of samples (n=50, 3 years after hatching) from each group were measured using the same method.

Standard length measurements were analyzed after arcsine-square root transformation. DAUF, DAUS, IW, and DAUE were analyzed after transforming the measurements relative to head length. The one-way ANOVA test was used to determine the significance between diploid and induced triploid among the various parameters (P<0.05, n=50). The five most significant variables were then used for stepwise discriminant analysis (n=50) to provide the maximum separation between groups (Bonar *et al.*, 1988). Differences between means were regarded as significant at P<0.05.

Fig. 1. Morphometric measurements between each landmark for diploid and induced triploid Far Eastern catfish, *Silurus asotus* (after Park *et al.*, 2004). Top: lateral view of the whole body; Bottom: dorsal view of head region. Measurements included standard length (*L*s); caudal peduncle height (CH); head width between the origin of the pectoral fins (HWOP); body width at the anterior insertion of the anal fin (BWAA); maxilla barbel length (MaxBL); mandible barbel length (ManBL); head length between the anterior edge of the upper lip and the midpoint of head width (HL); eye diameter (ED); interorbital width (IW); length of the dorsal fin (LD); direct distance between the anterior edge of the lower lip and the anterior insertion of the dorsal fin (DALAD), between the anterior edge of the upper lip and the most posterior aspect of the operculum (DAUPO), between the posterior insertion of the dorsal fin and the most posterior point of the lateral line (DPDPL), between the most posterior point of the lateral line and the anterior insertion of the anal fin (HPLAA), between the anterior insertion of the dorsal fin and the origin of the pectoral fin (DADOP), between the anterior insertion of the dorsal fin and the anterior insertion of the ventral fin (DADAV), between the anterior insertion of the dorsal fin and the anterior insertion of the anal fin (DADAA), between the anterior edge of the upper lip and the eye (DAUE), between the anterior edge of the upper lip and the first nostril (DAUF), and between the anterior edge of the upper lip and the second nostril (DAUS); the horizontal distance between the anterior edge of the lower lip and the most posterior aspect of operculum (DALPO), between the anterior edge of the lower lip and the origin of the pectoral fin (HALOP), and between the anterior edge of the lower lip and the anterior insertion of the ventral fin (HALAV); and the body depth at the anterior insertion of the anal fin (BDAA) and the midpoint of the anal fin base (BDMA).



2-5. Water temperature stress response

On May 2012, the high water temperature stress test commenced after provide a consistent diet and maintain a consistent water temperature in culture tanks of the diploid and the induced triploid Far Eastern catfish, *Silurus asotus*, and water temperature in culture tanks was maintained 20°C while two weeks. Water temperature in the two rectangular glass tanks (Dimensions W200×L69×H47 cm) were reached 25°C by heater and 30 samples of each ploidy was transfer immediately in each rectangular glass tanks. Blood samples were extracted from five randomly selected fish at 0 (pre), 1, 6, 12, 24, and 48 hrs post stress test. On June 2012, the low water temperature stress test was executed in above-mentioned same methods, but the difference between high water temperature test methods and low water temperature test methods was water temperature in the two rectangular glass tanks, and water temperature in those were reached 15°C by cooler. All experiments were triplicated.

Using syringes lined with the anticoagulant heparin blood was extracted and assayed at fixed intervals of 0, 1, 6, 12, 24 and 48 hrs from five experimental samples. Selected blood was filled into capillary tubes and analyzed after centrifuging at 200 ×g for 10 mins. Plasma was then collected and stored in a deep freezer (SW-UF-200; Samwon Freezing Engineering, Busan, Korea) at -80°C until analysis.

The cortisol concentration was measured using radioimmunoassay. Cortisol was determined in 50 μl samples using RIA kits (Coat-A-Count TKCO Cortisol RIA Kit; DPC, USA). Mixtures of sample in 100 ml antiserum were incubated for 45 min at 37°C, and then 1,000 ml separation reagent was added. The mixture was placed in a refrigerator at 4°C for 15 min and then centrifuged at 1,200 ×g for 15 min. Supernatant was assayed for gamma radiation using an automatic gamma counter (Cobra; Packard, USA). Plasma glucose concentration was analyzed according to methodology of Raabo and Terkildsen (1960: Kit 510, Sigma, St Louis, MO, USA), where production of H_2O_2 by glucose oxidase in the presence of *o*-dianisidine was evaluated as an absorbance increase at 450 nm. The lactic acid concentrations were analyzed using blood automatic analysis (Boehringer Mannhein Reflotron, Germanry).

Using the SPSS statistics package (SPSS 12.0, SPSS Inc., Chicago, IL, USA), one-way analysis of variance (ANOVA) were carried out to test for statistical significance ($P<0.05$) between diploid and induced triploid. Multiple comparisons were performed using Duncan's multiple range test (Duncan, 1955).

2-6. Blood and endocrine hormone analysis

On May 2012, 1 year broodstock were raised in recirculating water tank in Fisheries & Genetic Sciences Laboratory, Korea Maritime and Ocean University, Korea. Using passive integrated tag (PIT) method, the samples of diploid and induced triploid Far Eastern catfish, *Silurus asotus*, were bred for two years in one aquarium (Appendix 3). The mean body length was 45.2±3.28 cm, and mean body weight was 368±31.4 g, respectively. For determine difference of all measurements between spawning season and non-spawning, water temperature of each group were changed by seasonal temperature variation. Fluorescent light was 1000 lux and the light was on from 6:00 to 18:00. All measurements for spawning season were measured in May and those for non-spawning season were measured in Janurary, 2014, respectively.

Experimental diets were used commercial feed (Cheonhajeil Feed Coporation, Korea). Component of the feed was as follows; the contents of crude protein, crude fat and crude fiber were 40, 5 and 5%, and ash, calcium, phosphorus, mineral premix and vitamin premix were contained 14, 1, 1, 1 and 1% in experimental diet. Vitamin premix contained the following amount which were diluted in cellulose (g kg^{-1} mix; L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-

inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003). Mineral premix contained the following ingredients {g kg^{-1} premix; NaCl, 43.3; $MgSO_4 \cdot 7H_2O$, 136.5; $NaH_2PO_4 \cdot 2H_2O$, 86.9; KH_2PO_4, 239.0; $CaH_4(PO_4) \cdot 2H_2O$, 135.3; ferric citrate, 29.6; $ZnSO_4 \cdot 7H_2O$, 21.9; Ca-lactate, 304.0; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0}.

In the spawning season measured the 30 samples of the diploid and 30 samples of the induced triploid. In the non-spawning season measured each the 30 samples of the diploid and 30 samples of the induced triploid. Total of 120 samples were measured. Gonadosomatic index (GSI) was measured by following equation:

GSI (%) = (gonad weight/body weight) × 100.

Estradol, testosterone, thyroid stimulating hormone and thyroxine were measured by radioimmunoassay method. Cholesterols and insulin were measured by fluorophotometry (i-Chroma, Sun Kyung Medical, Korea). Blood analysis was measured by auto hematology analyzer (PE-6800, Prokan, China).

One-way and Two-way ANOVA were used to determine the significance ($P<0.05$) of the differences among the means responses of treatments (SPSS 9.0, SPSS Inc., USA).

2-7. Body composition analysis

Thirty fish were sampled from diploid and induced triploid Far Eastern catfish, *Silurus asotus*, respectively and were sacrificed for proximate analysis. Proximate analysis was conducted according to standard AOAC (1990)'s method. Crude protein was determined by the Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412, Flwail, Switzerland), crude lipid was determined using an ether-extraction method (Soxtec TM 2043 Fat Extraction System, Foss Tecator, Hoganas, Sweden), moisture was determined by oven drying at 105 ℃ for 24 hrs, and ash was determined using a muffle furnace at 550 ℃ for 4 hrs. Fatty acids was measured by injecting the gas chromatograpphy the purified fatty acids in forch method.

Sample lipids were extracted with chloroform-methanol (2:1 v/v) according to the method of Bligh and Dyer (1959). Each sample (30 g) was combined with 180 mL of chloroform-methanol (2:1 v/v) and homogenized for 2 mins. Sixty milliliter of chloroform and distilled water was added sequentially to the filtrate and shaking was carried out for 10 min. The extract was filtered through Whatman No. 2 filter paper. The filtrate was transferred to a 500 mL separatory funnel and partitioned into organic solvents by vigorously shaking the solution for 20-30 sec. Sufficient time was allowed for the layers to separate and the chloroform layer was drained into a flask over sodium sulfate to remove the traces of water. The extracts

were evaporated to dryness using a rotary vacuum evaporator (N-1000VW, EYELA, Tokyo, Japan) with the water bath heated at 40 °C.

Fatty acid methyl ester was prepared with 14% BF_3/methanol and analyzed with a gas chromatograph (CP-3380, Varian Inc., Palo Alto, CA, USA) using a flame-ionization detector, as described previously (Salem *et al.*, 1996). The chromatography utilized a CP-SIL 5 CB fused silica capillary column (L 60 m × ID 0.32 mm, film thickness 0.01 μm: Varian Inc., Palo Alto, CA, USA). Peaks were identified by comparison with fatty acid standards (GLC-462, Nu-Check-Prep, Elysian, MN, USA), and area and its percentage for each resolved peak were analyzed using a Galaxie chromatography software.

2-8. Determination of amitotic erythrocyte

Diploid and induced triploid Far Eastern catfish, *Silurus asotus*, were determined by reading PIT tag chip and were anesthetized with 600 ppm clove oil (Appendixes 1 and 3). Blood of diploid and induced triploid Far Eastern catfish were extracted using syringe coated heparin at cauda part of each sample. Observing Far Eastern catfish's erythrocyte, whole blood 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 and I

observed by an high-powered optical microscope (Axioskop, Carl Zeiss, Germany).

The amitotic erythrocytes were determined by transmission electron micrograph (JEM 1200 E-X II, 60-80 kv, JEOL, Tokyo, Japan). For electron microscopy, blood samples of diploid and induced triploid in experiment were pre-fixed in a cold 2.5% glutaraldehyde solution (pH 7.5) for 2 hrs. The erythrocytes of each group were centrifuged 1,000 rpm at 10 mins, and were pre-fixed for 2 hrs at 4°C in 2.5% glutaraldehyde solution buffered by 0.1 M phosphate buffer solution (PBS, pH 7.2). After washing with PBS for 10 mins, the samples were post-fixed in 1% osmium tetroxide (OsO_4) for 2 hrs at 4°C. Samples were rewashed with PBS, then serially dehydrated with ethanol from 50 to 100%, and embedded in Epon 812.

Sections (0.5 μm thick) were cut using an ultramicrotome (LKB, Nova, Sweden) and then stained with toluidine blue to determine the investigation region. The sections were double-stained with uranylacetate and lead citrate solution and examined using a transmission electron microscope. After processing, amitotic erythrocytes of diploid and induced triploid were observed and were counted using counter.

3. Results

3-1. Cell cycle and cell cycle protein expression

Figure 2 shows a DNA histogram of diploid Far Eastern catfish, *Silurus asotus*, with tail fin tissue and gill tissue generated by the software package. This histogram contains the gap 1 (G_1)-peak, the synthesis (S)-phase region and the gap 2 (G_2) + mitosis (M)-peak with the background correction. The percentages of the G_1-, the S- and the G_2+M-phase fractions were 95.8%, 1.2% and 3.0% in the tail fin tissue and 75.1%, 11.2% and 13.7% in the gill tissue, respectively (Figs. 2a and 2b). Figure 3 represents the histogram of induced triploid Far Eastern catfish with tail fin tissue and gill tissue. The percentages of the G_1-, the S- and the G_2+M-phase fractions were 97.4%, 0.6% and 2.0% in the tail fin tissue cells and 85.2%, 8.9% and 5.9% in the gill tissue cells, respectively (Figs. 3a and 3b).

The mean percentages of the G_1-, the S- and the G_2+M-phase fractions were 92.5%, 3.2% and 4.3% in tail fin tissue of diploid, and 93.4%, 2.6% and 4.0% in those of induced triploid, respectively (Table 1). There were no significant differences in the percentages of each cell cycle fraction between diploid and induced triploid. Nor were there any significant differences in the percentages of each cell cycle fraction between the diploid and the induced triploid of the tail fin tissue. However, the S- and G_2+M-phase fraction of

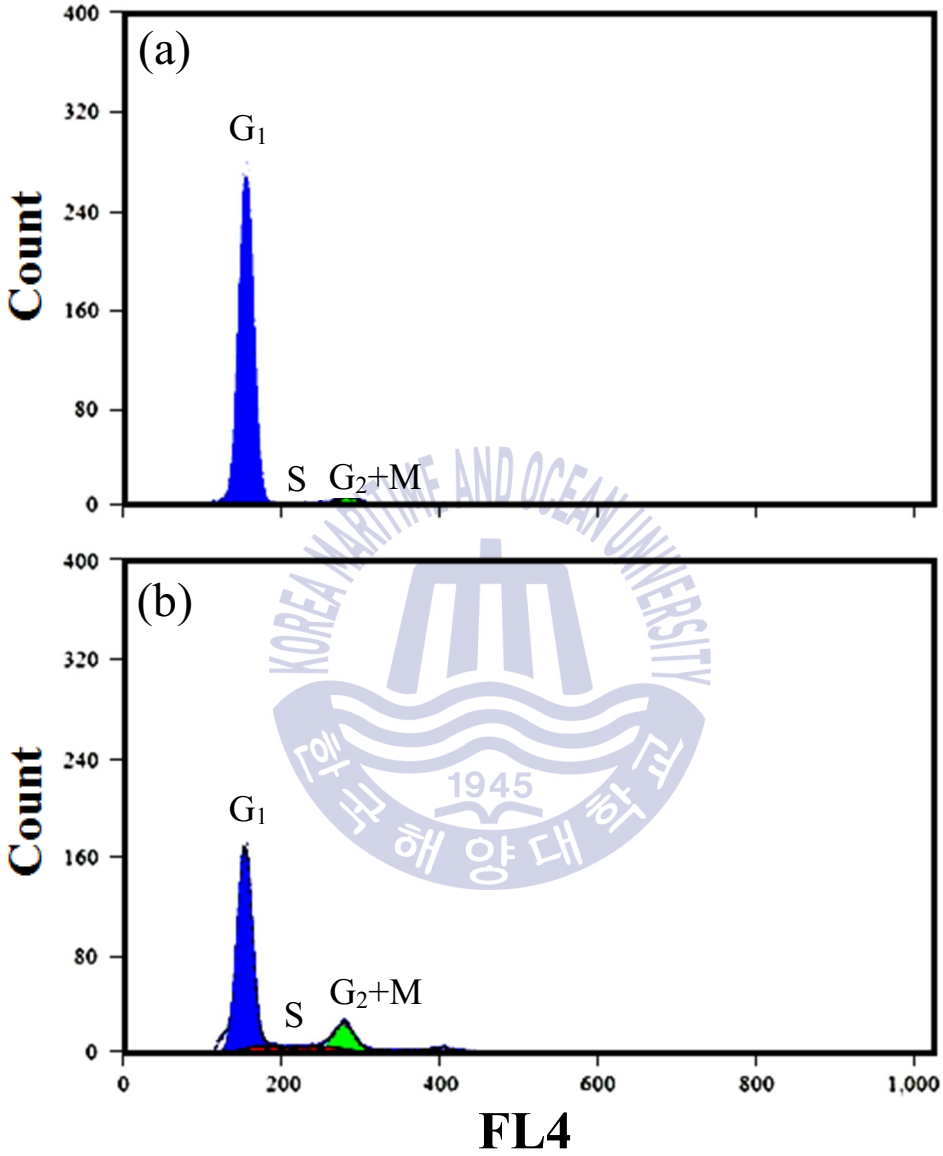


Fig. 2. DNA histogram of diploid Far Eastern catfish, *Silurus asotus*, in (a) tail fin tissue and (b) gill tissue. Each cell cycle fraction with background correction is indicated. Fluorescence 4 (FL4) is ray of red light.

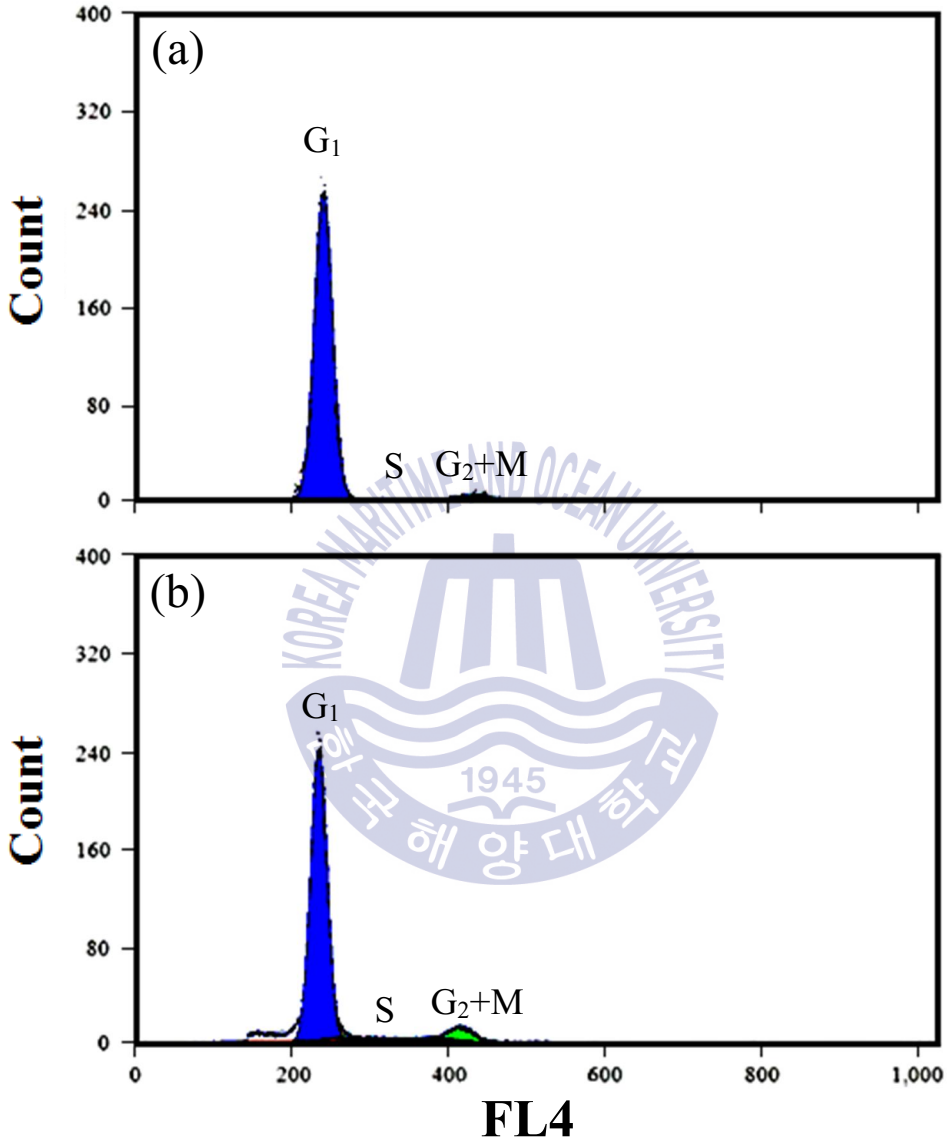


Fig. 3. DNA histogram of induced triploid Far Eastern catfish, *Silurus asotus*, in (a) tail fin tissue and (b) gill tissue. Each cell cycle fraction with background correction is indicated. Fluorescence 4 (FL4) is ray of red light.

Table 1. Relationship of cell fraction among ploidy and tissue in Far Eastern catfish, *Silurus asotus*

Mean fraction (%)	2n[1]			3n[1]		
	G_1	S	G_2+M	G_1	S	G_2+M
Tail fin	92.5±5.58[a]	3.2±0.71[a]	4.3±0.87[a]	93.4±5.71[a]	2.6±0.74[a]	4.0±1.05[a]
Gill	75.1±3.69[b]	11.1±2.66[b]	13.8±3.52[b]	85.2±5.98[b]	8.9±0.58[b]	5.9±1.51[b]

[1]Values are means ± SD. Values in same row having the different superscripts are significantly different (n=20, P<0.05).

diploid was higher than those of induced triploid, although those differences were not statistically significant (Table 1).

On the other hand, the mean percentages of the G_1-, the S- and the G_2+M -phase fractions between diploid and induced triploid had shown significantly difference in gill tissue ($P<0.05$), and the mean percentages of each phase fractions were 75.1%, 11.1% and 13.8% in gill tissue of diploid and 85.2%, 8.9% and 5.9% in those of induced triploid, respectively (Table 1). The differences of cell cycle between tail fin tissue and gill tissue were statistically significant in diploid and induced triploid Far Eastern catfish ($P<0.05$). Also, the differences between diploid and induced triploid Far Eastern catfish were statistically significant in tail fin tissue and gill tissue ($P<0.05$).

Figure 4 shows protein expression of cyclin D1 and cyclin E through western blot anlysis at diploid and induced triploid Far Eastern catfish to compare protein expression of cell cycle adjustment protein along organized damage. Regenerating site's length of gill tissue and tail fin tissue was 2-3 mm while 20 days. Cyclin D1 and cyclin E expressions after organized damage were lower than those before organized damage in both tissues of diploid and induced triploid Far eastern catfish, respectively. In all experimental groups, protein expressions of induced triploid were higher than those of diploid.

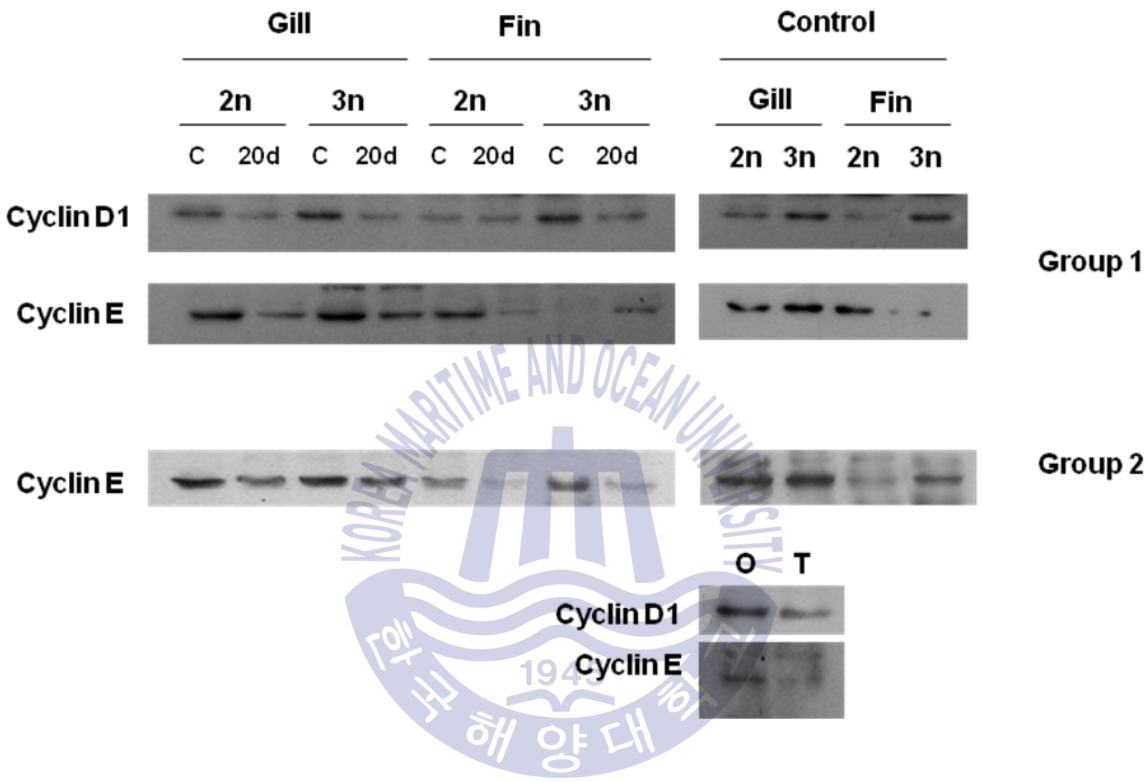


Fig. 4. Protein expression of cyclin D1 and cyclin E through western blot anlysis at diploid and induced triploid Far Eastern catfish, *Silurus asotus*. C: control, tissue before organized damage; 20 d: tissue at 20 days after organized damage; O: origin part of tail fin; T: terminal part of tail fin.

Significant difference of Cyclin D1 and cyclin E expressions were not determined between gill tissue and tail fin tissue. In tail fin tissue, cyclin D1 and cyclin E expressions of origin part were higher than those of terminal part.

3-2. Cellular nucleus

Structure of the retina in induced triploid Far Eastern catfish, *Silurus asotus*, were the same as those in the diploid Far Eastern catfish, analyzed from seven distinct component layers; pigment epithelium and bacillary layer, outer nuclear layer, outer plexible layer, inner plexible layer, ganglion cell layer, optic nerve fiber layer and inner limiting membrane (Figs. 5a and 5b). The thickness (%) of each layer in both induced triploids and diploid fish is shown in Table 2. Statistically significant differences in thickness was found in the outer nuclear layer, outer plexible layer, inner plexible layer and pigment epithelium and bacillary layer (*t*-test, n=12, d.f.=239, P<0.05) between the two tissue types. In the former four layers, the diploids showed larger values than the induced triploids (Table 2). There was also a difference in cellular structure between induced triploids and diploids. In induced triploids, the outer nuclear layer consisted of two strata of nuclei (Table 2; Fig. 5c), while in diploids the same layer consisted of three strata of nuclei (Table 2; Fig. 5d).

Fig. 5. Retina of diploid and induced triploid in Far Eastern catfish, *Silurus asotus*. Layer of retina of (a) diploid and (b) induced triploid (Scale bars indicate 50 μm). Outer layer cell nucleus (c, *1) of (c) diploid retina and outer layer cell nucleus (d, *2) of (d) induced triploid retina. Scale bars indicate 10 μm. Abbr. ILM, inner limiting membrane; ONF, layer of optic nerve fibers; LGC, layer of ganaglion cell; IPL, inner plexibel layer; OPL, outer plexible layer; ONL, outer nuclear layer; PE, pigment cell and bacillary layer. H & E staining.

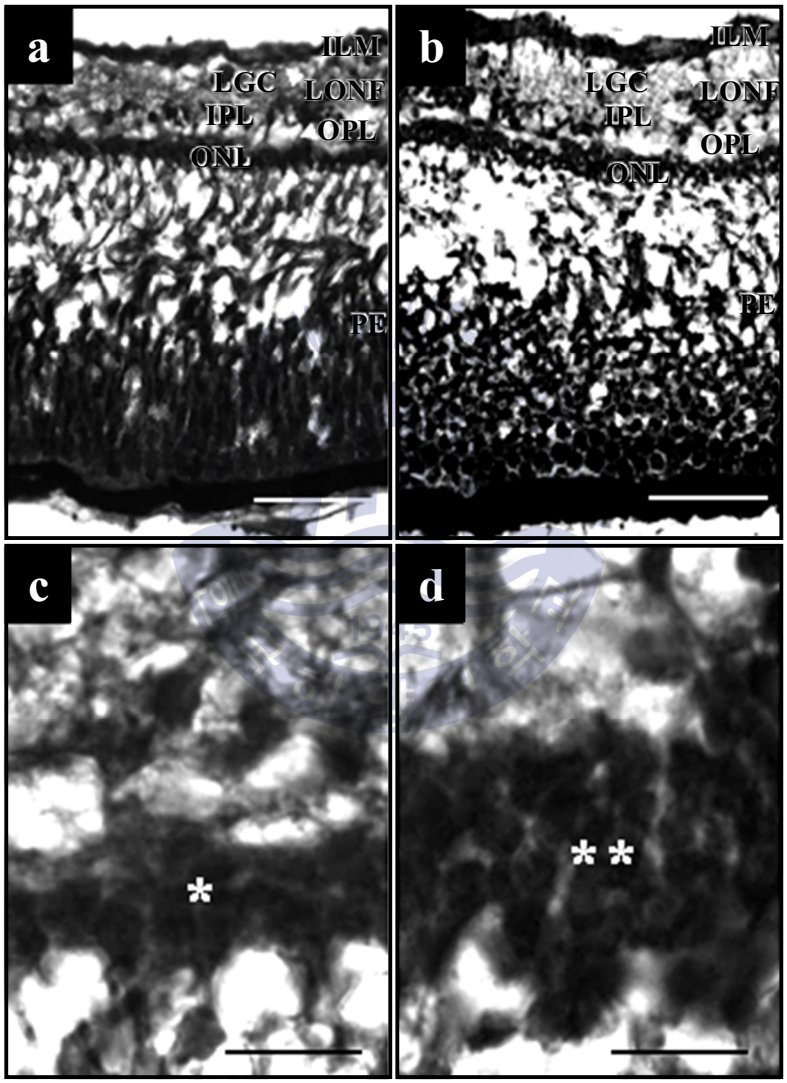


Table 2. Thickness in each component layer and the number of outer nuclear layer of retina in diploid and induced triploid Far Eastern catfish, *Silurus asotus*

	2n	3n	Ratio[1]
Thickness of retina (μm)[2]	251.79 ± 10.216[a]	246.68 ± 10.639[a]	0.98
Thickness of each layer of retina (%)[2]			
Inner limiting membrane (ILM)	4.05 ± 0.549[a]	4.31 ± 0.303[a]	1.06
Layer of optic nerve fibers (ONF)	3.75 ± 0.312[a]	3.45 ± 0.164[a]	0.92
Layer of ganglion cell (LGC)	7.99 ± 0.598[a]	8.25 ± 0.604[a]	1.03
Inner plexible layer (IPL)	6.28 ± 0.698[b]	5.54 ± 0.569[a]	0.88
Outer plexible layer (OPL)	5.60 ± 0.205[b]	4.76 ± 0.587[a]	0.85
Outer nuclear layer (ONL)	8.21 ± 0.642[b]	6.89 ± 0.463[a]	0.84
Pigment epithelium and bacillary layer (PE)	70.28 ± 8.687[b]	63.92 ± 7.003[a]	0.91
Number of outer nuclear layers in retina	3	2	1.50

[1]3n/2n.

[2]Values are means ± SD. Values in same row having the different superscripts are significantly different (n=12, d.f.=293, P<0.05).

The size and area of the secondary proximal tubule cell of the trunk kidney of induced triploid and diploid Far Eastern catfish and the size, area and number of nuclei in the secondary proximal tubule of the trunk kidney were compared. In relation to secondary proximal tubule cell size and area diploids were significantly larger than induced triploids (*t*-test, n=12, d.f.=239, P<0.05). In relation to size, area and volume of nucleus they were also significantly larger than induced triploid (*t*-test, n=12, d.f.=239, P<0.05) (Table 3). However, the number of nuclei within the secondary proximal tubule of trunk kidney was higher in diploids then induced triploids (*t*-test, n=12, d.f.=239, P<0.05) (Figs. 6a and 6b).

Table 4 shows the comparison of hepatocyte nuclear area and nuclear height of midgut epithelium between diploids and induced triploids samples. The hepatocyte nuclear area was 1.25 times larger (*t*-test, n=12, d.f.=239, P<0.05) in induced triploids (Figs. 6c and 6d), while the nuclear height of midgut epithelium was 9.91 ± 0.797 μm^2 (mean ± SD) in diploids and 12.27± 0.785 μm^2 (mean ± SD) in induced triploids, with induced triploid fish being 1.24 times larger than diploids (Figs. 6e and 6f).

Table 3. Cell and nuclear size of secondary proximal tubule of trunk kidney and its nuclear number in diploid and induced triploid Far Eastern catfish, *Silurus asotus*

	2n	3n	Ratio[1]
Secondary proximal tubule cell of trunk kidney[2]			
Major axis (μm)	10.34 ± 1.178[a]	12.19 ± 1.375[b]	1.18
Minor axis (μm)	8.33 ± 7.706[a]	9.72 ± 8.222[b]	1.67
Surface area (μm^2)	64.67 ± 6.275[a]	90.35 ± 9.978[b]	1.40
Secondary proximal tubule nucleus of trunk kidney[2]			
Major axis (μm)	3.26 ± 0.328[a]	4.11 ± 0.420[b]	1.26
Minor axis (μm)	3.01 ± 0.397[a]	3.42 ± 0.307[b]	1.14
Surface area (μm^2)	7.70 ± 0.755[a]	11.01 ± 8.181[b]	1.43
Volume (μm^3)	14.64 ± 7.658[a]	27.05 ± 2.106[b]	1.85
Nucleus number in secondary proximal tubule	15.70 ± 2.080[b]	9.80 ± 1.111[a]	0.63

[1]3n/2n.

[2]Values are means ± SD. Values in same row having the different superscripts are significantly different (n=12, d.f.=293, P<0.05).

Fig. 6. Comparison of secondary proximal tubule nuclei of trunk kidney, hepatocyte nuclear area and nuclear height of midgud epithelium in diploid and induced triploid Far Eastern catfish, *Silurus asotus*. Secondary proximal tubule nucleus (a, *1) of (a) diploid trunk kidney and secondary proximal tubule nucleus (b, *2) of (b) induced triploid trunk kidney. Scale bars indicate 10 μm. Hepatocyte of diploid (c, *1) and induced triploid (d, *2) (Scale bars indicate 5 μm). Midgut epithelium of diploid (e, *1) and induced triploid (f, *2) (Scale bars indicate 10 μm). H & E staining.

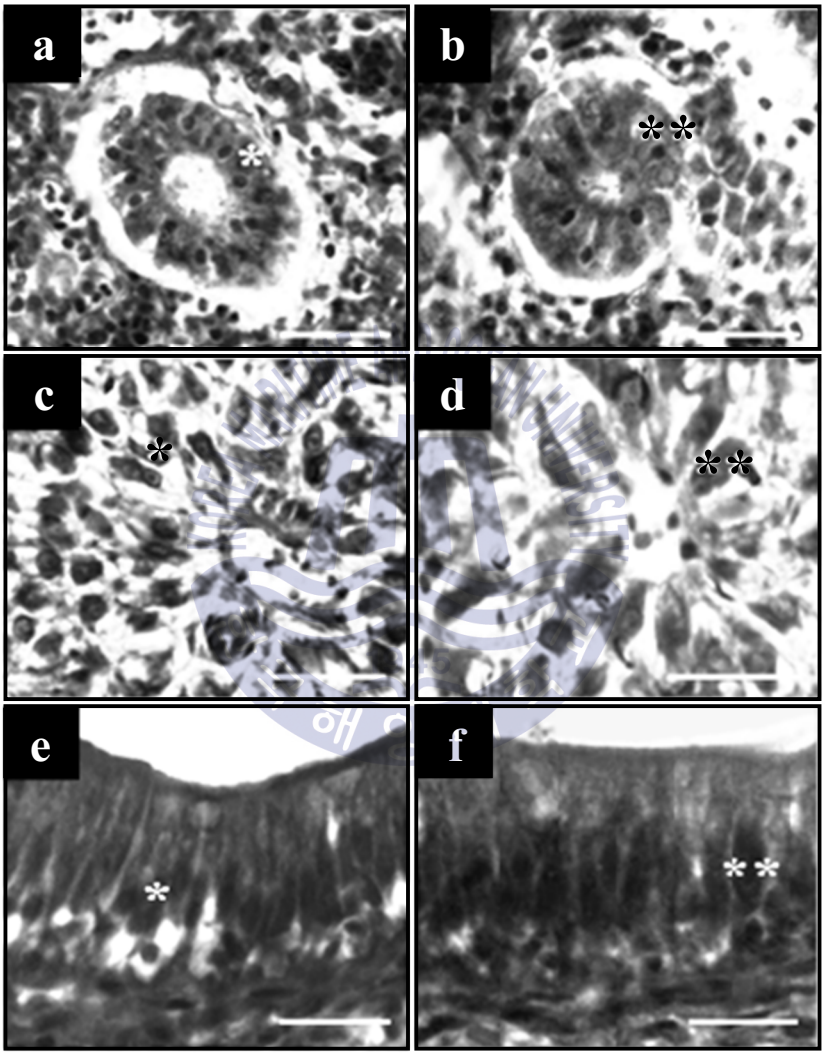


Table 4. Comparison of hepatocyte nuclear and nuclear height of midgut epithelium in diploid and induced triploid Far Eastern catfish, *Silurus asotus*

	2n[1]	3n[1]	Ratio[2]
Hepatocyte nuclear area (μm^2)	10.77 ± 0.643[a]	13.41 ± 1.210[b]	1.25
Nuclear height of midgut epithelium (μm)	9.91 ± 0.797[a]	12.27 ± 0.785[b]	1.24

[1]Values are means ± SD. Values in same row having the different superscripts are significantly different (n=12, d.f.=293, P<0.05).
[2]3n/2n.

3-3. Morphometric traits

At the end of the experiment, accumulated survival was 90% in the induced triploid Far Eastern catfish, *Silurus asotus*, group, but only 75% in the diploid Far Eastern catfish group. The average standard lengths of the diploid and induced triploid groups were 21.4 ± 2.91 cm and 19.9 ± 2.48 cm, respectively, 1 year after the beginning of the experiment and 33.9 ± 2.94 cm and 38.1 ± 1.70 cm, respectively, at the end of the experiment.

Table 5 shows the means of the morphometric dimensions of Far Eastern catfish at 1 year after hatching and results of ANOVA testing for differences among groups. Differences between the diploid and induced triploid groups significantly affected both the classical and truss dimensions. There were significant differences in these dimensions in the diploid group compared to the induced triploid group. The induced triploid group had drastically increased truss dimensions of BDAA/*L*s in the trunk region and showed higher truss dimensions of BDAA/*L*s than the diploid group. In addition, the induced triploid group had an increased classical dimension of HALOP/*L*s in the head region and a higher classical dimension of HALOP/*L*s than the diploid group. However, the diploid group had a higher ED/*L*s eye diameter than the induced triploid group. Significant differences between the diploid and induced triploid groups were not observed for the other morphometric dimensions ($P<0.05$).

Table 5. Means and standard deviations for morphometric dimensions of diploid and induced triploid Far Eastern catfish, *Silurus asotus*, 1 year after hatching and results of ANOVA testing for differences between groups[1]

Morphometric dimension	2n	3n	ANOVA
DALAD/*Ls*	30.32 ± 0.629	32.36 ± 0.783	NS
DPDPL/*Ls*	69.52 ± 3.220	71.70 ± 3.742	NS
HPLAA/*Ls*	55.55 ± 0.567	56.95 ± 2.180	NS
HALAV/*Ls*	36.90 ± 0.898	37.54 ± 1.168	NS
HALOP/*Ls*	19.01 ± 0.474	21.61 ± 0.251	*
DALPO/*Ls*	20.56 ± 0.253	21.64 ± 1.053	NS
DAVPO/*Ls*	19.04 ± 0.599	19.59 ± 0.495	NS
DADOP/*Ls*	15.50 ± 0.306	16.70 ± 0.404	NS
DADAV/*Ls*	17.54 ± 0.840	18.78 ± 1.296	NS
DADAA/*Ls*	18.75 ± 1.095	19.92 ± 1.296	NS
MaxBL/*Ls*	25.25 ± 1.351	29.52 ± 2.313	NS
ManBL/*Ls*	8.13 ± 0.679	8.76 ± 0.989	NS
ED/*Ls*	2.43 ± 0.114	2.17 ± 0.197	*
LD/*Ls*	7.64 ± 0.236	6.97 ± 0.448	NS
CH/*Ls*	4.49 ± 0.946	5.80 ± 0.595	NS
BDAA/*Ls*	13.70 ± 0.300	16.38 ± 0.630	*
BDMA/*Ls*	11.59 ± 0.610	12.54 ± 0.999	NS
BWAA/*Ls*	10.06 ± 0.534	11.23 ± 1.249	NS
HWOP/*Ls*	15.69 ± 1.091	16.01 ± 0.303	NS
DAUF/HL	13.81 ± 0.784	14.81 ± 0.160	NS
DAUS/HL	24.97 ± 3.562	25.99 ± 1.121	NS
IW/HL	48.85 ± 0.725	49.12 ± 1.106	NS
DAUE/HL	37.08 ± 1.646	35.51 ± 0.685	NS

[1]For dimensions, refer to text for details. Data were analyzed using one-way ANOVA on data transformed to the arcsine of the square root. ANOVA: *P<0.05; NS: Not significant.

Table 6 shows the means of morphometric dimensions of the fish at 3 years after hatching and results of ANOVA testing for differences among groups. The same general trends appeared. Specifically, the diploid group had increased classical dimensions of DALAD/*L*s and HALAV/*L*s in the head region, and had higher classical dimensions of DALAD/*L*s and HALAV/*L*s than the induced triploid group. Also, the diploid group had higher DAUF/HL, DAUS/HL, and IW/HL in the head region than the induced triploid group, and had higher ManBL/*L*s in mandible barbel length than the induced triploid group ($P<0.05$). However, there was no significance in any truss dimensions between the two groups.

Significant variables were DALAD, HALAV, DAUF, DAUS, IW, and ManBL (Table 7). The most useful combination of these variables for separating the two groups was DALAD, IW, and DAUF, which correctly classified 85% of the catfish as induced triploid or diploid, the maximum degree of separation obtained. Table 8 shows the classification function coefficients of the most significant variables. Classification functions (C) developed by stepwise discriminant analysis for Far Eastern catfish were

C = 7.269 (DALAD) - 6.538 (IW) + 34.573 (DAUF) - 46.151

for diploid catfish, and

Table 6. Means and standard deviations for morphometric dimensions of diploid and induced triploid Far Eastern catfish, *Silurus asotus*, 3 years after hatching and results of ANOVA testing for differences between groups[1]

Morphometric dimension	2n	3n	ANOVA
DALAD/*Ls*	31.52 ± 1.012	29.99 ± 1.405	*
DPDPL/*Ls*	71.03 ± 1.533	70.02 ± 2.830	NS
HPLAA/*Ls*	60.48 ± 2.882	60.94 ± 3.217	NS
HALAV/*Ls*	37.95 ± 1.980	34.64 ± 1.609	*
HALOP/*Ls*	19.61 ± 0.708	19.39 ± 1.494	NS
DALPO/*Ls*	19.01 ± 1.036	19.10 ± 1.192	NS
DAVPO/*Ls*	17.06 ± 0.926	16.68 ± 0.711	NS
DADOP/*Ls*	15.82 ± 2.074	15.01 ± 0.996	NS
DADAV/*Ls*	17.28 ± 1.706	16.51 ± 3.068	NS
DADAA/*Ls*	20.20 ± 1.375	20.45 ± 1.872	NS
MaxBL/*Ls*	20.89 ± 1.776	19.95 ± 1.534	NS
ManBL/*Ls*	8.44 ± 1.178	7.31 ± 0.735	*
ED/*Ls*	2.07 ± 0.214	1.96 ± 0.101	NS
LD/*Ls*	6.16 ± 1.061	5.74 ± 0.511	NS
CH/*Ls*	6.28 ± 0.721	7.25 ± 0.767	NS
BDAA/*Ls*	16.73 ± 1.047	16.30 ± 1.065	NS
BDMA/*Ls*	15.07 ± 0.938	15.49 ± 0.903	NS
BWAA/*Ls*	5.89 ± 0.569	7.76 ± 0.944	NS
HWOP/*Ls*	14.92 ± 1.356	14.34 ± 0.754	NS
DAUF/HL	17.37 ± 1.777	13.15 ± 1.310	*
DAUS/HL	25.23 ± 4.561	20.24 ± 2.378	*
IW/HL	55.51 ± 2.864	47.06 ± 6.153	*
DAUE/HL	36.19 ± 2.740	32.14 ± 2.152	NS

[1]For dimensions, refer to text for details. Data were analyzed using one-way ANOVA on data transformed to the arcsine of the square root. ANOVA: *P<0.05; NS: Not significant.

Table 7. The standardized canonical discriminant function coefficients of the most significant variables providing maximum separation between diploid and induced triploid Far Eastern catfish, *Silurus asotus*[1]

Standardized canonical discriminant function coefficients	Function 1
HALAV	0.232
DALAD	0.914
ManBL	0.439
IW	1.032
DAUF	2.169
DAUS	0.418

[1]For dimensions, refer to text for details. Data were analyzed using one-way ANOVA on data transformed to the arcsine of the square root.

Table 8. Classification function coefficients of the most significant variables providing maximum separation between diploid and induced triploid Far Eastern catfish, *Silurus asotus*[1]

Classification function coefficients	2n	3n
DALAD	7.269	10.709
IW	-6.538	-9.262
DAUF	34.573	7.112
(Constant)	-46.151	-49.886

[1]For dimensions, refer to text for details. Data were analyzed using one-way ANOVA on data transformed to the arcsine of the square root.

C = 10.709 (DALAD) - 9.262 (IW) + 7.112 (DAUF) - 49.886

for induced triploid catfish. Assignment of the stock data to one of these two equations resulted in correct classification 85% of the time (Table 9).

3-4. Water temperature stress response

The average cortisol concentration between diploid and induced triploid Far Eastern catfish, *Silurus asotus*, from the high temperature stress test is shown in Fig. 7a. The average cortisol concentration of control groups were 0.77 μg/dL, 0.66 μg/dL respectably and has been increased to 1.88 μg/dL, 1.66 μg/dL in 1 hr of high temperature exposure and became 2.80 μg/dL, 3.29 μg/dL in 6 hrs. After 12 hrs of high temperature exposure, it rather decreased to 2.26 μg/dL, 1.80 μg/dL a bit and both groups became 1.00 μg/dL in 24 hrs and 0.96 μg/dL, 0.89 μg/dL in 48 hrs. Generally, there was no significant difference between them but the change according to exposure was seen compared to 0 hr and the cortisol concentration was the highest at 6 hrs of progress for them.

The average cortisol concentration between diploid and induced triploid from the low temperature stress test is shown in Fig. 7b. The average cortisol concentrations of control groups were 0.80 μg/dL, 0.71 μg/dL respectably and has been rapidly increased to 14.76 μg/dL, 10.49 μg/dL in 1 hr of low temperature exposure and became 22.09 μg/dL, 17.66 μg/dL in 6 hrs.

Table 9. Classification results of the most significant variables on diploid and induced triploid Far Eastern catfish, *Silurus asotus*[1]

Ploid	Predicted group membership (%)		Total
	2n	3n	
2n	42 (85.1)	8 (14.9)	50 (100)
3n	7 (14.3)	43 (85.7)	50 (100)

[1]For dimensions, refer to text for details. Data were analyzed using one-way ANOVA on data transformed to the arcsine of the square root.

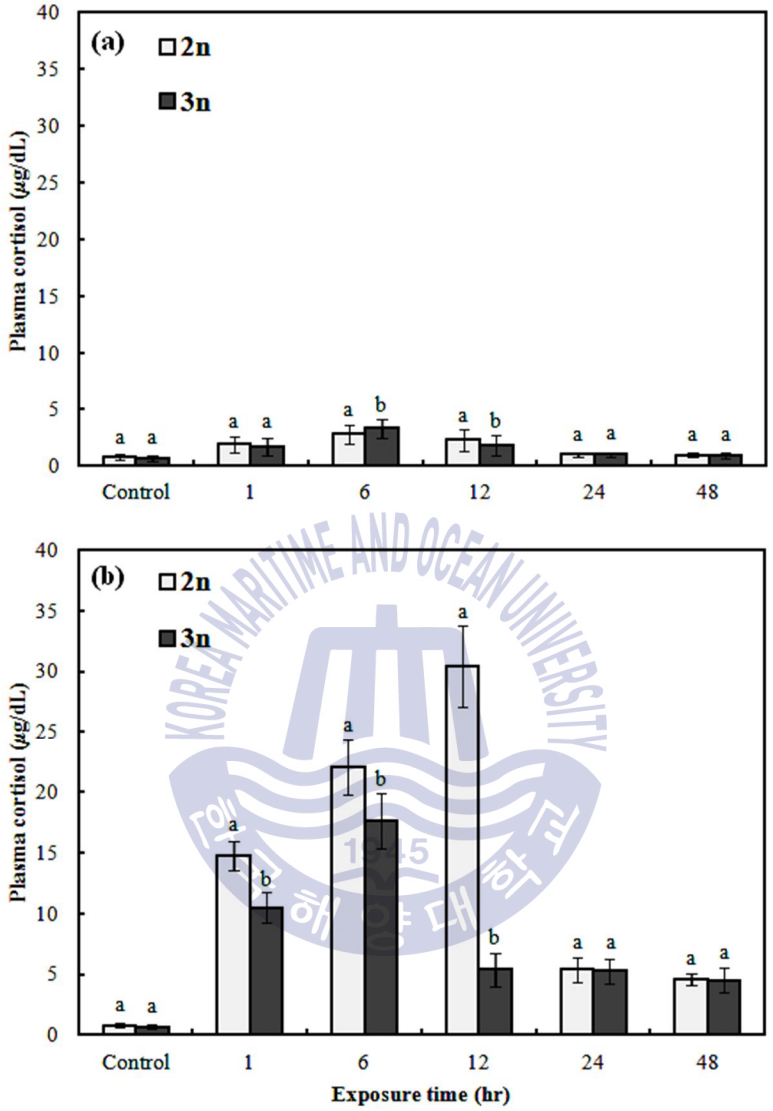


Fig. 7. Plasma cortisol concentration variations in blood plasma of diploid and induced triploid Far Eastern catfish, *Silurus asotus*, during 48 hrs of (a) high water temperature and (b) low water temperature stress period. Values represent means ± SE (n=30). Actually n=5 for each experiment because mean and SE are calculated separately for each group.

After 12 hrs of low temperature exposure, it became 30.43 μg/dL, 5.39 μg/dL respectably so that the cortisol concentration of diploid increased while induced triploid decreased rapidly. In 24 hrs, concentrations became 5.38 μg/dL, 5.27 μg/dL so the cortisol concentrations rapidly decreased for 2 diploid while 3 induced triploid had hardly any changes. In 48 hrs, the concentrations became 4.60 μg/dL, 4.53 μg/dL so both groups had little reduction but there was no significant difference in exposure time. Generally, there was a significant difference according to the exposure time for diploid and induced triploid and the cortisol concentration of induced triploid was generally lower than that of diploid. The diploid showed the highest cortisol concentration in 12 hrs of low temperature exposure and the induced triploid had the highest cortisol concentration in 6 hrs of low temperature exposure.

Plasma cortisol concentration of low water temperature stress was higher than those of high water temperature stress (P<0.05). The average concentration of plasma glucose between diploid and induced triploid at the high temperature test is seen in Fig. 8a. The average plasma glucose concentration of control groups were 30 mg/dL, 24 mg/dL and became with little increase, 31 mg/dL, 26 mg/dL in 1 hr, then 31 mg/dL, 35 mg/dL in 6 hrs. The concentration increased to 50 mg/dL, 59 mg/dL in 12 hrs of high temperature exposure and decreased to 31 mg/dL, 34 mg/dL in 24 hrs. In 48 hrs, it decreased to 30 mg/dL, 29 mg/dL little bit.

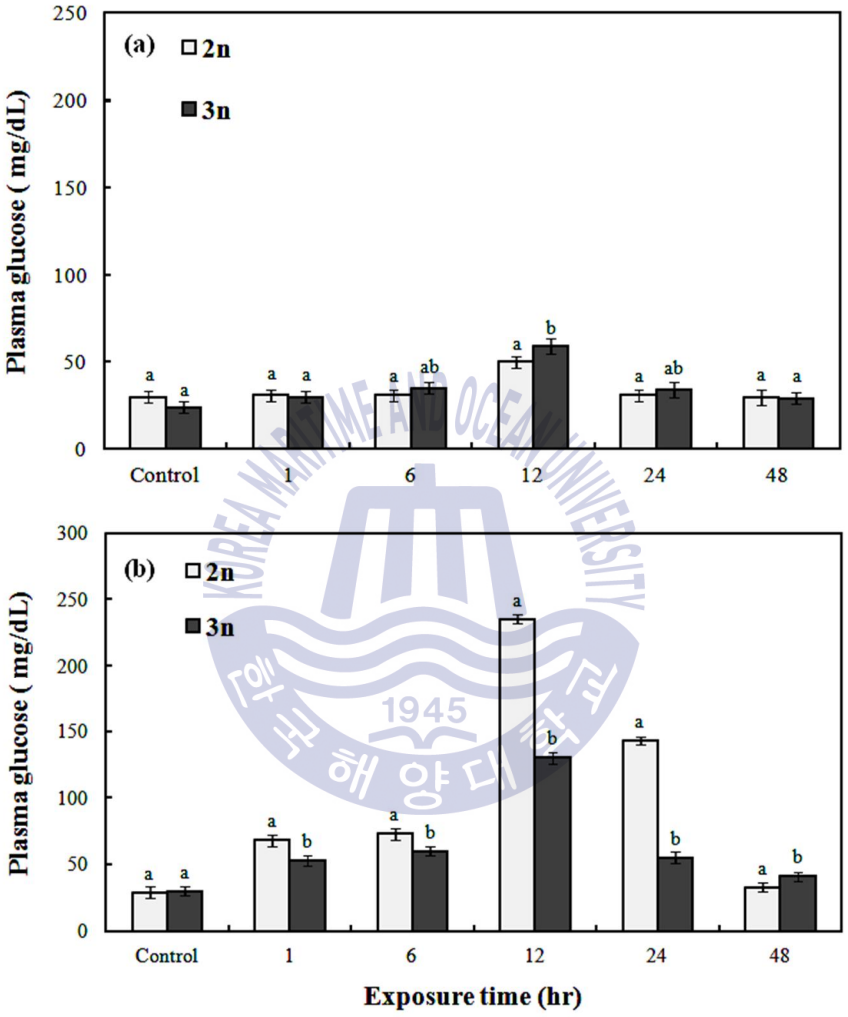


Fig. 8. Plasma glucose concentration variations in blood plasma of diploid and induced triploid Far Eastern catfish, *Silurus asotus*, during 48 hrs of (a) high water temperature and (b) low water temperature stress period. Values represent means ± SE (n=30). Actually n=5 for each experiment because mean and SE are calculated separately for each group.

In general, there was no significant difference between diploid and induced triploid but there was a little difference compared to 0 hr (P<0.05). The plasma glucose concentration was the highest for diploid and induced triploid in 12 hrs of high temperature exposure.

The average concentration of plasma glucose between diploid and induced triploid at the low temperature test is seen in Fig. 8b. The average plasma glucose concentrations of control groups were 29 mg/dL, 30 mg/dL and significantly increased to 68 mg/dL, 53 mg/dL in an hr and became 73 mg/dL, 60 mg/dL after 6 hrs. The concentration rapidly increased to 235 mg/dL, 130 mg/dL in 12 hrs and rapidly decreased to 143 mg/dL, 55 mg/dL in 24 hrs. In 48 hrs, it decreased to 33 mg/dL, 41 mg/dL. In general, there was significant difference between diploid and induced triploid and the general plasma glucose concentration of induced triploid was lower than the diploid (P<0.05). The highest plasma glucose concentrations of them were seen at 12 hrs after the test. Plasma glucose concentration of high water temperature stress was lower than those of low water temperature stress (P<0.05).

In case of lactic acid, there was a significant difference between high and low temperature stress test and the lactic acid concentration for the high temperature stress exposure was higher than that of low temperature and there was no significant difference between diploid and induced triploid

(Fig. 9). As shown in Fig. 9, the significant difference on exposure time, the lactic acid concentration after 24 hrs of high temperature exposure was highest 1.74 mmol/L, 1.64 mmol/L for diploid and induced triploid (Fig. 9a: $P<0.05$), and the lactic acid concentration after 24 hrs of diploid and induced triploid was highest 2.61 mmol/L, 2.50 mmol/L for the low temperature exposure (Fig. 9b: $P<0.05$).

3-5. Blood composition and endocrine hormone

Change in water temperature was measured for Far Eastern catfish, *Silurus asotus*, during the experimental time. Water temperature during the spawning season was 25±1.0°C, and during non-spawning season it was 13.5±4.64°C. The mean value of water temperature was 22.4±4.58°C (Fig. 10). Table 10 shows the result of two-way ANOVA test on gonadsomatic index (GSI), sex hormone, thyroid hormone, and hematological parameter between diploid and induced triploid Far Eastern catfish during the spawning and non-spawning seasons. Body weight (BW), subcutaneous fat weight/BW (FW), GSI, estradiol, testosterone, thyroid stimulating hormone, and thyroxine were shown to be significant between diploid and induced triploid. In addition, BW, FW, GSI, estradiol, and testosterone were shown to be significant between the periods of spawning season and non-spawning season.

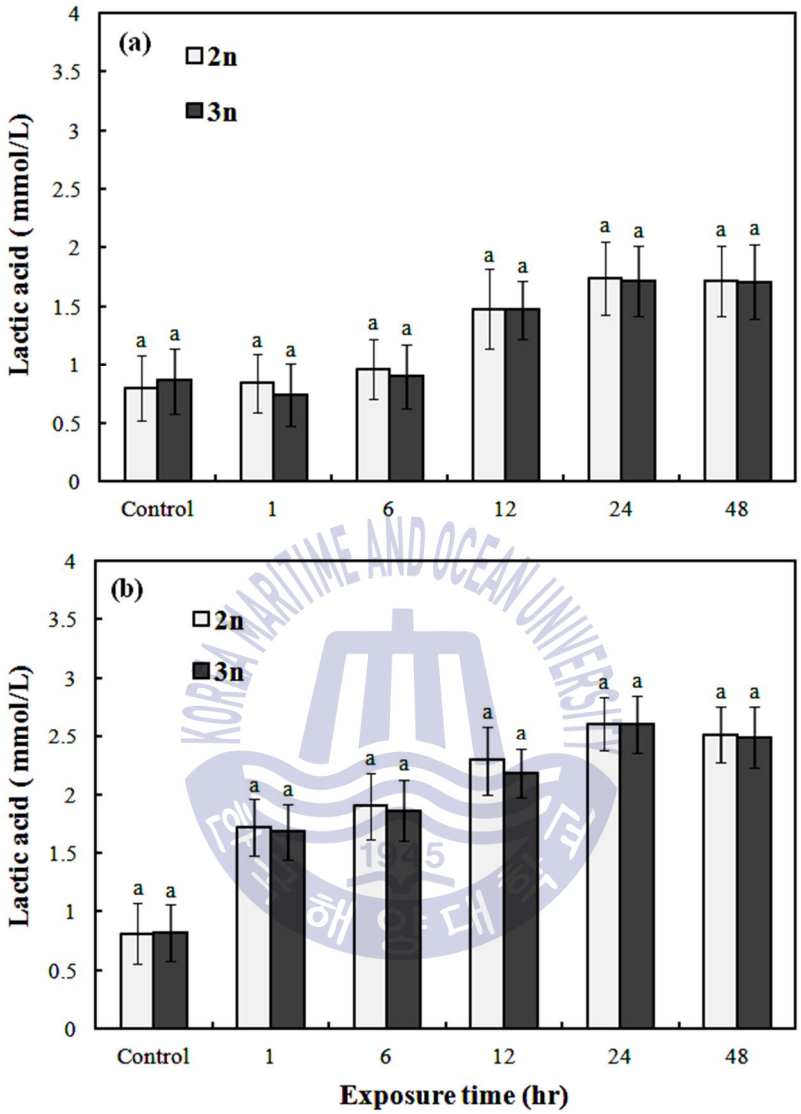


Fig. 9. Lactic acid concentration variations in blood plasma of diploid and induced triploid Far Eastern catfish, *Silurus asotus*, during 48 hrs of (a) high water temperature and (b) low water temperature stress period. Values represent means ± SE (n=30). Actually n=5 for each experiment because mean and SE are calculated separately for each group.

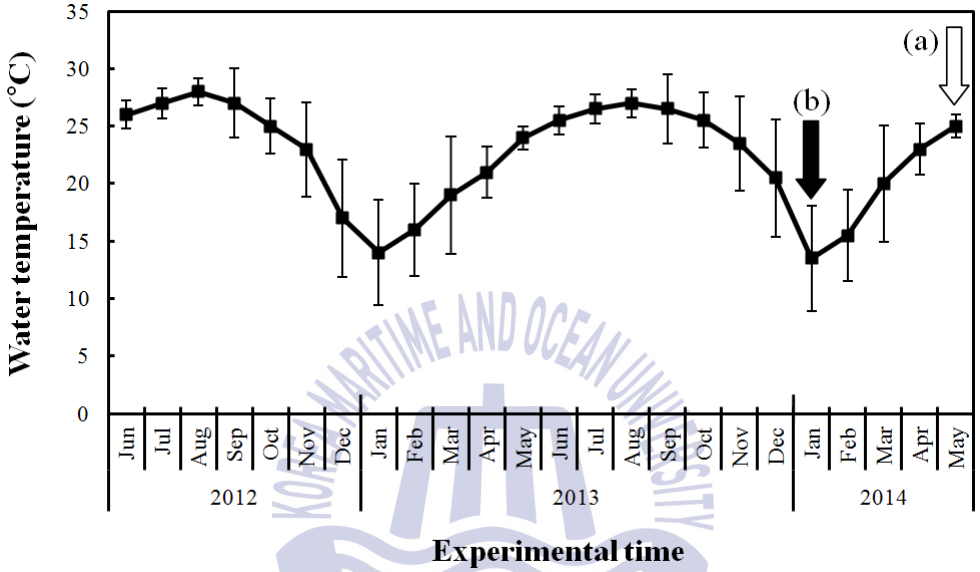


Fig. 10. Change of water temperature in Far Eastern catfish, *Silurus asotus*, while experimental time. Measured time of non-spawning season was January (a, white arrow) and measured time of spawning season was May (b, black arrow), 2014, respectively. The arrows on vertical bars are sampling time of diploid and induced triploid Far Eastern catfish. The values of vertical bars are standard deviation while 30 days of each month.

Table 10. Comparative analysis of gonadosomatic index (GSI), sex hormone and thyroid hormone between diploid and induced triploid Far Eastern catfish, *Silurus asotus*, in spawning and non-spawning season[1]

	Spawning[2]		Non-spawning[2]	
	2n	3n	2n	3n
Body weight (BW, g)	324.1±6.88^{b}	904.2±9.17^{d}	309.8±7.41^{a}	894.3±8.54^{c}
Subcutaneous fat weight/BW (%)	4.7±0.54^{a}	16.5±1.02^{c}	8.9±0.77^{b}	16.6±1.06^{c}
GSI (%)[3]	11.4±1.55^{c}	0.1±0.02^{a}	6.4±1.01^{b}	0.1±0.02^{a}
Estradiol (pg/L)	75.1±8.67^{c}	8.6±1.43^{a}	10.1±1.88^{b}	8.6±1.43^{a}
Testosterone (ng/L)	8.3±1.36^{c}	1.9±0.15^{a}	2.3±0.13^{b}	1.9±0.15^{a}
Thyroid stimulating hormone (μIU/L)	2.5±0.38^{a}	3.9±0.52^{b}	3.7±0.44^{b}	3.8±0.92^{b}
Thyroxine (μg/dL)	7.9±1.12^{a}	11.4±1.24^{b}	11.5±1.39^{b}	11.3±1.71^{b}

	Two-way ANOVA test		
	Ploidy	Season	Interaction
Body weight (BW, g)	P<0.05	P<0.05	P<0.05
Subcutaneous fat weight/BW (%)	P<0.03	P<0.04	P<0.04
GSI (%)[3]	P<0.04	P<0.05	P<0.05
Estradiol (pg/L)	P<0.04	P<0.04	P<0.14
Testosterone (ng/L)	P<0.05	P<0.04	P<0.11
Thyroid stimulating hormone (μIU/L)	P<0.01	P<0.08	P<0.10
Thyroxine (μg/dL)	P<0.01	P<0.06	P<0.12

[1]Values of each group were mean±standard error. Values in the same column not sharing common superscripts are significantly different among ploidy and season (P<0.05).
[2]All parameters of each group were measured in May, 2014 for spawning and January, 2014 for non-spwaning, respectively.
[3]Gonadosomatic index (GSI)=(gonad weight/body weight) x100.

Interactions between ploidy and seasons were shown in BW, FW, and GSI.

As shown in Table 10, GSI values of induced triploid Far Eastern catfish were less than those of diploid both spawning season and non-spawning season ($P<0.05$). In addition, gonads of induced triploid were sterility (Fig. 11), and GSI of induced triploid in spawning season and non-spawning season were not significantly different from each other ($P<0.05$). GSI of diploid in the spawning season were higher than that the non- spawning season ($P<0.05$). During non-spawning season, estradiol and testosterone were not different between diploid and induced triploid, but in spawning season, the values of two hormones in diploid were higher than those in induced triploid.

As shown in Table 10, estridiol and testosterone of diploid during the non- spawning season were shown to be higher compared to the spawning season ($P<0.05$). Testosterone and estradiol of induced triploid were not significantly different between the spawning season and non-spawning season, respectively ($P>0.05$). The difference in sex hormones was observed in diploid during the spawning season and the non-spawning season, but the difference was not observed in the induced triploid ($P<0.05$). Subcutaneous fat contents of induced triploid were shown to be much higher than those of diploid (Fig. 12).

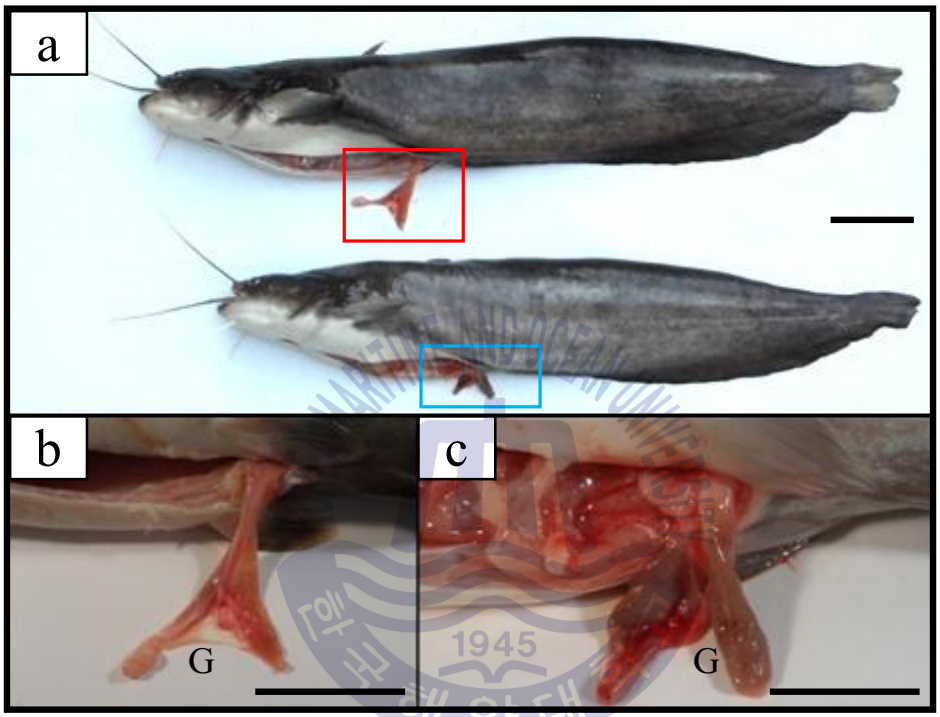


Fig. 11. External morphology of gonad in induced triploid Far Eastern catfish, *Silurus asotus*, in spawning season. a: external morphology and gonad of induced triploid; b: high power view of red line boxes in Fig. 24-a; c: high power view of blue line boxes in Fig. 24-a. Scale bars indicate 5 cm. G: gonad.

As shown in Table 11, erythrocyte count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were shown to be significant between diploid and induced triploid. All hematological parameters were not shown to be significant between the periods of spawning season and non-spawning season, and interaction was not shown between the ploidy and season. Erythrocyte count was reduced in induced triploid, and hematocrit of induced triploid was lower than diploid during spawning and non-spawning seasons. In spawning and non-spawning seasons, MCVs in the induced triploid was observed to be larger than the diploid, and MCH of induced triploid was larger than diploid during both seasons.

As shown in Fig. 13, the total cholesterol of induced triploid and diploid showed a significant difference during the spawning season, but there was no significant difference during the non-spawning season ($P<0.05$). H-cholesterol of induced triploid during the spawning season was shown to be higher, but diploid was higher during the non- spawning season ($P<0.05$). LDL-cholesterol of induced triploid during the spawning season was shown to be higher, but diploid was shown to be higher during the non- spawning season ($P<0.05$). Triglyceride of induced triploid was shown to be higher during both seasons ($P<0.05$).

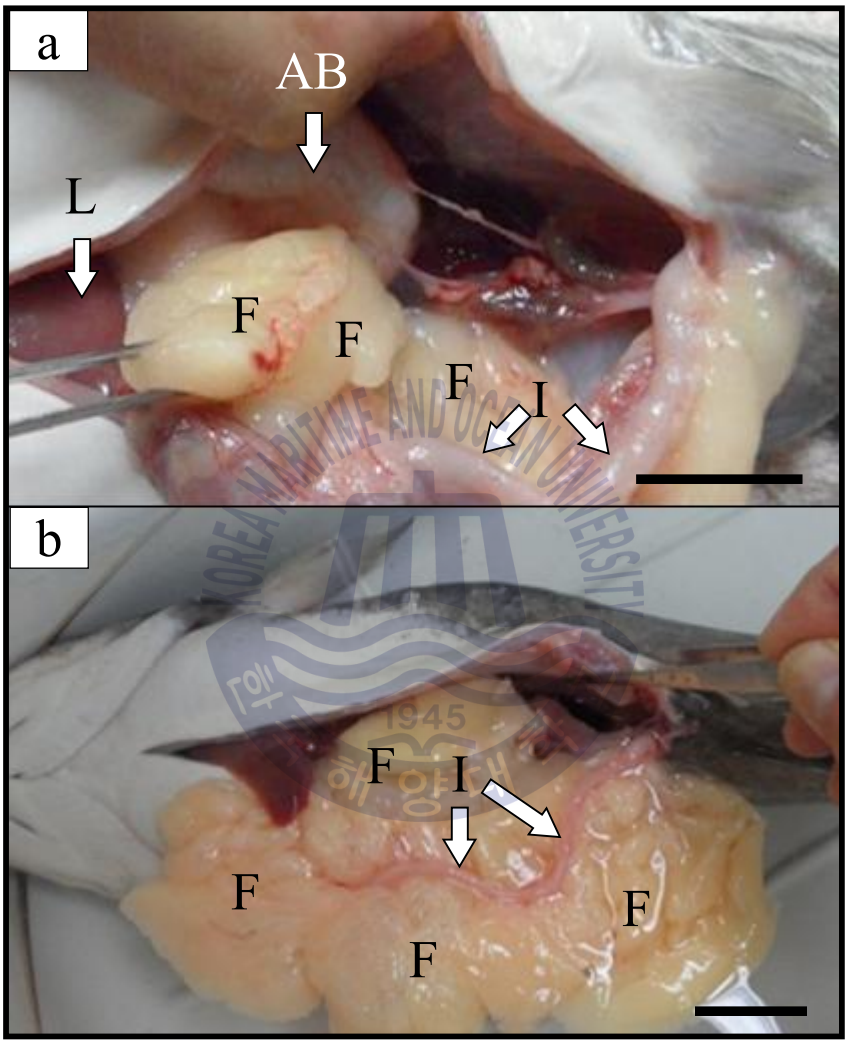


Fig. 12. Internal organs and subcutaneous fat of (a) diploid and (b) induced triploid Far Eastern catfish, *Silurus asotus*, in spawning season. Scale bars indicate 3 cm. AB: air bladder; F: subcutaneous fat; I: intestine; L: liver.

Table 11. Comparative analysis of hematological parameters between diploid and induced triploid Far Eastern catfish, *Silurus asotus*, in spawning and non-spawning season[1]

Hematological parameters	Spawning[2]		Non-spawning[2]	
	2n	3n	2n	3n
Erythrocyte count (cells/μL)	2.5±0.24^{b}	1.2±0.06^{a}	2.6±0.38^{b}	1.2±0.11^{a}
Hematocrit (%)	35.7±3.18^{b}	33.7±2.64^{a}	35.5±3.28^{b}	34.9±2.87^{ab}
Mean corpuscular volume (μm^3)	139.3±5.42^{a}	204.0±4.46^{b}	140.8±6.91^{a}	204.2±5.73^{b}
Total hemoglobin content (g/100 mL)	9.3±0.83^{a}	9.4±0.35^{a}	9.3±0.83^{a}	9.4±0.35^{a}
Mean corpuscular hemoglobin (pg)	36.5±2.98^{a}	55.5±0.67^{b}	37.1±3.69^{a}	54.9±1.87^{b}
Mean corpuscular hemoglobin concentration (%)	26.6±1.83^{a}	26.1±2.89^{a}	26.4±1.24^{a}	25.9±1.42^{a}

	Two-way ANOVA test		
	Ploidy	Season	Interaction
Erythrocyte count (cells/μL)	P<0.01	P<0.09	P<0.11
Hematocrit (%)	P<0.11	P<0.13	P<0.12
Mean corpuscular volume (μm^3)	P<0.03	P<0.14	P<0.10
Total hemoglobin content (g/100 mL)	P<0.09	P<0.10	P<0.14
Mean corpuscular hemoglobin (pg)	P<0.01	P<0.08	P<0.14
Mean corpuscular hemoglobin concentration (%)	P<0.08	P<0.09	P<0.20

[1]Values of each group were mean±standard error. Values in the same column not sharing common superscripts are significantly different among ploidy and season (P<0.05).

[2]All parameters of each group were measured in May, 2014 for spawning and January, 2014 for non-spwaning, respectively.

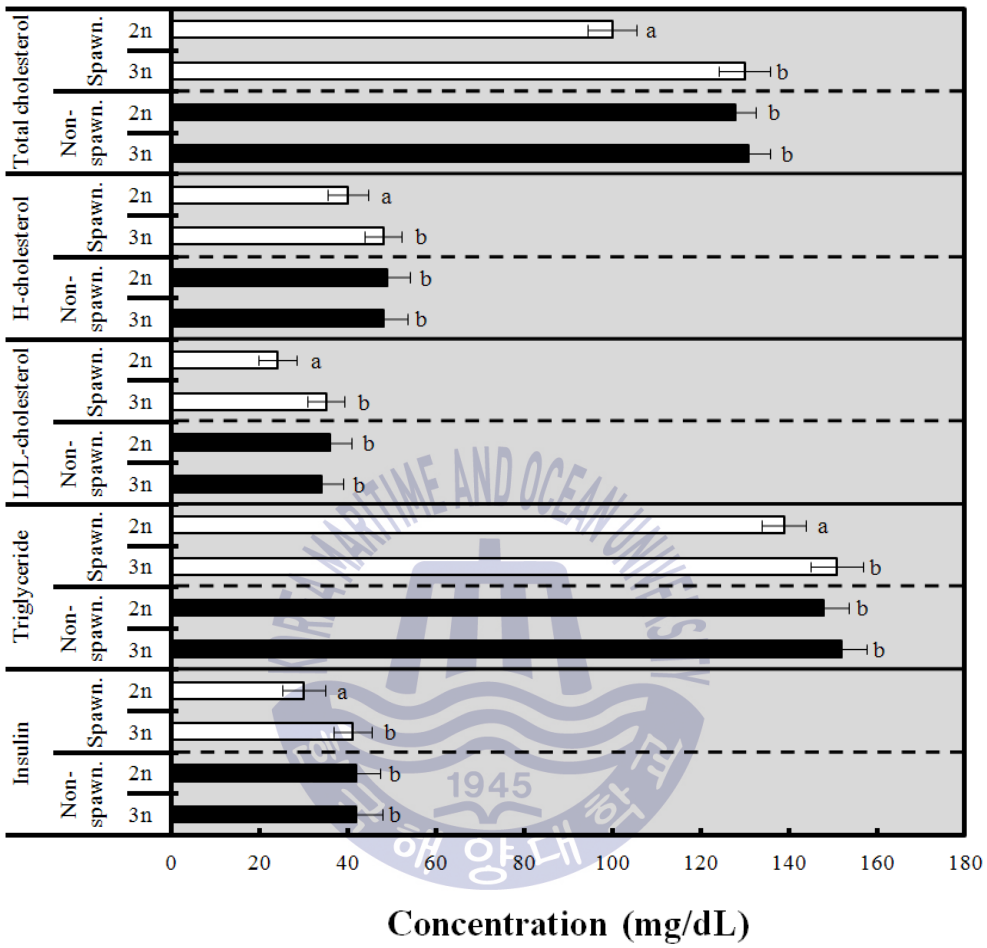


Fig. 13. Comparative analysis of total cholesterol, high cholesterol (H-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol), triglyceride and insulin concentrations between diploid and induced triploid Far Eastern catfish, *Silurus asotus*, in spawning (May) and non-spawning season (January) at 2014. The values of horizontal bars are means ± SD (n=30) in each group. Different letters on error bars are significantly different for each group (P<0.05).

Insulin was shown to be higher in induced triploid during the spawning season, but there was no significant difference between diploid and induced triploid during the non-spawning season (P<0.05).

3-6. Body composition

Table 12 shows the result of two-way ANOVA test on the body compositions of diploid and induced triploid Far Eastern catfish, *Silurus asotus*, during spawning and non-spawning season. Moisture and crude fat were shown to be significant between diploid and induced triploid, and all compositions were shown to be significant between the periods of spawning and non-spawning seasons. Moisture and crude fat showed interaction between ploidy and season. As shown in Table 12, moisture and crude fat of spawning season were significantly different between diploid and induced triploid, respectively (P<0.05), and moisture of non-spawning season was significantly different between diploid and induced triploid (P<0.05). Moisture, ash, and crude protein of diploid and induced triploid were significantly different between the spawning season and non-spawning season, respectively (P<0.05). Especially, the crude fat showed no significant difference between diploid and induced triploid, during the non-spawning season; but during the spawning season, crude fat of induced triploid was shown to be higher than that of the diploid (P<0.05).

Table 12. Comparative analysis of body compositions between diploid and induced triploid Far Eastern catfish, *Silurus asotus*, in spawning and non-spawning season[1]

Compositions	Spawning[2]		Non-spawning[2]	
	2n	3n	2n	3n
Moisture	73.4±2.64^{c}	71.6±6.22^{b}	71.8±1.54^{b}	69.8±1.36^{a}
Ash	3.3±1.03^{a}	3.6±2.05^{a}	5.4±1.10^{b}	5.4±1.19^{b}
Crude protein	18.5±2.00^{a}	8.9±0.85^{a}	7.1±1.45^{b}	8.7±1.37^{b}
Crude fat	4.9±1.93^{a}	16.7±3.06^{b}	15.8±1.57^{b}	16.1±0.73^{b}

	Two-way ANOVA test		
	Ploidy	Season	Interaction
Moisture	$P<0.04$	$P<0.02$	$P<0.05$
Ash	$P<0.06$	$P<0.05$	$P<0.11$
Crude protein	$P<0.08$	$P<0.05$	$P<0.12$
Crude fat	$P<0.02$	$P<0.05$	$P<0.05$

[1]Values of each group were mean±standard error. Values in the same column not sharing common superscripts are significantly different among ploidy and season ($P<0.05$).

[2]All parameters of each group were measured in May, 2014 for spawning and January, 2014 for non-spwaning, respectively.

Table 13 shows the significant difference in fatty acid between diploid and induced triploid Far Eastern catfish, during the spawning and non-spawning seasons. Fatty acid 14:0, palmitic acid (16:0), stearic acid (18:0), 22:0 and total saturated fatty acids (total SFA) were shown to be significant between diploid and induced triploid; and 14:0, 16:0, 18:0, and 20:0 were shown to be significant between the spawning and non-spawning seasons, respectively. The 14:0, 16:0, and 18:0 showed interaction between ploidy and season. The 16:1n-7, 18:1n-9, and total mono unsaturated fatty acids (total MUFA) showed significant difference between diploid and induced triploid. The 18:1n-9 and MUFA showed significant difference between the spawning and non-spawning seasons, and interaction was shown between ploidy and season, respectively. Total n-6 polyunsaturated fatty acids (total n-6 PUFA), 22:5n-3, 22:6n-3 and total n-3 polyunsaturated fatty acids (total n-3 PUFA) showed significant difference between diploid and induced triploid, respectively. The 18:2n-6, total n-6 PUFA, 22:5n-3, 22:6n-3 and total n-3 PUFA showed significant difference between the spawning and non-spawning seasons, and interaction was shown between ploidy and season, respectively.

Table 13. Significances of fatty acid between diploid and induced triploid Far Eastern catfish, *Silurus asotus*, in spawning and non-spawning season

Fatty acid	Two-way ANOVA test		
	Ploidy	Season	Interaction
C14:0	*P*<0.01	*P*<0.03	*P*<0.05
C16:0	*P*<0.01	*P*<0.02	*P*<0.04
C18:0	*P*<0.02	*P*<0.02	*P*<0.05
C20:0	*P*<0.11	*P*<0.05	*P*<0.13
C22:0	*P*<0.05	*P*<0.13	*P*<0.08
C24:0	*P*<0.11	*P*<0.09	*P*<0.13
Total sat.[1]	*P*<0.04	*P*<0.09	*P*<0.07
C16:1n-7	*P*<0.05	*P*<0.06	*P*<0.07
C18:1n-9	*P*<0.05	*P*<0.05	*P*<0.05
C18:1n-7	*P*<0.08	*P*<0.09	*P*<0.09
C20:1n-9	*P*<0.11	*P*<0.12	*P*<0.22
C22:1n-9	*P*<0.10	*P*<0.09	*P*<0.22
C24:1n-9	*P*<0.16	*P*<0.17	*P*<0.14
Total mono.[2]	*P*<0.02	*P*<0.04	*P*<0.05
C18:2n-6	*P*<0.01	*P*<0.02	*P*<0.04
C18:3n-6	*P*<0.12	*P*<0.11	*P*<0.12
C20:2n-6	*P*<0.12	*P*<0.12	*P*<0.13
C20:3n-6	*P*<0.15	*P*<0.16	*P*<0.14
C20:4n-6	*P*<0.13	*P*<0.16	*P*<0.19
C22:2n-6	*P*<0.10	*P*<0.11	*P*<0.15
C22:4n-6	*P*<0.10	*P*<0.10	*P*<0.17
C22:5n-6	-	-	-
Total n-6[3]	*P*<0.05	*P*<0.05	*P*<0.05
C18:3n-3	*P*<0.11	*P*<0.13	*P*<0.11
C20:3n-3	*P*<0.12	*P*<0.11	*P*<0.13
C20:5n-3	*P*<0.12	*P*<0.13	*P*<0.14
C22:5n-3	*P*<0.01	*P*<0.04	*P*<0.05
C22:6n-3	*P*<0.01	*P*<0.02	*P*<0.04
Total n-3[4]	*P*<0.01	*P*<0.02	*P*<0.03

[1]Total saturated fatty acids.
[2]Total monounsaturated fatty acids.
[3]Total n-6 polyunsaturated fatty acids.
[4]Total n-3 polyunsaturated fatty acids.

Spawning and non-spawning variations by ploidy in flesh fatty acid composition are shown in Table 14. In general, the fatty acid profiles exhibited notable similarities in both spawning and non-spawning Far Eastern catfish. In the case of spawning, there were significant differences in percentages of total SFA, total n-6 PUFA and total n-3 PUFA between diploid and induced triploid (P<0.05). SFA was mainly comprised of 16:0 and 18:0. Induced triploid showed higher percentages of 16:0 and 18:0, compared to diploid. The most commonly occurring MUFA were 16:1n-7 and 18:1n-9. The percentage of 18:1n-9 was lower while the percentage of 16:1n-7 was higher in induced triploid (P<0.05). Among PUFA, induced triploid showed lower percentage of 18:2n-6 and higher percentages of 22:5n-3 and 22:6n-3 (P<0.05). In the case of non-spawning, there were significant differences in the percentages of total SFA, total MUFA, total n-6 PUFA and total n-3 PUFA between diploid and induced triploid (P<0.05). However in spawning, induced triploid showed lower percentages of 16:0 and 18:0 and higher percentage of 18:1n-9, compared to diploid (P<0.05). Among PUFA, induced triploid showed higher percentage of 22:5n-3 and lower percentage of 22:6n-3 (P<0.05).

Table 14. Comparative analysis of fatty acid between diploid and induced triploid Far Eastern catfish, *Silurus asotus*, in spawning and non-spawning season[1]

Fatty acid	Spawning[2]		Non-spawning[2]	
	2n	3n	2n	3n
C14:0	3.2±0.21^{a}	3.6±0.41^{a}	4.3±0.63^{b}	5.2±0.30^{c}
C16:0	18.4±0.49^{b}	16.2±0.79^{a}	24.2±0.97^{d}	21.3±0.77^{c}
C18:0	8.8±2.15^{b}	11.7±0.07^{c}	7.9±0.58^{b}	6.3±0.18^{a}
C20:0	4.1±0.12^{b}	4.3±0.12^{b}	2.8±0.90^{a}	2.8±0.27^{a}
C22:0	0.8±0.02^{a}	2.5±0.25^{b}	0.5±0.38^{a}	0.9±0.13^{a}
C24:0	0.6±0.03^{a}	0.4±0.10^{a}	0.5±0.32^{a}	0.7±0.03^{a}
Total sat.[3]	35.8±1.83^{a}	38.7±1.05^{b}	40.2±1.58^{c}	37.2±0.88^{b}
C16:1n-7	7.9±0.38^{a}	12.1±3.37^{b}	8.2±0.42^{a}	8.3±0.27^{a}
C18:1n-9	23.7±1.37^{c}	15.0±1.23^{a}	15.2±4.34^{a}	19.6±0.87^{b}
C18:1n-7	4.0±0.27^{b}	3.0±0.33^{a}	4.0±0.77^{b}	4.3±0.14^{b}
C20:1n-9	0.2±0.01^{a}	0.3±0.01^{a}	0.2±0.11^{a}	0.2±0.01^{a}
C22:1n-9	0.4±0.01^{a}	1.3±0.95^{b}	0.2±0.09^{a}	0.4±0.02^{a}
C24:1n-9	0.1±0.01^{a}	0	0.1±0.05^{a}	0.1±0.00^{a}
Total mono.[4]	36.3±1.58^{c}	31.7±0.88^{b}	27.9±4.64^{a}	32.8±1.17^{b}
C18:2n-6	11.6±0.28^{b}	8.4±0.07^{a}	10.5±1.13^{b}	10.3±0.50^{b}
C18:3n-6	0.4±0.26^{a}	0.2±0.01^{a}	0.2±0.15^{a}	0.2±0.01^{a}
C20:2n-6	0.4±0.11^{a}	0.3±0.01^{a}	0.2±0.11^{a}	0.2±0.01^{a}
C20:3n-6	0.8±0.06^{a}	0.6±0.01^{a}	0.4±0.23^{a}	0.7±0.01^{a}
C20:4n-6	0.8±0.22^{a}	0.4±0.02^{a}	0.2±0.21^{a}	0.4±0.01^{a}
C22:2n-6	0.1±0.03^{a}	0.5±0.04^{a}	0.2±0.12^{a}	0.2±0.07^{a}
C22:4n-6	0.2±0.02^{a}	0.1±0.01^{a}	0.2±0.12^{a}	0.3±0.04^{a}
C22:5n-6	0	0	0	0
Total n-6[5]	14.3±0.47^{c}	10.5±0.06^{a}	11.9±1.29^{ab}	12.3±0.54^{b}
C18:3n-3	1.2±0.04^{a}	1.2±0.04^{a}	1.0±0.52^{a}	1.5±0.10^{a}
C20:3n-3	0.8±0.06^{a}	0	0.1±0.06^{a}	0.4±0.20^{a}
C20:5n-3	0.4±0.01^{a}	0.9±0.02^{a}	0.2±0.15^{a}	0.6±0.01^{a}
C22:5n-3	2.1±0.11^{ab}	3.1±0.01^{c}	1.8±1.02^{a}	2.4±0.13^{b}
C22:6n-3	9.0±0.62^{a}	13.3±0.47^{b}	16.6±1.43^{c}	12.8±0.64^{b}
Total n-3[6]	13.5±0.81^{a}	18.6±0.41^{c}	19.8±3.08^{c}	17.6±0.62^{b}

[1]Values of each group were mean ± SE. Values in the same column not sharing common superscripts are significantly different among ploidy and season (P<0.05).
[2]All parameters of each group were measured in May, 2014 for spawning and January, 2014 for non-spwaning, respectively.
[3]Total saturated fatty acids.
[4]Total monounsaturated fatty acids.
[5]Total n-6 polyunsaturated fatty acids.
[6]Total n-3 polyunsaturated fatty acids.

3-7. Occurrence of amitotic erythrocyte

The types of atypical cell were determined three types in induced triploid Far Eastern catfish, *Silurus asotus*, asymmetric division, irregular-shaped and absence of nucleus (Figs. 14a, 14b, 13c and 14d). Asymmetric division type means that nucleus of erythrocyte divided asymmetrically (Fig. 14b). Irregular-shaped type means that nucleus was built irregularly, and irregular-shaped nuclei were shown variously (Fig. 14c). Absence of nucleus type means that nucleus was not observed in erythrocyte (Fig. 14d). The atypical cell samples of Far eastern catfish were shown in Table 15. Cell number of asymmetric division in induced triploid was more than those in diploid. In addition, cell numbers of irregular-shaped and absence of nucleus in induced triploid were higher than those in diploid. The first result as cell number of asymmetric division in induced triploid had a about 4 times more than those in diploid, and absence of nucleus is about 4.5 times differential between diploid and induced triploid. The differential of irregular shaped cell's number between diploid and induced triploid is the highest among the three types; about 5.5 times. Therefore, the numbers of atypical cells in induced triploid Far Eastern catfish were approximately 5 fold than those of diploid. The difference of cell number among three types was no significance in diploid ($P>0.05$), but it was significance in induced triploid ($P<0.05$).

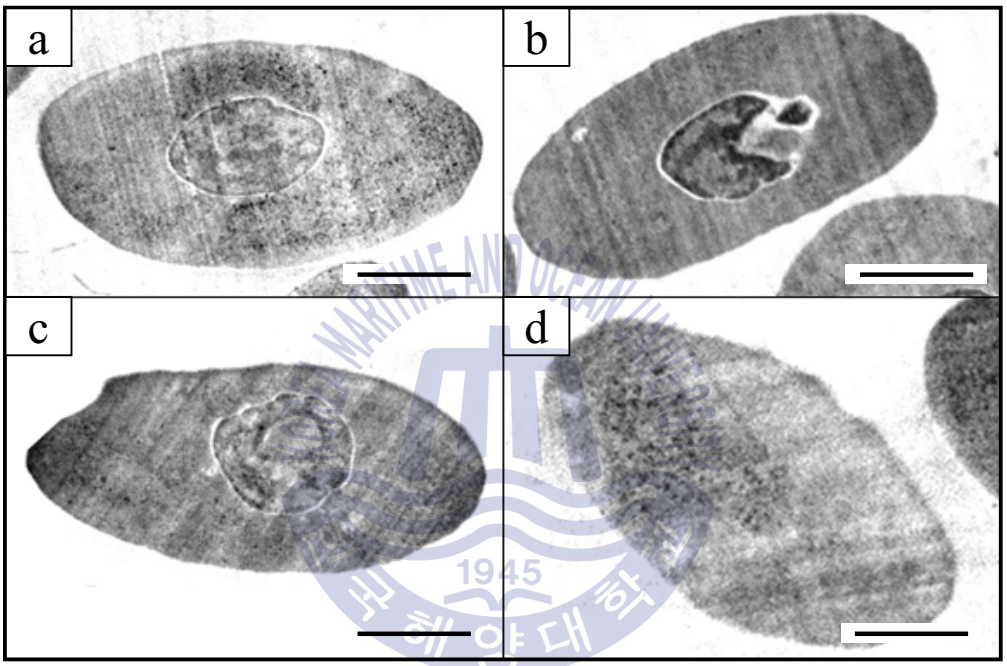


Fig. 14. Transmission electron micrograph of amitotic nucleus in erythrocyte of induced triploid Far Eastern catfish, *Silurus asotus*. Scale bars indicate 5 μm. a: normal; b: asymmetric division of nucleus; c: irregular-shaped; d: absence for nucleus.

Table 15. The quantity of different types of abnormal nucleus among 100 erythrocytes of induced triploid Far Eastern catfish, *Silurus asotus*[1]

Samples	Abnormal types of erythrocyte's nucleus						Total number of atypical cells	
	Asymmetric division		Irregular-shaped		Absence of nucleus			
	2n	3n	2n	3n	2n	3n	2n	3n
1	1	5	1	6	1	7	3	6
2	2	6	2	7	1	6	5	5
3	1	4	1	5	1	5	3	6
4	3	8	1	8	2	8	6	6
5	1	10	3	9	1	9	5	8
6	1	5	1	10	1	6	3	7
7	2	7	2	6	3	5	7	5
8	3	6	1	8	1	6	5	5
9	1	8	1	9	2	10	4	10
10	1	9	1	10	1	6	3	6
Average	1.6^{a}	6.8^{b}	1.4^{a}	7.8^{b}	1.4^{a}	6.8^{b}	4.4^{a}	21.4^{b}
Standard deviation	0.84	1.93	0.70	1.75	0.71	1.69	1.43	4.48

[1]According to Wang *et al.* (2010). Means in rows with the different superscript letter are significantly different ($P < 0.05$).

In induced triploid, the cell numbers of absence of nucleus were lowest (P<0.05), and the numbers of irregular-shaped cell were highest in three types (P<0.05).

In the shape of blood of induced triploids, the types of atypical cell in Far Eastern catfish were shown that shape of nucleus was transformed abnormally. In Far Eastern catfish, the numbers of atypical cells in induced triploid were higher than those of diploid. The absence of nucleus in diploid and induced triploid were shown in Far Eastern catfish, and this type in diploid samples was the lowest among three types in this species (P<0.05).

4. Discussion

There are a number of techniques for separating diploid and induced triploid Far Eastern catfish, *Silurus asotus*. The three most widely accepted methods now used are flow cytometric measurements of erythrocyte DNA (Thorgaard *et al.*, 1982), Coulter counter estimation of erythrocyte nuclear size (Thorgaard *et al.*, 1982), and measurement of erythrocyte nuclear volume by light microscopy (Wolters *et al.*, 1982). Unfortunately, the first two methods are very expensive, and light microscopy is time-consuming and insufficiently accurate for widespread use. Clearly, any method that would be as accurate as, more rapid, and less costly than these three techniques would be helpful to Far Eastern catfish culturists. So, flowcytometry was used in this study for determine success of induced triploid's induction and cell cycle of diploid and induced triploid.

The most common application of flowcytometric techniques related to the cell cycle is the determination of the fraction of cells in the gap 1 (G_1)-, synthesis (S)-, and gap 2 (G_2) + mitosis (M)-phases (Dean and Jett, 1974). This information is obtained from DNA distributions. In each distribution, the peak at ×1 DNA content (relative fluorescence, 50) is produced by diploid, G_1-phase cells. The peak at ×2 DNA content (relative fluorescence, 100) is produced by G_2+M phase cells, and the intermediate continuum is produced by S-phase cells in which varying amounts of DNA have replicated.

The areas under each of these regions of DNA distribution are proportional to the fractions of cells in the corresponding cell cycle phase (Dean and Jett, 1974). As mentioned Xoana *et al*. (2012), cyclin D1 and cyclin E are related to cell division and DNA synthesis. If protein expressions of cyclin D1 and cyclin E are higher, then cell cycle is shorter. In this study, proteins expressions of both cyclin in diploid were higher than those in induced triploid, and cell cycle of diploid was shorter than that of induced triploid.

Phase fraction analysis can be used in the assessment of cellular growth conditions (Dean and Jett, 1974). Numerous studies on growth rates of induced triploid fish have been published. Increased cell size does not appear to confer any growth advantage to induced triploids, due to the concomitant decrease in cell numbers. The rate of muscle fiber growth does not differ between induced triploids and diploids, whether juvenile or adult (Yamashita, 1993). Reduced gonadal growth in induced triploids may allow increased energy allocation to somatic growth, but any growth advantage may be offset by diminished levels of gonadal steroids, which have an anabolic effect (Benfey, 1999).

Seol *et al.* (2008) reported that haematological parameters and respiratory function in diploid and induced triploid of the Far Eastern catfish. The results of Seol *et al.* (2008) showed an increase in erythrocyte size in induced triploids, in agreement with the previously reported increase in the

cell volumes of polyploidy animals (Benfey, 1999). In teleost fish, the increase in erythrocyte size associated with induced triploidy has already been reported and the measurment of red blood cell dimensions was proposed as a rapid and inexpensive assay for induced triploidy (Ueno, 1984; Benfey, 1999). The increase in erythrocyte nuclear size in induced triploids is a consequence of their higher DNA content (Benfey, 1999).

As mentioned Seol *et al.* (2008), the haematocrit value, total haemoglobin content, and mean corpuscular haemoglobin concentration were not significantly different between diploid and induced triploid Far Eastern catfish, but the erythrocyte size, erythrocyte count, mean corpuscular volume, and mean corpuscular haemoglobin were increased in induced 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 induced triploid Far Eastern catfish, as reported in other fish species (Benfey, 1999; Seol *et al.*, 2008). The relationship between oxygen consumption and respiratory frequency was higher in induced triploids than in diploids, although diploid and induced triploid Far Eastern catfish showed similar oxygen consumption (Seol *et al.*, 2008). Therefore, the lower oxygen capability of induced triploid is in agreement with the haematological characteristics of induced triploid Far Eastern catfish (Seol *et al.*, 2008).

In this study, mitosis of diploid in each tissue was more active than those of induced triploid Far Eastern catfish in each tissue, and mitosis of gill tissue in each ploidy was more active than those of tail fin tissue in each ploidy. Merely, diploid and induced triploid Far Eastern catfish using this experiment was 3 years after hatched, and the measured time was spawning season, when diploid had matured gonad but induced triploid had maintained sterility of a gonad. Therefore, diploid had a higher metabolism and respiratory function than induced triploid (Seol *et al.*, 2008).

Our analysis of the retinal tissue provided similar structural characteristics to that of carps (Takashi, 1982; Park and Kim, 2000). The outer nuclear layer of the retina consisted of three strata of nuclei in diploids and two strata in induced triploid fish. Consequently the diploid nuclear layers were thicker. It is generally accepted that nuclear size increases in proportion with chromosome number (Aliah *et al.*, 1990). The number of nuclei in diploid visual cells (cells of pigment epithelium and bacillary layer) are therefore larger resulting in higher cell density. This fact suggests that diploids possess higher acuity of vision than induced triploids. This phenomenon has also been observed in induced triploid ayu, *Plecoglossus altivelis*, and induced triploid hybrid between mud loach, *Misgurnus mizolepis*, and cyprinid loach, *M. anguillicaudatus* (Park and Kim, 2000).

Swarup (1959) reported that the number of cells of the pronephric duct

of the three-spined stickleback, *Gasterosteus aculeatus*, was 26 in the diploid and 18 in the induced triploid, while Aliah *et al.* (1990) reported that the secondary proximal tubule of the trunk kidney in ayu contained 9 nuclei in diploids and 6 nuclei in induced triploids. In this study the size of secondary proximal tubule of trunk kidney was 15.7 ± 2.08 nuclei in diploids and 9.8 ± 1.11 nuclei in induced triploids, proving to be similar to previous studies (Aliah *et al.*, 1990; Park and Kim, 2000). Our investigations also revealed the hepatocyte nuclear area and height of midgut epithelium to be larger in induced triploids than in diploid indicating larger chromosome numbers. Nevertheless, the reason why this condition doesn't cause giantism is due to the inevitable tradeoff where larger cell size results in lower total cell numbers (Ueno, 1984; Aliah *et al.*, 1990; Park and Kim, 2000).

A consequence of induced triploidy is the increase of nuclear size because of the higher number of chromosomes. In addition, the maintenance of the nucleo-cytoplasmic ratio implies that, in induced triploids, the cells of most of the organs (brain, retina, kidney, liver, testis, ovaries) and tissues (blood, cartilages, muscles, epithelia) are larger than those of their diploid counterparts (Benfey, 1999). On the other hand, the organs and tissues of induced triploid individuals appear to have a reduced number of cells compared with diploids so that the entire size remains alike that of a diploid fish (Tiwary *et al.*, 2004; Maxime, 2008). The induced triploid and diploid

characteristics identified in this study are identical to other similar comparative studies (Aliah *et al.*, 1990; Park and Kim, 2000).

The classical dimensions for which diploid and induced triploid 1-year-old Far Eastern catfish showed significant differences were HALOP/*L*s and ED/*L*s; those for 3-years-old Far Eastern catfish were DALAD/*L*s, HALAV/*L*s, DAUF/HL, and DAUS/HL. For more than 30 years, most morphometric investigations of fish have based character selection on the classical dimensions of length, depth, and width, primarily in the head and tail regions, as described by Hubbs and Lagler (1947). These dimensions are concentrated along the anterior-posterior body axis and the head and caudal regions and, therefore, produce uneven and biased coverage of the body form (Li *et al.*, 1993).

Truss dimensions for which diploid and induced triploid 1-year-old Far Eastern catfish showed significant differences were HALOP and BDAA/*L*s; that for 3-years-old Far Eastern catfish was IW/HL. The truss dimension consists of a systematically arranged set of distances measured among a set of preselected anatomical landmarks, which are points identified on the basis of local morphological features and chosen to divide the body into functional units (Strauss and Bond, 1990). These dimensions, which include components of body depth and length along the longitudinal axis of the fish, have theoretical advantages over classical morphometric characteristics in

discriminating among groups (Humphries *et al.*, 1981; Strauss and Bookstein, 1982; Winans, 1984; Currens *et al.*, 1989). Both truss and classical dimensions are used to describe fish body shape (Hubbs and Lagler, 1947; Strauss and Bookstein, 1982; Park *et al.*, 2001). Currens *et al.* (1989), studying the body shapes of starved chinook salmon, *Oncorhynchus tshawytscha*, and rainbow trout, *O. mykiss*, using both truss and classical dimensions, pointed out that the depth of the trunk region was most affected and the caudal region least affected. Therefore, measurements of the caudal region of fish are more useful for understanding interspecific variation than are measurements of the trunk region.

Differences between diploid and induced triploid fish 1 year after hatching were in the head region, body depth, and eye diameter; significant variables 1 year after hatching were HALOP/*L*s, BDAA/*L*s, and ED/*L*s. That is, induced triploid fish had longer head regions and body depths as well as smaller eye diameters than diploid fish. However, differences between diploid and induced triploid fish 3 years after hatching differed from those 1 year after hatching. Because the most significant variables 3 years after hatching were DALAD/*L*s, HALAV/*L*s, ManBL/*L*s, DAUF/HL, DAUS/HL, and IW/HL, differences between diploid and induced triploid fish 3 years after hatching were in the head region, mandible barbel length, distance between upper lip and nostrils, and distance between eyes.

Bonar *et al.* (1988) evaluated the separation of induced triploid and diploid grass carp, *Ctenopharyngodon idella*, by external morphology, using classification functions of body depth, gape width, and cheek height. The separation rate was only 65% correct. In the present study, the separation rate was 85% correct. In an attempt to further increase the accuracy of separation, the lowest discriminant coefficient (DALAD) among the most significant variables was excluded; however, this did not improve the accuracy of separation.

The trends in plasma cortisol, plasma glucose and lactic acid concentrations of diploid and induced triploid Far Eastern catfish observed in this experiment are indicative of stressed reactions. Plasma cortisol and plasma glucose are recognized as useful indicators of stress in fish (Schreck, 1982). As expected trends in plasma cortisol levels increased significantly at the beginning of a chronic stress situation (Barton and Iwama, 1991) but then declined back to initial values thereafter (Pickering and Stewart, 1984; Tort *et al.*, 1996). Our results show that plasma cortisol level increases faster than glucose concentrations. This result was similar to a study carried out by Chang and Hur (1999) and Park *et al.* (2008a, 2009).

Plasma cortisol and glucose levels in red drum, *Sciaenops ocellatus*, simultaneously exposed to MS-222 and quinaldine anesthetic, were reported to be elevated (Massee *et al.*, 1995). Barton and Iwama (1991) stated that

“Usually, phenomenon that plasma cortisol concentration of fishes rises by stress is first order reaction, phenomenon that plasma glucose concentration rises is result of second-order first order reaction by hormone rise reaction by stress.”. This trend has been reported in the gray mullet, *Mugil cephalus*, and kelp grouper, *Epinephelus bruneus* (Chang and Hur, 1999; Park *et al.*, 2008a).

Das *et al.* (2004) suggested that the greater use of glucose for increased cell metabolism during early exposure may have overwhelmed the increase in blood glucose, even though glycogenolysis would have increased during this period (Martinez-Alvarez *et al.*, 2002). However, because of dysfunctional cell metabolism the lower use of glucose later in the exposure time (after 48 hrs) resulted in an increase in blood glucose levels.

One of the more traditional stress indicators has been blood lactic acid (Pickering and Pottinger, 1989). If experimental animal was added to chronic stress, then result of lactic acid concentration is high (Wedemeyer *et al.*, 1990). The accumulation of lactic acid in muscle or blood (hyperlacticemia) is now well accepted as an indicator of anaerobic metabolism due to fright or severe exertion (Turner *et al.*, 1983). However, the view that lactic acidosis is the ultimate cause of death that sometimes occurs after severe exercise has been challenged (Wood *et al.*, 1983).

The only controlled experiment to have assessed physiological aspects

of the stress response in induced triploid fish is that of Biron and Benfey (1994), who found no difference between induced triploids and diploids in hematocrit and plasma cortisol and glucose profiles after an acute handling stress. In light of abundant anecdotal information that induced triploids do not cope well with poor water quality, a common source of chronic stress in aquaculture, detailed study of the response of induced triploids to chronic stress is warranted. Poorer survival due to chronic stress may be reflected in reduced energy stores and/or increased rates of depletion of these stores during stressful conditions. Although substrate utilization during aerobic metabolism does not differ between induced triploids and diploids, it may be that induced triploids differ in their ability to withstand sustained anaerobic metabolism (Ojolick *et al.*, 1995).

As shown in Table 19, induced triploid showed increased haploid and enhanced cell size, but the cell number was decreased; and induced triploid did not increase in body size. Kim *et al*. (2001) and Seol *et al*. (2008) suggested that giantism of induced triploid did not appear due to the decreasing cell number of induced triploid. In a previous study by Seol *et al*. (2008), erythrocyte count of diploid was higher than the induced triploid number. In the case of induced triploid, the nucleus of the red blood cell has major axis which is 1.33 times larger and the minor axis is 1.26 times larger than the diploid (Seol *et al*., 2008). This fact is well accepted, and the

measure of erythrocyte size is frequently used as the sole criterion for determining ploidy level in a fish (Benfey, 1999). Ojolick *et al*. (1995) clearly showed that induced triploid rainbow trout, *Oncorhynchus mykiss*, has increased mortality rate, compared to diploids, when reared at chronic high temperatures where oxygen solubility is reduced and oxygen demand is increased.

The followings were observed: initial survival of freshwater fish, transformation, and the effect of thyroxine on the development and growth (Donaldson *et al.*, 1979; Lam and Sharma, 1985; Weatherley and Gill, 1987). Thyroxine was observed to be higher in induced triploid during the spawning season ($P<0.05$). Induced triploids can be expected to show better growth compared to the diploid. In diploid, thyroxine is increased during non-spawning season than during the spawning season.

Shirai *et al*. (2001) investigated the influence of spawning and season on lipid content, lipid classes, and fatty acid composition, and found that the percentages of 22:6n-3 in pohosphatidylchline and phosphatidyl ethanolamine from ovary were higher during spawning than after spawning seasons; meanwhile, 20:5n-3 and 22:6n-3 percentages in ovarian total lipids were similar. Huynh *et al*. (2007) reported that spawning herring also had significantly higher PUFA content in the organ tissues, particularly in the milt and ovary, and notable DHA having the greatest proportion. Perez *et al*.

(2007) suggested that major fatty acids (16:0, 18:1n-9, 20:5n-3 and 22:6n-3), in gonadal and muscular polar, and neutral lipid in both males and females were increased during pre-spawning to mid-spawning, and declined thereafter. PUFA are necessary for the homeostasis in fish.

Induced triploid's total fatty acid level was higher than diploid, and induced triploid at low temperature showed the highest fatty acid levels (Qin *et al*., 1998). In Table 22, total SFA of induced triploid was shown to be higher than diploid, during the spawning season; but during the non-spawning season, diploid appeared to be higher ($P<0.05$). Total MUFA of diploid was shown to be higher than induced triploid during the spawning season, but during the non-spawning season, induced triploid was shown to be higher ($P<0.05$). Total n-6 PUFA of diploid was shown to be higher than induced triploid during the spawning season, but during the non-spawning season, induced triploid was shown to be higher ($P<0.05$). Total n-3 PUFA of induced triploid was shown to be higher than diploid during the spawning season, but during the non-spawning season, diploid was shown to be higher ($P<0.05$).

As it was mentioned by Nobuya *et al*. (2001), the lipid contents of Far Eastern catfish's ovary during the spawning season were shown to be higher than those during the non-spawning season. In our study, the total lipid acids of induced triploid were shown to be higher than that of the induced triploid,

during spawning season. Lipid source influences the growth and body composition of Far Eastern catfish (Kim *et al.*, 2010). Therefore, higher content of fatty acid in induced triploid will induce higher growth rate of induced triploid. This study revealed the increase in fatty acids of induced triploid, by sterilization in induced triploid.

In this experiment, occurrence of atypical cells by induction of triploid was observed in Far Eastern catfish. It is generally believed that erythrocytes in fish are terminally differentiated and can no longer undergo any further division. The peripheral blood of fish, however, possesses erythroblasts, which may have the ability to undergo mitosis (Wang *et al.*, 2010). If the cells with multiple nuclei are in the process of mitosis, chromatin condensation should take place in cells and the electron density of the nuclei should increase. The other events in the process of mitosis, such as pairing of homologous chromosomes, would also occur, which could be observed histologically. In animals, amitosis may occur in the epithelium, connective tissue, muscle and liver (Shi *et al.*, 2000), but in amphibians, amitosis occurs widely in erythrocytes (Hu *et al.*, 2005; Wang *et al.*, 2010).

The type of irregular-shaped nucleus in induced triploid Far Eastern catfish was the highest in all groups of this experiment. This type of nuclear division was also found in other polyploid fish species (Zhou *et al.*, 2002). Although some researchers reported that multiple atypical cells seldom

appeared in diploids and tetraploids (Gao *et al.*, 2007), some others showed that the percentage of atypical erythrocytes increased with the increase in ploidy level (Han *et al.*, 2007).

Some cell nuclei in induced triploid fish were constricted at the middle or towards one side, which resulted in nuclear membrane invagination. Lengthy evolution has allowed the tension of the membrane to adapt to the volume of the diploid nucleus, which could lead to the division of polyploid nuclei to recovering their inherent ploidy (Wang *et al.*, 2010). For example, the hybrid of the pentaploid crucian carp, *Carassius carassius*, and blunt snout bream, *Megalobrama amblycephala*, showed triple nucleated erythrocytes (Liu *et al.*, 2007).

Structural changes in induced triploid erythrocytes may disrupt some of their functions such as the ability of gas transportation (Johari *et al.*, 2008). Because of entirely the same nutritional and environmental conditions in Johari *et al.* (2008), it is hypothesized that the increase of abnormalities in induced triploid erythrocytes may not be due to these conditions. These changes have also been reported in induced triploid brook trout produced by hydrostatic pressure shock and thus shock technique can't make these abnormalities (Wlasow *et al.*, 2004). This phenomenon has presumably direct relation to increased ploidy level.

On the other hand, red blood cells with divided nuclei have been

observed in coho salmon, *O. kisutch*, due to the lack of folic acid in its diet (Smith, 1968). The presence of folic acid is essential for normal formation of red blood cells. The lack of this vitamin in fish can also result in the decrease of total number of erythrocytes, so observation of these abnormalities in induced triploids may be due to disruption in function of gene replicates related with folic acid synthesis or metabolism in DNA molecule (Smith, 1968; Johari *et al.*, 2008). Folic acid content of used feed in our experiment was not investigated. So, future investigation is necessary to design relationship between induction of triploid and folic acid.

The results of comparative analysis of various characteristics between diploid and induced triploid have been able to verify certain factual information, which in conjunction with other characteristics, can be used as indicators in the identification of triploidization, ploidy level and growth condition in Far Eastern catfish. In the result of cell cycle analysis, mitosis of diploid in each tissue was more active than those of induced triploid Far Eastern catfish in each tissue, and mitosis of gill tissue in each ploidy was more active than those of tail fin tissue in each ploidy. The period of cell cycle is the period of mitotic cycle, and shorter mitotic cycle period means a rapid growth, higher metabolism and higher respiratory function (Dean and Jett, 1974).

Induced triploid Far Eastern catfish had shorter head regions and

mandible barbel lengths than diploid fish, and diploid fish had a greater distance between the upper lip and nostrils as well as between the eyes than induced triploid fish. The induced triploid fish had high growth rates of the head region and body depth during the first year after hatching. These rates then decreased and diploid fish had higher growth rates of the head region and the mandible barbel than induced triploid fish. Eventually, the body shape of induced triploid fish was characterized by a smaller head and shorter mandible barbel than diploid fish.

The results of blood analysis were similar to previous study (Seol *et al*., 2008). In the result of endocrine hormone analysis, estradiol and testosterone and gonadosomatic index of diploid were higher than those of induced triploid in spawning and non-spawning season, and those of diploid were decreased from spawning season to non-spawning season. Thyroid stimulating hormone, thyroxine total cholesterol, H-cholesterol, LDL-cholesterol and triglyceride of induced triploid were higher than those of diploid in spawning season. Thyroid stimulating hormone and thyroxine were related with growth, and three cholesterols and triglyceride were related with lipid-synthesization. That is, induced triploid is concentrate more on growth and lipid-synthesization, because induced triploid is sterile, and convert the energy used in the reproductive (Kim *et al.*, 1990, 2001; Benfey, 1999).

This study was determined sterility and other characteristics of induced triploid Far Eastern catfish by histology, morphology and physiology. The reason why this condition doesn't cause giantism is proved by histological characteristics. Morphometric analysis identified the morphometric characteristics that can be easily used to determine diploid and induced triploid Far Eastern catfish. The results extend our knowledge of the morphometric changes that occur in diploid and induced triploid Far Eastern catfish during growth and may be useful for application to the aquaculture industry. In addition, comparative analysis of sex hormone and growth hormone between diploid and induced triploid were proved to convert the energy used in the reproductive growth of the body. Therefore, the result of this study could use evidence of previous studies about induced triploid Far Eastern catfish.

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APPENDIX 1
Anesthetic effects of clove oil and MS-222

1-1. Background and objective

The skin of the Far Eastern catfish, *Silurus asotus*, is covered with mucus. It is difficult to handle the fish out of the water because the skin of the catfish is slick (Suzuki *et al.*, 2003). Anesthesia is commonly used in fisheries for both experimental and practical purposes. It is used primarily to immobilize animals to facilitate the handling process, to save time and to avoid stressing the animals (Summerfelt and Smith, 1990). Among its principal uses, anesthesia facilitates the following operations: weighing and measuring; marking and tagging; studying fish physiology and behavior; performing surgery; collecting fish in tidal pools and with scuba; photography; preparing fish for live shipment and transport; manually spawning; injecting vaccines and antibiotics; and collecting blood and other tissues (Park *et al.*, 2011).

Anesthesia can decrease the stress levels in fish subjected to blood sampling, immobilization, handling, the injection of vaccines and antibacterial substances, medical treatment for diseases, artificial spawning, transport, and sorting (Park *et al.*, 2011). Also, the physiological response of

fish is almost similar to that of other animals, while the degree of stress (intensity and duration) of the biochemistry of the blood and tissues is a different, little known aspect (Selye, 1973; Schreck, 1981). The received stress on the fish is primarily inspired by the nervous system, and the endocrine boundaries cortisol and catecholamine. As a result of secondary causes, excessive secretion of the osmotic adjustment and changes in the carbohydrate metabolism, blood circulation and population of cells takes place, decreasing the growth rate of the primary infection or disease, which leads to symptoms such as increased, this phenomenon usually taking place simultaneously (Schreck, 1981).

Recently, clove oil has received a lot of interest in relation to fish. It is a safe and inexpensive anesthetic. It does not require a withdrawal period like lidocaine hydrochloride, since there is no element to pollute the environment; and it has been evaluated as being suitable to be used in the field of aquaculture (Park *et al*., 2009). In addition, its anesthetic effect on freshwater prawn, *Macrobrachium resenbergii*, and American lobster, *Homrtus anericus*, as crustaceans has been confirmed (Coyle *et al*., 2005; Waterstrat and Pinkham, 2005).

The objective of this appendix is to determine optimum anesthetic concentrations of clove oil and MS-222 in Far Eastern catfish.

1-2. Materials and Methods

The anesthetic experiment of Far Eastern catfish, *Silurus asotus*, began on May 2012 and ended on July 2011. I randomly selected 20 specimens from each group for each combination of anesthetic and concentration. The experimental fish were adapted to a 400-L glass tube. The temperature of the water in the tube was controlled so that it was equal to the temperature of the water in the anesthetic and recovery phases of the experiment. All the fish were fasted for 24 hrs before the study. For the anesthetic experiment, one specimen was randomly selected from the breeding tube with a net. Anesthesia was administered in a 50-L plastic rectangular parallelepiped tube under the control of an aeration system. Large-sized specimens were sourced from 1-year-old hatchlings with an average body mass of 302.1 ± 15.22 g (mean ± S.D.) and a standard length of 31.5 ± 4.19 cm (mean ± S.D.). Small-sized specimens were sourced from 2-month-old hatchlings with an average body mass of 50.1 ± 5.91 g (mean ± S.D.) and a standard length of 15.5 ± 1.58 cm (mean ± S.D.).

The anesthetic effects of clove oil (Sigma, St Louis, MO, USA, clove oil containing 85% eugenol) and of tricaine methanesulfonate (MS-222: Sigma, St Louis, MO, USA) was investigated at the five concentrations of 200, 300, 400, 500 and 600 ppm. The stock solution of clove oil was dissolved in 95% methanol (Sigma, St Louis, MO, USA) at a ratio of 1:10. The decision-based

table for the anesthetic effect (Table 1-1) was constructed in agreement with the Table in Park *et al.* (2011). The anesthesia time was measured from the time when the fish were placed in the anesthetized water to the time at which the stage A6 state was attained. In this stage, the fish were perfectly sedated, with minimum opercular movements. The recovery time was measured from the time when the fish were placed in the recovery water to the time at which the stage R6 state was attained. In this stage, the fish again exhibited normal swimming and responsiveness to visual stimulation (Table 1-1). When the fish were anesthetized in an anesthetic tube, they were immediately moved to the recovery tube. The anesthesia levels and recovery times of the fish were measured in seconds using a stopwatch.

1-3. Results and Conclusion

During the anesthetic experiments in Far Eastern catfish, *Silurus asotus*, with clove oil and MS-222, no fish died due to the stress associated with anesthesia. Table 1-2 shows the parameters describing the anesthesia times for clove oil and MS-222 at each concentration of the two anesthetics and

Table 1-1. Stages of anesthesia induction and recovery in clove oil and MS-222 efficacy tests performed in Far Eastern catfish, *Silurus asotus* (modified from Summerfelt and Smith, 1990)

Anesthesia	
Stage	Characteristic behavior
A1	Normal swimming; opercular movement and normal general movement
A2	Swimming speed slowed; rolling from side to side
A3	Partial loss of equilibrium; swimming erratic
A4	Complete loss of equilibrium; swimming perfectly inside out; pectoral fin, pelvic fin and dorsal fin movement stop
A5	Little sedation; anal fin and tail fin movement stop
A6	Perfect sedation; only opercular movement
A7	Opercular movement ceased
Recovery	
Stage	Characteristic behavior
R1	Resume opercular movement
R2	Preferential movement of pectoral fin and tail fin
R3	Dorsal fin, pelvic fin and anal fin movement
R4	Swimming perfectly inside out
R5	Swimming erratic; recovery of balance
R6	Normal swimming; responsiveness to visual stimuli

Table 1-2. Effects of clove oil dose and MS-222 on anesthesia in large-sized and small-sized Far Eastern catfish, *Silurus asotus*

Dose (mgL^{-1})	Exposure time (sec)[1]			
	Clove oil		MS-222	
	Large size[2]	Small size[2]	Large size	Small size
200	173 ±26.9[a]	85 ±19.9[a]	235 ±18.4[a]	119 ±21.5[a]
300	103 ± 6.7[b]	57 ± 3.4[b]	170 ±23.2[b]	77 ±13.3[b]
400	70 ±11.3[c]	42 ± 6.5[c]	100 ±15.9[c]	56 ± 4.5[c]
500	62 ± 8.7[d]	40 ± 2.7[d]	82 ± 8.7[d]	50 ± 6.1[c]
600	47 ± 6.2[e]	32 ± 2.0[e]	61 ± 9.2[e]	40 ± 4.2[d]

Two-way ANOVA

	Clove oil				MS-222			
	DF	Mean square	F-value	P-value	DF	Mean square	F-value	P-value
Dose	4	37225.5	229.3	< 0.0001	1	188361.5	190.2	< 0.0001
Size	1	68933.9	554.3	< 0.0001	4	297981.3	300.9	< 0.0001
Interaction	10	4135.7	33.2	< 0.0001	10	6342.1	6.4	< 0.0001

[1]Each value is the mean ± standard deviation of a triplicate experiment (total n=60). Values in the same column not sharing common superscripts are significantly different (P<0.05).

[2]Large-sized specimens were sourced from 1-year-old hatchlings with an average body mass of 302.1 ± 15.22 g and a standard length of 31.5 ± 4.19 cm. Small-sized specimens were sourced from 2-month-old hatchlings with an average body mass of 50.1 ± 5.91 g and a standard length of 15.5 ± 1.58 cm.

each body size of the experimental fish. The anesthesia time was significantly affected by the body size of the fish and by the concentration of each anesthetic. The time decreased consistently with the concentration of each anesthetic and was greater for the large fish ($P<0.05$). The anesthesia time of the large fish decreased as the concentration of clove oil increased ($P<0.05$). The trend in the anesthesia times in the small fish as a function of the concentration of clove oil was similar to that observed in the large fish. Furthermore, the anesthesia time was lower in the small fish than in the large fish at each concentration of clove oil ($P<0.05$). The trends in the anesthesia times in MS-222 for each fish size were similar to the trends in the anesthesia times in clove oil for the same fish size.

Table 1-3 contains the parameters associated with the recovery times for clove oil and MS-222 at each concentration and fish size. The recovery times in clove oil and MS-222 showed patterns similar to the patterns of the anesthesia times. The recovery times were significantly affected by the fish size and by the concentration of each anesthetic ($P<0.05$). At each concentration of clove oil and MS-222, the recovery time was greater for the large fish ($P<0.05$). As the concentration of clove oil and MS-222 increased, the recovery time increased in the large-sized fish ($P<0.05$). As the concentration of clove oil increased, the recovery time decreased ($P<0.05$) in

Table 1-3. Effects of clove oil and MS-222 doses on recovery in large-sized and small-sized Far Eastern catfish, *Silurus asotus*

Dose (mgL^{-1})	Recovery time (sec)[1]			
	Clove oil		MS-222	
	Large size[2]	Small size[2]	Large size	Small size
200	190 ± 26.2^{a}	133 ± 13.2^{ab}	377 ± 46.6^{a}	102 ± 11.9^{a}
300	183 ± 10.1^{ab}	131 ± 14.2^{ab}	285 ± 41.6^{b}	108 ±05.5^{b}
400	179 ± 10.1^{ab}	129 ± 09.4^{ba}	274 ± 61.8^{bc}	109 ±08.0^{b}
500	173 ± 8.7^{b}	129 ± 13.6^{ba}	206 ± 28.4^{c}	113 ±09.5^{c}
600	170 ± 10.3^{b}	129 ± 10.3^{ba}	204 ± 29.6^{c}	114 ±09.5^{c}

Two-way ANOVA

	Clove oil				MS-222			
	DF	Mean square	F-value	P-value	DF	Mean square	F-value	P-value
Dose	4	379.8	2.72	< 0.0001	1	7618.860	26.055	< 0.0001
Size	1	141179.8	1011.23	< 0.0001	4	154729.7	529.150	< 0.0001
Interaction	10	870.7	6.23	< 0.0001	10	802.087	2.743	< 0.0001

[1]Each value is the mean ± standard deviation of a triplicate experiment (total n=60). Values in the same column not sharing common superscripts are significantly different (P<0.05).

[2]Large-sized specimens were sourced from 1-year-old hatchlings with an average body mass of 302.1 ± 15.22 g and a standard length of 31.5 ± 4.19 cm. Small-sized specimens were sourced from 2-month-old hatchlings with an average body mass of 50.1 ± 5.91 g and a standard length of 15.5 ± 1.58 cm.

the small fish. In contrast, the recovery time of these fish increased ($P<0.05$) as the concentration of MS-222 increased.

The results from anesthetic experiment could be useful to aquaculturists and for other purposes that require the sedation of the fish. This experiment showed that clove oil and MS-222 are effective at anesthetizing Far Eastern catfish. This experiment was showed the relationship between the anesthetic effect and the size of the Far Eastern catfish. These anesthetics meet the requirement of ideal anesthesia because they offer an anesthesia time within 3 mins, because they offer a recovery time within 5 mins, and because both anesthetics are cost-effective, efficient, safe and non-toxic to the fish and the user (Park *et al.*, 2003).

APPENDIX 2

Stress response and wound healing after surgical incisions

2-1. Background and objective

Fish surgery is the removal of tissue from the fish body, agency, healing and removal of the gland wave tumor, and treatment of the wound transplantation of immunological healing, and implant tag chip; and is used for such means as the cleavage of fins for fish marking and transplantation, remote telemetry device, and electrode for behavioral and physiological studies (Summerfelt and Smith, 1990). Fish surgical instruments and equipment for surgery, fish with the use of technical antibiotics, and anesthetics used in parallel, are used to increase the success rate of a field of science and technology (Marty and Summerfelt, 1990; Summerfelt and Smith, 1990). The objective of this study is find out the stress response and wound healing of surgical incisions for Far Eastern catfish, *Silurus asotus*.

2-2. Materials and Methods

Far Eastern catfish, *Silurus asotus*, at 1 year after hatching were used in this experiment. The experiment started on May 2012. Prompt surgery to minimize the effects of stress in 600 ppm clove oil (Containing 85% eugenol;

Sigma, St Louis, MO, USA) contain anesthesia was performed, and the anesthetized temperature was 20°C. After the start of surgery, the time to recover from surgery of the experiment fish exposed to the air was considered to be less than 5 mins, so that anesthesia and recovery were complete, and the sutures could take. The far eastern catfish's dorsal fin, and the lateral line connecting the midpoint of the shortest distance, and were defined. Using scissors for surgery, the surgical site, including the epidermis and the muscles, were cut deeply, and the length of incision was about a 2-3 cm wound, parallel to the sideline. The wounded samples were sutured using suture needle (Ophthalmic suture needle No. 0, Ailee, Korea), and a simple-instrument method of incision with 3-4 stitches. To complete, the surgical site was sutured using a brush, and the wound was applied with vaseline (Vaseline intensive care, Unilever, USA). The surgical sutured group and control group after recovery from anesthesia were reared in a filtration system in a tank. The water temperature during breeding was maintained at 20 ± 0.5°C. Investigation of the survival rate, herniation rate, and adhesion rate after surgery were immediately observed, until 42 days after surgery.

In order to investigate the stress response of fish after surgical treatment, stress hormones were investigated, such as plasma cortisol. Blood samples were extracted from five randomly selected fish, using syringes lined with the anticoagulant heparin. Blood was extracted from five experimental

samples, at fixed intervals of pre (0), 1, 6, 12, 24, 48, 72 and 96 hrs after surgery. The collected blood was placed in capillary tubes, and analyzed, after centrifugation at 200 ×g for 10 mins. The plasma was then collected, and stored in a deep freezer (SW-UF-200; Samwon Freezing Engineering, Busan, Korea) at -80°C, until analysis. The cortisol concentration was measured with a radioimmunoassay (RIA) in 50 μL samples, using an RIA Kit (Coat-A-Count TKCO Cortisol RIA Kit, DPC, USA). Mixtures of samples in 100 μL of antiserum were incubated for 45 mins at 37 °C, and then 1,000 μL of separation reagent was added. The mixture was placed in a refrigerator at 4°C for 15 mins, and then centrifuged at 1,200 ×g for 15 mins. The supernatant was assayed for γ-radiation, using an automatic γ-counter (Cobra, Packard, USA).

2-3. Results and Conclusion

The necropsy of adhesion and herniated rate of Far Eastern catfish, *Silurus asotus*, are shown in Table 2-1. Three experimental fishes were observed with hernia at 1 day after surgery, and six samples were observed with hernia from 2 days and 3 days after surgery. From 3 days after surgery, no object was observed with hernia during the experimental time.

Table 2-1. Necropsy of adhesion and herniated rate at the incision site during 42 days in Far Eastern catfish, *Silurus asotus*[1]

Days after suture	Herniation (%)	Percent of		
		Adhesion	Slight adhesions	Substantial adhesions
1	3/90 (3.3)	90/90 (100)	0/0 (0)	0/0 (0)
2	3/87 (3.4)	87/87 (100)	0/0 (0)	0/0 (0)
3	3/84 (3.6)	84/84 (100)	0/0 (0)	0/0 (0)
4	0/84 (0)	84/84 (100)	0/0 (0)	0/0 (0)
7	0/84 (0)	75/84 (85.2)	12/84 (14.8)	0/0 (0)
14	0/84 (0)	39/84 (48.1)	45/84 (51.9)	0/0 (0)
21	0/84 (0)	18/84 (22.2)	66/84 (77.8)	0/0 (0)
28	0/84 (0)	0/0 (0)	84/84 (100)	0/0 (0)
35	0/84 (0)	0/0 (0)	39/84 (48.1)	45/84 (51.9)
42	0/84 (0)	0/0 (0)	0/0 (0)	84/84 (100)

[1]All samples were anesthetized with 1,000 ppm clove oil. Died samples included the percentage of herniation and necropsy of adhesion. Each value is the mean percentage of triplicate experiments (n=90).

At 7 days after surgery, the surgical site was not healing as a whole, and there were no adhesions. The healing began to progress towards adhesion at 7 days after surgery, and the movement of fishes at 7 days after surgery was more active, than that at 1 day after surgery. At 14 days after surgery, 52% of all samples were observed with slight adhesion, and the ratio of slight adhesion increased until 28 days after surgery. At 28 days after surgery, all samples were observed with slight adhesion, and due to the lack of epidermal melanin, traces of off-white appeared in the surgical site. At 35 days after surgery, 52% of all samples were observed with substantial adhesion as complete adhesions in the surgical site, and all experimental samples were completely adhered at 42 days after surgery. Variations of plasma cortisol concentrations during 96 hrs after surgery are shown in Fig. 2-1. Cortisol concentrations of fish after surgical treatment increased, as time passed.

Plasma cortisol concentrations differed significantly between the not anesthetized group, and the clove oil anesthetized group. Mean plasma cortisol concentration level of the not anesthetized group and clove oil anesthetized group were 1.0±0.15 μg/dL and 0.9±0.20 μg/dL before the experiment, respectively (Fig. 2-1). Plasma cortisol concentrations of the not anesthetized group increased from 15.5±1.51 μg/dL at 1 hr, to 38.2±1.43 μg/dL at 6 hrs (P<0.05).

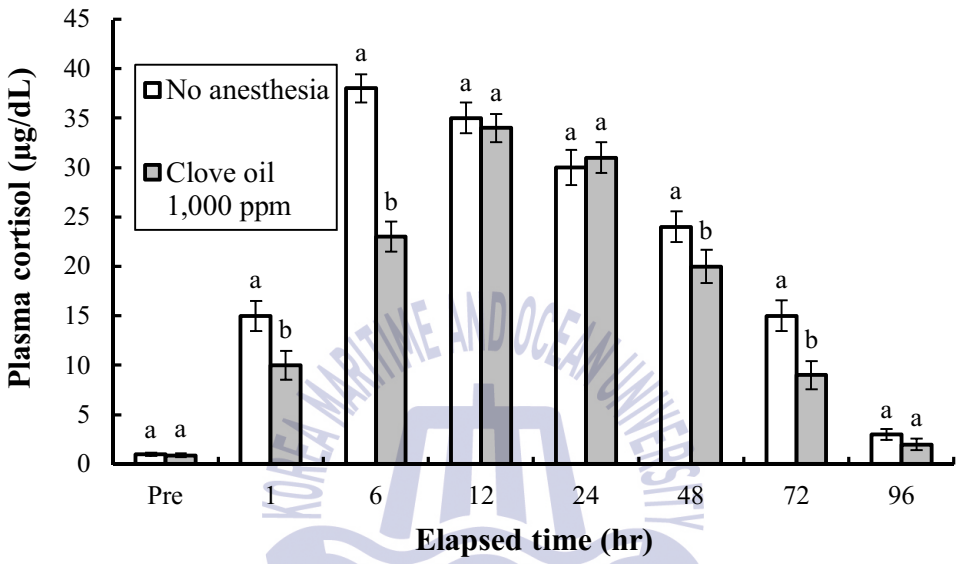
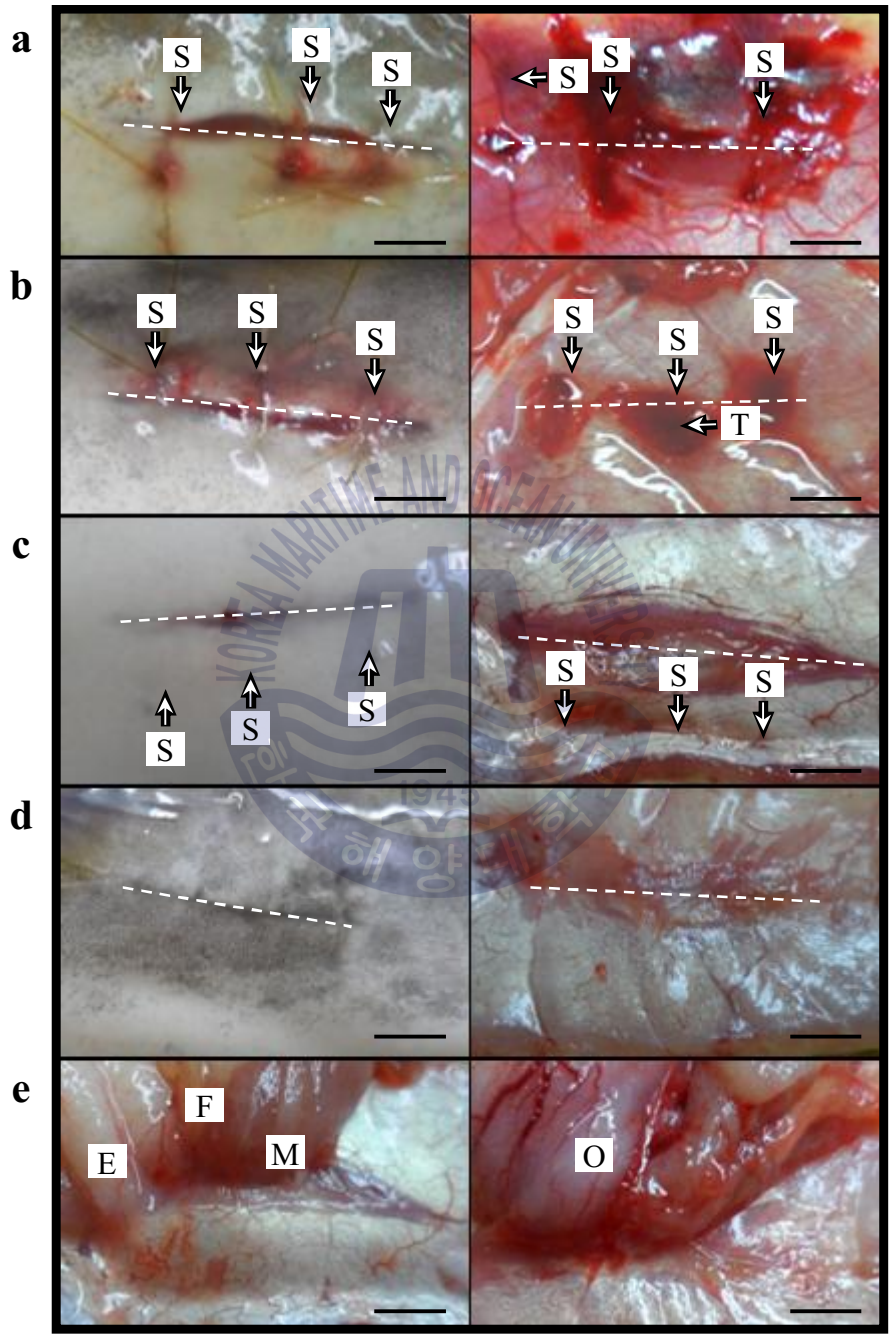


Fig. 2-1. Variations of the plasma cortisol concentrations in the blood plasma of the Far Eastern catfish, *Silurus asotus*, during 96 hrs after suture, with the no anesthesia and clove oil (1,000 ppm) anesthesia groups. Pre means control group before surgical incision. Vertical bars are means ± SE (n=90). Actually, n=30 for each experiment, because the means and SE were calculated separately for each group. Different letters on error bars are significantly different for each time (P<0.05).

Plasma cortisol concentrations of the clove oil anesthetized group increased from 10.4±1.44 μg/dL at 1 hr, to 34.3±1.67 μg/dL at 12 hrs (P<0.05). Plasma cortisol concentration of the not anesthetized group and clove oil anesthetized group at 96 hrs recovered to be 3.2±0.56 μg/dL and 2.1±0.60 μg/dL, but were higher than that of the pre group (before surgery; P<0.05). The cortisol concentration of the not anesthetized group was highest at 6 hrs, and that of the clove oil anesthetized group was highest at 12 hrs. The cortisol concentrations of the not anesthetized group were higher, than those of the clove oil anesthetized group, from 48 hrs to 96 hrs. The cortisol concentrations of the not anesthetized group showed faster increase and slower recovery, than those of the clove oil anesthetized group.

Figure 2-2 show the external morphology of the surgical site. Immediately after surgery, the movement of fish was slightly slow, for surgery on the side of body. At 14 days after surgery, stitching fiber was still considerable, and surgical site bleeding was observed. In addition, the surgical site showed a slight adhension, and was not healed as a whole (Fig. 2-2a). At 28 days after surgery, the movement of fish was more active; sealing trace of the surgical site was clear, but stitching fiber was minimally observed (Fig. 2-2b). At 35 days after surgery, trace of the suture had almost disappeared, when observed on the outside of the fish.

Fig. 2-2. External morphology of the surgical site's healing progress in Far Eastern catfish, *Silurus asotus*. Left: outside view; Right: inside view. (a) Surgical site at 14 days after suture; (b) Surgical site at 28 days after suture; (c) Surgical site at 35 days after suture; (d) Surgical site at 42 days after suture; and (e) Adhesion of the surgical site with fat (left), testis (left), midgut (left and right) and stomach (right), at 42 days after suture. Scale bars indicate 1 cm. White dotted line: abdominal incision part. E: testis; F: fat; M: midgut; O: stomach; S: stitching site; T: thrombus.



In addition, sutures were not found; but the result of observation on the inside of the fish showed hemorrhagic conditions, and recovery of the wound was not yet healed (Fig. 2-2c). Finally, at 42 days after surgery, no stitching fiber was visible, and the sutured wounds were distinctly recovered (Fig. 2-2d). At 42 days after surgery, fat and testis were recovering adhesion from the wound treatment, and adhesion to the intestine and the wound was observed (Fig. 2-2e).

In this study, the stress response by surgical surgery investigated the cellular reaction and neuroendocrine, and their interaction. Primarily, the activity of the hypothalamus - pituitary is high, which causes the secretion of cortisol in the blood (Chang and Hur, 1999). The reaction of the secondary water-ion imbalance, the heart rate, the increased oxygen consumption and the increase of energy mobilization, plasma glucose appear in the elevated (Tomasso *et al.*, 1980; McDonald and Miligan, 1997). This reaction is used as a typical indicator of the neuroendocrine response. In this experiment, in stress hormone levels, the first increase is cortisol concentration and plasma glucose, and plasma lactic acid concentration is gradually increased in the order of the secondary reaction. This means that plasma glucose and plasma cortisol are usefully employed as a stress index of fish (Park *et al.*, 2008a). In this study, the plasma cortisol and glucose concentrations were significantly increased after surgery. A rapid increase in plasma cortisol levels compared

to glucose can be seen; such a result is found to have been studied in the same way in Chang and Hur (1999) and Park *et al.* (2008a).

The results of surgical incision experiment that was healing normally wounds external to perform a surgical operation of catfish and physiological studies common endocrinological, pharmaceutical and it can be applied. Therefore, this experiment proved relieved effect of stress by anesthetics and stability of biopsy of gonad by surgical incisions for injection of PIT tag chips and distinction of maturity of gonad, and improved breeding system of diploid and induced triploid Far Eastern catfish using PIT tagging method.

APPENDIX 3

Long-term effects of passive integrated transponder tags

3-1. Background and objective

Recently, Kim *et al.* (2012) described the effects of dietary protein and lipid levels on the growth and body composition of juvenile Far Eastern catfish, *Silurus asotus*. In those studies, the values used to determine differences between the control and experimental groups were not the values of individual fish in each group but rather the mean values of each group because of difficult in identifying individual fish. To overcome this problem, it is necessary to use selective breeding systems and to develop a method for monitoring the changes in experimental factors in which individual fish can be identified. Recent advances in tagging technology have made it possible to identify individuals, and provide information on the survival and growth of individual fish (Lee *et al.*, 2009). Therefore, tagging technology could be introduced into breeding systems and for experimental research involving Far Eastern catfish.

The ability to identify individual fish is essential in research focusing on growth, migration, mortality, and stock identification (Konstantinov, 1978; Park and Lee, 2001). It is vital that the tagging system enables convenient,

rapid, and non-lethal identification of the fish (Thomas *et al.*, 1999). These requirements are satisfied by the use of a passive integrated transponder (PIT) tag. Since their introduction to monitor fish movement, PIT tags have been used to study the behaviors of small mammals, birds, invertebrates, reptiles, and amphibians (Prentice *et al.*, 1990; Gibbons and Andrews, 2004). PIT tags are useful for studying animal behavior because they are small, inexpensive, last indefinitely, and provide individual, unambiguous marks for the tagged organisms (Lee *et al.*, 2009).

The objective of this study is to investigate the most suitable site of PIT tags inserted into three sites of Far Eastern catfish.

3-2. Materials and Methods

Far Eastern catfish, *Silurus asotus*, at 6 months after hatching were used for this experiment. The passive integrated transponder (PIT, HS 5900L, Biomark Inc., Boise, ID, USA) tag experiment started on 1 November 2011 and lasted for 2 years. The fish were measured using a digital vernier caliper (CD-20CP, Mitytoyo, Kawasaki, Japan) and an electronic balance, and the mean ± standard deviation of body length and body weight were 20.8 ± 2.14 cm and 97 ± 5.6 g, respectively. I used glass implantable transponders of 12.04 mm in length, 2.04 mm in diameter, and weighing 88.4 mg in air

(TX1400L; Biomark Inc., Boise, ID, USA). Tagging was carried out after anaesthetization with clove oil (Sigma, St Louis, MO, USA) at a concentration of 600 mg/l for 1 min. The fish were divided into four experimental groups with 20 fish per group, in triplicate. In three groups, the PIT tag was inserted into the dorsal muscle (dorsal group; site a in Fig. 3-1), the abdominal cavity (abdominal cavity group; site b in Fig. 3-1), or the tail muscle (tail group; site c in Fig. 3-1). The fourth group was a control group in which no tag was inserted.

A single PIT tag was inserted into the intended site using a replaceable needle implanter (MK-7, Biomark Inc., Boise, ID, USA). The syringe needle was 5 cm long, and the angle of injection was 8-10°. The needle was injected to a depth of 3 cm, and the depth of the needle point below the epidermis was 7-8 mm. The tags were scanned to confirm their readability using a mini portable reader (P/N 800-0249-01, 125 kHz; Destron Technology, South St Paul, MN, USA) and fish were weighed before being returned to the rearing tank. Povidone iodine was applied to the injection site. Except for the induction of anesthesia and recovery time (35 sec), the tagging procedure took <30 sec per fish.

Water was partially replaced with sand-filtered aerated seawater (pH: 7 ± 0.5; dissolved oxygen: 8 ± 0.7 mg/l; ammonia: 0.006 mg/l) every weekend.

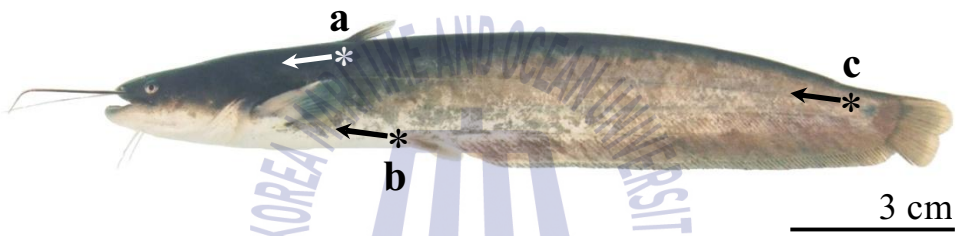


Fig. 3-1. Inject locations and directions of PIT chip on the Far Eastern catfish, *Silurus asotus*. a: dosal location; b: abdominal cavity location; c: tail location; * (black and white): inject locations of each site; black and white arrows: inject direction.

All fish were hand-fed twice daily with commercial feed (E-Wha Oil & Fat Ind., Co., Busan, Korea: 50% crude protein, 8% crude fat, 4% crude fiber, and 15% ash) at 1%–3% of their body weight during the experiment. Measurements were carried out every 2 months for 2 years (November 2010 to November 2012) on the same day of the month. Measurements, including body weight, standard length, survival rate, tag retention, and tag readability were recorded under anesthesia. The cumulative survival rate was recorded every day in each group.

Analyses of growth were done using individual fish. The growth rate (GRW, %), condition factor (CF), and specific growth rate (SGR, %/day) of each fish were calculated as follows:

$$GRW\ (\%) = (\text{final standard length} - \text{initial standard length}) \times 100/\text{initial standard length}$$

$$CF = \text{body weight} \times 100\ [(\text{standard length})^3]^{-1}$$

$$SGR\ (\%/\text{day}) = (\log_e W_2 - \log_e W_1) \times 100/(d_2 - d_1),$$

where W_1 and W_2 are the individual weights (g) at days 1 (d_1) and 2 (d_2), respectively. In all experimental groups, the time from d_1 to d_2 was 4 months (120-123 days).

The effects of tag location on growth parameters were examined by one-way analysis of variance (ANOVA), and multiple comparisons were performed using Duncan's multiple-range test. Statistical analyses were done

using SPSS statistical software version 12.0 (Duncan, 1955; SPSS Inc., Chicago, IL, USA).

3-3. Results and Conclusion

Table 3-1 lists the survival rate in each of the four experimental groups, as well as the tag retention and readability rates in Far Eastern catfish, *Silurus asotus*, tagged with passive integrated transponder (PIT) in the abdominal cavity, dorsal muscle, or tail muscle. At 4 months after tagging, the survival rate was lower in the abdominal group than in the other groups. The tag retention rate was significantly lower in the tail group than in the other groups ($P<0.05$). However, the tag readability rate was not significantly different among the experimental groups ($P>0.05$). At 8 months after tagging, the survival rate was not significantly different between the abdominal group and the other groups ($P>0.05$). Although the tag retention rate was significantly lower in the tail group than in the other groups ($P<0.05$), the tag readability rate was not significantly different among the three experimental groups. At 12 months after tagging, the survival rate was significantly lower in the abdominal and tail groups than in the dorsal group ($P<0.05$). The trends in the tag retention and readability rates at 12 months were similar to those at 8

Table 3-1. Survival rate, tags retention rate and readability of Far Eastern catfish, *Silurus asotus*, tagged with PIT tags in the abdominal cavity, dorsal muscle, tail muscle and untagged control groups

Month after tagged	Exp. group[1]	Variables[2]					
		Number of survivals	Survival rate (%)	Number of retained tags	Tags retention rate (%)	Number of readable tags	Tags readability (%)
0 (Nov., 2010)	CN	60/60	100	-	-	-	-
	DS	60/60	100	60/60	100	60/60	100
	AC	60/60	100	60/60	100	60/60	100
	TS	60/60	100	60/60	100	60/60	100
4 (Mar., 2011)	CN	59/60	98.3	-	-	-	-
	DS	58/60	96.7	60/60	100	60/60	100
	AC	55/60	91.6	60/60	100	60/60	100
	TS	56/60	93.3	59/60	98.3	59/59	100
8 (Jul., 2011)	CN	58/59	98.3	-	-	-	-
	DS	57/58	98.3	58/58	100	58/58	100
	AC	54/55	98.2	55/55	100	55/55	100
	TS	55/56	98.2	54/56	96.4	54/54	100
12 (Nov., 2011)	CN	58/58	100	-	-	-	-
	DS	56/57	98.2	57/57	100	57/57	100
	AC	52/54	96.3	54/54	100	54/54	100
	TS	52/54	96.3	52/54	96.3	52/52	100
16 (Mar., 2012)	CN	58/58	100	-	-	-	-
	DS	56/56	100	56/56	100	56/56	100
	AC	51/52	98.1	52/52	100	52/52	100
	TS	50/52	96.2	51/52	98.1	51/51	100
20 (Jul., 2012)	CN	57/58	98.3	-	-	-	-
	DS	56/56	100	56/56	100	55/56	98.2
	AC	51/51	100	51/51	100	49/51	96.1
	TS	50/50	100	48/50	96.0	45/48	93.8
24 (Nov., 2012)	CN	57/57	100	-	-	-	-
	DS	55/55	100	55/55	100	52/55	94.5
	AC	49/49	100	49/49	100	46/49	93.9
	TS	45/45	100	42/45	93.3	35/42	83.3

[1]AC: abdominal cavity; CN: control (untagged, no injection); DS: dorsal muscle; TS: tail muscle.

[2]The samples of died and slipped tags and unreadable tags on previous measurement were excluded from next measurement of survival rate.

months. At 16 months after tagging, the survival rate was significantly lower in the abdominal group than in the dorsal and control groups, and was significantly lower in the tail group than in the abdominal group ($P<0.05$).

The trends in tag retention and readability rates at 16 months were similar to those at 8 and 12 months. At 20 months after tagging, the survival rates were not significantly different among the experimental groups ($P>0.05$). The tag retention rate was lower in the tail group than in the other groups. At this time, the tag readability rates had shown the first significant decrease since implanting the tags in all three experimental groups ($P<0.05$). At 24 months after tagging, the trends in survival and tag retention rates were similar to those at 20 months. The tag retention and readability rates were lower in the tail group than in the other groups. The number of surviving fish, retained tags, and readable tags were lower in the tail group than in the other groups. The tag retention and readability rates were significantly higher in the dorsal group than in the other groups throughout the experimental time ($P<0.05$).

Figure 3-2 shows the cumulative survival rates of each group during experimental time. The cumulative survival rate at 1 year was lower in the abdominal group than in the other groups, and was higher in the control group than in the other groups.

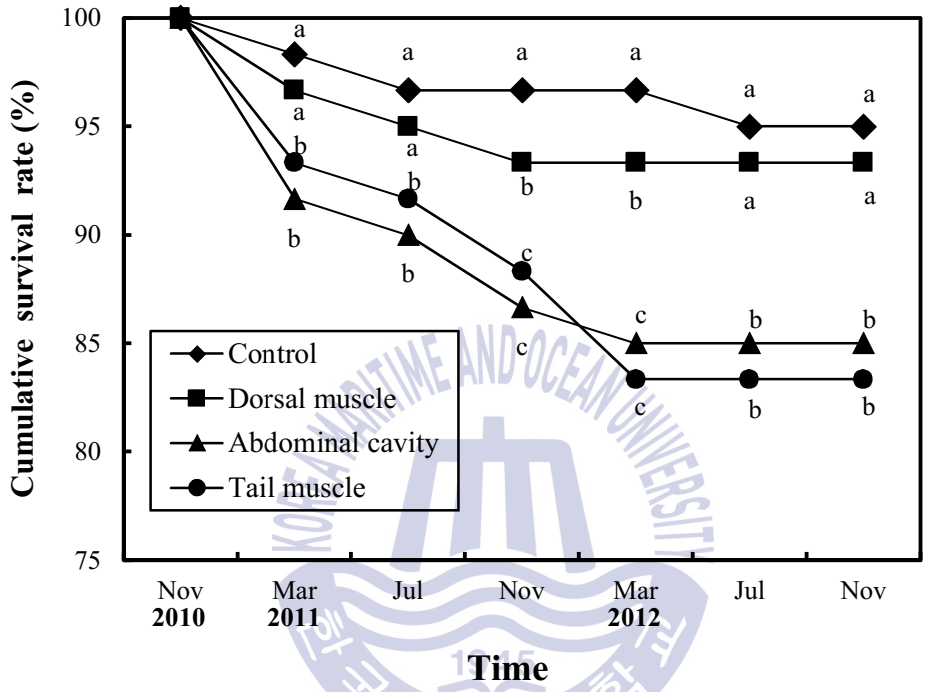


Fig. 3-2. The cumulative survival rates of Far Eastern catfish, *Silurus asotus*, tagged with PIT tags during experimental time. Each value is mean of each experimental group, and survival samples of slipped tags and unreadable tags were included in each value.

The cumulative survival rate beyond 1 year was lower in the tail group than in the other groups, but was not significantly different between the abdominal group and tail group. The cumulative survival rate was not significantly different between the dorsal group and the control group.

Table 3-2 lists the growth characteristics of the Far Eastern catfish in all four groups. Standard length, body weight, GRW, CF, and SGR during the experimental time were not significantly different among the four experimental groups ($P>0.05$). GRW and SGR in each group were significantly lower in the winter season (from November to February; at 4 and 16 months after tagging) than in other seasons ($P<0.05$). CF was not significantly different among the four experimental groups during the experimental time ($P>0.05$).

PIT tag experiment has demonstrated that PIT tagging of the dorsal muscle, the tail muscle, and the abdominal cavity in Far Eastern catfish can reliably identify individuals. In the result of this experiment, all PIT tags implanted into the dorsal and tail muscles remained *in situ*. The PIT tags implanted into the abdominal cavity were found in various locations that differed from their initial sites. In a previous study, transmitters implanted into the abdominal cavity were mainly located in three regions that differed from the initial sites (Marty and Summerfelt, 1988).

Table 3-2. Growth of Far Eastern catfish, *Silurus asotus*, tagged with PIT tags in the abdominal cavity, dorsal muscle, tail muscle and untagged control groups

Month after tagged	Exp. group[1]	Variables[2]				
		Standard length (cm)	Body weight (g)	Growth rate (GRW, %)	Condition factor (CF)	Specific growth rate (%)
0 (Nov., 2010)	CN	20.8±2.14	097±05.6	-	1.08±0.045	-
	DS	20.9±3.38	099±04.9	-	1.08±0.036	-
	AC	20.9±3.45	098±05.4	-	1.07±0.061	-
	TS	20.9±2.18	096±05.1	-	1.05±0.054	-
4 (Mar., 2011)	CN	21.8±3.55	108±07.8	06.6±0.65	1.03±0.057	0.15±0.043
	DS	21.7±4.01	107±08.0	06.7±0.75	1.01±0.048	0.15±0.032
	AC	21.5±5.11	106±08.5	06.1±0.89	1.00±0.094	0.15±0.067
	TS	21.6±4.12	109±08.4	06.3±0.69	1.01±0.064	0.15±0.045
8 (Jul., 2011)	CN	24.4±3.76	142±11.7	08.6±0.56	0.98±0.067	0.16±0.063
	DS	24.4±4.78	143±11.5	08.6±0.58	0.98±0.054	0.16±0.042
	AC	24.1±6.08	141±13.8	08.7±0.87	1.01±0.069	0.16±0.085
	TS	24.2±4.11	143±11.9	08.1±0.78	1.01±0.071	0.16±0.057
12 (Nov., 2011)	CN	28.6±3.94	236±13.4	17.2±1.43	1.01±0.056	0.42±0.054
	DS	28.5±4.59	233±13.8	16.8±1.50	1.01±0.074	0.41±0.048
	AC	28.1±5.64	227±15.5	16.6±1.88	1.02±0.062	0.40±0.054
	TS	28.5±4.43	235±13.0	17.8±1.49	1.02±0.085	0.41±0.076
16 (Mar., 2012)	CN	30.5±3.45	290±15.2	07.0±0.77	1.02±0.099	0.17±0.071
	DS	30.4±3.98	291±15.7	07.3±0.86	1.04±0.057	0.19±0.052
	AC	29.8±5.88	279±17.9	07.1±0.91	1.05±0.104	0.17±0.046
	TS	30.3±3.81	290±14.8	07.1±0.84	1.04±0.094	0.18±0.069
20 (Jul., 2012)	CN	33.3±3.76	396±16.9	09.2±0.81	1.07±0.072	0.26±0.047
	DS	33.2±3.51	395±17.1	09.2±0.88	1.08±0.084	0.25±0.051
	AC	32.5±5.61	371±20.5	09.1±0.90	1.08±0.091	0.24±0.071
	TS	33.1±4.01	394±18.0	09.2±0.87	1.09±0.072	0.26±0.065
24 (Nov., 2012)	CN	38.0±3.99	550±25.1	14.4±1.01	1.03±0.087	0.30±0.047
	DS	37.8±3.78	550±26.8	13.9±1.04	1.02±0.098	0.28±0.064
	AC	37.5±5.88	548±31.2	13.3±1.39	1.02±0.125	0.25±0.087
	TS	37.6±4.19	543±27.7	13.6±1.06	1.02±0.105	0.27±0.055

[1]AC: abdominal cavity; CN: control (untagged, no injection); DS: dorsal muscle; TS: tail muscle.

[2] Each value were mean±SD of survival samples on each experimental group.
GRW (%) = (final standard length-initial standard length) × 100/initial standard length.
CF = body weight×100 $\{(\text{standard length})^3\}^{-1}$.
Specific growth rate (%) = $(\log_e W_2 - \log_e W_1) \times 100/(d_2-d_1)$. W_1 and W_2 are individual weights (g) at day $d_1$and d_2, respectively. In all experimental groups, periods from d_1 to d_2 are 4 months (120-123 days).

It is unclear why the PIT tags were found inside the air bladder in some fish because there were no marks of PIT tag injection in the air bladder, and the air bladder in Far Eastern catfish is sealed in the abdominal cavity. Furthermore, some tags were unreadable from 16 months after implantation. In this experiment, long-term implantation of the tag did not significantly affect mortality rates compared with control fish. Considering the rates of tag retention, readability and survival, implanting PIT tags into dorsal muscle is preferable over other sites in the contexts of research and selective breeding of Far Eastern catfish.

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LECTURE:

1. Biology of Genetically Modified Organism: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, March-June, 2013.
2. Marine Animal Systematics and Experiment: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, September-December, 2013.
3. Fishery Biosciences: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, September-December, 2013.
4. Biology of Genetically Modified Organism: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, March-June, 2014.
5. Marine Animal Systematics and Experiment: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, September-December, 2014.

6. Fundamentals of Aquatic Ecology: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, September-December, 2014.
7. Cell Biology: Course of bachelor, Division of Marine Bioscience, Korea Maritime and Ocean University, Korea, March-June, 2015.
8. Marine Animal Systematics and Experiment: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, September-December, 2015.
9. Fundamentals of Aquatic Ecology: Course of bachelor, Division of Marine Environment and Bioscience, Korea Maritime and Ocean University, Korea, September-December, 2015.

AWARDS:

1. Award of Excellent Presentation: Fishery Sciences Association of Korea. Fall Meeting, No. 2012-07, Busan Exhibition & Convention Center (BEXCO), 16 November, 2012.
2. Award of Excellent Presentation: Fishery Sciences Association of Korea. Fall Meeting, No. 2013-30, BEXCO, 22 November, 2013.
3. Grand Prize of Excellent Presentation: International Conference of Fishery Sciences Association, BEXCO, 2 July, 2014.
4. Presentation Award: Korean Society of Developmental Biology. 33th Annual Meeting, Seoul National University Hospital, 12 September, 2014.

5. Award of Excellent Poster Presentation: International Conference of Fishery Sciences Association, BEXCO, 30 October, 2015.