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Intersexuality of *Scomberomorus niphonius* from the Coastal Area around Jeju Island, Korea (Teleostei: Scombridae)

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ABSTRACT : This study histologically describes the intersexuality of *Scomberomorus niphonius* collected from the coastal area near Jeju Island. A total of 126 *S. niphonius*, collected from March to July 2012 with a total length of 62.4 cm (±17.5) and a total weight of 1,701.9 g (±1,528.9) were used for analyses. From a histological perspective, two types of intersex were confirmed. One type had scattered germ cells from the opposite sex within the gonad. The second type developed germ cells from the opposite sex in the connective tissue of the outer gonadal membrane. The intersexuality was 14.3% (n=18/126), with females (21.3%; n=16/75) exhibiting a higher rate than males (3.9%; n=2/51). There was no displayed correlation between intersexuality and the total length and weight.

Key words : Intersexuality, *Scomberomorus niphonius*

INTRODUCTION

In an aquatic ecosystem, the environmental risk assessment of living things needs to be researched to keep the ecosystem healthy and to aid in its recovery (Tayler et al., 1998; Lee et al., 2010).

There are four main steps in the risk assessment for environmental factors: 1) hazard identification, 2) dose-response assessment, 3) exposure assessment, and 4) risk characterization. The first method is for monitoring general changes in an ecosystem (NRC, 1983).

A biomarker refers to a cell indicator or an individual physiology that detects exogenous factors affecting a living organism, biochemistry, and structure. Among the physiological biomarkers, reproductive indexes are an important point to detect long-term and continuous effects of pathogenic processes (Huggett et al., 1992).

Chemical water pollutants are generally divided into three categories; heavy metals, persistent organic pollutants (POPs), and endocrine disrupting chemicals (EDCs). EDCs disturb the reproductive endocrine system and change the manifestation or function of sex in aquatic animals, as either androgenic or estrogenic effectors. EDCs mimic sex hormones and may cause aberrant outcomes including reproductive, infertility problems, and intersex in wildlife (Tyler & Routledge, 1998; Kwon et al., 2006; Ju et al., 2009; Lee et al., 2009).

This study is to analyze the intersex characteristics depending on sex and size of *Scomberomorus niphonius* collected from the coastal area near Jeju Island.

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MATERIALS AND METHODS

1. Materials

The 126 specimens of *S. niphonius*, with a total length of 62.4 cm (±17.5) and a total weight of 1,701.9 g (±1,528.9), were collected from the coastal area near Jeju Island in Korea by mid water trawling from March to July 2012 (Table 1).

2. Methods

1) The sex ratio and intersex

The sex ratio and intersex ratio were categorized by gonadal specimen. Intersex includes only when the opposite sex germ cell is observed but no other sex characteristic.

2) The histological analysis

The experimental fish were measured with their total length and weight after being checked for any defects. Gonads were removed and weighed to the nearest 0.1 g using digital calipers. The tissue samples were fixed in Bouin's solution for 24 hours and washed in running water for 36 to 48 hours. The samples were then prepared in paraffin sections after being dehydrated with alcohol. The sections were cut at 4 to 6 µm with a microtome. The resulting sections were placed on slides, stained with Mayer's hematoxylin-eosin (H-E), and observed under a microscope.

RESULTS

1. The histological characteristics of intersex

From the histological perspectives, two types of intersex were observed. The first type shows the opposite germ

Table 1. Analyzed specimen number and sex ratio of *Scomberomorus niphonius*

Total length (cm)	Number			Sex ratio (F/F+M)	Chi-square	*P* value
	Total	Female	Male			
38.9−40.0	2	-	2	-	-	-
40.1−50.0	42	19	23	0.45	0.381	0.537
50.1−60.0	28	13	15	0.46	0.143	0.705
60.1−70.0	9	8	1	0.89	5.444	0.020
70.1−80.0	26	17	9	0.65	2.462	0.117
80.1−90.0	6	6	-	-	-	-
90.1−100.0	10	9	1	0.90	6.400	0.011
100.1−103.5	3	3	-	-	-	-
Total	126	75	51	0.59	4.571	0.033

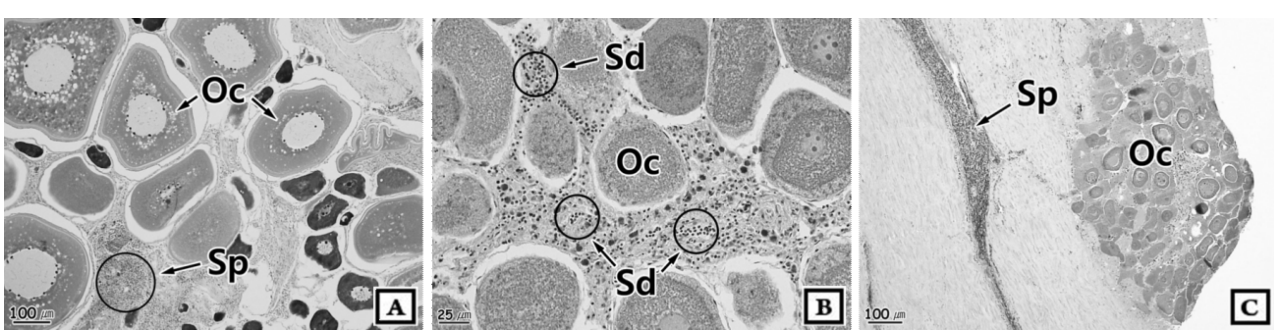

Fig. 1. Photomicrograph of intersex gonad of *Scomberomorus niphonius*. A and B, Female; C, Male. Oc: Oocytes, Sd: Spermatids, Sp: sperm.

cells scattered randomly throughout the gonad, which were found in all females (Fig. 1A, B). The second type shows mature germ cells from the opposite sex in the connective tissue of the outer gonadal membrane, which were found in all males (Fig. 1C). Intersex fish are seen to possess degenerated oocyte in their ovary (Fig. 1B) and previtellogenic oocyte in the intersex testis (Fig. 1C).

2. Sex and intersexuality

14.3% (n=18/126) of intersex fish were found in total. Intersexuality of the female and male was 21.3% (n=16/75) and 3.9% (n=2/51) respectively; it was indicated female higher than males (Table 2).

Table 2. Intersexuality of *Scomberomorus niphonius*

	Intersexuality (%)		
	Total	Female	Male
Total	14.3 (n=18/126)	21.3 (n=16/75)	3.9 (n=2/51)

3. Size and intersexuality

Collected specimens were measured and classified every 10.0 cm for the total length and 500.0 g for the total weight. Intersexuality in accordance with the total length and weight did not display apparent correlation (Table 3 and 4).

DISCUSSION

Morphological sex character in teleost is fundamentally genetic like in other vertebrate, but environmental factors at the early life stages can influence sex differentiation of male and female (Devlin & Nagahama, 2002).

Table 3. Intersexuality and total length of *Scomberomorus niphonius*

Total length (cm)	Intersexuality (%)		
	Total	Female	Male
38.9－50.0	13.6 (n=6/44)	26.3 (n=5/19)	4.0 (n=1/25)
50.1－60.0	10.7 (n=3/28)	15.4 (n=2/13)	6.7 (n=1/15)
60.1－70.0	11.1 (n=1/9)	12.5 (n=1/8)	-
70.1－80.0	11.5 (n=3/26)	17.7 (n=3/17)	-
80.1－90.0	33.3 (n=2/6)	33.3 (n=2/6)	-
90.1－100.0	10.0 (n=1/10)	11.1 (n=1/9)	-
100.1－103.5	66.7 (n=2/3)	66.7 (n=2/3)	-

Table 4. Intersexuality and total weight of *Scomberomorus niphonius*

Total weight (g)	Intersexuality (%)		
	Total	Female	Male
336.5－500.0	18.8 (n=3/16)	33.3 (n=3/9)	-
500.1－1,000.0	9.8 (n=5/51)	15.8 (n=3/19)	6.3 (n=2/32)
1,000.1－1,500.0	10.0 (n=1/10)	11.1 (n=1/9)	-
1,500.1－2,000.0	50.0 (n=2/4)	50.0 (n=2/4)	-
2,000.1－2,500.0	20.0 (n=2/10)	50.0 (n=2/4)	-
2,500.1－3,000.0	6.3 (n=1/16)	8.3 (n=1/12)	-
3,000.1－3,500.0	16.7 (n=1/6)	16.7 (n=1/6)	-
4,500.1－5,000.0	25.0 (n=1/4)	33.3 (n=1/3)	-
5,000.1－5,500.0	16.7 (n=1/6)	16.7 (n=1/6)	-
7,500.1－7,600.0	100.0 (n=1/1)	100.0 (n=1/1)	-

Reproductive biological characteristics are mainly used to understand how the marine environment affects fish on the population level. Among various reproductive biological characteristics, being intersex is a stable and long-term indicator. Thus, the intersexuality analysis provides useful monitoring on sensitive aquatic ecosystem indicating chemical stressors of chemical pollutants including endocrine disrupting chemicals (EDCs) (Drysdale & Bortone, 1989; Borton et al., 1991; Huggett et al., 1992; Bortone & Davis, 1994; Jobling et al., 1998, 2002; Vigano et al., 2001; Lee et al., 2010).

Hermaphroditism is found in about 400 fish species, which are mostly tropical or subtropical. Generally hermaphroditism in fish occurs if male gonadal tissues is increased, and the opposite sex gonadal tissues degenerate. The gonad structure of hermaphrodite fish was divided into delimited and undelimited type. In the delimited type the testicular tissue and ovarian tissue are divided by a membrane of connective tissue. In the undelimited type the testicular tissue and ovarian tissue are divided, but 1) the connective membrane does not exist and 2) the testicular tissue and ovarian tissue are mixed (Sadovy & Shapiro, 1987). The two types of histological intersex are identified in the present study. These kinds of histological intersex are similar to *Acanthogobius flavimanus*, *Chelon haematocheilus*, *Hemibarbus labeo*, *Leiognathus nuchalis, Mugil cephalus*, and *Synechogobius hasta* (Lee et al., 2010).

The exposure of fish to EDCs can cause sexual development disorders such as feminizing of males or masculine effects on females in an endocrine system of aquatic animals (Gimeno et al., 1998a, b; Iguchi, 1998; Ackermann et al., 2002; Metrio et al., 2003; Quinn et al., 2004). The masculine effects of EDCs are reported in tributyltin (TBT), polychlorinated biphenyls (PCBs), and zinc (Holm et al., 1991; Matta et al., 1998; Ju et al., 2009; Lee et al., 2009). Phenols are known for one of the EDCs that feminize males (Gimeno et al., 1997; Gray & Metcalfe, 1997; Gray et al., 1999).

Alkylphenols, a type of EDCs, induce developing an oviduct to *Cyprinus carpio* in genetically male fish (Gimeno et al., 1997). Estrogens and alkylphenols do the same to *Rutilus rutilus* males (Trevor et al., 2001). PCBs are inhibited to ovarian development in the sex differentiation of *Oncorhynchus mykiss* (Matta et al., 1998). They also induce intersexuality to *Scaphirhynchus albus* males (Harshbarger et al., 2000). Bis-tributyltin oxide (TBTO) causes *Gasterosteus aculeatus* degeneration of the oocytes (Holm et al., 1991). Nonylphenols and octylphenols causes intersexuality to male medaka (*Oryzias latipes*) (Gray & Metcalfe, 1997; Gray et al., 1999), and the degeneration of the oocytes or sperm maturation to zebra fish (*Danio rerio*) (Weber et al., 2003).

Intersexuality, caused by EDCs, in South Korea was noticed in teleost including *Acanthogobius flavimanus*, *Chelon haematocheilus*, *Hemibarbus labeo*, *Leiognathus nuchalis, Mugil cephalus*, and *Synechogobius hasta* collected near costal area near industrial cities in Ulsan, Onsan, Shihwa, Anshan, Yeosu, and Gwangyang (Lee et al., 2010).

This study found 14.3% of intersexuality in *Scomberomorus niphonius*, whereas females were 21.3% and males were 3.9% showing females have a higher intersex rate than males. The exposure of fish to EDCs that masculinize female fish is known to be the cause, but further studies in detail are necessary.

The effect that EDCs have on ecology varies according to the species, age, and life stage of fish (Niimi, 1983). These chemicals are mostly lipid-soluble, which allows great persistence within the ecosystem, and thus medium levels of transportation and biological accumulation through the food chain (Longnecker et al., 1997; Nilsson, 2000; Safe et al., 2000). Even when the EDCs are at low concentrations, they can have a harmful influence on humans or other animals that are at higher levels of the food web (Tyler et al., 1998; Trevor et al., 2001).

However, this analysis showed no correlation between intersexuality to the total length and weight of *Scomberomorus niphonius*. These results could be due to a temporary exposure to EDCs than EDCs accumulation throughout the food web or continuous exposure. This study was limited to the hazard identification stage, which is an environmental risk assessment stage suggested by the NRC (1983). In order to find out more about the pollutants and the causal relationship identified in this study, further research is needed.

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REFERENCES

Ackermann GE, Schwaiger J, Negele RD, Fent K (2002) Effects of long-term nonylphenol exposure on gonadal development and biomarkers of estrogenicity in juvenile rainbow trout *Oncorhynchus mykiss*. Aquat Toxicol 6:203-221.

Borton D, Carroll S, Goldberg S, Harrington K, Seltzer B, Dikon A (1991) Relationship between severity of illness and nosocomial infection: A trending model. Am J Infect Control 1:123.

Bortone SA, Davis WP (1994) Fish intersexuality as indicator of environmental stress: monitoring fish reproductive systems can serve to alert humans to potential harm. BioScience 4:165-172.

De Metrio G, Corriero A, Desantis S, Zubani D, Cirillo F, Deflorio M, Bridges CR, Eicker J, de la Serna JM, Megalofonou P, Kime DE (2003) Evidence of a high percentage of intersex in the Mediterranean swordfish *Xiphias gladius* L.. Mar Pollut Bull 46:358-361.

Devlin RH, Nagahama Y (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208:191-364.

Drysdale DT, Bortone SA (1989) Laboratory induction of intersexuality in the mosquitofish *Gambusia affinis*, using paper mill effluent. Bull Environ Contam Toxicol 43:611-617.

Gimeno S, Komen H, Gerritsen AGM, Bowmer T (1998) Feminisation of young males of the common carp *Cyprinus carpio*, exposed to 4-*tert*-pentylphenol during sexual differentiation. Aquat Toxicol 43:77-92.

Gimeno S, Komen H, Jobling S, Sumpter J, Bowmer T (1998) Demasculinisation of sexually mature male common carp *Cyprinus carpio*, exposed to 4-*tert*-pentylphenol during spermatogenesis. Aquat Toxicol 43:93-109.

Gimeno S, Komen H, Venderbosch PWM, Bowmer T (1997) Disruption of sexual differentiation in genetic male common carp (*Cyprinus carpio*) exposed to an alkylphenol during different life stages. Environ Sci Technol 31:2884-2890.

Gray MA, Metcalfe CD (1997) Induction of testis-ova in Japanese medaka *Oryzias latipes* exposed to *p*-nonylphenol. Environ Toxicol Chem 16:1082-1086.

Gray MA, Niimi AJ, Metcalfe CD (1999) Factors affecting the development of testis-ova in medaka *Oryzias latipes,* exposed to octylphenol. Environ Toxicol Chem 18: 1835-1842.

Harshbarger JC, Coffey MJ, Young MY (2000) Intersexes in Mississippi River shovelnose sturgeon sampled below Saint Louis, Missouri, USA. Mar Environ Res 50:247-250.

Holm G, Norrgren L, Linden O (1991) Reproductive and histopathological effects of long-term experimental exposure to bis(tributyltin)oxide (TBTO) on the three-spined stickleback *Gasterosteus aculeatus* Linnaeus. J Fish Biol 38:373-386.

Huggett RJ, Kimerle RA, Mehrle PM Jr., Bergman HL (1992) Biomarkers - Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Lewis Publishers, London, pp 1-347.

Iguchi T (1998) Environmental endocrine disruptors. Jap J Clin Med 56:2953-2962.

Jobling S, Coey S, Whitmore JG, Kime DE, Van Look KJW, McAllister BG, Beresford N, Henshaw AC, Brisghty G, Tyler CR, Sumpter JP (2002) Wild intersex roach *Rutilus rutilus* have reduced fertility. Biol Reprod 67:515-524.

Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP (1998) Widespread sexual disruption in wild fish. Environ

Sci Technol 32:2498-2506.

Ju SM, Park JJ, Lee JS (2009) Induction of intersex and masculinization of the equilateral venus *Gomphina veneriformis* (Bivalvia: Veneridae) by zinc. Ani Cell Sys 13:339-344.

Kwon JY, Lee CH, Kim JY, Kim SH, Kin DJ, Han HK, Lim HK, Byun SG (2006) Disruption of sex differentiation by exogenous sex steroid hormones in Korean rockfish, *Sebastes schlegeli*. Dev Reprod 10:247-254.

Lee JS, Kim JW, Park JJ, Ju SM, Park JS, Lee DG, Yun TW, Choi K, Yoon J, Eom I (2010) Sex ratio and intersexuality in coastal fishes near industrial complex of Korea. J Fish Pathol 23:211-219.

Lee JS, Cho HS, Jin YG, Park JJ, Shin YK (2009) Reproductive disrupting effect of organotin compound in the ark shell *Scapharca broughtonii* (Bivalvia: Arcidae). Ani Cell Sys 13:223-227.

Longnecker MP, Rogan WJ, Lucier G (1997) The human health effects of DDT (dichlorodiphenyl-trichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organochlorines in public health. Annu Rev Public Health 18:211-244.

Matta MB, Cairncross C, Kocan RM (1998) Possible effects of polychlorinated biphenyls on sex determination in rainbow trout. Environ Toxicol Chem 17:26-29.

Nilsson R (2000) Endocrine modulators in the food chain and environment. Toxicol Pathol 28:420-431.

NRC (National Research Council) (1983) Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, D.C., pp 1-192.

Quinn B, Gagne F, Costello M, McKenzie C, Wilson J, Mothersill C (2004) The endocrine disrupting effect of municipal effluent on the zebra mussel (*Dreissena polymorpha*). Aquat Toxicol 66:279-292.

Sadovy Y, Shapiro DY (1987) Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1987:136-156.

Safe SH, Welsch F, Janszen DB (2000) Endocrine disruptors and human health: Is there a problem? An update. Environ Health Perspect 108:487-493.

Trevor PRG, Susan J, Carole K, Steven M, Geoff B, Michael JW, John PS, Charles RT (2001) Exposure of juvenile roach *Rutilus rutilus* to treated sewage effluent induces dose-dependent and persistent disruption in gonadal duct development. Environ Sci Techol 35: 462-470.

Tyler CR, Routledge EJ (1998) Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment, oestrogenic effects in fish in English rivers with evidence of their causation. Pure Appl Chem 70:1795-1804.

Tyler CR, Jobling S, Sumpter JP (1998) Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol 28:319-361.

Weber LP, Hill RL Jr., Janz DM (2003) Developmental estrogenic exposure in zebrafish (*Danio rerio*): II. Histological evaluation of gametogenesis and organ toxicity. Aquat Toxicol 63:431-446.