

Spatial Variation in the Spawning Season of Bluegill *Lepomis macrochirus* in Lake Biwa, Japan

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Yoshimasa Yamamoto and Fuh-Kwo Shiah (2012) Spatial variation in the spawning season of bluegill *Lepomis macrochirus* in Lake Biwa, Japan. *Zoological Studies* 51(8): 1446-1453. Reproductive condition of bluegill *Lepomis macrochirus* was examined at 3 sites in Lake Biwa, Japan, in an attempt to investigate spatial variation in their spawning season. Analytical results showed that the gonadosomatic index (GSI) of fish at all sites increased from mid-May to early June. Although the GSI of fish at the southernmost and mid-latitude sites decreased from early to late June, the GSI decline was much more severe in fish at the southernmost site. In contrast, fish at the northernmost site exhibited no significant difference in GSI between early and late June. The proportion of females containing mature eggs decreased with latitude in early June but increased with latitude in late June, implying that bluegill at the southernmost site actively spawned in June and were in the final stage of spawning in late June, whereas fish at the northernmost site were still actively spawning in late June. These results suggest that the spawning season of bluegill in Lake Biwa varies from site to site; fish at lower latitudes begin to spawn earlier, and those at higher latitudes may have a longer spawning season. <http://zoolstud.sinica.edu.tw/Journals/51.8/1446.pdf>

Key words: Bluegill, Lake Biwa, Gonadosomatic index, Oocyte, Spawning season.

Temperature and day length are recognized as factors that exert predominant influences on the reproductive activity of fish (Jobling 1995, Wootton 1998). Patterns of seasonal changes in these factors vary with latitude, which may be responsible for latitudinal variation in the spawning season of some species. For example, bluegill *Lepomis macrochirus* Rafinesque in the US tend to spawn earlier in southern states (Spotte 2007), implying that these factors largely determine the spawning season of bluegill.

Identifying a latitudinal variation in the spawning season of a certain species may require broad-scale comparisons, such as at a countrywide or global level. However, according to a recent survey in Lake Biwa (surface area = 670 km², mean depth = 41 m) in Japan, bluegill at lower latitudes have larger ovaries than those at higher

latitudes in the pre-spawning season (Yamamoto et al. 2011), suggesting the possibility that the spawning season of bluegill varies even within a single water body. Temperature is assumed to be responsible for this phenomenon, given that reproductive activity of bluegill strongly depends on temperature (Nakamura et al. 1969, Nakao et al. 2006), and the thermal environment of the lake tends to vary with latitude (Akitomo et al. 2009, Yamamoto and Kao 2012). The occurrence of a latitudinal thermal gradient in Lake Biwa can likely be attributed to its topographical characteristics; the lake consists of a deep northern basin (surface area = 618 km², mean depth = 43 m) and a shallow southern basin (surface area = 52 km², mean depth = 3.5 m). Due to its shallow depth, the southern basin naturally warms up more rapidly in spring, and heat advection apparently contributes

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to warming of southern portions of the northern basin. Accordingly, in the entire lake, the water tends to warm up from lower latitudes, and bluegill are expected to spawn earlier at lower latitudes. Elucidating the relationship between latitude and spawning season of bluegill in Lake Biwa would provide further insights into how bluegill adjust their reproductive activity to adapt to various thermal conditions.

The objective of this study was to investigate regional difference in the spawning season of bluegill in Lake Biwa. Fish samples were collected from 3 sites at different latitudes, and variation in the reproductive condition was evaluated based on female reproductive traits.

MATERIALS AND METHODS

Lake Biwa, the largest lake in Japan, is located at 34°58'-35°31'N and 135°52'-136°17'E (Fig. 1). Bluegill were introduced into Japan from the US in 1960. Having inhabited Lake Biwa for over 4 decades, bluegill were recently found to be propagating in coastal regions of the lake (Mizuno

et al. 2007, Yamamoto et al. 2010).

Bluegill were sampled by hook and line at 3 sites (A, B and C) on the shores of the lake (Fig. 1). Sampling sites were within 100 m of the lake's shoreline. Since bluegill are easily captured by angling, and numerous fish were often found in schools at the sampling sites, angling allowed us to capture adult fish within a short time. Fish were sampled 3 times at each site from late spring to early summer 2011 on 11-12 May, 2-3 June and 27-29 June. Captured fish were immediately euthanized with an overdose of ethyl 3-aminobenzoate methanesulfonate salt.

Specimens were measured for standard length to the nearest 0.01 mm using a digital caliper (CD-8"CSX, Mitsutoyo, Kanagawa, Japan) and body weight to the nearest 0.1 g using a portable digital scale (MP-1000, Ashiba, Taipei, Taiwan); fish were then sexed by gonad inspection. For females, ovaries were isolated and weighed to the nearest 0.002 g using a portable digital scale (1210N, Tanita, Tokyo, Japan), and the gonadosomatic index (GSI) was calculated as $GSI = \text{ovary weight}/\text{body weight} \times 100$.

The reproductive condition of bluegill collected in June was also evaluated based on the largest oocyte size. Oocytes were sampled from the midsection of ovaries using a plastic spoon and preserved in vials filled with 5% Lugol's solution. No oocytes were sampled when the ovaries were obviously immature based on their color; immature ovaries were either whitish-pink or whitish-yellow, whereas mature ovaries were typically yellow. Oocytes were observed under an inverted microscope (Axio Observer A1, Carl Zeiss, Göttingen, Germany) at $\times 50$ magnification. Images of at least 5 oocytes of the largest size-class were captured with a digital CCD camera (AxioCam MRm, Carl Zeiss), and their diameters were measured using AxioVision 4.7 software (Carl Zeiss). Diameters of the elliptical oocytes were expressed as geometric means of the major and minor axes. Diameters of the 3 largest oocytes were averaged to represent the oocyte diameter of a specimen.

Ovary weight, GSI and oocyte diameter of the specimens were expressed as a function of standard length. Differences in ovary weight and GSI among sites and periods were assessed by analysis of covariance (ANCOVA) on standard length. However, analysis of variance (ANOVA) or *t*-test was used to assess differences in the means of GSI or largest oocyte diameters among sites or periods if at least 1 target group failed to exhibit

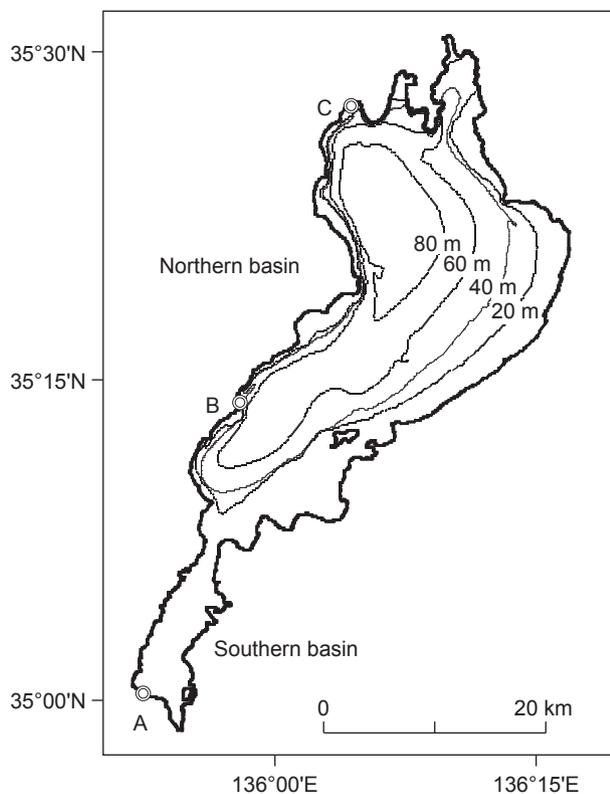


Fig. 1. Map of Lake Biwa showing the sampling sites. Lines on the map indicate the depth profile of the lake.

a significant correlation between GSI or largest oocyte diameter with standard length. The level of statistical significance was set to $p < 0.05$.

RESULTS

Overall, 283 female samples were collected. Ranges of the standard length well overlapped among sites and periods. Means of standard

length ranged 91.1-114.2 mm (Table 1).

Figure 2A-C show the relationship between ovary weight and standard length of bluegill. The \log_{10} -transformed ovary weight was significantly correlated with \log_{10} -transformed standard length ($r = 0.677-0.983$, $n = 14-45$, $p < 0.001$). ANCOVA revealed significant differences in ovary weight vs. standard length regressions among sites throughout the study (mid-May: $F_{2,93} = 5.66$, $p < 0.01$; early June: $F_{2,107} = 3.22$, $p < 0.05$; late

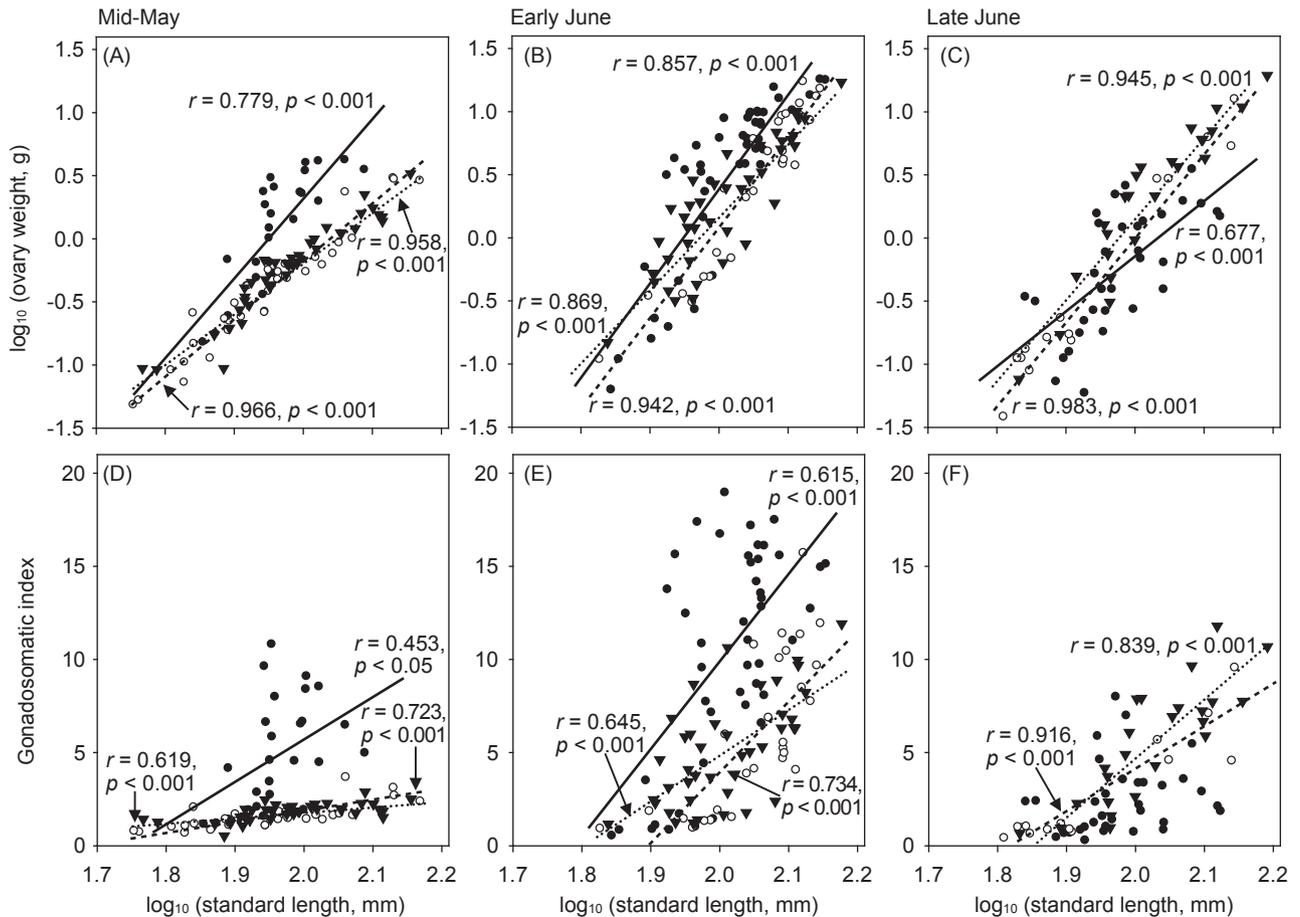


Fig. 2. Relationships between ovary weight and standard length (A-C) and between the gonadosomatic index and standard length (D-F) of bluegill. Closed circles and the solid line, open circles and the broken line, and closed triangles and the dotted line respectively refer to sites A, B, and C.

Table 1. Standard length (mm) of bluegill collected at each site

	Site A		Site B		Site C	
	Mean ± S.E.	n	Mean ± S.E.	n	Mean ± S.E.	n
Mid-May	92.5 ± 2.15	28	91.1 ± 3.91	33	97.7 ± 3.21	38
Early June	104.6 ± 2.54	45	114.2 ± 3.64	27	103.2 ± 2.90	41
Late June	96.7 ± 2.81	33	91.5 ± 7.39	14	107.2 ± 4.38	24

June: $F_{2,65} = 3.30, p < 0.05$). At site A, although the regressions did not significantly differ between mid-May and early June (slope, $F_{1,69} = 0.586, p = 0.45$; intercept, $F_{1,70} = 1.65, p = 0.20$), the regressions significantly differed between early and late June ($F_{1,74} = 7.45, p < 0.01$). At sites B and C, significant differences in the regressions were detected between mid-May and early June (site B: $F_{1,56} = 20.4, p < 0.001$; site C: $F_{1,75} = 8.38, p < 0.01$), but not between early and late June (site B: slope, $F_{1,37} = 1.05, p = 0.31$, intercept, $F_{1,38} = 0.045, p = 0.83$; site C: slope, $F_{1,61} = 1.56, p = 0.21$; intercept, $F_{1,62} = 0.13, p = 0.72$).

Figure 2D-F show the relationship between GSI and standard length of bluegill. GSI significantly increased with \log_{10} -transformed SL ($r = 0.453-0.916, n = 14-45, p < 0.05$), except for the result at site A in late June ($r = 0.216, n = 33, p = 0.23$). Regressions between GSI and standard length significantly differed among sites throughout

the study (mid-May: ANCOVA, $F_{2,93} = 7.35, p < 0.01$; early June: ANCOVA, slope, $F_{2,107} = 2.64, p = 0.076$, intercept, $F_{2,109} = 30.1, p < 0.001$; late June: ANOVA, $F_{2,68} = 10.4, p < 0.001$). Figure 3A-C show changes in the GSI of bluegill. At site A, GSI significantly increased from mid-May to early June (slope, $F_{1,69} = 1.81, p = 0.18$; intercept, $F_{1,70} = 11.6, p < 0.01$), and decreased from early to late June (t -test, $p < 0.01$). At site B, significant differences in the regressions were detected between mid-May and early June ($F_{1,56} = 33.9, p < 0.001$) and between early and late June ($F_{1,37} = 4.15, p < 0.05$). At site C, a significant difference in the regressions was detected between mid-May and early June ($F_{1,75} = 22.1, p < 0.001$), but not so between early and late June (slope, $F_{1,61} = 0.792, p = 0.38$; intercept, $F_{1,62} = 0.0047, p = 0.95$). GSI values that bluegill can exhibit decreased with latitude; the highest GSI values at sites A, B, and C were 18.9, 15.7, and 11.9, respectively.

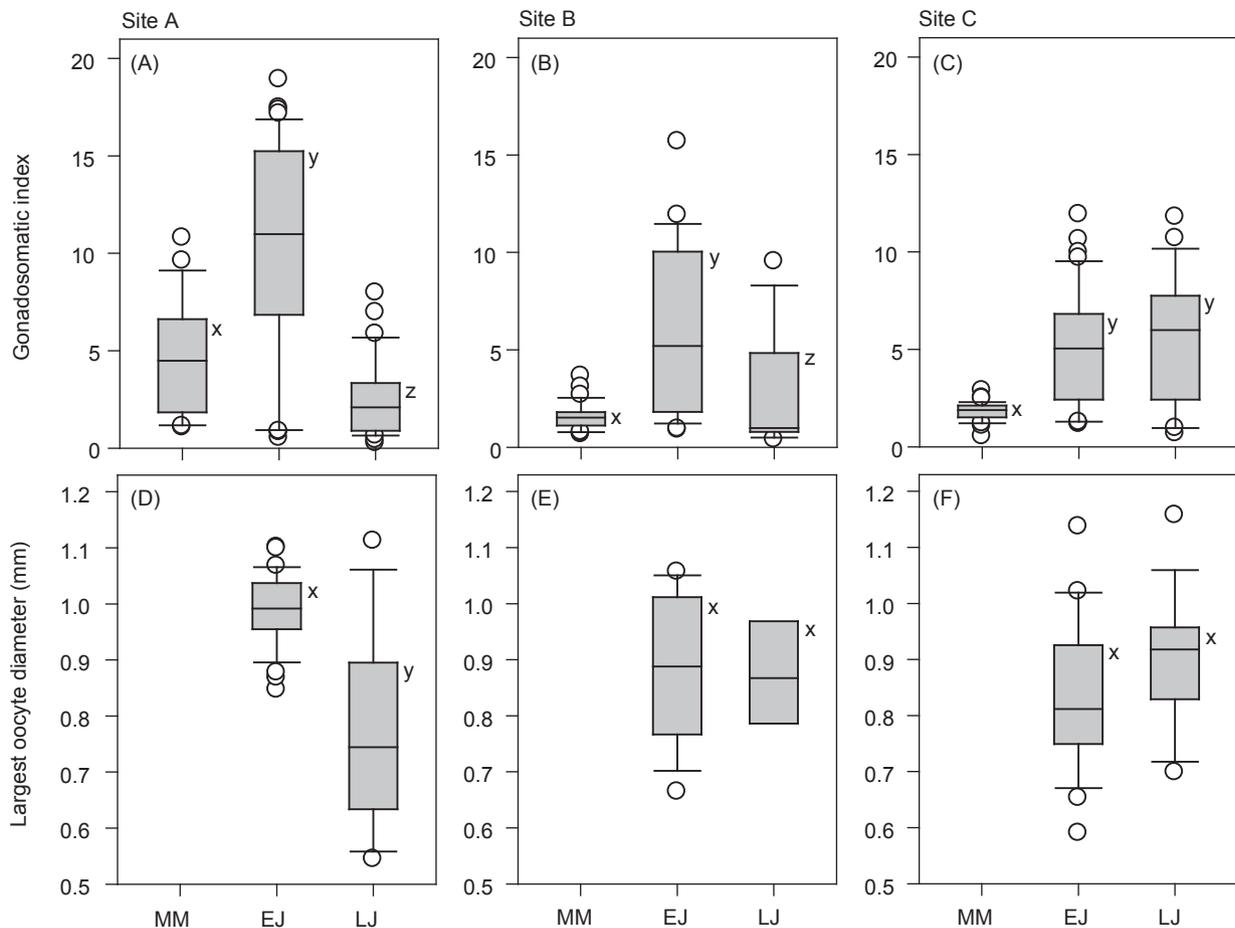


Fig. 3. Changes in the gonadosomatic index (A-C) and the largest oocyte diameter (D-F) of bluegill. MM, mid-May; EJ, early June; LJ, late June. In each box plot, the horizontal line indicates the median, the box gives the 25th and 75th percentiles, and whiskers represent the 10th and 90th percentiles. Open circles are outliers. Data labeled with the same letters do not significantly differ ($p > 0.05$).

In early June, percentages of specimens that were considered to be immature based on their whitish ovaries were 20.0% at site A, 29.6% at site B, and 29.3% at site C; these respective values changed to 63.6%, 64.3%, and 29.2% in late June (data not shown). GSI values of such immature fish were significantly lower than those of fish whose oocytes were sampled (*t*-test, $p < 0.001$); in addition, ranges of GSI of the 2 groups did not overlap each other except for specimens collected at site A in late June and site C in early June (data not shown). The largest oocyte diameter increased curvilinearly with GSI, and a double-reciprocal plot indicated significant correlations between these

measurements ($r = 0.484-0.907$, $n = 5-36$, $p < 0.05$) (Fig. 4).

Figure 5 shows the relationship between the largest oocyte diameter and standard length. In early June, a significant correlation between the largest oocyte diameter and standard length was detected in bluegill at sites A ($r = 0.362$, $n = 36$, $p < 0.05$) and C ($r = 0.433$, $n = 29$, $p < 0.05$), but not in fish at site B ($r = 0.293$, $n = 19$, $p = 0.22$). The largest oocyte diameters significantly differed among sites (ANOVA, $F_{2,81} = 18.5$, $p < 0.001$). In late June, the largest oocyte diameter insignificantly increased with fish body size at sites B and C (site B: $r = 0.141$, $n = 5$, $p = 0.82$; site C:

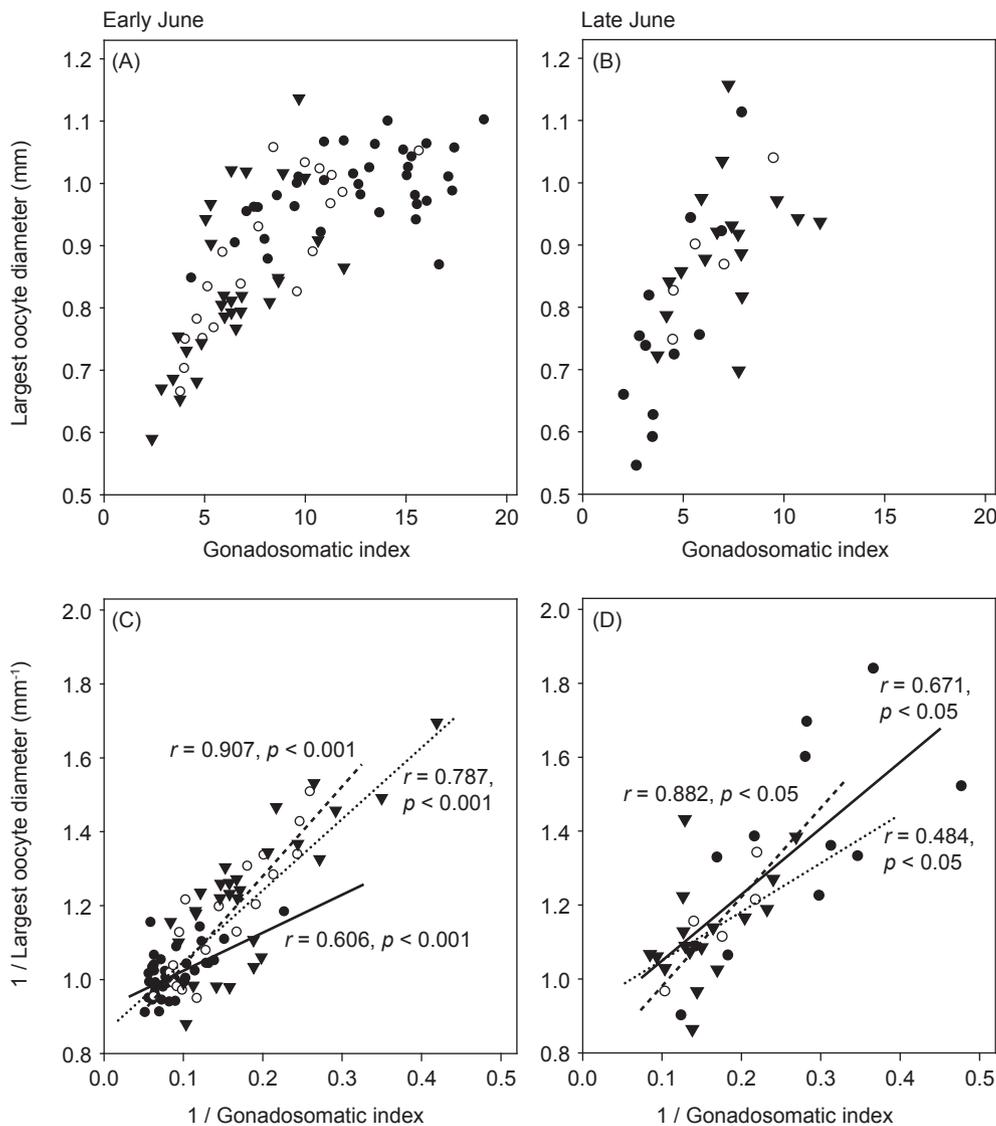


Fig. 4. Relationship between the largest oocyte diameter and the gonadosomatic index of bluegill (A, B) and results of double-reciprocal plot (C, D). Symbols are the same as those in figure 2.

$r = 0.324$, $n = 17$, $p = 0.20$. In contrast, the largest oocyte diameter insignificantly decreased with the fish body size at site A ($r = -0.156$, $n = 12$, $p = 0.63$). The largest oocyte diameters significantly differed among sites (ANOVA, $F_{2,31} = 3.78$, $p < 0.05$). Although no significant differences were detected at sites B and C, the mean largest oocyte diameters of bluegill at site A in early June were significantly larger than those in late June (t -test, $p < 0.001$) (Fig. 3D-F).

DISCUSSION

Ranges of ovary weight and GSI of bluegill collected at sites B and C in May 2011 well overlapped, rendering relationships between these measurements and latitude less clear compared to the results in May 2010 (Yamamoto et al. 2011). The similarity in values of these measurements suggests that fish in these sites were similar in their maturation. Given that temperature profoundly influences reproductive

activity in bluegill (Nakamura et al. 1969, Nakao et al. 2006), this result may be attributed to insignificant temperature difference between the 2 sites. Topographical factors may be important when considering the thermal environment of the northern basin of Lake Biwa. In the present study, we collected fish samples on the west coast of the northern basin, where the bottom is characterized by steep slopes. In contrast, Yamamoto et al. (2011) collected fish samples on the east coast of the northern basin, which has gentle slopes. Although the water of Lake Biwa warms up from lower latitudes in spring, the water along the west coast of the northern basin may be less subject to warming up due to the deep environments compared to the east coast of the same basin. Such coast-specific thermal environments may differently affect the maturation of bluegill between the east and west coasts of the northern basin.

Natural fish populations often display a clear seasonal pattern of GSI, with highest values at the onset of the spawning season (Mann 1980, Lenhardt 1992, Fox and Crivelli 1998). Despite its tendency to increase with fish body size, GSI can widely vary among individuals of similar sizes (Mann 1980, Yamamoto et al. 2011), implying that the status of fish varies individually. In mid-May, some fish with standard lengths of 87.76-105.34 mm at site A exhibited higher GSIs than those of larger fish. This finding suggests that some young females may be more reproductively active than older fish, at least for a certain period of time. The maturation of ovaries markedly progressed from mid-May to early June, as inferred by large increases in GSI, whereas the GSIs of young adult fish collected at site B were mostly much smaller than those at sites A and C. One hypothesis explaining this observation is that reproductively active young fish at site B migrated to a nearby lagoon. Owing to small and shallow environments, lagoons more rapidly warm up than does the main lake, thus facilitating earlier spawning of bluegill (Yamamoto and Shiah 2013). A recent survey suggested the possible migration of bluegill inhabiting the lake to lagoons from late spring to early summer (Shibata et al. 2011). A lagoon lies adjacent to site B, but no lagoons are near sites A and C. Since fish samples were collected by hook and line, it is possible that we failed to adequately sample the adult population of bluegill. However, the assumption that reproductively active females at site B were attracted to the nearby lagoon may explain both the absence of reproductively active young

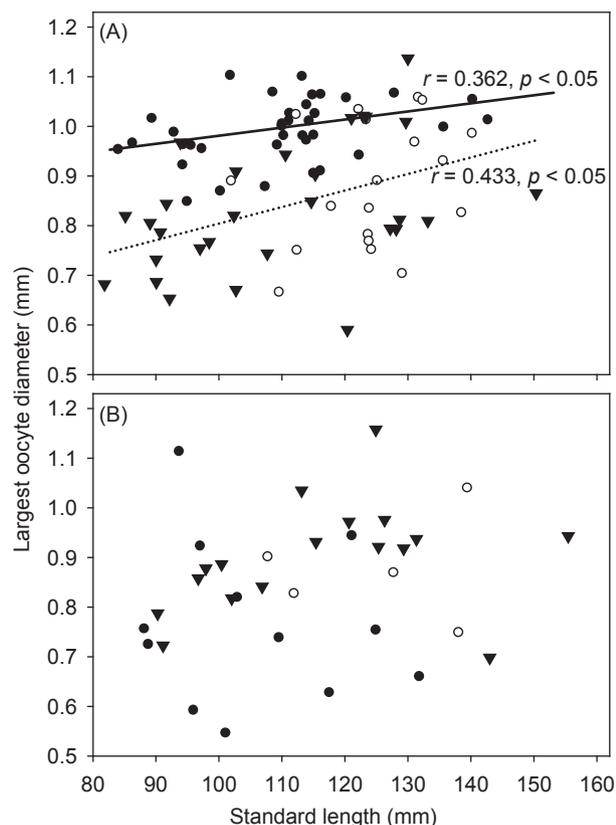


Fig. 5. Relationship between the largest oocyte diameter and standard length of bluegill in early (A) and late June (B). Symbols are the same as those in figure 2.

females there and the indistinct latitudinal variation in the GSI of bluegill.

The rapid reduction in GSI of fish at site A from early to late June suggests that bluegill there actively spawned in June. Since some fish with standard lengths of 88.27-97.18 mm exhibited higher GSIs than those of larger fish in late June, spawners in the final stage of the spawning season might be composed mainly of relatively young fish. The range of GSI values of fish collected at site B decreased from early to late June owing to the absence of fish with a GSI exceeding 10 in the latter samples. Although spawning progression during June may explain this result, the result was based on a very small sample size of reproductively active females in late June and consequently should be interpreted with caution. Substantially equal ranges of the ovary weight and GSI of fish at site C between early and late June imply that, contrary to fish at sites A and B, the spawning activity of fish at this site was maintained at high levels throughout June.

The presence of oocytes at various developmental stages in ovaries complicates evaluation of the maturation status of multiple spawning fishes, and previous studies often noted the size of the largest oocyte as a measure of maturation (West 1990). For bluegill, Banner and Hyatt (1975) reported that the diameters of the largest 'eggs' removed from the genital opening ranged 0.85-0.92 mm 2-3 days before spawning, 0.92-1.10 mm as ovulation approached, and 0.85-1.36 mm at maturity with the capability of being fertilized. Nakamura et al. (1971) reported that the mean diameter of oviposited eggs of bluegill was 1.23 mm, and neither the body size of spawners nor the frequency of spawning was related to egg size. If oocytes with diameters of 0.85 mm or larger are considered to be mature eggs for convenience, the respective proportions of females containing mature eggs at sites A, B, and C were 77.8%, 37.0%, and 24.4% in early June and 9.1%, 21.4%, and 50.0% in late June. The great reduction in the proportion of fish with mature eggs at site A from early to late June suggests that the spawning season of bluegill at this site had almost come to an end by late June. In contrast, fish at site C were assumed to be in the middle of spawning in late June, considering the high proportion of fish with mature eggs in this period.

GSI and the largest oocyte size have often been used to indicate the maturation status of fish (West 1990). In the present study, a close relationship between GSI and the largest oocyte

size was demonstrated in bluegill, and the same conclusion about regional variation in the spawning activity of the fish in Lake Biwa was naturally drawn from changes in these measurements. Site-specific patterns of the changes in GSI and the proportion of females with mature eggs suggest that the spawning season of bluegill in the lake can vary from site to site, in terms of timing and/or duration. It is reasonable to assume that the spawning of bluegill at lower latitudes begins earlier since the water temperature of the lake increases from lower latitudes (Yamamoto et al. 2011). Notably, Nakao et al. (2006) suggested the possibility that the spawning season of bluegill at the northern edge of Lake Biwa lasts longer than that in the southern basin. Given that bluegill are multiple spawners, the length of the spawning season of bluegill is assumed to be connected with the spawning frequency and/or the length of the inter-spawning interval. Nakamura et al. (1971) observed the spawning patterns of 12 captive bluegill at the age of 3-4 yr, and reported that 7 fish spawned (at least) once, 4 fish spawned 2-4 times with an inter-spawning interval of 7-9 d, and the remaining 1 spawned 3 times over 26 d, implying that spawning frequency and the inter-spawning interval of bluegill can considerably vary among individuals (see also Nakamura et al. 1969). In the case of the pumpkinseed *L. gibbosus* (Linnaeus), a closely related species of bluegill, females from French populations spawned more frequently over longer spawning seasons than those from a Canadian population under captive conditions, and under natural conditions, the Canadian population exhibited a sharp reduction in the GSI compared to the French populations (Fox and Crivelli 1998). Whether the proportion of multiple spawners and the length of the inter-spawning interval of bluegill in Lake Biwa vary with latitude remains to be elucidated.

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REFERENCES

- Akitomo K, K Tanaka, M Kumagai, C Jiao. 2009. Annual cycle of circulations in Lake Biwa, part 1: model validation. *Limnology* **10**: 105-118.
- Banner A, M Hyatt. 1975. Induced spawning of bluegill sunfish. *Progr. Fish-Cult.* **37**: 173-180.
- Fox MG, AJ Crivelli. 1998. Body size and reproductive allocation in a multiple spawning centrarchid. *Can. J. Fish. Aquat. Sci.* **55**: 737-748.

- Jobling M. 1995. Environmental biology of fishes. London: Chapman and Hall.
- Lenhardt M. 1992. Seasonal changes in some blood chemistry parameters and in relative liver and gonad weights of pike (*Esox lucius* L.) from the River Danube. *J. Fish Biol.* **40**: 709-718.
- Mann RHK. 1980. The growth and reproductive strategy of the gudgeon, *Gobio gobio* (L.), in two hard-water rivers in southern England. *J. Fish Biol.* **17**: 163-176.
- Mizuno T, H Nakao, LBM Fish Survey Group, T Nakajima. 2007. Risk analysis of habitat utilization by bluegill (*Lepomis macrochirus*) in the Lake Biwa basin. *Jpn. J. Conserv. Ecol.* **12**: 1-9. (in Japanese with English summary)
- Nakamura N, S Kasahara, T Yada. 1969. Studies on the usefulness of the bluegill sunfish, *Lepomis macrochirus* Rafinesque, as an experimental standard animal. I. On the breeding habits. *J. Fac. Fish. Anim. Husb. Hiroshima Univ.* **8**: 1-11. (in Japanese with English summary)
- Nakamura N, S Kasahara, T Yada. 1971. Studies on the usefulness of the bluegill sunfish, *Lepomis macrochirus* Rafinesque, as an experimental standard animal. II. On the developmental stages and growth from the egg through one year. *J. Fac. Fish. Anim. Husb. Hiroshima Univ.* **10**: 139-151. (in Japanese with English summary)
- Nakao H, K Fujita, T Kawabata, K Nakai, H Sawada. 2006. Breeding ecology of bluegill *Lepomis macrochirus*, an invasive alien species, in the north basin of Lake Biwa, central Japan. *Jpn. J. Ichthyol.* **53**: 55-62. (in Japanese with English summary)
- Shibata J, Z Karube, M Oishi, M Yamaguchi, Y Goda, N Okuda. 2011. Physical structure of habitat network differently affects migration patterns of native and invasive fishes in Lake Biwa and its tributary lagoons: stable isotope approach. *Populat. Ecol.* **53**: 143-153.
- Spotte S. 2007. Bluegills: biology and behavior. Bethesda, MD: American Fisheries Society.
- West G. 1990. Methods of assessing ovarian development in fishes: a review. *Aust. J. Mar. Freshw. Res.* **41**: 199-222.
- Wootton RJ. 1998. Ecology of teleost fishes, 2nd ed. London: Kluwer.
- Yamamoto Y, SJ Kao. 2012. Relationship between latitude and growth of bluegill *Lepomis macrochirus* in Lake Biwa, Japan. *Ann. Zool. Fennici* **49**: 36-44.
- Yamamoto Y, FK Shiah. 2013. Potential importance of lagoons as breeding grounds for bluegill *Lepomis macrochirus* in Lake Biwa, Japan. *Limnology* **14**: 31-37.
- Yamamoto Y, H Tsukada, D Nakai. 2010. Latitudinal gradient in the body weight of bluegill *Lepomis macrochirus* in Lake Biwa, Japan. *Zool. Stud.* **49**: 625-631.
- Yamamoto Y, H Tsukada, FK Shiah. 2011. Latitudinal gradient in the ovary weight of bluegill *Lepomis macrochirus* in Lake Biwa, Japan. *Ethol. Ecol. Evol.* **23**: 291-299.