Reproductive biology of the amphidromous goby *Sicyopterus japonicus* (Gobiidae: Sicydiinae)

by

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ABSTRACT. - To understand the reproductive biology of the amphidromous goby, *Sicyopterus japonicus*, juvenile and adult gobies of this species were sampled in the Ota River, Wakayama, Japan. Sampling was conducted monthly from June 2003 to October 2004, and once each season from April 2005 to March 2006 at 7 sampling stations from the estuary to the upper reaches of the river. The gonadosomatic index values of females (N = 171) ranged from 0.0 to 20.8 and showed high values in July and August (summer, mean: 6.5) that indicated they have a short spawning season. There were no relationships between gonadosomatic index and standard length, age, or distance from the river mouth. This indicated that they do not have a spawning aggregation in a certain area in the river. 30 randomly selected oocytes from each ovary of 48 females were measured to observe the monthly changes of oocyte diameters. "Small oocytes" of 5-100 μm diameters were observed all year round, while "large oocytes" > 200 μm were only observed in July and August. Only specimens collected in July and August were classified as being mature by histological examination. Potential batch fecundities calculated by the percentage of large oocytes in the gonads were estimated to be 10,800-52,500 (N = 10), and standard length was correlated with potential batch fecundity. Egg clutches of *S. japonicus* were observed throughout the sampling sites except in the upper- and lower-most reaches in August 2004 to 2008. Spawning places were found in rapids and egg clutches were attached to the undersides of stones. Although *S. japonicus* in the Ota River had similar characteristics with tropical Sicydiinae species, such as large clutches deposited on the undersurface of the stones, their spawning season was shorter than that of tropical species that have almost year-round spawning.

RÉSUMÉ. - Biologie de la reproduction du gobie amphidrome *Sicyopterus japonicus* (Gobiidae : Sicydiinae).

Afin de comprendre la biologie de la reproduction du gobie amphidrome, *Sicyopterus japonicus*, des juvéniles et des adultes de cette espèce ont été échantillonnés dans la rivière Ota, Wakayama, Japon. L'échantillonnage a eu lieu tous les mois de juin 2003 à octobre 2004, et une fois par saison d'avril 2005 à mars 2006, au niveau de 7 stations d'échantillonnage de l'estuaire au cours supérieur de la rivière. L'indice gonadosomatique des femelles (N = 17) variait de 0 à 20, 8 et les valeurs les plus élevées ont été obtenues en juillet et en août (été, moyenne : 6,5), indiquant qu'elles ont une période de ponte courte. Aucun lien entre l'indice gonadosomatique, la longueur standard, l'âge ou la distance à l'embouchure n'a été décelé. Cela indique donc qu'il n'y a pas de regroupement à un endroit donné de la rivière au moment de la reproduction. 30 ovocytes, sélectionnés au hasard, provenant de chaque ovaire de 48 femelles ont été mesurés afin d'observer les changements mensuels du diamètre des ovocytes. Les "petits ovocytes", de 5 à 100 μm de diamètre, ont été observés tout au long de l'année tandis que les "gros ovocytes", d'un diamètre > 200 μm, n'ont été observés qu'en juillet et en août. Seuls les spécimens collectés en juillet-août ont pu être classés comme des individus matures à partir de l'observation histologique. La fécondité potentielle du lot a été calculée en fonction du pourcentage de gros ovocytes dans les gonades et a été estimée à 10800-52500 (N = 10). La longueur standard était corrélée à la fécondité potentielle du lot. Les pontes de *S. japonicus* ont été observées au niveau de chacun des sites d'échantillonnage sauf dans les zones les plus en amont et les plus en aval en août 2004 et 2008. Les zones de reproduction ont été observées au niveau des zones de rapides et les pontes fixées sous les roches. Bien que *S. japonicus* de la rivière Ota possède des caractéristiques biologiques semblables à celles de Sicydiinae tropicaux, comme de larges pontes déposées sous les roches, sa période de reproduction s'avère être beaucoup plus courte que celle des espèces tropicales qui, elles, se reproduisent presque tout au long de l'année.

Key words. - Sicydiinae - *Sicyopterus japonicus* - Gonadosomatic index - Spawning season - Gonad - Clutch size.

The Sicydiinae gobies include about 9 described genera and 80-90 species that are distributed from the Indian Ocean to the Pacific Ocean and the West Indies adjacent to the Atlantic Ocean (Keith, 2003; Pezold *et al.*, 2006; Froese and Pauly, 2009; Keith and Lord, 2011). They live mainly on tropical oceanic islands and have an amphidromous life cycle (Keith, 2003). The Sicydiinae gobies have attracted interest due to their ability to climb waterfalls (Fukui, 1979; Fitzsimons *et al.*, 2003) and more recently because of their long larval duration in the ocean (e.g., Radtke *et al.*, 2001; Iida *et al.*, 2008; Lord *et al.*, 2010). Many Sicydiinae gobies are distributed in a limited area and may be endemic species, for example, *Sicyopterus sarasini* in New Caledonia (Lord and Keith, 2008), and *Cotylopus rubripinnis* in Mayotte (Keith *et al.*, 2005), while others are distributed across wider regions such as *Lentipes concolor* in Hawai'i (Devick *et al.*,

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1995) and *Stiphodon percnopterygionus* in the northeastern Pacific (Akihito *et al*., 2000). Sicydiinae gobies are known to have long reproductive seasons, some reproduce all year round, and to have large clutch sizes (e.g., Manacop, 1953; Fitzsimons *et al*., 1993; Kinzie, 1993; see review in Keith, 2003).

Sicyopterus japonicus is a temperate Sicydiinae goby, which inhabits East Asia from Taiwan to Fukushima, Japan (Akihito *et al*., 2000; Iguchi *et al*., 2005). This species is the only species in the Sicydiinae subfamily living at temperate latitudes. It has been reported that they are herbivorous (Dôtu and Mito, 1955) and have seasonality in their life history characteristics such as recruitment from the ocean to the river, grazing, over-wintering and growth (Iida *et al*., 2008, 2009).

Reproductive biology is an important part of life history studies. *Sicyopterus japonicus* has been reported to have a large number of eggs laid in a single clutch (Dôtu and Mito, 1955) and a short spawning season in summer (Iida *et al*., pers. obs.). Iida *et al*. (unpubl. manuscript) has examined the overall life history of this species recently, but there has been no detailed study specifically on the reproductive biology of *S. japonicus*. Because *S. japonicus* inhabits a temperate region, its reproductive biology might be different from other Sicydiinae gobies in tropical and subtropical regions because of different climate patterns. To understand the life history and the reproductive biology of *S. japonicus*, an annual field survey was conducted in the Ota River in Wakayama, Japan. This study analyses the reproductive characteristics of this species using histological techniques and field observations made over a 5 years period.

MATERIALS AND METHODS

Sampling, measurement and aging

S. japonicus specimens were collected monthly from June 2003 to October 2004 (except June 2004), and April, August, and October 2005, and March 2006 in the Ota River (27 km long), Wakayama Prefecture, Japan. Sampling was conducted at 7 stations 1-23 km from the river mouth every 2-5 km using an electric shocker, lances and hand nets (Fig. 1), with the lower-most station being St. 1. The altitudes of St. 1 and St. 7 were 5 and 110 m, respectively. At each sampling, water temperature was measured to the nearest tenth of a degree using a mercury thermometer. To compare life history characteristics of *S. japonicus* among seasons, we designated April-June as spring, July-September as summer, October-December as fall and January-March as winter based on the fluctuation of water temperature in the Ota River.

Standard length (SL) and body weight (BW) of specimens were measured to the nearest tenth of a millimetre with a ruler and hundredth of a gram with digital scales, respectively. Specimens larger than 45 mm SL were sexed by external examination of the shape of the genital papilla or pigmentation of the anal fin (Akihito *et al*., 2000). Specimens smaller than 45 mm SL were defined as sex-unidentified because these specimens did not have externally visible morphological differences. Specimens were fixed either in 10% formalin for gonadal analysis or the heads were fixed in 99% ethanol separately for otolith analysis.

To examine the relationship between age and gonado-somatic index, the annual rings of otoliths (N = 59) were counted. Sagittal otoliths were extracted from each speci-

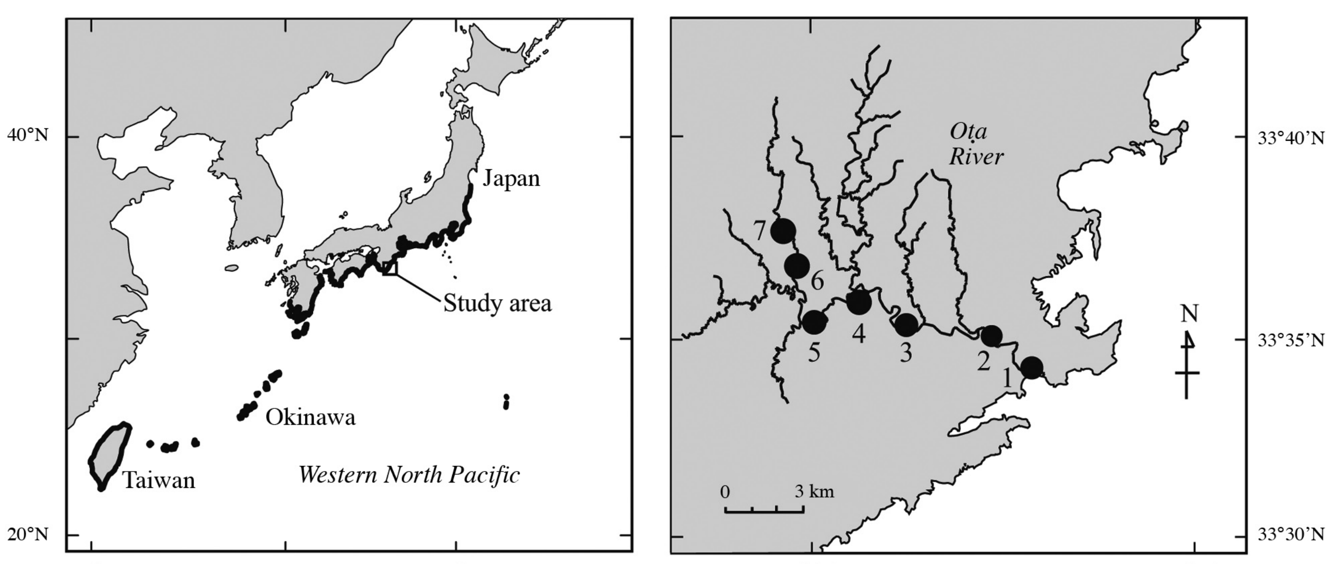


Figure 1. - Map of the East Asian region showing the geographic distribution of *Sicyopterus japonicus* (thick black lines, that show coastlines only) and the location of the study area (right panel). The sampling stations that were surveyed are the black dots numbered 1-7.

men, mounted on glass slides with euparal (Chroma-Gesellschaft Schmid GmbH & Co.), and were examined at 50-500x using a stereomicroscope (Nikon, SMZ-U). They were photographed using a digital camera (Nikon, digital camera DXM1200F) to count opaque zones. Details of the otolith analyses are described in Iida *et al*. (unpubl. manuscript).

Gonadosomatic index

For gonadal examination, a total of 171 females (42.4-108.0 mm SL) were subsampled, using about 8 specimens per month from June 2003 to October 2004 (except October 2003), and April, August and October 2005 and March 2006, and gonads were weighed to the nearest hundredth of a gram with digital scales. The gonadosomatic index (GSI) was calculated as: GSI = 100 x GW/BW, where GW is the gonad weight (g). At least 0.01g of tissue was required for measurement, so the gonads of males were too small to be weighed.

Observation of oocytes

Subsampled gonads of about 3-6 specimens per month from November 2003 to October 2004 (48 in total) that were fixed in formalin were broken up using tweezers under a stereomicroscope (Nikon, SMZ-U) and were photographed using a digital camera (Nikon, digital camera DXM1200F). The longest diameters of 30 randomly sampled oocytes of each specimen were measured on digitized images to the nearest micrometer using ImageJ software Ver. 1.43g (NIH, National Institute of Health, USA: http://rsbweb.nih.gov/ij/index.html). As there were both large and small oocytes in the gonads (described in the result section), they were divided into two general categories of "small oocytes" for smaller than 100 μm in diameter and "large oocytes" for larger than 100 μm. The total number of oocytes of 10 females (50.5-95.0 mm SL) collected in July and August 2005 was calculated using a gravimetric method as follows. The whole gonad was removed from the fish, blotted and weighed, and then subsamples of oocytes were taken. The oocytes in a subsample were weighed with digital scales and counted under a stereomicroscope. The total number of oocytes was calculated by the weight and number of oocytes in the subsample, and the total weight of the ovary. The number of large and small oocytes was counted and the percentage of each type was calculated. The mean (95% confidence interval) percentage of large oocytes was 48.0 (42.1-53.9) %, and these large oocytes likely represent the proportion of oocytes that are ready to be spawned in one batch (Miller, 1984). To estimate the potential batch fecundity of each fish (number of eggs laid in a single spawning event), the number of total oocytes was multiplied by 48%.

Histological study

Wet-mount preparations of ovarian tissue were examined to determine the maturation stage. The thin sections of ovarian tissue (6 μm) stained with hematoxylin and eosin were prepared from 2 to 6 females of various sizes and stages of gonadal maturation per month from November 2003 to October 2004 by routine histological procedures (N = 33 in total). Ovaries were assigned into two stages as follows: (1) immature (ovaries with pre-vitellogenic oocytes), and (2) mature (ovaries with vitellogenic oocytes) based on the criteria of Ha and Kinzie (1996).

Spawning places and egg clutches

Searches for egg clutches of *S. japonicus* was conducted at St. 1-7 in the Ota River in August and September from 2004 to 2008 by overturning rocks at each sampling site. The searches were not quantitative and were conducted ca. 25 times for about one hour at each station and by one or two persons by snorkelling each time. Egg clutches were only found at St. 2-6. We checked whether a guarding male was under each of 29 stones that were found to have an egg clutch from 2005 to 2008. Egg clutches attached to stones were photographed along the river bank after being found at each sampling station using a digital camera and then all eggs in the clutch were removed using tweezers before being fixed in 5% formalin. Subsamples of about 8% eggs of each clutch were taken before fixation and preserved in 5% formalin separately in small vials for later estimations of clutch sizes. Total area of each egg clutch was measured by digitized images using ImageJ software. Clutch sizes (total number of eggs in each egg clutch) of 8 subsampled nests were estimated using the total area of the clutch and from the number of subsampled eggs within 1 cm^2 of each clutch.

Statistical analyses

A Kruskal-Wallis test was used to compare GSI among each month. To test whether GSI was related to sampling station and/or season, a multiple regression analysis was carried out with GSI as a dependent variable and Tukey's HSD tests were used for multiple comparison tests. Pearson's correlation coefficient tests were carried out for examining the relationships of SL with GSI, SL and estimated number of oocytes. Significant differences were determined at the 0.05 probability level. JMP 5.0.1J (SAS Institute Inc.) and Prism 4.0c (GraphPad Software Inc.) were used for statistical analyses.

RESULTS

Gonadosomatic index

The GSI values of 171 females ranged from 0.0 to 20.8. The GSI was significantly different among months (Kruskal-

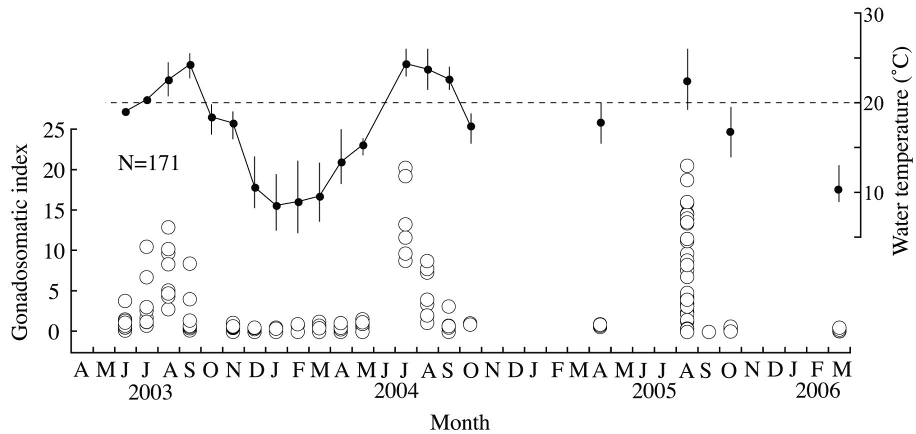


Figure 2. - Monthly changes of water temperature and gonadosomatic index of female *Sicyopterus japonicus* collected in the Ota River from June 2003 to March 2006. Closed circles and vertical lines indicate means and ranges of water temperature, respectively. Dotted horizontal line indicates 20°C as a reference level for the spawning season.

Wallis test, $p < 0.0001$) and was high during summer (July-August, range: 0.2-20.5, mean: 5.5) and low from fall to spring (October-June, range: 0.0-1.6, mean: 0.4) (Fig. 2). There was a clear seasonal change in water temperature in the river ranging from 5.5°C (St. 5 and 6 in February 2004) to 26.0°C (St. 4 in July and August 2004) (Fig. 2). Water temperature increased during April to June, showing the highest temperatures from July to September, and decreased during October and November, showing the lowest temperatures from December to March. Water temperature was generally lower in the upper river reaches than in the lower reaches. The highest GSI values were shown in July and August and were almost synchronized with the highest water temperature (Fig. 2). This suggests that the months from July to September, when many specimens had GSI values higher than 3.0, is probably the spawning season. There was no clear relationship between SL and GSI values in the spawning season (Pearson's correlation coefficient, $r^2 = 0.018$, $p = 0.24$, Fig. 3A). Specimens 2-6 years old showed GSI values higher than 3.0 (Fig. 3B). The specimens having higher GSI values (> 3.0) were collected throughout the river from St. 1 to St. 7 (Fig. 3C). There was a significant relationship between GSI and season (Multiple regression, $p < 0.0001$, Tab. I), but not between GSI and sampling station ($p = 0.50$, Tab. I). Summer was significantly different from the other three seasons (Tukey's HSD test, $p < 0.05$, Tab. I), but there were no other differences among seasons (each $p > 0.05$, Tab. I).

Table I. - Multiple regression analysis of *Sicyopterus japonicus* with gonadosomatic index (GSI) as dependent variable and sampling station and season. Different letters indicate significant differences.

Term	d.f.	*F*	*P*
Sampling station	6	0.92	0.49
Season	3	29.05	< 0.0001
Residual error	170		
Season	Adjusted mean		
Spring	3.37	a	
Summer	9.10	b	
Fall	3.27	a	
Winter	3.35	a	

Gonads and ovarian eggs

The oocyte diameters of *S. japonicus* females were variable, with small oocytes (< 100 μm) being observed all year and large oocytes (> 100 μm) being observed mainly in July and August along with a few in September (Fig. 4). Almost all large oocytes were > 200 μm. From the histological study of ovaries, all specimens collected from October to May were premature with only pre-vitellogenic oocytes based on their deeply stained cytoplasm surrounding the nucleus (Fig. 4). All specimens observed in July and August (N = 10) were mature having ovaries with oil globules and vitellogenic oocytes within the cytoplasm (Fig. 4). The small pre-vitellogenic oocytes were also observed among vitellogenic oocytes in July and August. Only one of three specimens was mature in September.

Total numbers of oocytes of 10 females were estimated to be from 22,540 to 109,290 oocytes (mean: 56,030) and the number of large oocytes likely to be spawned in the current spawning season was estimated from 10,820 to 52,460 (mean: 26,900). The SL of females was positively correlated with the number of large oocytes (N = 10, Pearson's correlation coefficient, $r^2 = 0.69$, $p < 0.01$).

Spawning places and clutch sizes

During August and September 2004 to 2008, more than 50 egg clutches in the nests of *S. japonicus* were observed at St. 2-6. The light yellow eggs were attached with adhesive filaments to the undersurfaces of stones (15-28 cm in longest diameter of the stones). More than two clutches were sometimes attached under a particular stone. The stones were embedded in small pebbles in areas with rapids and 10-80 cm deep. Egg masses were attached with adhesive

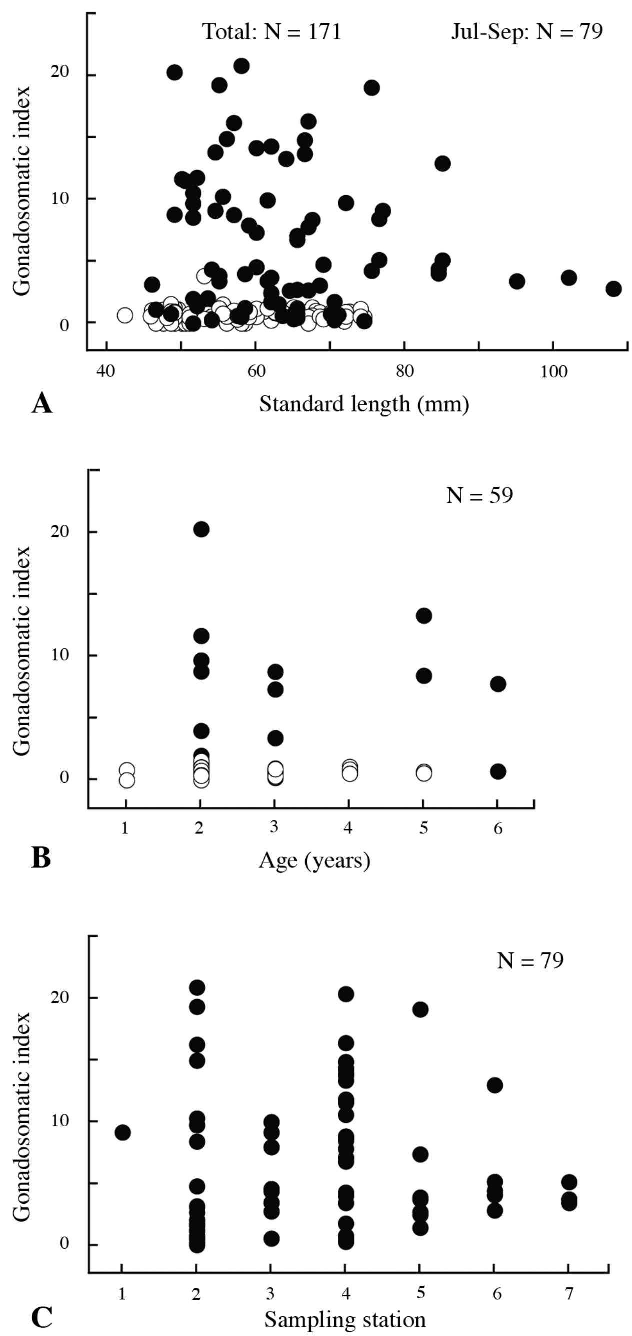
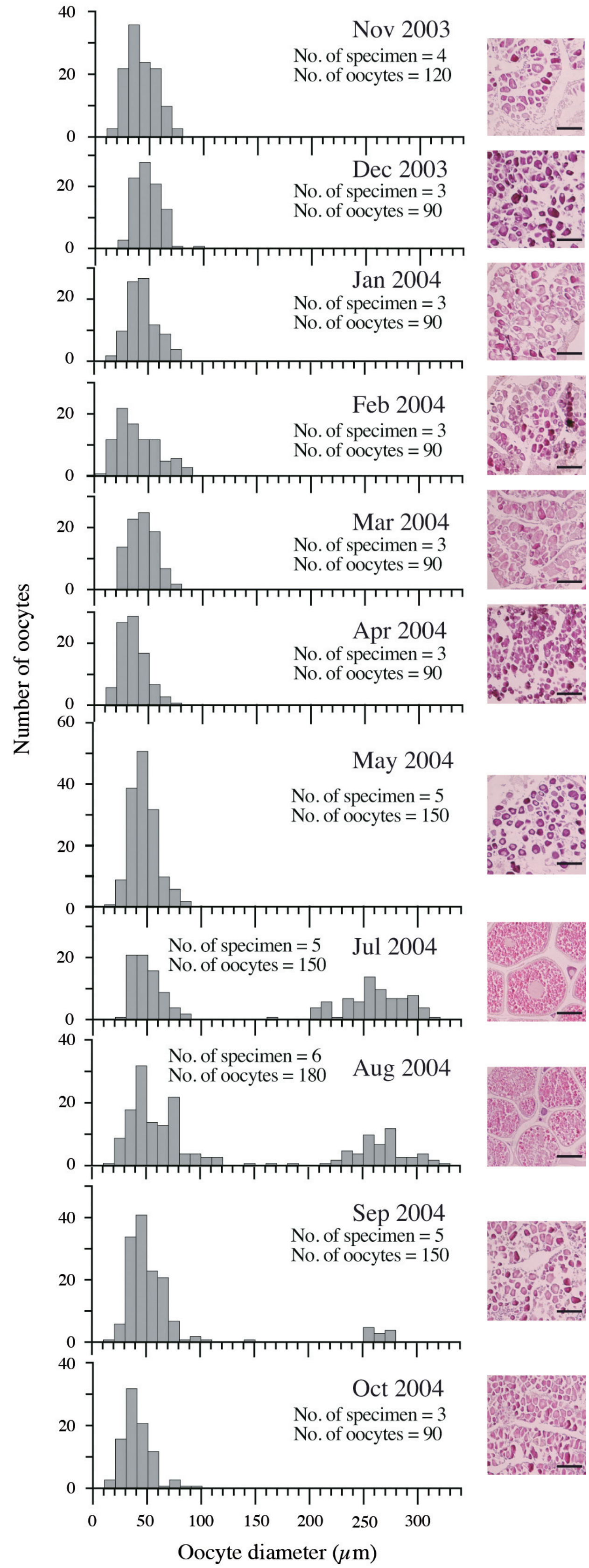


Figure 3. - Relationship between “gonadosomatic index” and (**A**) “standard length” and (**B**) age of all fish including those not collected in the spawning season, and (**C**) sampling station during the spawning season, of female *Sicyopterus japonicus* collected in the Ota River from June 2003 to March 2006. Closed circles indicate specimens collected during the spawning season (July-September).

Figure 4 (right). - Oocyte diameter (left panels) and histological sections (right panels) of gonads of *Sicyopterus japonicus* collected from November 2003 to October 2004 in the Ota River. Scale bars are 100 μm.



filaments to each other and the areas of each clutch ranged from 7.4-26.6 cm^2 (N = 8). Clutch sizes for the 8 nests that were sampled at St. 2 and St. 5 were estimated to range from 11,700 to 76,300 eggs. Eggs were spherical and their diameters were 0.5 mm. An adult guarding male *S. japonicus* was observed to be under at least 17 nests (59%) of the 29 stones that were found to have an egg clutch.

DISCUSSION

Ovarian egg clutches

The ovarian egg clutches (22,540-109,290) and the numbers of large oocytes (10,820-52,460) of the 10 females (50.5-95.0 mm SL) examined in this study were smaller than that of an 87 mm SL female of this species previously reported (225,000, Dôtu and Mito, 1955). This difference may have resulted from differences of SL of females and variation of batch fecundities among individuals. It is also possible that Dôtu and Mito (1955) counted both large and small oocytes together, whereas our estimates were based only on large oocytes. Although several dead *S. japonicus* were seen under stones in the river during our sampling in winter, probably because of low temperature and less feeding, no dead *S. japonicus* were observed in the river during and after the spawning season in our underwater observations during this study. This, and the finding of females from age of 2 to 6 that were mature, indicate that it is probable that *S. japonicus* is a repeat spawner with an iteroparous life history.

The ovarian egg clutch sizes and numbers of large oocytes of *S. japonicus* in the present study are similar to what has been observed in other Sicydiinae gobies [50,000-70,000 for *Sicyopterus lagocephalus* (Keith, 2003), 60,000-150,000 for *Sicydium punctatum* (Keith, 2003), 1,000-10,000 for *Stiphodon percnopterygionus* (Yamasaki and Tachihara, 2006)] and are larger than what has been reported for other taxa of gobies [e.g., 170-410 for *Padogobius martensi* (Marconato *et al*., 1989) and 480-4,820 for *Rhinogobius* sp. CB (species complex Cross-Band type, Tamada and Iwata, 2005)] and other temperate amphidromous fish [e.g., 5,000-20,000 for ayu, *Plecoglossus altivelis* (Matsuyama and Matsuura, 1982) and 400-1,700 for sculpin, *Cottus hangiongensis* (Goto and Iguchi, 2001)], except for the amount of 2,000-40,000 oocytes that was estimated for the eleotrid, *Eleotris fusca* (Maeda *et al*., 2008). These large clutch sizes are one of the major characteristics of Sicydiinae gobies.

Spawning season

The spawning season of *S. japonicus* in the Ota River in Wakayama was found to be from July to September, because the GSI values of female gobies were > 3.0 only during that three month period that is related to the warmest part of the summer season, when large oocytes were only seen, and gonads were mature based on the histological study. In addition to these results, newly hatched larvae were collected in the same river from July to September (Iida *et al*., unpubl. data). Their spawning season was during the period when the water temperature in the Ota River was highest for the year (24°C). At lower latitudes, the spawning season of this species tended to be longer, being at least 4 months from July to October in Taiwan (23°N, Ju, 2001) and 4 months from May to August on Okinawa Island (26°N, Yamasaki *et al*., 2011). Mean water temperature during the spawning season was about 20°C in Wakayama (this study) and was over 22°C in Okinawa (Yamasaki and Tachihara, 2006). Although there is no water temperature data from the study in Taiwan, spawning of *S. japonicus* seems to have occurred at temperatures > 20°C. As it is well known that reproduction is typically correlated with environmental changes such as water temperature (e.g., Hoar, 1965; Mackay, 1974), water temperature probably affects the duration of the spawning season of *S. japonicus*.

The spawning season of *S. japonicus* was shorter than other Sicydiinae gobies in tropical and subtropical regions, e.g., all year for *Sicyopterus lagocephalus* in the Philippines (Manacop, 1953), *Sicyopterus stimpsoni* in Hawai'i (Fitzsimons *et al*., 1993), *Sicydium punctatum* in Dominica (Bell *et al*., 1995) and 7-9 months for *Lentipes concolor* in Hawai'i (Kinzie, 1993) and *Stiphodon percnopterygionus* in Okinawa (Yamasaki and Tachihara, 2006). In comparison with other temperate amphidromous fish, the duration of the spawning season of *S. japonicus* was similar, e.g., end of August to September in northern parts of Japan and end of October to December in southern parts of Japan for *P. altivelis* (Nishida, 1995), and April to July for *Rhinogobius* sp. DA (Dark type) (Takahashi and Yanagisawa, 1999). These observations indicate that the spawning season of *S. japonicus* is similar to other temperate amphidromous fish, but not to the more closely related Sicydiinae species, probably because of the temperate region where they live.

Spawning places and male behaviour

During the spawning season, many egg clutches were observed and collected throughout the river, except in the upper-most station and at the station near the river mouth. As there was no relationship between GSI and sampling stations, and that egg clutches were collected across wide reaches of the river (St. 2-6), *S. japonicus* probably does not have a particular spawning area within the river or form aggregations for locating potential mates, as it has been reported for other diadromous fish [e.g., *Awaous guamensis* from the Pacific area (Kido and Heacock, 1992; Keith, 2003); *P. altivelis* in Japan (Nishida, 1995); *Galaxias maculates* in Australia and New Zealand (McDowall, 1988; Chapman *et al*., 2006)]. Spawning in their usual habitat and not to have a habitat segregation in a particular place in the river was also reported for other Sicydiinae gobies, e.g., *Sicyopterus stimpsoni* and *Lentipes concolor* (Fitzsimons *et al*., 1993; Kinzie, 1993). However, it has been reported that groups of *S. japonicus* climb the rocks of rapids and waterfalls to move toward the upper reaches of rivers in summer using their sucker-like pelvic fin and lips (Fukui, 1979), and this movement might be correlated with spawning or habitat changes after spawning. Further research is needed to understand these habitat changes and the dynamics of their spawning ecology.

The egg clutches of *S. japonicus* were attached to the undersurface of embedded stones as was mentioned in the previous report about *S. japonicus* (Dôtu and Mito, 1955) and has been reported for other gobies (e.g., *Rhinogobius* spp., Takahashi and Yanagisawa, 1999; *Lentipes concolor*, Kinzie, 1993). As mentioned above, *S. japonicus* probably does not have a preference for a spawning area among the reaches (upper or lower), they may have a special preference for spawning in places with small pebbles and large stones onto which they can attach their egg clutches. Since courtship behaviour was reported (Iida *et al*., unpubl. manuscript) and male gobies were often seen under the stones during observations as mentioned in the previous study (Dôtu and Mito, 1955), *S. japonicus* probably has courtship behaviour and parental care for eggs by males as has been reported for many other gobies (e.g., Marconato *et al*., 1989; Takahashi, 2000; Takegaki, 2000).

Conclusion

This study presented details about the reproductive biology of *S. japonicus* in the Ota River at a latitude of 34°N, which is the highest latitude at which a Sicydiinae goby has been studied. *S. japonicus* has a shorter spawning season compared to other tropical and subtropical Sicydiinae gobies, but the length of their spawning season is similar to other temperate amphidromous fish. The clutch size of *S. japonicus* was similar to that of other Sicydiinae gobies and larger than that of temperate amphidromous fish. In conclusion, we found that *S. japonicus* has combined characteristics of tropical Sicydiinae gobies and temperate amphidromous fish probably reflecting their taxonomic affinities and the latitude of their species range. Future studies are needed on the reproductive ecology of this species in other rivers at a range of different latitudes to fully understand the possible relationship between environmental conditions such as water temperature and the life history of this species.

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