

Clutch and Egg Sizes of Two Migratory Forms of the Threespine Stickleback *Gasterosteus aculeatus* in Eastern Hokkaido, Japan

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Manabu Kume (2011) Clutch and egg sizes of two migratory forms of the threespine stickleback *Gasterosteus aculeatus* in eastern Hokkaido, Japan. *Zoological Studies* 50(3): 309-314. Clutch and egg sizes were compared between the Japan Sea and Pacific Ocean forms of the threespine stickleback collected from the Bekanbeushi River in eastern Hokkaido, Japan. The Japan Sea form produced many small-sized eggs, whereas the Pacific Ocean form produced fewer large-sized eggs, suggesting the existence of a trade-off between clutch and egg sizes in both forms. A previous study revealed that the 2 forms diverged in their breeding habitats by using different salinity regimes within a single watershed. These findings suggest that the Japan Sea and Pacific Ocean forms have evolved different reproductive strategies and characteristics in response to differing salinities and properties of their respective environments. <http://zoolstud.sinica.edu.tw/Journals/50.3/309.pdf>

Key words: Egg, Reproductive strategy, Salinity adaptation, Anadromy, *Gasterosteus aculeatus*.

The trade-off between offspring number (e.g., clutch size) and offspring size (e.g., egg size) is a common feature in both animals and plants (Roff 1992, Goto and Iguchi 2001). In animals, empirical evidence for this trade-off is generally based upon negative correlations between clutch and egg sizes (Roff 1992), suggesting a potential trade-off between offspring number and the quality of zygotes (Smith and Fretwell 1974, Einum and Fleming 2000). This trade-off is based on the assumption that the amount of material available for investing in offspring is limited. Hence, females optimize the rates of gain from investing in individual offspring against the costs to the total number of offspring produced, so that the fitness of both the female and its offspring are maximized (Smith and Fretwell 1974, Roff 1992). In teleost, the trade-off between clutch and egg sizes has received substantial empirical attention and is well described across (Duarte and Alcaraz 1989, Elgar 1990, Kolm et al. 2006) and within species (Baker

1994, Einum and Fleming 2000). Variations in reproductive characteristics reflect adaptations to various factors, such as water temperature, salinity, and parental care (Iguchi and Yamaguchi 1994, Johnston and Leggett 2002, Kolm and Ahnesjö 2005).

The threespine stickleback species complex (*Gasterosteus aculeatus*) is widely distributed along coastal regions throughout the Northern Hemisphere (Wootton 1984). This species complex contains various morphs that are often reproductively isolated from other morphs. Thus, the threespine stickleback species complex provides a great model system for elucidating the ecological mechanisms underlying phenotypic evolution and speciation (Schluter 2000, McKinnon and Rundle 2002, Hendry et al. 2009). The threespine stickleback found in coastal regions of Japan includes a genetically divergent species pair: the Japan Sea and Pacific Ocean forms (Higuchi and Goto 1996, Kitano et al. 2007).

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These 2 forms are thought to have diverged when the Sea of Japan was geographically isolated from the Pacific Ocean about 2×10^6 yr ago (Higuchi and Goto 1996, Kitano et al. 2007). After the last glacial period, the Japan Sea and Pacific Ocean forms were brought into secondary contact. The life-history patterns of the 2 forms differ (Higuchi and Goto 1996): the Japan Sea form is comprised of anadromous populations, while the Pacific Ocean form has anadromous and freshwater life-history features. Breeding habitats of the Japan Sea and Pacific Ocean forms are also divergent (Kitano et al. 2009, Kume et al. 2010): the Japan Sea form predominantly breeds in brackish waters, while the Pacific Ocean form primarily uses freshwater habitats to breed. In addition, the 2 forms diverge in body shape and feeding traits in each one's native habitat (Kitano et al. 2007, Kume et al. 2010). Thus, if this habitat divergence is promoting ecologically significant phenotypic adaptations, I would predict that divergences in both morphological and reproductive traits are occurring between the 2 forms, because reproductive traits are directly associated with the fitness of both females and juveniles. In the present study, I compare female reproductive characteristics (gonadosomatic index (GSI), clutch size (CS), and egg size (ES)) between the anadromous Japan Sea and Pacific Ocean populations of the threespine stickleback and discuss the evolutionary processes of their reproductive strategies in relation to the different salinity regimes in each form's breeding habitat.

MATERIALS AND METHODS

The Japan Sea and Pacific Ocean forms of *G. aculeatus* were collected from the Bekanbeushi River in eastern Hokkaido, Japan. In this river, the Japan Sea form was observed to predominantly use the brackish-water lake, Lake Akkeshi, to breed, whereas the Pacific Ocean form was found to primarily breed in the lower to middle reaches of the river (Kume et al. 2005 2010, Kitano et al. 2009). Specimens were identified as either the Japan Sea or Pacific Ocean form based on morphological characteristics: the Japan Sea form is smaller, possesses shorter caudal lateral plates, and has more-numerous gill rakers (Higuchi and Goto 1996, Kitano et al. 2007). From May to June 2003 and 2005, mature females (Kume et al. 2005) were collected at Kamuiwa (station B; northwestern part of Lake Akkeshi;

43°4'N, 144°52'E) for the Japan Sea form using a haul seine and in the Ohbetsu River (station A; a tributary of the Bekanbeushi River; 43°5'N, 144°53'N) for the Pacific Ocean form using a small stationary set net.

All samples were preserved in 10% formalin. We measured the standard length (SL; to the nearest 0.05 mm) with calipers and body weight (BW; to the nearest 0.01 g) with a digital balance. After the ovaries were excised from females, we measured the gonad weight (GW; to the nearest 0.01 g) using a digital balance and calculated the GSI, defined as $GSI = (GW / BW - GW) \times 100$ (Wootton 1998). CS and ES were compared between the 2 forms. The total number of eggs was counted in each ovary. I measured the diameter of ~200 unfertilized but fully mature eggs (to the nearest 0.01 mm) chosen at random from each female using calipers, and calculated the mean egg size in each female.

In several teleost including *G. aculeatus*, allometric relationships between reproductive characteristics and body size were shown (Duarte and Alcaraz 1989, Wootton 1998). Thus, allometric relationships between reproductive characteristics (GW, CS, and ES) and SL were tested by an analysis of variance (ANOVA). Before being analyzed by ANOVA, GW, CS, ES, and SL were log₁₀-transformed. If significant correlations between reproductive characteristics and SL were recognized by ANOVA, slopes of the regression lines of the 2 forms were compared by an analysis of covariance (ANCOVA). In addition, if no significant differences in the regression coefficients (slopes) were recognized by ANCOVA, the intercepts of the regression lines were compared by ANCOVA after the slopes of the regression lines were corrected. On the other hand, if no significant correlations between reproductive characteristics and SL were recognized by ANOVA, mean values of the reproductive characteristics were compared between the 2 forms by a *t*-test. Data analyses were performed by using R vers. 2.6.1 software (R Development Core Team 2008).

RESULTS

Reproductive investment in ovaries was compared between females of the Japan Sea and Pacific Ocean forms of *G. aculeatus*. No significant correlation was recognized between log₁₀GW (Y) and log₁₀SL (X) in either form (Japan Sea form, $Y = -1.72 + 0.77X$, $R^2 = 0.006$, $F_{1,25} =$

0.001, $p = 0.98$; Pacific Ocean form, $Y = -5.50 + 2.93X$, $R^2 = 0.21$, $F_{1,27} = 1.23$, $p = 0.28$). The mean GSI of the Japan Sea form was significantly smaller than that of the Pacific Ocean form ($t = 2.14$, $p = 0.037$; Table 1).

The mean numbers of eggs per female were 446 (range, 329-547) eggs ($n = 13$) for the Japan Sea form and 506 (range 325-694) eggs ($n = 10$) for the Pacific Ocean form (Table 1). A significant correlation was found between $\log_{10}CS$ (Y) and $\log_{10}SL$ (X) of females of each form (Japan Sea form, $Y = -5.95 + 4.59X$, $R^2 = 0.630$, $F_{1,10} = 7.24$, $p = 0.021$; Pacific Ocean form, $Y = -6.62 + 4.82X$, $R^2 = 0.75$, $F_{1,7} = 10.0$, $p = 0.013$; Fig. 3). There were no significant differences in regression coefficients (slopes) between the 2 forms ($F_{1,19} = 0.053$; $p > 0.05$), but the intercept of the regression line was significantly higher for the Japan Sea form than for the Pacific Ocean form (ANCOVA, $F_{1,20} = 11.3$, $p < 0.005$; Fig. 1). These results indicate that the size-adjusted CS of the Japan Sea form was

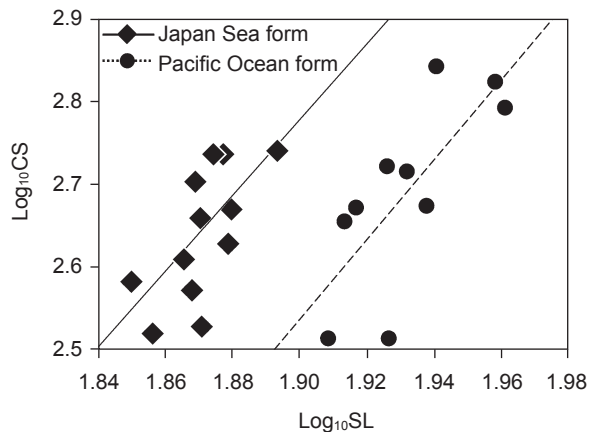


Fig. 1. Relationship between the clutch size (CS) and standard length (SL) of the Japan Sea and Pacific Ocean forms of the threespine stickleback.

larger than that of the Pacific Ocean form.

There was a positive relationship between $\log_{10}ES$ (Y) and $\log_{10}SL$ (X) in the Japan Sea form ($Y = -1.49 + 1.02 X$, $R^2 = 0.59$, $F_{1,10} = 22.6$, $p = 0.02$). Meanwhile, no correlation was recognized in the Pacific Ocean form ($Y = -0.04 + 0.14X$, $R^2 = 0.04$, $F_{1,7} = 0.38$, $p = 0.55$), as was shown in several teleost including *G. aculeatus* (Duarte and Alcaraz 1989, Baker 1994). The mean ES of the Japan Sea form was remarkably smaller than that of the Pacific Ocean form ($t = 15.0$, $p < 0.0001$; Table 1).

DISCUSSION

Differences in female reproductive characteristics between the Japan Sea and Pacific Ocean forms of *G. aculeatus* were found in this study. Japan Sea females produced relatively larger numbers of smaller eggs than did Pacific Ocean females. Anadromous populations of the 2 forms, which cohabit in the Biwase River of eastern Hokkaido, Japan, were also shown to have similar relationships between clutch and egg sizes (Higuchi 1996). Among anadromous Japan Sea and Pacific Ocean populations around Japan, geographical variations in both clutch size and egg size were recognized: 54-61 eggs in the adjusted clutch size (eggs/10 mm) and 1.46-1.57 mm in mean egg diameter for Japan Sea females, and 59 eggs in the adjusted clutch size (eggs/10 mm) and 1.61-1.86 mm in mean egg diameter for Pacific Ocean females (Tables 1, 2). These findings suggest that the tendency of the Japan Sea form to produce smaller eggs than the Pacific Ocean form is a common feature. However, differences in clutch size between the 2 forms were unclear. Thus, further studies are required to compare

Table 1. Comparison of reproductive characteristics (mean \pm S.D.) between mature Japan Sea and Pacific Ocean females of the threespine stickleback

Reproductive characteristics	Japan Sea form	Pacific Ocean form	t-test	
			t value	p value
GSI	15.3 \pm 4.56 (27)	19.4 \pm 8.74 (29)	2.143	0.037
CS	446 \pm 78 (13)	506 \pm 127 (10)	1.326	0.206
adjusted CS*	61	59	-	-
ES (mm)	1.36 \pm 0.050 (13)	1.69 \pm 0.054 (10)	15.17	< 0.0001
SL (mm)	73.4 \pm 3.36 (13)	85.1 \pm 3.64 (10)	6.130	< 0.0001

GSI: gonad somatic index, CS: clutch size, ES: egg size, SL: standard length. *Adjusted CS = mean CS \times 10 / mean SL. Figures in parentheses indicate sample size.

clutch-egg sizes relationships between them.

In terms of the relationship between clutch and egg sizes in aquatic organisms, a relatively smaller number of larger-sized eggs was hypothesized to represent an adaptation to a poor food supply as in the upper reaches of a river (freshwater environments) by promoting survival in the early developmental period, while a larger number of smaller-sized eggs is thought to represent an adaptation to a rich food supply as in a river mouth (brackish and/or seawater) (Roff 1992, Wootton 1998). In freshwater fish, for example, it is well known that fish breeding in the upper reaches of rivers tend to produce a smaller number of larger eggs than fish breeding in the lower reaches (Goto and Iguchi 2001). *Gasterosteus aculeatus* is widely distributed at high latitudes (Wootton 1984), where the primary productivity in the sea and brackish waters is higher than that in fresh water. Consistent with the work in other fish, freshwater resident populations of *G. aculeatus* have larger and fewer eggs than anadromous populations (Snyder 1990, Mori 1991, Baker 1994). In the Bekanbeushi River, the Japan Sea form predominantly breeds in a brackish-water lake (with salinities of 20-33 psu), whereas the Pacific Ocean form mainly breeds in the middle reaches of the river in relatively low-salinity environments (with salinities of 0-10 psu) (Kitano et al. 2009, Kume et al. 2010). Thus, this hypothesis may apply to the Japan Sea and Pacific Ocean forms of *G. aculeatus*: with a relatively higher food supply in brackish-water environments than in fresh waters, the Japan Sea form may have

improved its adaptation by laying a larger number of smaller-sized eggs.

An alternative hypothesis is that the different relationships between clutch and egg sizes in the Japan Sea and Pacific Ocean forms may reflect adaptations to different predation regimes during the early developmental stage. In general, parent fish and their offspring in the lower reaches are at higher risks of predation than those in the upper reaches in a single watershed (Wootton 1998). Hence, it was predicted that fish which produce many small-sized eggs are more adaptive in a habitat with high predation, because survival possibilities of their eggs and juveniles are increased (Goto and Iguchi 2001). In the Bekanbeushi River, 5 species of piscivorous fish, including *Salvelinus leucomaenis leucomaenis* and *Myoxocephalus stelleriwerre*, were observed at the river mouth to the lower reaches (see table 3 in Kume et al. 2010). However, no predatory fish were found in the upper reaches, although some nonpiscivorous fish, such as *Tribolodon hakonensis* and *Phoxinus phoxinus sachalinensis*, were observed (Kume et al. 2010). If there is a relatively higher predation risk for eggs and juveniles of the Japan Sea form relative to that for the Pacific Ocean form, the former may have advanced its adaptation by laying a greater number of smaller-sized eggs. However, I could not determine whether piscivorous fish fed on eggs and juveniles of the threespine stickleback. Therefore, further studies on the feeding habits of potential predatory fishes need to be performed to test this prediction.

The Japan Sea and Pacific Ocean forms

Table 2. Comparison of female reproductive characteristics among anadromous populations of threespine stickleback shown in previous studies

Location	SL (mm)	GSI	CS	adjusted CS*	ES (mm)	References
Japan Sea form						
Kahoku-gata (Ishikawa)	74.6 (64.0-80.3)	22.6	401 (203-578)	54	1.46 (± 0.084 S.D.)	Mori (1987, 1991)
Biwase River (Hokkaido)	69.8 (59.7-75.6)	-	- (189-503)	-	1.57 (1.53-1.72)	Higuchi (1996)
Kyonsanpukudo (Korea)	76 (70-82)	21.5	-	-	-	Mori (1991)
Pacific Ocean form						
Lake Harutori (Hokkaido)	80.2 (75.3-85.5)	28.05	471 (308-655)	59	-	Mori (1990), Higuchi (1996)
Biwase River (Hokkaido)	81.1 (72.2-90.6)	-	481 (385-642)	59	1.86 (1.73-1.91)	Higuchi (1996)

GSI: gonad somatic index, CS: clutch size, ES: egg size. *Adjusted CS = mean CS × 10 / mean SL. Figures in parentheses indicate the range. Bars indicate no data.

of *G. aculeatus* have different life-history styles: freshwater resident populations of the Pacific Ocean form are found in many lakes and rivers, whereas no population with a freshwater resident life-history style was found for the Japan Sea form (Mori 1987, Higuchi and Goto 1996). In fact, except for the Japan Sea form of *G. aculeatus*, freshwater resident populations were frequently derived from anadromous populations (Mori 1990, Bell and Foster 1994, Higuchi et al. 1996). In a rearing experiment, juvenile fish of the Japan Sea form had higher mortalities than those of the Pacific Ocean form after 2 or 3 month in freshwater environments; the former was particularly susceptible to thyroid goiter (Yamada 2003). This period of higher mortality under freshwater rearing conditions coincided with the period that Japan Sea juveniles begin their sea-run migration in nature (Kume and Mori 2009). Thus, a lack of freshwater resident populations of the Japan Sea form in natural waters may be attributed to their low freshwater adaptability during the young developmental period. These facts suggest that the life-history style of the Japan Sea form has evolved to be dependent on seawater environments, whereas that of the Pacific Ocean form has evolved to be dependent on freshwater environments. Such differences in evolutionary processes of the life-history style of each form being dependent on the salinity regime in each breeding habitat may be reflected in differences in female reproductive characteristics. Further comparative studies of female reproductive characteristics among sympatric and allopatric populations of both forms are needed to clarify the evolutionary processes of the divergent reproductive strategies of the 2 forms in terms of their adaptations to different environmental conditions (e.g., salinity) in each breeding habitat.

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REFERENCES

- Baker JA. 1994. Life history variation in female threespine stickleback. In MA Bell, SA Foster, eds. The evolutionary biology of the threespine stickleback. Oxford, UK: Oxford Univ. Press, pp. 144-187.
- Bell MA, SA Foster, eds. 1994. The evolutionary biology of the threespine stickleback. Oxford, UK: Oxford Univ. Press.
- Duarte CM, M Alcaraz. 1989. To produce many small or few large eggs: a size-independent reproductive tactic of fish. *Oecologia* **80**: 401-404.
- Einum S, IA Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature* **405**: 565-567.
- Elgar MA. 1990. Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos* **59**: 283-287.
- Goto A, K Iguchi, eds. 2001. Evolutionary biology of egg size in aquatic animals. Tokyo: Kaiyu-sha. (in Japanese)
- Hendry AP, DI Bolnick, D Berner, CL Peichel. 2009. Along the speciation continuum in stickleback. *J. Fish Biol.* **75**: 2000-2036.
- Higuchi M. 1996. Genetic structure and morphological, ecological study of threespine stickleback, *Gasterosteus aculeatus*, around Japan. PhD dissertation, Hokkaido Univ., Sapporo, Japan. (in Japanese)
- Higuchi M, A Goto. 1996. Genetic evidence supporting the existence of two distinct species in the genus *Gasterosteus* around Japan. *Environ. Biol. Fish.* **47**: 1-16.
- Higuchi M, A Goto, F Yamazaki. 1996. Genetic structure of threespine stickleback, *Gasterosteus aculeatus*, in Lake Harutori, Japan, with reference to coexisting anadromous and freshwater forms. *Ichthyol. Res.* **43**: 349-358.
- Iguchi K, M Yamaguchi. 1994. Adaptive significance of inter- and intrapopulation egg size variation in Ayu *Plecoglossus altivelis*. *Copeia* **1994**: 184-190.
- Johnston TA, WC Leggett. 2002. Maternal and environmental gradients in the egg size of an iteroparous fish. *Ecology* **83**: 1777-1791.
- Kitano J, S Mori, CL Peichel. 2007. Phenotypic divergence and reproductive isolation between sympatric forms of Japanese threespine sticklebacks. *Biol. J. Linn. Soc.* **91**: 671-685.
- Kitano J, JA Ross, S Mori, M Kume, FC Jones, YF Chan et al. 2009. A role for a neo-sex chromosome in stickleback speciation. *Nature* **421**: 1079-1083.
- Kolm N, I Ahnesjö. 2005. Do egg size and paternal care coevolve in fishes? *J. Fish Biol.* **66**: 1499-1515.
- Kolm N, NB Goodwin, S Balshine, JD Reynolds. 2006. Life history evolution in cichlids 2: directional evolution of the trade-off between egg number and egg size. *J. Evol. Biol.* **19**: 76-84.
- Kume M, T Kitamura, H Takahashi, A Goto. 2005. Distinct spawning migration patterns in sympatric Japan Sea and Pacific Ocean forms of threespine stickleback *Gasterosteus aculeatus*. *Ichthyol. Res.* **52**: 189-193.
- Kume M, J Kitano, S Mori, T Shibuya. 2010. Ecological divergence and habitat isolation between two migratory forms of Japanese threespine stickleback (*Gasterosteus aculeatus*). *J. Evol. Biol.* **23**: 1436-1446.
- Kume M, S Mori. 2009. Sea-run migratory behaviour in the Japan Sea form of three-spined stickleback *Gasterosteus aculeatus* in the tidal pool of eastern Hokkaido Island, Japan. *J. Fish Biol.* **75**: 2845-2850.

- McKinnon JS, HD Rundle. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.* **17**: 480-488.
- Mori S. 1987. Divergence in reproductive ecology of the three-spined stickleback, *Gasterosteus aculeatus*. *Jpn. J. Ichthyol.* **34**: 165-175.
- Mori S. 1990. Two morphological types in the reproductive stock of three-spined stickleback, *Gasterosteus aculeatus*, in Lake Harutori, Hokkaido Island. *Environ. Biol. Fish.* **27**: 21-31.
- Mori S. 1991. Reproductive characteristics of the three-spined sticklebacks in Japan. *Inlandw. Biol.* **6**: 1-10. (in Japanese)
- R Development Core Team. 2008. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Roff DA. 1992. The evolution of life histories. Theory and analysis. New York: Chapman and Hall.
- Schluter D. 2000. The ecology of adaptive radiation. Oxford, UK: Oxford Univ. Press.
- Smith CC, SD Fretwell. 1974. The optimal balance between size and number of offspring. *Am. Nat.* **108**: 499-506.
- Snyder RJ. 1990. Clutch size of anadromous and freshwater threespine sticklebacks: a reassessment. *Can. J. Zool.* **68**: 2027-2030.
- Wootton RJ. 1984. A functional biology of sticklebacks. London: Croom Helm.
- Wootton RJ. 1998. Ecology of teleost fishes (2nd ed). London: Kluwer Academic Publishers.
- Yamada M. 2003. Evolutionary process and introgression of mitochondrial DNA in threespine stickleback, *Gasterosteus aculeatus*, around Japan. PhD dissertation, Hokkaido Univ., Sapporo, Japan.