J. Fish. Sci. Technol. 8(4), 207-212, 2005

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Directional Asymmetry of Gonadal Development in Ayu (*Plecoglossus altivelis*)

In-Seok Park*, Chang Ik Zhang[1], Young Ja Kim[2] and In Chul Bang[3]

**Division of Marine Environment and Bioscience, College of Ocean Science and Technology, Korea Maritime University, Busan 606-791,Korea*

[1]*Department of Marine Production Management, Pukyong National University, Busan 608-737, Korea*

[2]*Research Institute of Marine Science and Technology, Korea Maritime University, Busan 606-791, Korea*

[3]*Department of Life Science, College of Natural Science, Soonchunhyang University, Asan 333-745, Korea*

Directional (sinistral) asymmetry (DA) occurs when the traits of one side of a supposedly bilaterally symmetrical organism differ in a random way from those of the other side. We examined asymmetries in gonadal growth traits within both sexes of hatchery reared ayu, *Plecoglossus altivelis*. The pattern of the gonadal growth from hatching to 320 days after hatching was a type of DA except for the growth in gonad weight from 140 to 180 days after hatching, although the ovary of the right side tended to exhibit more pronounced DA phenomenon.

Key words: Ayu (*Plecoglossus altivelis*), Directional asymmetry, Sinistral, Gonadal development, Histological analysis, Growth pattern

Introduction

Directional asymmetry (DA) occurs when the traits of one side of a supposedly bilaterally symmetrical organism, where the left and right halves of the body are basically mirror images of one another, differ in a random way from those of the other side (Graham et al., 1998). It is expressed as the difference in the number, size, shape or other feature of the traits between the left and right side. Phylogenetic analyses of asymmetry variation offer a powerful tool for exploring the interplay between ontogeny and evolution because (i) conspicuous asymmetries exist in many higher metazoans with widely varying modes of development, (ii) patterns of bilateral variations within species may identify genetically and environmentally triggered asymmetries, and (iii) asymmetries arising at different times during development may be more sensitive to internal cytoplasmic inhomogeneities compared to external environmental stimuli (Palmer, 1996).

Ayu, *Plecoglossus altivelis* Temminck and Schlegel belong to the Order Salmoniformes and the Family Plecoglossidae, and are an amphidromous species found in the Far Eastern Asia region including Korea, Japan, Taiwan and China (Chyung, 1977; Kim and Park, 2002). They are considered a gourmet delicacy in the Far Eastern Asia and are used for a profitable, though fairly primitive, form of fish culture in Korea (Bang et al., 2000; Cho et al., 2003). In this paper we examine DA in gonadal growth traits within both sexes of hatchery reared ayu.

*Corresponding author: ispark@hhu.ac.kr

Materials and Methods

Fish collection and rearing method

The experimental materials were laboratory-bred offspring of the ayu, from the Fishery Genetics and Breeding Laboratory Aquarium (Division of Marine Environment and Bioscience, Korea Maritime University, Korea), obtained by the standard method of artificial propagation. Newly hatched larvae were sampled at 3-day intervals from the 1st to the 30th day after hatching, at 5-day intervals from the 30th to the 60th day after hatching and at 10 to 30-day intervals from the 150th to the 320th day after hatching. Fifty individuals were randomly selected from each sampling. Water temperature was maintained at 23±0.5°C.

Observations and Measurements

Total length and body weight for 50 freshly sampled larvae at different stages were measured to the nearest 0.01 g and 0.01 cm. Prior to measuring, the larvae were anaesthetized with 300 ppm lidocain-HCl/$NaHCO_3$ at 23°C. Using data on total length and body weight of ayu from samples for 320 days after hatching, we estimated the growth equations for total length and body weight with respect to time (days). Growth rates for the first 60 days after hatching were fit separately from those for 61-320 days. For the histological observation of gonads, samples were fixed in 10% neutral formalin after measurement. After fixing in Bouin's fixative for 24 hr, gonads extracted from specimens of the 1^{st} to the 120^{th} day post-hatch group (head and caudal region excluded) and the 140^{th} to the 320^{th} day post-hatch group, were processed for histological sectioning by routine dehydration and paraffin embedding procedures. Cross-sections of 4-6 μm thickness were stained with Mayer's hematoxylin and eosin phloxine B solution for the examination.

External morphological characteristics of gonads at 20, 120, 200 and 320 days after hatching were drawn and ovaries and testes at 320 days after hatching, fixed in 10% neutral formalin, were photographed. Lengths and weights of ovary and testis from sampled fish were measured after hatching. The growth patterns of the two gonads showed exponential curves, and thus we employed an exponential growth model for explaining the gonadal development of ayu.

Statistical analysis

To examine the asymmetry of the gonadal development of ayu, statistical tests on the difference between the left and right gonads were performed to assess whether the intercepts and slopes of the growth curves for the two groups were statistically different. An one way analysis of variance (ANCOVA) was used to test the null hypothesis. Also, Student's t-test was employed to test for the differences in length and weight between the right and left gonads for each developing stage and to detect which traits exhibit the strongest asymmetric mechanism.

Results and Discussion

Fig. 1a shows the linear growth pattern in total length for ayu. Presumably, the different growth equations were due to switching food items from Artemia nauplius to artificial feed. Growth pattern of the body weight of ayu was represented an exponential curve from 70 to 320 days after hatching as shown in Fig. 1b. Lengths and weights of ovary and testis of ayu were gradually regressed on days after hatching (Fig. 2). There was no difference in gonadal growth in the first 100 days after hatching. This is consistent with the observation that the gonadal differentiation type for ayu is gonochoristic. Ovarian differentiation was identified by the presence in the

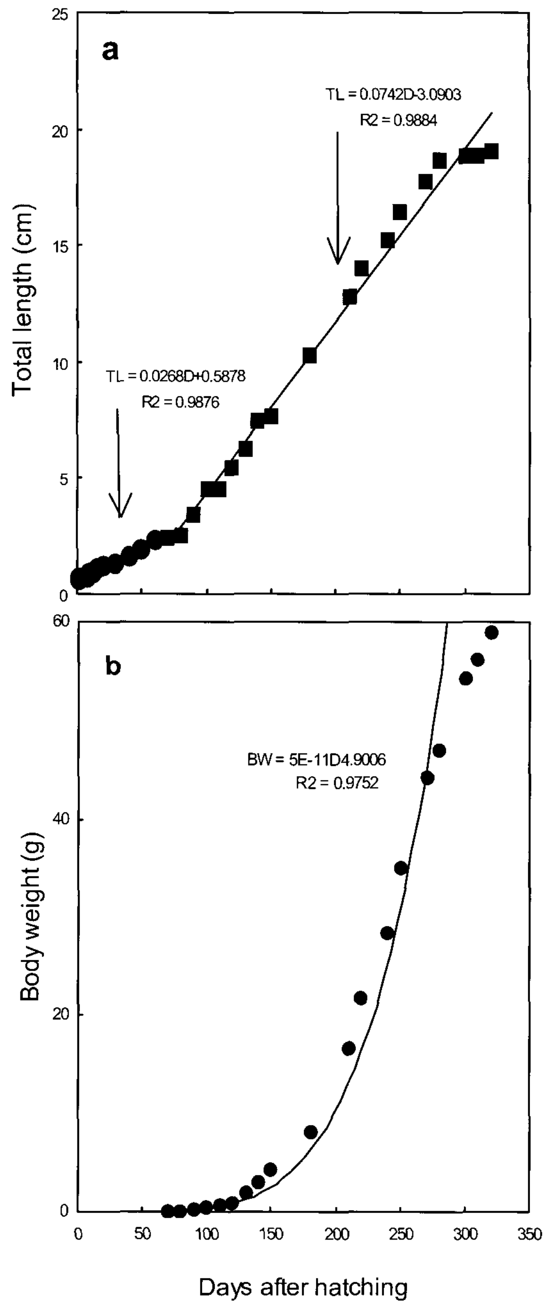

Fig. 1. Early growth of ayu, *Plecoglossus altivelis*, in total length (a) and body weight (b) from hatching to 320 days after hatching.

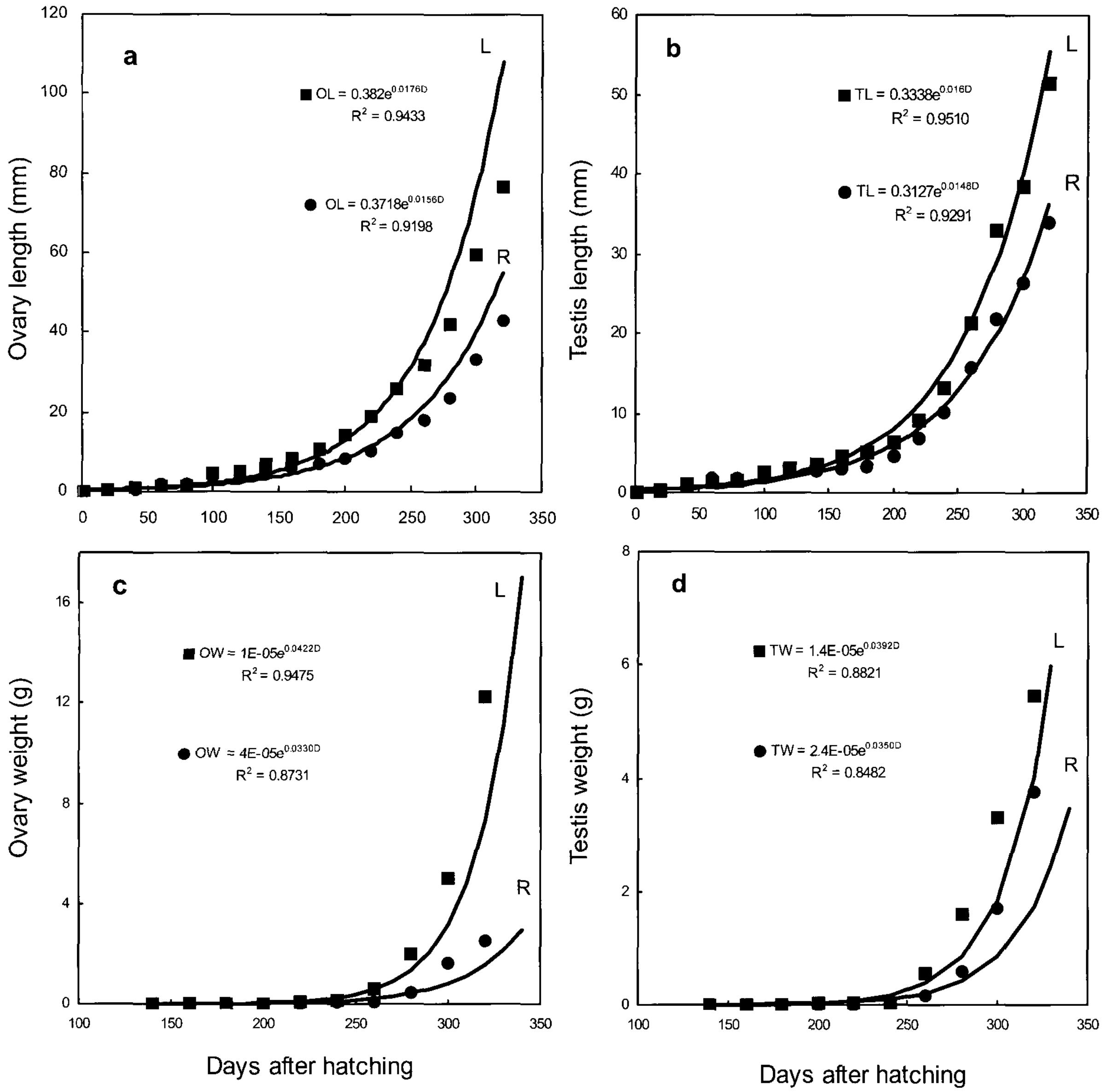

Fig. 2. Early growth of ovary length (a) and testis length (b) from hatching to 320 days after hatching, and ovary weight (c) and testis weight (d) from 140 days to 320 days after hatching in ayu, *Plecoglossus altivelis*. R, Right side (●); L, Left side (■).

ovarian cavity of meiotic oocytes in 90-day old fry, and the testicular differentiation was identified by the presence of spermatogonial cells and efferent duct in 100-day old fry as reported by Sasaki et al. (1987) and Bang et al. (2000).

Directional asymmetry (DA) patterns of bilateral variation within species may identity genetically triggered asymmetries (Palmer, 1996), and this DA and developmental instability have been estimated using the residual variance from directionally asymmetric traits in mandibles (horse mouse) and leaves (soybean) (Graham et al., 1998). Gonadal growth of all four traits were apparently exponential (Figs. 2 a-d). Results of the ANCOVA show that the intercepts of all the traits were significantly different between the right and left gonads except for the testis weight ($P<0.05$) (Table 1). The slopes of the testis weight were not statistically different ($P>0.05$). Also, the Students t-test showed that the lengths of ovary and testis between the right and left were highly significant for all the developing stages ($P<0.001$) (Table 2). However, weights of ovary and testis

Table 1. ANCOVA results for testing the difference between left and right gonads in ayu

	Intercept				Slope			
	Left	Right	t-value	*P*	Left	Right	t-value	*P*
Length								
Ovary	-0.9623	-0.9893	-2.1809*	<0.05	0.0176	0.0156	-1.2418	>0.05
Testis	-1.0977	-1.1624	-25.0246**	<0.001	0.0160	0.0148	-0.8005	>0.05
Weight								
Ovary	-11.4114	-10.2101	-2.6608*	<0.05	0.0422	0.0030	-1.6161	>0.05
Testis	-11.1664	-10.6408	-1.1433	>0.05	0.0392	0.0350	-0.5898	>0.05

* Significant at α=0.05, ** Significant at α=0.01.

Table 2. Result of Student's t-test for the difference between lengths of left and right gonads of ayu

	Undifferentiated gonads			
	Left	Right	t-value	*P*
0	0.12	0.11	7.83*	<0.001
20	0.34	0.30	17.31*	<0.001
40	1.07	0.64	146.87*	<0.001
60	1.70	1.31	81.51*	<0.001
80	1.80	1.53	60.90*	<0.001

	Ovary				Testis			
	Left	Right	t-value	*P*	Left	Right	t-value	*P*
100	4.60	3.50	39.50*	<0.001	2.50	2.12	17.73*	<0.001
120	5.30	4.22	34.41*	<0.001	3.14	2.53	31.75*	<0.001
140	6.74	4.97	41.38*	<0.001	3.61	2.75	36.96*	<0.001
160	8.81	5.85	45.87*	<0.001	4.48	3.05	50.96*	<0.001
180	10.81	6.76	57.53*	<0.001	5.17	3.41	51.35*	<0.001
200	14.30	8.12	64.78*	<0.001	6.39	4.48	44.31*	<0.001
220	17.04	10.13	63.15*	<0.001	9.09	6.74	35.28*	<0.001
240	25.91	14.58	67.37*	<0.001	13.21	10.09	31.83*	<0.001
260	31.89	18.18	41.78*	<0.001	21.20	15.59	34.59*	<0.001
280	41.79	23.56	61.64*	<0.001	32.91	21.86	45.46*	<0.001
300	59.45	33.28	59.34*	<0.001	38.47	26.23	40.79*	<0.001
320	76.61	42.87	59.91*	<0.001	51.29	33.90	43.88*	<0.001

*Sgnificant at α=0.01.

Table 3. Results of Student's *t*-test for the difference between weights of left and right gonads in ayu

	Ovary				Testis			
	Left	Right	t-test	*P*	Left	Right	t-test	*P*
140	0.01	0.01	0.79	>0.05	0.01	0.01	0.57	>0.05
160	0.01	0.01	1.37	>0.05	0.01	0.01	0.99	>0.05
180	0.01	0.01	1.72	>0.05	0.01	0.01	1.37	>0.05
200	0.03	0.01	12.00*	<0.001	0.02	0.01	4.86*	<0.001
220	0.05	0.01	21.12*	<0.001	0.02	0.01	6.06*	<0.001
240	0.17	0.07	53.00*	<0.001	0.05	0.03	13.77*	<0.001
260	0.62	0.11	163.54*	<0.001	0.54	0.18	123.33*	<0.001
280	2.01	0.48	184.41*	<0.001	1.60	0.58	305.18*	<0.001
300	5.03	1.59	175.08*	<0.001	3.31	1.70	143.46*	<0.001
320	12.25	2.53	118.16*	<0.001	5.44	3.77	40.75*	<0.001

* Significant at α=0.01.

between the right and left were highly significant only for the developing stages for 200 days after hatching (P<0.001) (Table 3).

During this experimental period, the gonadal growth of ayu showed significant differences (P< 0.001), and thus it seems that the pattern of gonadal growth of ayu is DA in general except for growth in gonad weight from 140 to 180 days after hatching. Ovary length in ayu showed the strongest asymmetric mechanism among the four traits, having about 1.7 times greater ovary of the right side than that of the left side (Table 2). From the results of Figs. 3-4, the

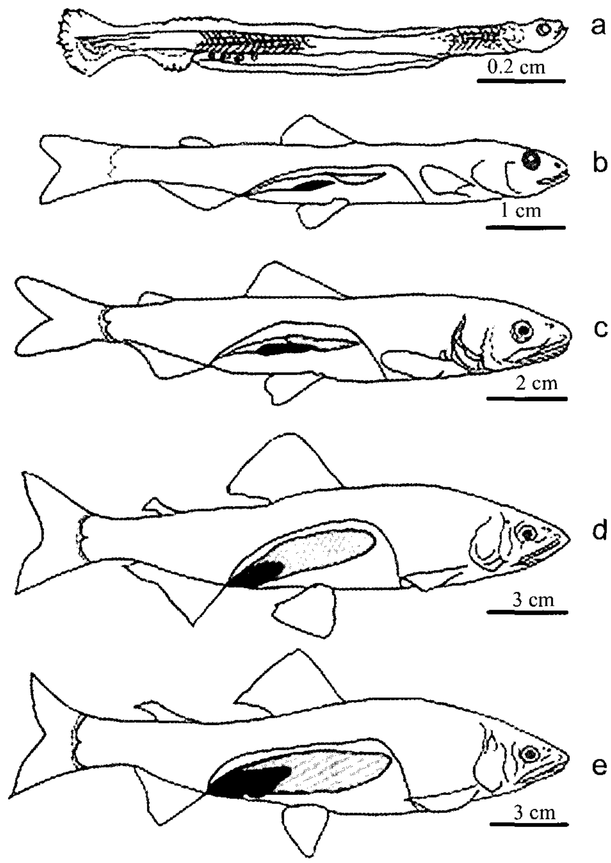

Fig. 3. Bilateral asymmetry in gonad development pattern of ayu, *Plecoglossus altivelis*. (a) Post-hatching 20 days old larva with undifferented gonad, mean TL 1.18 cm; (b) Post-hatching 120 days old female juvenile, mean TL 5.45 cm; (c) Post-hatching 220 days old female adult, mean TL 13.97 cm; Post-hatching 320 days old female (d) and male (e) adult, mean TL 19.04 cm. Right side gonad (■), Left side gonad (▒).

pattern of DA in ayu gonads are apparently sinistral, although the ovary of the right side tended to exhibit more pronounced DA phenomenon. This DA does not have obvious functional significance. DA regularly occurs in the gonad of birds and other vertebrates (Møoller, 1994) and various other vertebrate charac-ters (Alvarez, 1995). Right-biased directionality of ayu gonad expression in this study is unlike those of left-biased directionality of pelvic girdle expression in three-spined stickleback, *Gasterosteus aculeatus* which is known to occur usually in association with pelvic reduction (Bell et al., 1985; Reimchen, 1997; Mazzi and Bakker, 2001).

Our present result of DA of gonadal development in ayu suggests that this phenomenon is not the result of developmental noise or accident, but rather a

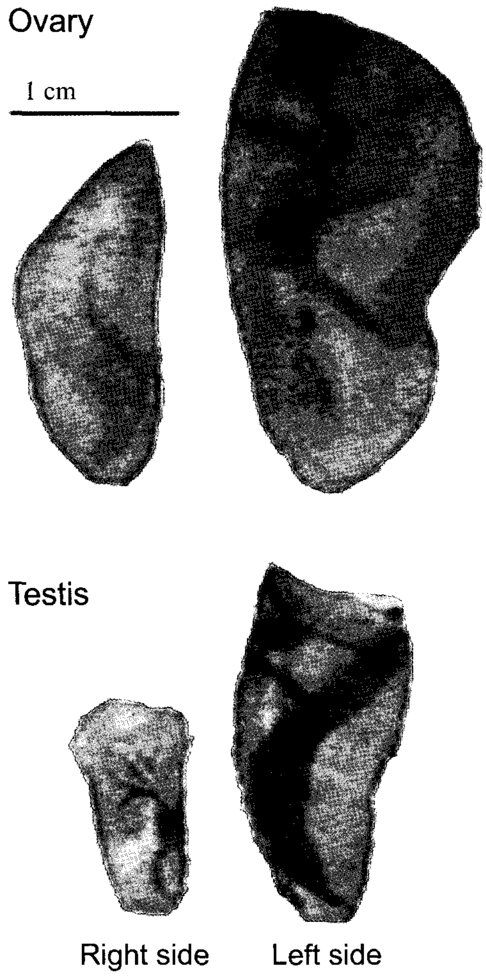

Fig. 4. External morphology of ovary and testis of ayu, *Plecoglossus altivelis* at 320 days after hatching (mean TL, 19.04 cm) (prespawning period). R, Right side gonad; L, Left side gonad.

developmentally controlled DA, which can be an example of a genetically controlled discontinuous character (Matsukuma, 1996). This is the first report to our knowledge on DA in fish. We suggest that this DA in ayu may represent a stochastic fixation in phylogeny and thus may be useful in systematics research as noted by Francis et al. (1985).

Acknowledgments

Comments from anonymous reviews greatly improved the quality of this manuscript. This research was supported by a project grant (KRF-2004-005-F00003) from Korea Research Foundation Grant to I.-S. Park, Fishery Genetics and Breeding Laboratory. The assistance of the project staff is gratefully acknowledged.

References

Alvarez, F. 1995. Functional directional asymmetry in fellow deer (*Dama dama*) antlers. J. Zool., 236, 563-569.

Bang, I.C., S.Y. Park, Y.A. Lee, C.H. Kim and K.K. Kim. 2000. Early gonadogenesis and sex differentiation in sweet fish, (*Plecoglossus altivelis*). Aquaculture, 13, 215-222.

Bell, M.A., R.C. Francis and A.C. Havens. 1985. Pelvic reduction and its directional symmetry in threespine sticklebacks from Cook Inlet Region, Alaska. Copeia, 2, 437-444.

Cho, S.H., Y.S. Lim, J.H. Lee, J.H. Lee, J.K. Lee, S.G. Park and S.M. Lee. 2003. Effects of feeding rate and feeding frequency on survival, growth, and body composition of ayu post-larvae *Plecoglossus altivelis*. J. World Aquacult. Soc., 34, 85-91.

Chyung, M.K. 1997. The Fishes of Koera. Iljisa, Seoul, pp. 727.

Francis, R.C., A.C. Havens and M.A. Bell. 1985. Unsusal lateral plate variation of threespine sticklebacks, (*Gasterosteus aculeatus*) from Knik Lake, Alaska. Copeia, 3, 619-624.

Graham, J.H., J.M. Emlen, D.C. Freeman, L.J. Leamy and J.A. Kieser. 1998. Directional asymmetry and the measurement of developmental instability. Biol. J. Linn. Soc., 64, 1-16.

Kim, I.S. and J.Y. Park. 2002. Freshwater Fishes of Korea. Kyohak-sa, Seoul, pp. 465.

Matsukuma, A. 1996. Transposed hinges: A polymorphism of bivalve shells. J. Moll. Stud., 62, 415-431.

Mazzi, D. and T.C.M. Bakker. 2001. Acid stress increases pelvic spine asymmetry in juvenile three-spined sticklebacks. J. Fish Biol., 59, 582-592.

MØller, A.P. 1994. Directional selection on directional asymmetry: testes size and secondary sexual characters in birds. Proc. Roy. Soc. London. Ser. B, 258, 147-151.

Palmer, A.R. 1996. From symmetry to asymmetry: Phylogenetic patterns of asymmetry variation in animals and their evolutionary significance. Pro. Natl. Acad. Sci. USA, 93, 14279-14286.

Reimchen, T.E. 1997. Parasitism of asymmetrical pelvic phenotypes in stickleback. Can. J. Zool., 75, 2084-2094.

Sasaki, T., F. Takashima and A. Takahashi. 1987. Sex differentiation and its manipulation in ayu. Suisanzoshoku, 34, 249-251.

(Received June 2005, Accepted December 2005)