Reproductive biology of three amphidromous gobies, *Sicyopterus japonicus*, *Awaous melanocephalus*, and *Stenogobius* sp., on Okinawa Island

by

Nozomi YAMASAKI (1, 2), Masashi KONDO (1), Ken MAEDA* (3, 4)
& Katsunori TACHIHARA (4)

ABSTRACT. - Reproductive biology and early development of three amphidromous gobies (*Sicyopterus japonicus*, *Awaous melanocephalus*, and *Stenogobius* sp.) were investigated in streams on Okinawa Island in southern Japan. Gonad examination, observation of spawning habits, and seasonal occurrence of newly hatched drifting larvae suggested the following: *S. japonicus* and *Stenogobius* sp. mature at approximately 40 mm and 35 mm in standard length (SL), respectively; *A. melanocephalus* matures at approximately 40 mm SL, but the smallest parent observed at a spawning nest was 70 mm SL; females of the larger *S. japonicus* and *A. melanocephalus* may spawn several hundred thousand eggs in a single clutch, while the batch fecundity of *Stenogobius* sp. is comparatively smaller (max. 72,000); the spawning seasons of *S. japonicus* and *A. melanocephalus* are May-August and June-November, respectively; and *Stenogobius* sp. can spawn throughout the year, but it is usually inactive from January to April. The eggs of *S. japonicus* and *A. melanocephalus* are small (0.4 mm in diameter) and almost spherical, while those of *Stenogobius* sp. are elliptical (1.0 mm long). The three species hatch as similar small undeveloped larvae, but they can be differentiated by melanophore arrangement. The small eggs, higher fecundity, undeveloped phase at hatching, and spawning during warmer seasons are all considered to be characteristics that are common to the subfamily Sicydiinae and the genera *Awaous*, *Stenogobius*, and *Eleotris*, inhabiting tropical and subtropical insular streams. However, minor variations are observed between species or genera in the timing and duration of the spawning season, construction of spawning nests, egg laying habits, egg morphology, and the size of newly hatched larvae and their yolk sacs.

RÉSUMÉ. - Biologie de la reproduction de trois gobies amphidromes de l'île d'Okinawa : *Sicyopterus japonicus*, *Awaous melanocephalus* et *Stenogobius* sp.

La biologie de la reproduction et le développement précoce de trois gobies amphidromes des rivières de l'île d'Okinawa (au sud du Japon) ont été étudiés. L'observation des gonades, des comportements lors de la reproduction et l'observation saisonnière de la présence de larves dérivantes suggèrent les résultats suivants : *S. japonicus* et *Stenogobius* sp. sont respectivement matures à environ 40 mm et 35 mm de longueur standard (SL) ; *Awaous melanocephalus* est mature à environ 40 mm SL, mais les parents les plus petits observés près du nid mesuraient 70 mm SL ; les femelles de *S. japonicus* et *A. melanocephalus*, qui sont des espèces plus grandes, pondent certainement plusieurs centaines de milliers d'œufs dans une seule ponte, alors que la fécondité de *Stenogobius* sp. est plus faible comparée aux deux espèces précédentes (max. 72000) ; les saisons de reproduction sont de mai à août pour *S. japonicus*, de juin à novembre pour *A. melanocephalus* et *Stenogobius* peut se reproduire tout au long de l'année bien qu'il soit généralement inactif de janvier à avril. Les œufs de *S. japonicus* et de *A. melanocephalus* sont petits (0,4 mm de diamètre) et presque sphériques alors que ceux de *Stenogobius* sp. sont elliptiques (1,0 mm de long). Les trois espèces éclosent sous la forme de petites larves peu développées d'aspect similaire mais pouvant être différenciées selon l'arrangement des mélanophores. Des petits œufs, une fécondité élevée, une larve peu développée à l'éclosion et la reproduction se produisant au moment des saisons plus chaudes sont tous des éléments caractéristiques communs à la sous-famille des Sicydiinae et aux genres *Awaous*, *Stenogobius* et *Eleotris* que l'on trouve dans les rivières tropicales et subtropicales. Cependant, il existe des petites variations entre espèces ou genres en ce qui concerne le moment et la durée de la saison de reproduction, la construction des nids, le comportement de ponte, la morphologie des œufs et la taille des larves à l'éclosion ainsi que la taille de la vésicule vitelline.

Key words. - Spawning season - Fecundity - Spawning habit - Larval morphology - Drifting larva - Gobiidae.

The freshwater fish fauna of tropical and subtropical insular streams are often dominated by sicydiine and gobionelline gobies (Gobiidae), with some sleepers (Eleotridae), pipefishes (Syngnathidae), eels (Anguillidae), mullets

(1) Graduate School of Engineering and Science, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Nakagami-gun, Okinawa 903-0213, Japan.
(2) Present address: 398 Uechi, Shimoji, Miyakojima-shi, Okinawa 906-0304, JAPAN [stiphodon@hotmail.com]
(3) Marine Genomics Unit, Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa 904-0412, Japan. [goby@live.jp]
(4) Faculty of Science, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Nakagami-gun, Okinawa 903-0213, Japan. [ktachiha@sci.u-ryukyu.ac.jp]
* Corresponding author

(Mugilidae), and flagtails (Kuhliidae) (e.g., Marquet and Galzin, 1992; Keith *et al*., 1999, 2010; Fitzsimons *et al*., 2002; Maeda and Tachihara, 2006). Sicydiine gobies are generally considered to be amphidromous and are most commonly distributed in tropical and subtropical insular streams (Keith *et al*., 2011). Among the members of the subfamily Gobionellinae, the genera *Awaous* and *Stenogobius* have been suggested to be closely related to the subfamily Sicydiinae (Thacker, 2003). Together with the sicydiine gobies, the members of these two genera are considered to typify the gobiid inhabitants of tropical and subtropical insular streams (Watson, 1991, 1992).

Sicyopterus japonicus (Sicydiinae), *Awaous melanocephalus*, and *Stenogobius* sp. (Gobionellinae) are common amphidromous gobies in the freshwater streams of the Ryukyu Archipelago, a subtropical island chain in southern Japan. While we were unable to identify this *Stenogobius* species due to the lack of intensive taxonomical examination of this genus in this region (Watson, 1991), it is considered to be a single species and is referred to as "Tanekawa-haze" in Japan (Akihito *et al*., 2002). Although these three species are relatively abundant in the Ryukyu Archipelago, aspects of their reproductive biology have not been examined with the exception of the morphology of eggs and larvae of *A. melanocephalus* (Yamasaki and Tachihara, 2007). The timing of the spawning season, spawning habits, fecundity, and the morphology of the eggs and larvae of *S. japonicus* have been reported in populations from Kyushu and Honshu in central Japan (Dotu and Mito, 1955; Watanabe *et al*., 2007; Iida *et al*., 2009). However, these life history characteristics are required to be investigated in the Ryukyu Archipelago, because they may vary according to geography (Iida *et al*., 2009). Among the other members of the Sicydiinae, *Awaous* and *Stenogobius*, aspects related to the reproductive biology and/or early development have only been reported in *Sicyopterus lagocephalus* from the Philippines (Manacop, 1953) and Réunion Island (Valade *et al*., 2009), *Sicyopterus stimpsoni*, *Lentipes concolor*, *Awaous guamensis*, and *Stenogobius hawaiiensis* from Hawaii (Maciolek, 1977; Kinzie, 1993; Ha and Kinzie, 1996; Way *et al*., 1998; Lindstrom, 1999), and *Stiphodon percnopterygionus* from the Ryukyu Archipelago (Yamasaki and Tachihara, 2006). Consequently, knowledge of reproductive biology and early life history of amphidromous gobies is fragmentary. It is therefore necessary to undertake additional studies of amphidromous gobioid taxa to clarify aspects of life histories and reproductive biology, including the possibility of geographic variation.

In the present study, the reproductive biology, including size at first maturation, timing and duration of the spawning season, fecundity, and spawning habits of the three gobiid species, *S. japonicus*, *A. melanocephalus*, and *Stenogobius* sp. were investigated in the streams on Okinawa Island, Ryukyu Archipelago. In addition, we also described the morphology of eggs and larvae of *S. japonicus* and *Stenogobius* sp. obtained under captive rearing conditions.

MATERIALS AND METHODS

Gonad examination

Three amphidromous gobies, *Sicyopterus japonicus*, *Awaous melanocephalus*, and *Stenogobius* sp., were collected from 27 streams in northern Okinawa Island from 1995 to 2005. After fixing in 10% formalin, the gonads were removed and weighed after the standard lengths (SL) and body weights were measured. The gonadosomatic index (GSI) was calculated as follows: GSI = Weight of gonad / Body weight × 100. Tissue samples of ovaries and testes were dehydrated and embedded in paraffin, sectioned at 7-8 μm, and stained with Mayer's hematoxylin and eosin. The ovaries were then classified into the three developmental stages using criteria modified from Ha and Kinzie (1996): (1) "immature", ovary containing only immature pre-vitellogenic oocytes; (2) "mature", ovary containing vitellogenic and/or hydrated oocytes; (3) "post-spawning", ovary with atretic oocytes. For evidence of previous spawning, each ovary was also examined for presence of postovulatory follicles. The testes were classified into two developmental stages using criteria modified from Ha and Kinzie (1996): (1) "immature", testis without mature sperm; (2) "mature", testis with mature sperm in testicular follicles. To estimate potential batch fecundity, an ovary specimen was removed and weighed. The number of vitellogenic oocytes in the ovary specimen was counted and standardized by weight for the entire ovary.

Spawning sites

Searches were conducted for gobioid egg masses in four of the streams (stream length, 3.6-12.4 km) on Okinawa Island in 1997 and from 2003 to 2010. On finding an egg mass of *Sicyopterus japonicus*, *Awaous melanocephalus*, and *Stenogobius* sp., the distance from the stream mouth, water temperature, water depth, and the area occupied by the egg masses were recorded. The egg masses were identified to the species based on observation of the parent fish tending the egg mass or apparently being under the nest. When we failed to observe the parent, the egg masses were identified based on the construction of the nest, arrangement of eggs on the substratum, and general morphology of the eggs (the specific characteristics were obtained based on the observation of the egg masses identified with the parent; those are described in "Discussion"). The SL of parent was then measured with a dial calliper after capture or estimated by underwater observation. A subsample of one *S. japonicus* egg mass was removed from an area measuring 0.22 cm^2 and fixed in 5% formalin; the number of the eggs within entire egg mass could then be estimated by standardization of the number of eggs contained within this subsample. Close-up photographs of five egg masses from *A. melanocephalus* and four egg masses from *Stenogobius* sp. were taken with a scale, and the numbers of eggs within the egg masses were estimated based on the number of eggs in a subsample of the egg masses (0.25 or 1.00 cm^2) in the photographs and the area occupied by the entire egg mass. In addition, the numbers of eggs in another 17 *A. melanocephalus* egg masses were estimated based on the area occupied by the egg mass and the mean egg density calculated in the five *A. melanocephalus* egg masses mentioned above (983 eggs/cm^2).

Morphology of eggs and reared larvae

The egg morphologies were described based on egg samples (n = 7-10/egg mass) removed from two *Sicyopterus japonicus* egg masses collected on 26 May 1997 and 10 August 2003 and from two *Stenogobius* sp. egg masses collected on 13 July 2004 and 3 July 2008. To describe the morphology of larvae, an egg mass of *S. japonicus* collected on 23 June 2004 and an egg mass of *Stenogobius* sp. collected on 3 July 2008 were brought to the laboratory. The egg masses were maintained, together with the substratum to which the egg mass was attached, in a tank containing freshwater with gentle aeration until hatching. The hatched larvae were then transferred to another tank, and seawater was added to increase the salinity to 13-30 ppt. The water temperature used for rearing *S. japonicus* was 22.5-24.5°C and that used for *Stenogobius* sp. was 23.5-24.5°C. Larval descriptions were based on samples collected from the tanks each evening until the larval yolk had been completely consumed (n = 4-11/day). The eggs and larvae were observed under a stereomicroscope (magnification 50-63, SNZ800, Nikon, Tokyo) and an optical microscope (magnification 60-150, ECLIPSE E600, Nikon, Tokyo) immediately after fixation in 5% formalin. The vertical and horizontal diam-

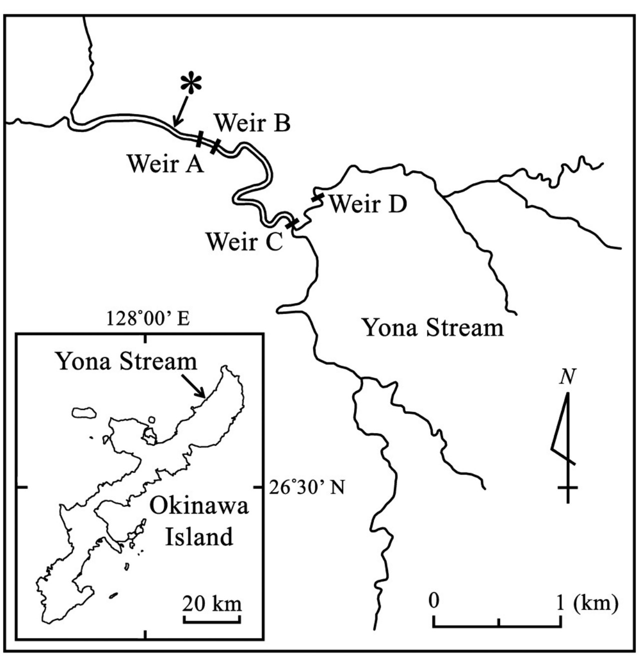

Figure 1. - Map showing a collection site of drifting fish larvae (asterisk) and location of the weirs in the Yona Stream, Okinawa Island. Gobioid fish fauna was investigated in the reaches between the collection site and the weir D.

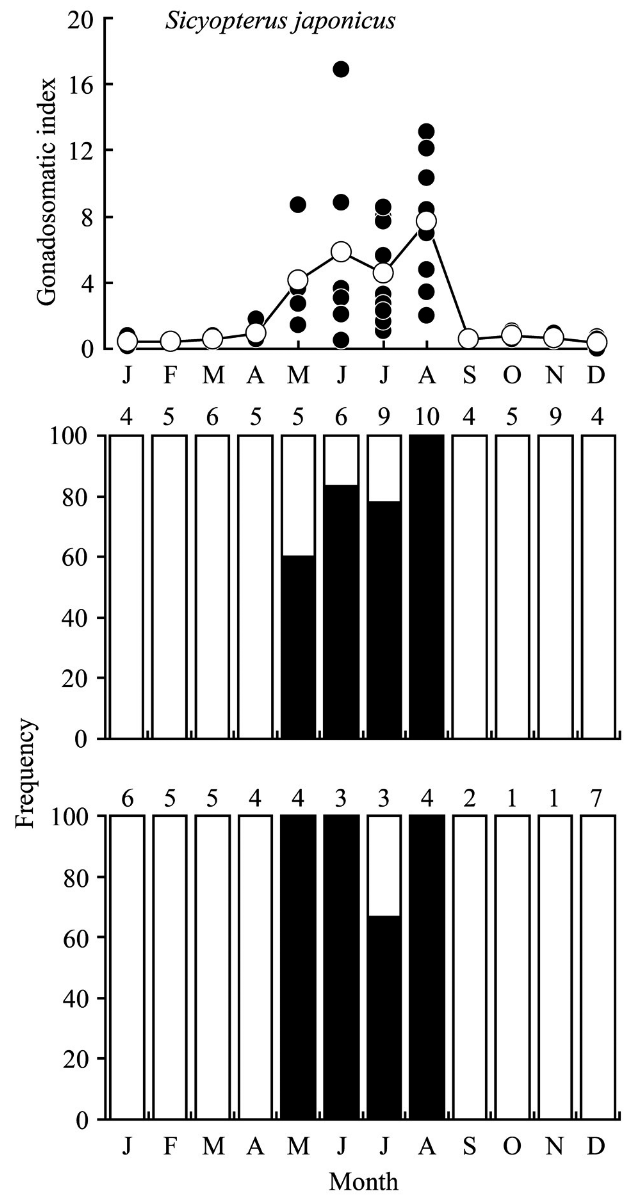

Figure 2. - Monthly changes in female gonadosomatic index (GSI) (top: n = 69) and the frequency of gonadal development stages (middle: females, n = 69; bottom: males, n = 45) in *Sicyopterus japonicus* collected in the streams on Okinawa Island from 1996 to 2005. Numbers above bars indicate the number of samples for a given month. Solid circles: individual GSI; open circles: mean monthly GSI; solid bars: mature; open bars: immature.

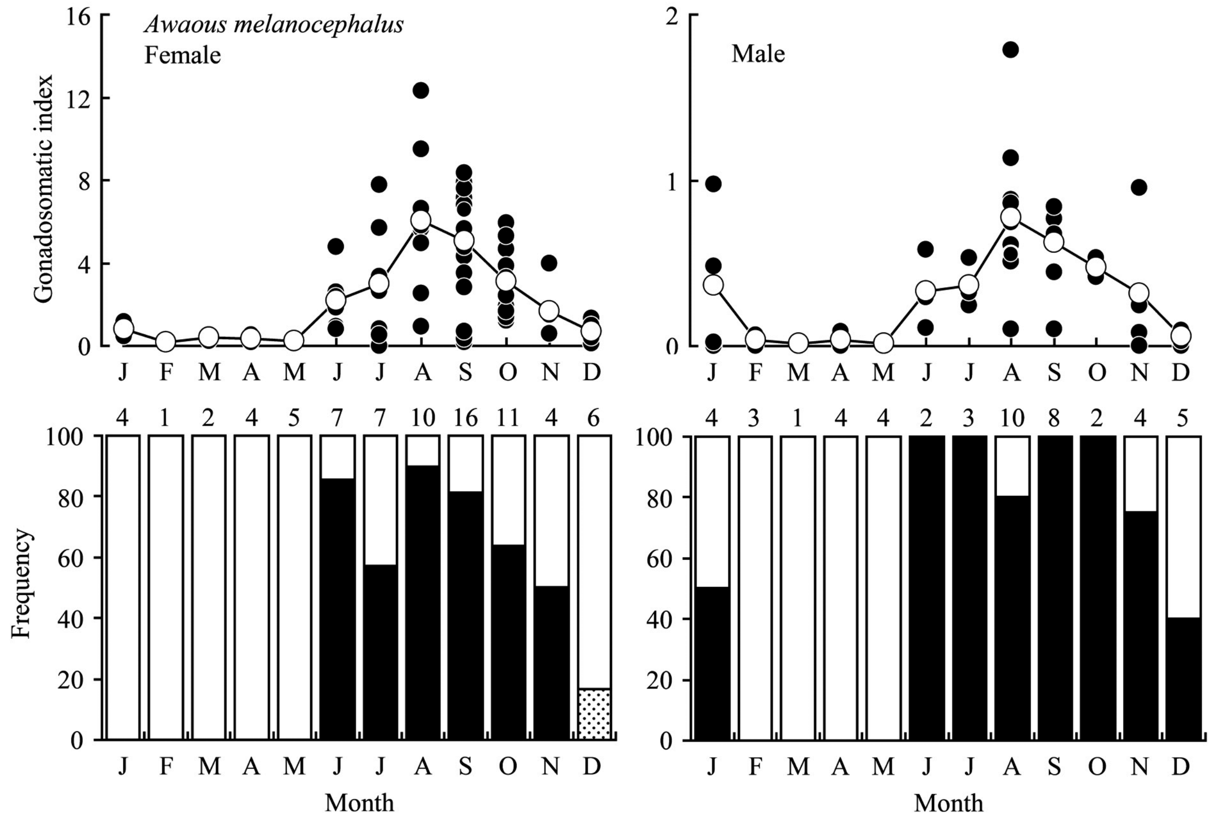

Figure 3. - Monthly changes in gonadosomatic index (GSI) (top: females, n = 77; males, n = 52) and the frequency of gonadal development stages (bottom: females, n = 77; bottom: males, n = 50) in *Awaous melanocephalus* collected in the streams on Okinawa Island from 1995 to 2005. Numbers above bars indicate the number of samples for a given month. Solid circles: individual GSI; open circles: mean monthly GSI; solid bars: mature; open bars: immature; dotted bar: post-spawning.

eter of eggs was measured with the micropyle located at the top (see Fig. 7 of Maeda *et al*., 2008). The notochord length (NL), total length (TL), and yolk-sac diameter (YSD) of the larvae were measured under the stereo- and optical microscopes with an ocular micrometer.

Collection and morphology of drifting larvae

Larvae were collected with a plankton net (Kitahara-type, mouth diameter 23 cm, length 85 cm, mesh XX-13) in the Yona Stream on northern Okinawa Island (Fig. 1) twice a month (the first half and the second half) from October 2004 to September 2005, to examine the seasonal occurrence patterns of the gobioid larval drift. Tidal fluctuations have been observed in the Yona Stream (ca. 5.5 km long) up to 0.8 km from the stream mouth. The net was set at the centre of the stream in a run immediately above the upper limit of the tidally influenced area (Fig. 1). The water depth and stream width at the collection site were 25-50 cm and 180-400 cm, respectively. Since Maeda and Tachihara (2010) demonstrated that drifting larvae of all taxa collected were predominantly encountered several hours after dusk, a one-hour sample effort with the net (consisting of two consecutive 30-minutes sessions to prevent the mesh from clogging up) was initiated when the intensity of illumination decreased to 10 lux (17:55-19:55), which was measured with an illuminometer (SLX-1332, Sansyo, Tokyo). All of the materials thus collected were fixed in 5% formalin. After each of the two 30-minute samples were diluted with water to 300 ml, the material was gently agitated and a 10% subsample (30 ml) was screened for fish larvae under a stereomicroscope in the laboratory. The number of whole larvae within the two 10% subsamples from the two 30-minute sampling sessions was multiplied by 10 to obtain an estimate of the total number of larvae caught during one hour. Averages of the two one-hour sampling sessions conducted every month were then used to describe the seasonal occurrence patterns. The TL and YSD of randomly selected, undamaged larvae were measured under a stereomicroscope with an ocular micrometer.

Gobioid inventories

Gobioid adult fish fauna in the Yona Stream was investigated in the reaches above the site where drifting larvae were collected. There were three weirs located 1.0 km (weir A), 1.1 km (weir B), and 2.9 km (weir C) from the stream mouth (Fig. 1). The stream forked immediately above weir C, and the north tributary had a weir 0.4 km from the fork (weir D).

Surveys of the occurrence of gobioid adults were conducted by snorkelling between the collection site and weir A once or twice a month from November to December 2004 and from April to August 2005, and between the collection site and weir D in October 2005. We noted the number of individuals when we found 1-10 adult individuals of gobioid species, and recorded as “abundant” when we observed more than 10 adults. In the event that only juveniles were found, the occurrence of juveniles was noted.

RESULTS

Gonad examination

The mean GSI of *Sicyopterus japonicus* females was less than 1.0 from January to April, increasing to a mean of 4.1 in May. GSI was also greater than 4.0 in June, July and August, before decreasing to 0.6 in September and remaining less than 1.0 thereafter (Fig. 2). Mature ovaries were found from May to August (Fig. 2). Mature testes in *S. japonicus* males were found from May to August (Fig. 2), but the testes were very small and had GSI values of less than 0.5.

The mean GSI of *Awaous melanocephalus* females was less than 1.0 from January to May, increasing to a mean of 2.2 in June. Mean GSI then peaked at 6.1 in August before gradually decreasing to 0.7 in December (Fig. 3). Mature ovaries were found from June to November, and a post-spawning ovary composed of atretic and pre-vitellogenic oocytes was observed in December (Fig. 3). Postovulatory follicles were observed in the ovaries of two females collected in July and August. With the exception of two male specimens that had relatively high GSI values in January (1.0 and 0.5), the GSI values of *A. melanocephalus* males were less than 0.1 from January to May. Mean GSI values began to increase in June (mean GSI 0.3), before peaking at 0.8 in August, and then gradually decreasing to 0.1 in December (Fig. 3). More than 70% of males had mature testes from June to November, although mature testes were also observed in January and December (Fig. 3).

The GSI values of two female *Stenogobius* sp. collected in January were 2.8. With the exception of a female specimen collected in April which had a GSI of 11.0, GSI values were less than 2.0 from February to April. Mean GSI values increased to more than 2.0 from May to December, but did not show a clear peak (Fig. 4). Mature ovaries were found throughout the year, although more than 60% of females collected in February and March had immature ovaries (Fig. 4). The testes of *Stenogobius* sp. males were very small and the GSI values of males were always less than 0.5, except for four males collected in July that had GSI values ranging from 0.5 to 0.7. Mature testes were observed from April to December (Fig. 4).

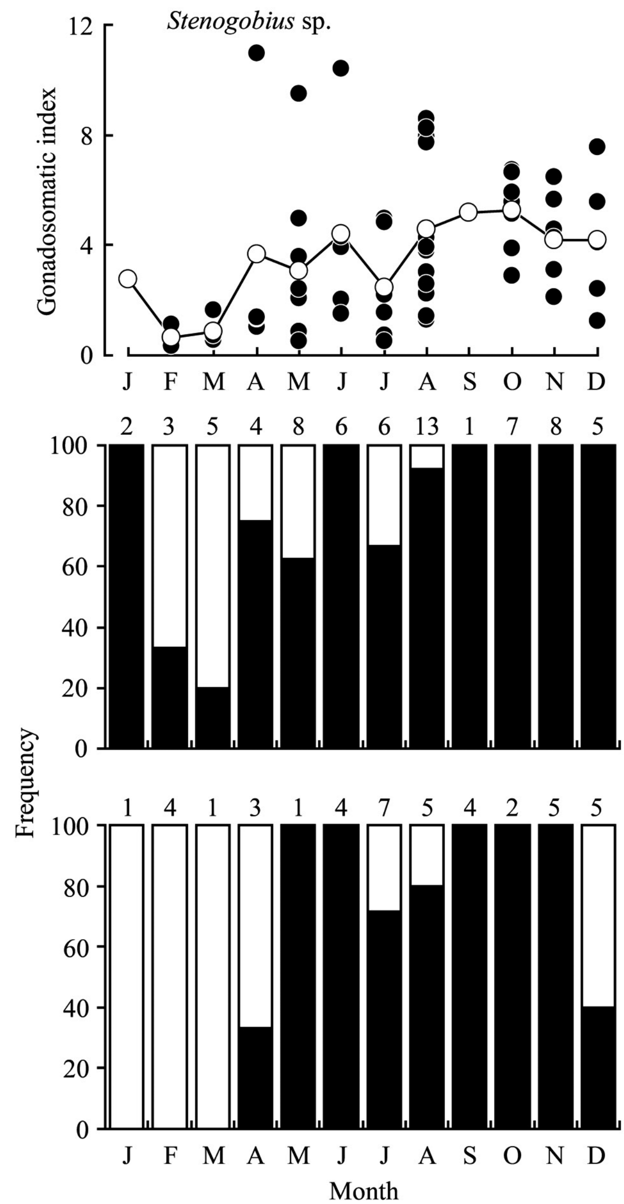

Figure 4. - Monthly changes in female gonadosomatic index (GSI) (top: n = 68) and the frequency of gonadal development stages (middle: females, n = 68; bottom: males, n = 42) in *Stenogobius* sp. collected in the streams on Okinawa Island from 1996 to 2005. Numbers above bars indicate the number of samples for a given month. Solid circles: individual GSI; open circles: mean monthly GSI; solid bars: mature; open bars: immature.

The smallest females of *S. japonicus, A. melanocephalus*, and *Stenogobius* sp. with mature ovaries and GSI values exceeding 3.0 measured 43.7, 41.5, and 34.6 mm SL, respectively (Fig. 5). The GSI values of more than half of the females that were larger than these SL collected during their spawning season (*S. japonicus*, May-August; *A. melanocephalus*, June-November; and *Stenogobius* sp., all months) were greater than 3.0, while females smaller than these SL

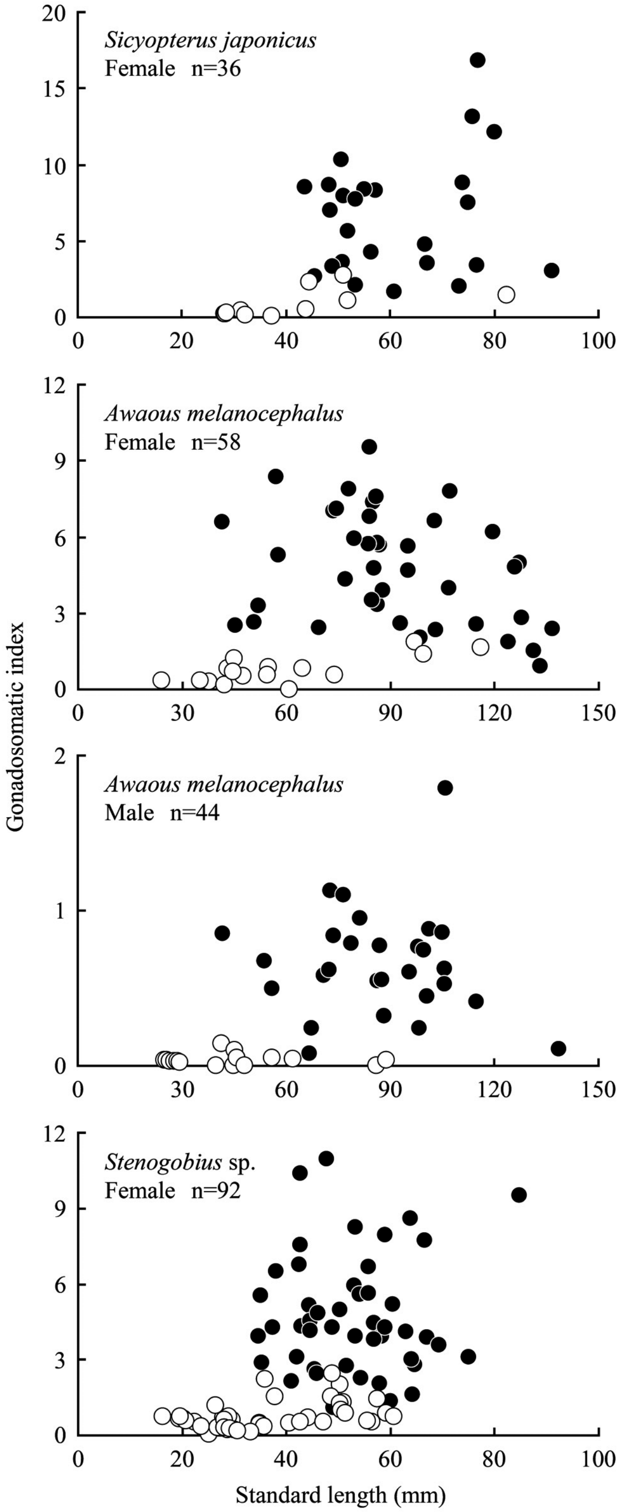

Figure 5. - Relationships between standard length and gonadosomatic index of *Sicyopterus japonicus* females, *Awaous melanocephalus* females and males, and *Stenogobius* sp. females collected in streams on Okinawa Island during the spawning seasons from 1995 to 2005 (*S. japonicus*: May to August; *A. melanocephalus*: June to November; *Stenogobius* sp.: all months). Solid circles: mature; open circles: immature.

always had lower GSI values (< 1.2). The smallest males of *S. japonicus, A. melanocephalus*, and *Stenogobius* sp. with mature testes measured 40.2, 41.8, and 36.5 mm SL, respectively, and more than 75% of the males larger than these SL had mature testes during the spawning season. The GSI values of more than 70% of the *A. melanocephalus* males with mature testes were greater than 0.5, and the GSI values of males with immature testes were always less than 0.2 (Fig. 5).

The potential batch fecundities of *S. japonicus*, *A. melanocephalus*, and *Stenogobius* sp., estimated by the number of vitellogenic oocytes, were 12,000-94,000 (43.7-80.0 mm SL), 3,000-290,000 (41.5-127.8 mm SL), and 2,000-72,000 (34.6-84.7 mm SL), respectively. Larger females of all three species had considerably more vitellogenic oocytes than smaller ones (Fig. 6).

Spawning habits

We observed 23 *Sicyopterus japonicus* egg masses from May to August (1-6 egg masses / day). The spawning grounds were located in freshwater riffles and pools (water depth: 14-130 cm) extending from immediately above the upper margin of the tidally influenced area (0.9-1.3 km from the stream mouth) to the upper reaches (6.0-9.0 km from stream mouth) of the stream. Water temperatures ranged from 25 to 29°C. Egg masses, which were attached to each other to form clusters by adhesive filaments, were attached to the undersides of stones (Fig. 7A), and were usually accompanied by a solitary adult (45-87 mm SL); however, we often failed to observe these adults, because they immediately disappeared when the nest stone was removed. Spawning nests, which were generally hidden spaces beneath stones that were surrounded by gravel and stones, could usually not be seen if the stones to which the egg mass were attached were not turned over. The area occupied by the observed egg masses was 6-28 cm^2. The number of eggs contained in the largest egg mass was estimated to be 203,597, and the number of larvae that would have hatched from this egg mass was estimated to be 138,000, since dead eggs were observed in a part of the egg mass.

We found 43 egg masses of *Awaous melanocephalus* from July to October (1-6 egg masses/day). The spawning grounds were located in riffles and pools (water depth: 7-125 cm) extending from around the upper margin of the tidally influenced area (0.4 km from the stream mouth) to the lower and middle reaches of the freshwater area (1.0-2.5 km from the stream mouth). Water temperatures ranged from 22 to 30°C. Eggs were laid densely in a monolayer mass (Fig. 7B) on a variety surfaces of the substrates on the relatively open nests, including the under and lateral sides of stones on the nest, and the ground (gravel or stones) of the nest beneath such stone; the ceiling, lateral sides, and bottom surface on the holes in a concrete block; and the upper surfaces of stones exposed on

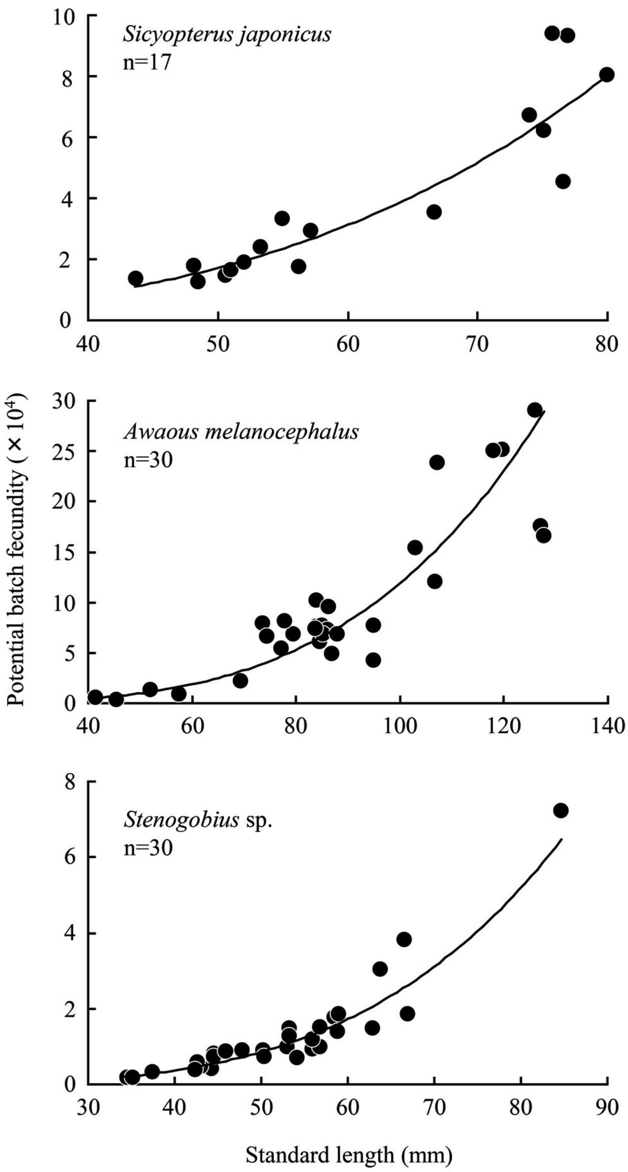

Figure 6. - Relationships between standard length and potential batch fecundity (BF) of *Sicyopterus japonicus*, *Awaous melanocephalus*, and *Stenogobius* sp. collected in streams on Okinawa Island from 1995 to 2005. Regression curves for *S. japonicus*, *A. melanocephalus*, and *Stenogobius* sp. were expressed as $BF = 0.046SL^{3.3}$ (r = 0.94), $BF = 0.0066SL^{3.6}$ (r = 0.94), and $BF = 0.0026SL^{3.8}$ (r = 0.95), respectively.

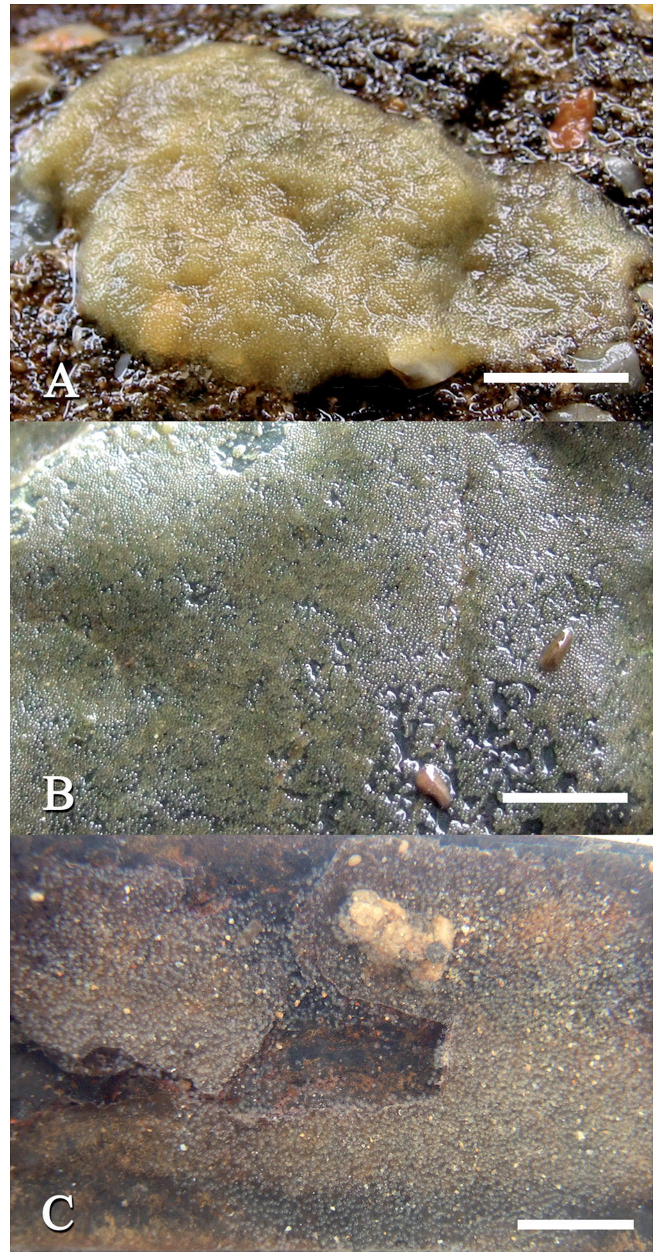

Figure 7. - Egg masses of *Sicyopterus japonicus* (**A**), *Awaous melanocephalus* (**B**), and *Stenogobius* sp. (**C**). Scale bar = 1 cm.

the streambed. Solitary adults (70-135 mm SL) were usually found in the nest, and observed tending the egg mass. On the four occasions that these individuals were caught and sexed based on the morphology of the genital papilla, they were found to be males. The area occupied by egg masses was 40-246 cm^2. The number of eggs contained in a single egg mass was estimated to be 38,829-259,530, and the number of larvae hatching from an egg mass was estimated to be approximately 200,000.

We found six egg masses of *Stenogobius* sp. in July and October (1-3 egg masses/day). The spawning grounds were located in pools and gentle riffles (water depth: 10-40 cm) extending from the upper reaches of the tidally influenced area (0.3 km from the stream mouth) to the lower reaches of the freshwater area (0.9 km from the stream mouth). Water temperatures ranged from 22 to 27°C. The eggs were laid densely in a monolayer mass (Fig. 7C) on the undersurfaces of stones or artificial wastes (concrete and rubber blocks) placed on or somewhat submerged in the gravels and sands of the streambed. On four of the six occasions when adults were observed in the vicinity of the egg masses, these individuals were identified as being solitary males (40-65 mm SL); we failed to measure and sex the parent on the other two occasions. Egg masses occupied an area of 5-17 cm^2. The number of eggs in a single egg mass was estimated to be 485-8,074.

Morphology of eggs and larvae

Eggs of *Sicyopterus japonicus* were approximately spherical (Fig. 8A), with the vertical diameter measured with the micropyle located at the top (range 0.36-0.40 mm, mean ± SD = 0.38 ± 0.01 mm; n = 20) being somewhat smaller than the horizontal diameter (0.41-0.46 mm, 0.43 ± 0.01 mm; n = 20). The larvae, measuring 1.39 ± 0.13 mm TL at hatching (n = 4), grew to 2.00 ± 0.05 mm TL by 3 days after hatching (AH) (n = 4; Tab. I). Newly hatched larvae had a yolk sac (23.0 ± 3.9% TL; Tab. I), and the yolk was completely absorbed by 5 days AH. The mouth, which had not yet formed at hatching (Fig. 8B), opened by 3 days AH (Fig. 8E). Eyes were fully pigmented by 3 days AH (Fig. 8E). Pectoral-fin buds appeared by 2 days AH (Fig. 8D). Newly hatched larvae had melanophores on the tip of the snout, on the anterior and posterior margins of the yolk sac, and ventrally on the trunk and tail (Fig. 8B). Melanophores on the tail located 6-8 myomeres posterior to anus at hatching (Fig. 8B), moved anteriorly and united with melanophores on the trunk by 3 days AH (Fig. 8E).

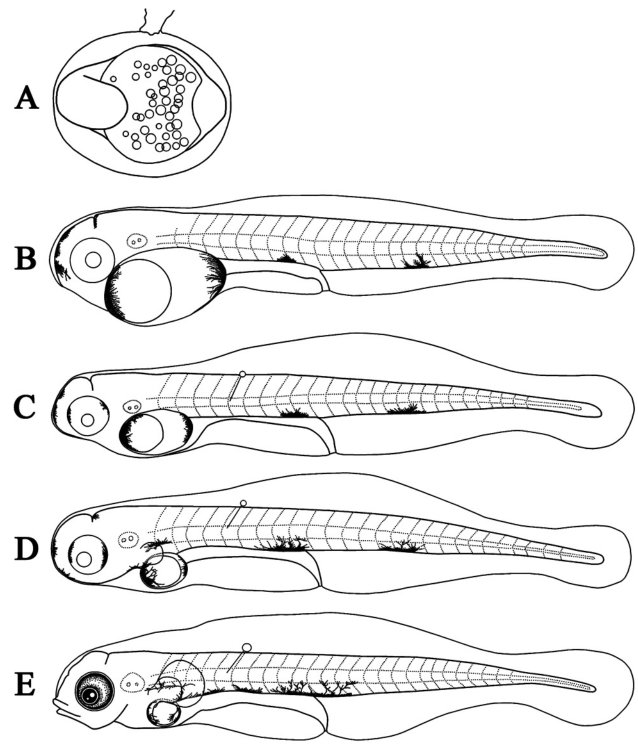

Figure 8. - Egg and larval development of *Sicyopterus japonicus*. **A**: egg, 0.34 mm in vertical diameter; **B**: newly hatched, 1.59 mm in total length (TL); **C**: 1 day old, 1.84 mm TL; **D**: 2 days old, 1.98 mm TL; **E**: 3 days old, 1.94 mm TL.

Eggs of *Stenogobius* sp. were elliptical (Fig. 9A) with the vertical diameter ranging from 0.89 to 1.16 mm (mean ± SD = 1.02 ± 0.09 mm; n = 17) and the horizontal diameter ranging from 0.35 to 0.40 mm (0.38 ± 0.02 mm; n = 17). The larvae, measuring 1.65 ± 0.05 mm TL at hatching (n = 11), grew to 2.09 ± 0.01 mm TL by 3 days AH (n = 7; Tab. I). Newly hatched larvae had a yolk sac (16.7 ± 1.3% TL; Tab. I), and the yolk was completely absorbed by 4 days AH. The mouth, which had not yet formed at hatching (Fig. 9B), opened by 2 days AH (Fig. 9D). Eyes were fully pigmented by 3 days AH (Fig. 9E). Pectoral-fin buds appeared by 1 day AH (Fig. 9C). Newly hatched larvae had melanophores on the tip of the snout, on the anterior and posterior margins of the yolk sac, along dorsal midline from head to trunk and tail, and along ventral midline of the trunk and tail (Fig. 9B). Dorsal melanophores decreased markedly by 1 day AH (Fig. 9C) and disappeared entirely by 2 days AH (Fig. 9D).

Table I. - Notochord length, total length, and yolk-sac diameter of *Sicyopterus japonicus* and *Stenogobius* sp. larvae measured under rearing conditions in the laboratory.

	Notochord length (mm)		Total length (mm)		Yolk-sac diameter (% TL)		No. of larvae examined
	Range	mean ± SD	Range	mean ± SD	Range	mean ± SD	
S. japonicus							
Newly hatched	1.24-1.53	1.32 ± 0.14	1.32-1.59	1.39 ± 0.13	19.5-26.9	23.0 ± 3.9	4
1 day after hatching	1.54-1.76	1.65 ± 0.09	1.64-1.84	1.74 ± 0.09	11.1-13.0	12.1 ± 0.7	6
2 days after hatching	1.76-1.98	1.89 ± 0.07	1.84-2.01	1.93 ± 0.06	5.1-8.8	6.8 ± 1.3	6
3 days after hatching	1.84-1.94	1.89 ± 0.05	1.94-2.06	2.00 ± 0.05	4.8-6.2	5.5 ± 0.7	4
Stenogobius sp.							
Newly hatched	1.50-1.62	1.56 ± 0.05	1.60-1.74	1.65 ± 0.05	14.9-18.3	16.7 ± 1.3	11
1 day after hatching	1.72-1.98	1.89 ± 0.08	1.82-2.10	1.98 ± 0.08	6.0-8.8	7.5 ± 0.9	10
2 days after hatching	1.90-2.00	1.95 ± 0.03	2.00-2.12	2.06 ± 0.03	3.8-6.9	4.7 ± 0.9	10
3 days after hatching	1.98-2.02	1.99 ± 0.01	2.08-2.12	2.09 ± 0.01	1.9-4.8	3.0 ± 1.0	7

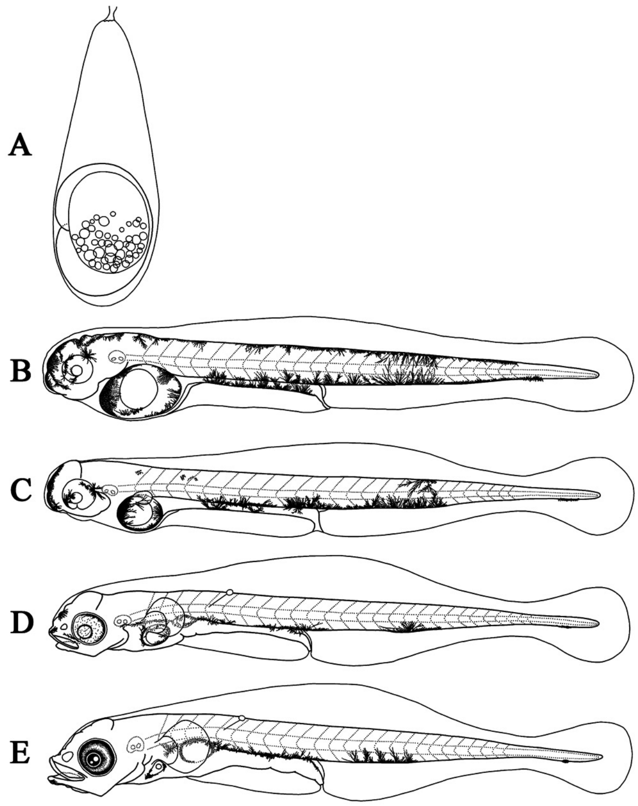

Figure 9. - Egg and larval development of *Stenogobius* sp.. **A**: egg, 0.96 mm in vertical diameter; **B**: newly hatched, 1.66 mm in total length (TL); **C**: 1 day old, 1.94 mm TL; **D**: 2 days old, 2.10 mm TL; **E**: 3 days old, 2.10 mm TL.

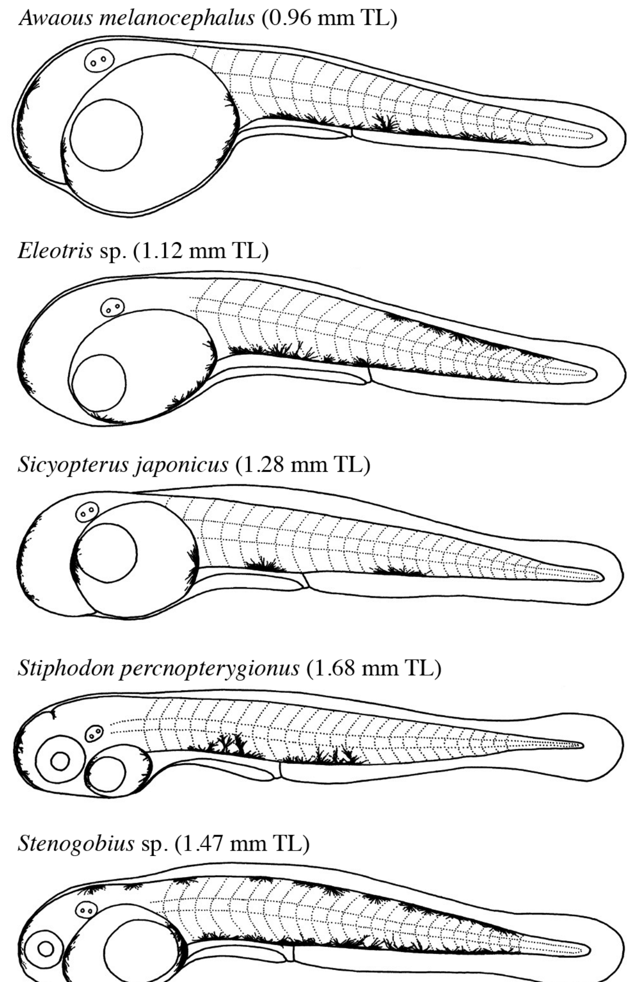

Figure 10. - Small undeveloped drifting larvae collected in the Yona Stream on Okinawa Island. TL: total length.

Morphology of drifting larvae

All fish larvae collected with the plankton net belonged to the suborder Gobioidei. Based on Maeda and Tachihara (2010), the larvae were sorted depending on whether they were small, undeveloped without pigmented eyes, mouth, or pectoral fins (< 1.8 mm TL), or not (> 2.0 mm TL). The latter group was identified as *Redigobius bikolanus* (mean ± SD, 2.4 ± 0.2 mm TL, n = 73; YSD = 8.6 ± 1.2% TL, n = 53), *Rhinogobius giurinus* (2.6 ± 0.1 mm TL, n = 24; YSD = 6.2 ± 1.2% TL, n = 15), other *Rhinogobius* spp. (4.0 ± 0.1 mm TL, n = 55; YSD = 5.3 ± 1.2% TL, n = 35), and a *Luciogobius* species that had not been described in Maeda and Tachihara (2010) (3.4 ± 0.1 mm TL, n = 18; YSD = 11.1 ± 1.4% TL, n = 12). The *Luciogobius* larvae were identified based on the occurrence of conspicuous melanophores along the dorsal and ventral midlines extending from the head to the tail, and relatively large number of the myomeres; all of which are characteristics of larvae belonging to the genus *Luciogobius* (Okiyama, 1988). Specifically, the larvae had 33 myomeres, with 1, 2, and 1 melanophores along the dorsal midline of the head, trunk, and tail, respectively, and with continuous melanophores on the ceiling of the oral cavity, dorsally on the gut, ventrally on the trunk and on the anterior half of the tail. Melanophores were also present around the tip of the lower jaw, at the angles of the lower jaws, along ventral midline from around the cleithral symphysis to the anus, dorsally on the gas bladder and the yolk, and ventrally around the tip of the notochord.

The small undeveloped larvae were subdivided into the five types based on the arrangement of the melanophores and could be identified as *Awaous melanocephalus*, *Eleotris* spp., *Sicyopterus japonicus*, *Stiphodon percnopterygionus*, and *Stenogobius* sp., based on descriptions on *S. japonicus* and *Stenogobius* sp. larvae in the present study, together with the descriptions in Yamasaki and Tachihara (2007), Yamasaki *et al*. (2007), and Maeda *et al*. (2008) (Key is given in "Discussion"). *Awaous melanocephalus* had melanophores on the tip of snout and on the anterior and posterior margins of the yolk sac, and a continuous series of melanophores extending ventrally from the trunk to the mid-tail (Fig. 10). Arrangement of the melanophores of *Eleotris* spp. was similar to that of

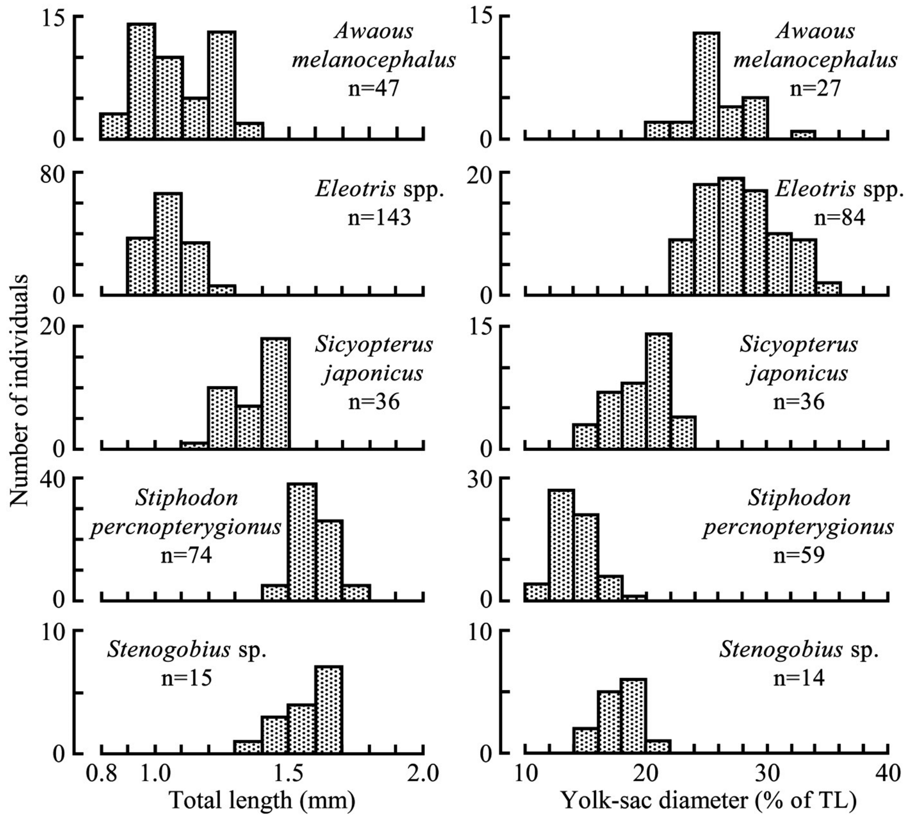

Figure 11. - Total length (left) and yolk-sac diameter (right) frequency distributions of small undeveloped type drifting larvae collected in the Yona Stream on Okinawa Island.

A. melanocephalus, but, in addition to the melanophores that *A. melanocephalus* possessed, *Eleotris* spp. also had melanophores dorsally on the tail (Fig. 10). *Sicyopterus japonicus* and *Stiphodon percnopterygionus* had melanophores on the tip of snout, on the anterior and posterior margins of the yolk sac, and ventrally on the trunk and tail (Fig. 10). The ventral melanophores on the tail were located immediately posterior to the anus in *Stiphodon percnopterygionus* (Fig. 10), whereas there was a gap of 5-6 myomeres between the anus and the ventral melanophores on the tail in *Sicyopterus japonicus* (Fig. 10). In addition to melanophores on the tip of the snout and on the anterior and posterior margins of the yolk sac, *Stenogobius* sp. exhibited the heaviest pigmentation, with a series of melanophores extending along the dorsal midline from the head to tail and the ventral midline from the trunk to tail (Fig. 10).

Awaous melanocephalus and *Eleotris* spp. were the smallest (*A. melanocephalus*, 1.1 ± 0.1 mm TL, n = 47; *Eleotris* spp., 1.1 ± 0.1 mm TL, n = 143) and had a relatively large yolk sac (*A. melanocephalus*, YSD = 26.4 ± 2.4% TL, n = 27; *Eleotris* spp., YSD = 27.8 ± 3.0% TL, n = 84). *Sicyopterus japonicus* was mid-sized with respect to TL (1.4 ± 0.1 mm, n = 36) and YSD (19.6 ± 2.2% TL, n = 36). *Stiphodon percnopterygionus* and *Stenogobius* sp. were larger (*S. percnopterygionus*, 1.6 ± 0.1 mm TL, n = 74; *Stenogobius* sp., 1.6 ± 0.1 mm TL, n = 15) and had a relatively small yolk sac (*S. percnopterygionus*, YSD = 14.1 ± 1.6% TL, n = 59; *Stenogobius* sp., YSD = 17.8 ± 1.8% TL, n = 14) (Fig. 11).

Seasonal occurrence of drifting larvae

The larvae of *Redigobius bikolanus*, *Rhinogobius giurinus*, other *Rhinogobius* spp., and *Luciogobius* sp. were collected from May to September, July to September, February to May, and in October, April and May, respectively (Fig. 12). The small undeveloped larvae occurred from May to December, but the months of occurrence differed slightly depending on the species (Fig. 12). *Stiphodon percnopterygionus* was collected in all months between May and December. The occurrence of *Eleotris* spp. and *Stenogobius* sp. were similar to *S. percnopterygionus*, but *Eleotris* spp. was not collected in December and *Stenogobius* sp. was not collected in November or December. *Awaous melanocephalus* and *Sicyopterus japonicus* occurred for a shorter period, with *A. melanocephalus* collected from August to October and *S. japonicus* collected in June and August.

Gobioids in Yona Stream

Nineteen adult and juvenile gobioid species were observed in the reaches above the site where drifting larvae were collected in 2004 and 2005. The taxonomy and tentative species names within the genus *Rhinogobius* follow

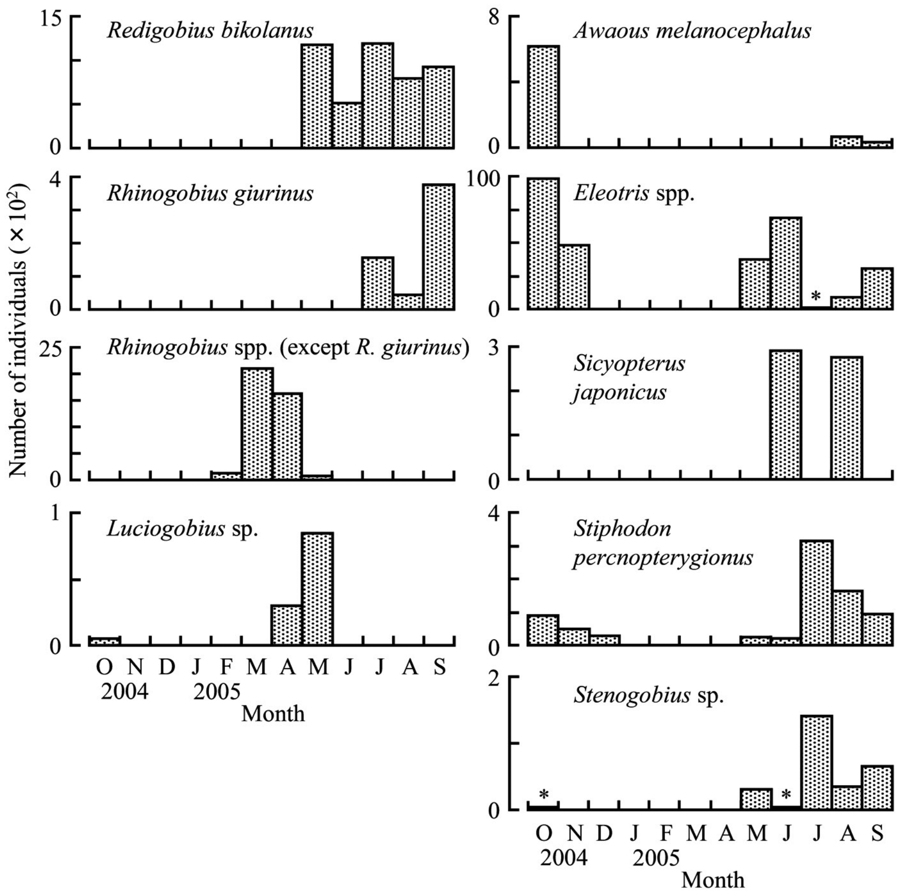

Figure 12. - Monthly changes in the number of drifting larvae collected with a plankton net in the Yona Stream on Okinawa Island from October 2004 to September 2005. Numbers of individuals are represented as averages of two one-hour evening samplings within a given month. Sampling was started when the illumination intensity dropped to 10 lux. Asterisks show a small bar, indicating when a few larvae were collected.

Akihito *et al*. (2002). Thirteen species (*Eleotris fusca*, *Eleotris acanthopoma*, *Sicyopterus japonicus*, *Stiphodon percnopterygionus*, *Awaous melanocephalus*, *Stenogobius* sp., *Redigobius bikolanus*, *Rhinogobius giurinus*, *Rhinogobius* sp. CB, *Rhinogobius* sp. DA, *Rhinogobius* sp. MO, *Rhinogobius* sp. BB, and *Tridentiger kuroiwae*) were abundant (more than ten adult individuals observed on at least one of the surveys), while *Ophieleotris* sp., *Hypseleotris cyprinoides*, *Sicyopterus lagocephalus*, and *Glossogobius celebius* were considered to be scarce (maximum number of adult individuals observed ranged from one to four). Maximum number of *Luciogobius ryukyuensis* adults observed by snorkelling was three; however, this species may not be scarce, because they are usually hidden within gravel. *Lentipes armatus* was only observed as five juveniles.

DISCUSSION

Size at first maturation

Gonad examinations suggested that both males and females of *Sicyopterus japonicus* mature at approximately 40 mm SL. The SL of the smallest adult attending a nest (45 mm; not sexed) supports this result. This size is similar to that of *Sicyopterus lagocephalus* in the Philippines, in which the smallest mature males and females have SL of 41-45 and 36-40 mm, respectively (Manacop, 1953). The smallest male and female *Awaous melanocephalus* specimens with mature gonads measured approximately 40 mm SL, whereas the smallest parent on the nest (probably a male) was approximately 70 mm SL. The disparities in the size may be due to egg masses tended by smaller males (40-70 mm SL) being more difficult to locate, but smaller males may also not be nest-holders as they engage in other reproductive behaviours. Further intensive field surveys are required in order to address this uncertainty. Such disparities in the size at first maturation and the minimum size of parents attending nests have also been observed in *Awaous guamensis* from Hawaiian streams. In *A. guamensis* males and females mature at approximately 73 mm SL and the smallest female observed attending nest measures approximately 110 mm SL (Ha and Kinzie, 1996). Based on the gonad examinations, males and females of *Stenogobius* sp. are both considered to mature at around 35 mm SL. The smallest male tending an egg mass (40 mm SL) was only slightly larger than this size.

Fecundity

Maximal potential batch fecundity of *Sicyopterus japonicus*, which was estimated based on the number of vitellogenic oocytes (94,000; 75.8 mm SL), was less than half the number of eggs found in the largest egg mass observed in the present study (ca. 203,600). Dotu and Mito (1955) reported the number of ovarian eggs in a *S. japonicus* specimen collected in Kyushu (87 mm SL) to be 224,960. As this species grows to more than 100 mm SL (Watanabe *et al.*, 2007), it is possible that the clutches of larger females may contain even more eggs. The potential batch fecundity of this species was thus similar to *Sicyopterus lagocephalus* from the Philippines, in which a female (66 mm SL) was reported to have 46,000 mature ovarian eggs (Manacop, 1953).

The maximal potential batch fecundity of *Awaous melanocephalus* (290,000; 126 mm SL) was similar to the maximum number of eggs (ca. 259,500) estimated for an egg mass found in the streams. Ha and Kinzie (1996) reported that a large *Awaous guamensis* female (217 mm SL) in a Hawaiian stream tended an egg mass containing 689,500 eggs. However, the maximum fecundity of *A. melanocephalus* may not be as large as that of *A. guamensis*, because the former species (< 150 mm SL, Akihito *et al.*, 2002) does not attain the size as the latter species. On the other hand, the potential batch fecundity of the smaller *A. melanocephalus* females (41.5-45.5 mm SL) was estimated as being only 3,000-5,000. It appears that fecundity in the genus *Awaous* varies markedly with female body size.

The number of eggs in *Stenogobius* sp. nests found in the present study (ca. 500-8,100) was considerably less than the potential batch fecundity estimated by the number of vitellogenic oocytes (max. 72,000). Although it is highly likely that we failed to locate larger egg masses in this species, it is also possible that females do not place all of their mature eggs in a single egg mass.

Egg morphology and spawning habits

The eggs of *Sicyopterus japonicus* (present study) and *Awaous melanocephalus* (Yamasaki and Tachihara, 2007) fall within the smallest class of fish eggs, as in all of the other sicydiine gobies reported to date (*Sicyopterus lagocephalus*, *Sicyopterus stimpsoni*, *Sicydium punctatum*, *Stiphodon percnopterygionus*, and *Lentipes concolor*; Manacop, 1953; Maciolek, 1977; Fitzsimons *et al.*, 1993; Kinzie, 1993; Bell and Brown, 1995; Yamasaki and Tachihara, 2006) and some of the eleotrid fish (*Eleotris* and *Hypseleotris*; Auty, 1978; Dotsu *et al.*, 1998, 2004; Maeda *et al.*, 2008). All of these eggs are almost pyriform or nearly spherical, with vertical diameters measured with the micropyle located at the top not exceeding 1.3 times the length of the horizontal diameter, although the egg shapes exhibit slight variations between species. For example, the vertical diameter of *S. japonicus* eggs was somewhat smaller than the horizontal diameter. On the other hand, the eggs of *Stenogobius* sp. are remarkably larger and their shape (elliptical; vertical diameter was more than twice of the horizontal diameter) differs markedly from the eggs of the aforementioned gobioid species. However, the size and morphology of newly hatched *Stenogobius* sp. larvae were similar to those of the aforementioned gobioid species with smaller eggs, i.e., larvae are very small (≤ 1.8 mm TL) and undeveloped, and lack a mouth, pigmented eyes, and pectoral-fin buds (Manacop, 1953; Auty, 1978; Kinzie, 1993; Bell and Brown, 1995; Dotsu *et al.*, 1998, 2004; Lindstrom, 1999; Yamasaki and Tachihara, 2006; Maeda *et al.*, 2008).

Spawning habits among the small-egg spawners, including *Stenogobius* sp., is heterogeneous. When we found the egg mass in the streams of the Ryukyu Archipelago, we were able to identify the species or genus based on the construction of the nest, arrangement of eggs on the substratum, and general morphology of the eggs, even if we failed to observe the parent tending the egg mass. The egg mass of *S. japonicus* is unique in that it is deposited as a cluster, while the egg masses of all other known gobioids are deposited in a single layer on the substratum (Miller, 1984), except for *S. lagocephalus* (Kondo *et al.*, unpubl. data); *Eleotris acanthopoma* and *E. fusca* deposit their eggs somewhat sparsely in intricate patterns separated by groove-like gaps (Maeda *et al.*, 2008); the eggs of *S. percnopterygionus, A. melanocephalus*, and *Stenogobius* sp. are deposited uniformly and densely in a single layer, but the egg mass of *A. melanocephalus* is distinguished by the larger size of the egg mass (> 40 cm^2) and with the nest being slightly exposed; the egg masses of *S. percnopterygionus* and *Stenogobius* sp. are smaller (< 20 cm^2) and the nests are concealed within the streambed, but the species can be identified easily based on egg shape (*S. percnopterygionus* is pyriform while *Stenogobius* sp. is elliptical). The uniquely clustered egg deposits of *S. japonicus* may enable this species to use smaller nests and to hide the nest. Indeed, the density of eggs in an *S. japonicus* egg mass (7,245 eggs/cm^2) is very high, when compared to *A. melanocephalus* (840-1,156 eggs/cm^2) and *Eleotris* spp (75-668 eggs/cm^2; Maeda *et al.*, 2008), both of which produce similarly sized eggs on relatively open nests.

Since the egg masses of the three species (*S. japonicus*, *A. melanocephalus*, and *Stenogobius* sp.) were found in habitats where adults were commonly observed, including the middle and upper reaches), these species were not considered to undertake regular migrations before spawning, as has been suggested in *A. guamensis* from Hawaii. Adults of *A. guamensis* have been reported to aggregate to spawn at the first riffle closest to the stream mouth (Ha and Kinzie, 1996; Kinzie, 1997). While post-spawning mortality has been suggested to be high in *A. guamensis* (Ha and Kinzie, 1996; Kinzie, 1997), we were unable to find any evidence of post-spawning mortality in *A. melanocephalus* on Okinawa

Island. While *A. guamensis* in Hawaiian streams are considered to be polygynous, with males overseeing a large territory containing several nests tended by the females that laid the eggs therein (Ha and Kinzie, 1996; Kinzie, 1997), the mating system employed by the *A. melanocephalus* populations we observed generally matched the pattern of a solitary male tending one egg mass as described by Miller (1984).

Identification of drifting larvae

Morphology of the newly hatched *Sicyopterus japonicus* larvae reared in the laboratory was similar to that of *Stiphodon percnopterygionus* (Yamasaki and Tachihara, 2006), except that the ventral melanophores on the tail of *S. japonicus* was located more posteriorly than it was in *S. percnopterygionus*. Newly hatched *Stenogobius* sp. larvae possessed a unique series of melanophores that extended dorsally from the head to the tail and ventrally on the trunk and tail. Thus, although Maeda and Tachihara (2010) were unable to identify the small undeveloped larvae to species, together with the descriptions in Yamasaki and Tachihara (2007), Yamasaki *et al*. (2007), and Maeda *et al*. (2008), we now have a set of morphological characters with which these larvae can be identified. Using the key below, we could identify the small undeveloped larvae collected in the present study as five taxa (*A. melanocephalus, Eleotris* spp., *S. japonicus*, *S. percnopterygionus*, and *Stenogobius* sp.). Although *Eleotris* larvae most likely comprises *E. fusca* and *E. acanthopoma*, these two species could not be distinguished from each other (Maeda *et al*., 2008). It is unlikely that larvae of *Ophieleotris* sp., *Hypseleotris cyprinoides*, *Lentipes armatus*, and *S. lagocephalus* were included among the drifting larvae collected in the present study. Their numbers are likely to be insignificant because very few adults were found to inhabit the Yona Stream in 2004-2005.

Key to the small undeveloped drifting larval species (sensu Maeda and Tachihara, 2010) commonly found in streams on Okinawa Island.

1. Head and/or body with melanophores dorsally 2
- Dorsum lacking melanophores 3

2. Dorsal melanophores from head and trunk to tail . . . . *Stenogobius* sp.
- Dorsal melanophores only on tail . . . . *Eleotris* spp.

3. Continuous series of melanophores ventrally on trunk and tail; 0.8-1.4 mm TL; YSD 21-36% TL . . . . *Awaous melanocephalus*
- Ventral melanophores on trunk and tail, discontinuous around anus; 1.1-1.8 mm TL; YSD < 26% TL 4

4. Ventral melanophores on tail separated from anus by a gap of 5-6 myomeres; 1.1-1.5 mm TL; YSD 16-26% TL . . . . *Sicyopterus japonicus*
- Ventral melanophores on tail located just posterior to anus; 1.4-1.8 mm TL; YSD 12-22% TL . . . . *Stiphodon percnopterygionus*

Drifting larvae belonging to the genus *Rhinogobius* were sorted into two types, *R. giurinus* and other *Rhinogobius* spp., with the latter larvae probably consisting of the three species, *Rhinogobius* sp. CB, *Rhinogobius* sp. MO, and *Rhinogobius* sp. DA. Although a significant number of *Rhinogobius* sp. BB adults were found in the middle and upper reaches of the Yona Stream, no larvae of this species were found from the drifting larvae samples collected in the lower reaches in the present study. This is because the larvae of this land-locked species stay in the fluvial habitat in the middle and upper reaches during all of the pelagic stages (Hirashima and Tachihara, 2000).

Even though *Luciogobius ryukyuensis* inhabited the reaches above the collection site, all of the drifting *Luciogobius* larvae collected were identified as belonging to a different *Luciogobius* species based on the numbers of the myomeres (38 in *L. ryukyuensis* vs. 33 in *Luciogobius* sp.) and melanophores along the dorsal midline (5-8 vs. 4, morphology of *L. ryukyuensis* larvae is based on Kondo *et al*., unpubl. data). In addition to *L. ryukyuensis*, the larvae of *Tridentiger kuroiwae* were also not among the drifting fish collected, even though this species was abundant above the collection site. The absence of *L. ryukyuensis* and *T. kuroiwae* larvae may be because these species spawn in a very restricted site near the lower margin of their habitat (Kondo *et al*., unpublished data), which in the Yona Stream may be below the collection site.

Spawning season

The mean water temperature in the streams of northern Okinawa Island generally fluctuates between 13-14°C in January and February and 25-27°C in July and August (Nishijima *et al*., 1974). The spawning season of *Sicyopterus japonicus* on Okinawa Island appears to extend from May to August, based on the occurrence of mature ovaries and testes (May to August), egg masses (June to August), and drifting larvae (June and August). This species has been reported to spawn from July to September in the Ota River near the southern tip of Honshu, a temperate island of Japan where the water temperature increases to > 20°C in July and decreases to < 20°C in October (Iida *et al*., 2009). On Okinawa Island, spawning in this species began in May when the mean water temperature rose above 20°C, however, it ceased by September when the mean water temperature was still relatively high (24-27°C; Nishijima *et al*., 1974). Conversely, spawning in *Stiphodon percnopterygionus*, *Eleotris acanthopoma*, and *E. fusca* was maintained long after water temperatures exceeded 20°C (May to December) (Yamasaki and Tachihara, 2006; Maeda *et al*., 2007). Although factors that control the relatively shorter spawning season in *S. japonicus* have not yet been elucidated, this characteristic is considered to be related to the life history of this species, which, uniquely among members of Sicydiinae, has adapted to inhabiting in the freshwater streams of both subtropical and temperate islands (Iida *et al*., 2009).

Although drifting larvae of *Awaous melanocephalus* were only collected from August to October in the Yona Stream, the discovery of egg masses in July and females with mature ovaries from June to November suggests that the spawning season of this species spans from June to November. Gonadal studies suggested that *Stenogobius* sp. is capable of spawning in Okinawan streams throughout the year. However, the absence of drifting larvae of this species in the Yona Stream between November and April suggests that reproduction may be inactive in the colder seasons (especially from January to April). The inactivity of reproduction in the colder season is corroborated by the absence of small undeveloped drifting larvae from February to April (few larvae were collected in January) in the Teima Stream on Okinawa Island (Maeda and Tachihara, 2010).

Conclusion

The reproductive biology of amphidromous gobioids has been well characterized in Hawaiian streams. In studies on *Awaous guamensis* in Hawaiian streams, Ha and Kinzie (1996) and Kinzie (1997) reported that adults can attain a large in size (> 300 mm SL), employ an unusual mating system (e.g. females tend egg masses), that the eggs are small in size, that adults migrated downstream where they aggregate and spawn in restricted spawning grounds, and that mortality after spawning is high. However, in the absence of any information on *Awaous* species from other regions or related genera, such as *Stenogobius*, it was not possible to determine how the reproductive biology of Hawaiian *Awaous* species was distinctive, i.e., whether these characteristics were restricted to gobioids in Hawaiian streams, or to this species or genus, or whether they are shared with related taxa. The information presented here provides a basis to compare the characteristics of *A. guamensis* from Hawaii with *A. melanocephalus* and *Stenogobius* sp. from Okinawa Island. These comparisons reveal that, with the exception of the small egg size, the aforementioned characteristics of Hawaiian *A. guamensis* do not hold for *A. melanocephalus* and *Stenogobius* sp. on Okinawa Island. The small eggs, higher fecundity, undeveloped phase at hatching, extended pelagic larval stage, and spawning during warmer seasons are all considered to be characteristics that are common to the subfamily Sicydiinae and the genera *Awaous*, *Stenogobius*, and *Eleotris*. As suggested by Maeda *et al*. (2008), these characteristics may be essential to inhabit insular streams scattered in the tropical and subtropical oceans. Nonetheless, the minor variations observed between species or genera in the timing and duration of the spawning season, construction of spawning nests, egg laying habits, egg morphology, and the size of newly hatched larvae and their yolk sacs, are considered to reflect differences in the nature of the microhabitats and habits of each species.

Acknowledgments. - We wish to thank the former members of the Laboratory of Fisheries Biology and Coral Reef Studies, University of the Ryukyus, who supported our surveys, especially K. Hirashima, K. Sasaki, N. Ogata, and M. Kawahira. We are also grateful to C. Lord (University of Tokyo) for preparing the title and abstract in French.

REFERENCES

AKIHITO, SAKAMOTO K., IKEDA Y. & SUGIYAMA K., 2002. - Gobioidei. *In*: Fishes of Japan with Pictorial Keys to the Species, English Edition (Nakabo T., ed.), pp. 1139-1310 and 1596-1619. Tokyo: Tokai Univ. Press.

AUTY E.H., 1978. - Reproductive behaviour and early development of the empire fish *Hypseleotris compressus* (Eleotridae). *Aust. J. Mar. Freshw. Res*., 29: 585-597.

BELL K.N.I. & BROWN J.A., 1995. - Active salinity choice and enhanced swimming endurance in 0 to 8-d-old larvae of diadromous gobies, including *Sicydium punctatum* (Pisces), in Dominica, West Indies. *Mar. Biol*., 121: 409-417.

DOTSU Y., SUZUKI T. & YANAGI M., 1998. - Spawning-induction, embryonic development and larvae of the carp-like sleeper *Hypseleotris cyprinoides*, gobiid fish. *Trans. Nagasaki Biol. Soc*., 49: 15-21. [in Japanese]

DOTSU Y., MIYAKI K., MATSUO T., ONO Y. & TAKAHAMA H., 2004. - Induced spawning, larval rearing and development of the sleeper, *Eleotris oxycephala* Temmink et Schlegel, gobioid fish. *Bull. Fac. Fish. Nagasaki Univ*., 85: 1-8. [in Japanese]

DOTU Y. & MITO S., 1955. - Life history of a gobioid fish, *Sicydium japonicum* Tanaka. *Sci. Bull. Fac. Agr. Kyushu Univ*., 15: 213-221. [in Japanese]

FITZSIMONS J.M., NISHIMOTO R.T. & YUEN A.R., 1993. - Courtship and territorial behavior in the native Hawaiian stream goby, *Sicyopterus stimpsoni*. *Ichthyol. Explor. Freshw*., 4: 1-10.

FITZSIMONS J.M., PARHAM J.E. & NISHIMOTO R.T., 2002. - Similarities in behavioral ecology among amphidromous and catadromous fishes on the oceanic islands of Hawai'i and Guam. *Environ. Biol. Fish*., 65: 123-129.

HA P.Y. & KINZIE R.A. III, 1996. - Reproductive biology of *Awaous guamenesis*, an amphidromous Hawaiian goby. *Environ. Biol. Fish*., 45: 383-396.

HIRASHIMA K. & TACHIHARA K., 2000. - Embryonic development and morphological changes in larvae and juveniles of two land-locked gobies, *Rhinogobius* spp. (Gobiidae), on Okinawa Island. *Jpn. J. Ichthyol*., 47: 29-41. [in Japanese]

IIDA M., WATANABE S. & TSUKAMOTO K., 2009. - Life history characteristics of a Sicydiinae goby in Japan, compared with its relatives and other amphidromous fishes. *Am. Fish. Soc. Sym*., 69: 355-373.

KEITH P., VIGNEUX E. & BOSC P., 1999. - Atlas des Poissons et des Crustacés d'eau douce de la Réunion. Partimoines naturels 39. 136 p. Paris: Museum National d'Histoire Naturelle.

KEITH P., MARQUET G., LORD C., KALFATAK D. & VIGNEUX E., 2010. - Atlas des Poissons et des Crustacés d'eau douce du Vanuatu. 253 p. Paris: Société Française d'Ichthyologie.

KEITH P., LORD C., LORION J., WATANABE S. TSUKAMOTO K., COULOUX A. & DETTAI A., 2011. - Phylogeny and biogeography of Sicydiinae (Teleostei: Gobiidae) inferred from mitochondrial and nuclear genes. *Mar. Biol*., 158: 311-326.

KINZIE R.A. III, 1993. - Reproductive biology of an endemic, amphidromous goby *Lentipes concolor* in Hawaiian streams. *Environ. Biol. Fish*., 37: 257-268.

KINZIE R.A. III, 1997. - Evolution and life history patterns in freshwater gobies. *Micronesica*, 30: 27-40.

LINDSTROM D.P., 1999. - Molecular species identification of newly hatched Hawaiian amphidromous gobioid larvae. *Mar. Biotechnol*., 1: 167-174.

MACIOLEK J.A., 1977. - Taxonomic status, biology, and distribution of Hawaiian *Lentipes*, a diadromous goby. *Pac. Sci*., 31: 355-362.

MAEDA K. & TACHIHARA K., 2006. - Fish fauna in the Teima Stream, Okinawa Island. *Biol. Mag. Okinawa*, 44: 7-25. [in Japanese]

MAEDA K. & TACHIHARA K., 2010. - Diel and seasonal occurrence patterns of drifting fish larvae in the Teima Stream, Okinawa Island. *Pac. Sci*., 64: 161-176.

MAEDA K., YAMASAKI N. & TACHIHARA K., 2007. - Size and age at recruitment and spawning season of sleeper, genus *Eleotris* (Teleostei: Eleotridae) on Okinawa Island, southern Japan. *Raffles Bull. Zool. Suppl*., 14: 199-207.

MAEDA K., YAMASAKI N., KONDO M. & TACHIHARA K., 2008. - Reproductive biology and early development of two species of sleeper, *Eleotris acanthopoma* and *Eleotris fusca* (Teleostei: Eleotridae). *Pac. Sci*., 62: 327-340.

MANACOP P.R., 1953. - The life history and habits of the goby, *Sicyopterus extraneus* Herre (Anga) Gobiidae with an account of the goby-fry fishery of Cagayan River, Oriental Misamis. *Philip. J. Fish*., 2: 1-57.

MARQUET G. & GALZIN R., 1992. - Systematic, repartition and biomass of the French Polynesia freshwater fishes. *Cybium*, 16: 245-259.

MILLER P.J., 1984. - Tokology of gobioid fishes. *In*: Fish Reproduction: Strategies and Tactics (Potts G.W. & Wooton R.J., eds), pp. 119-152. London: Academic Press.

NISHIJIMA S., SHOKITA S. & KAWANABE H., 1974. - A general description of the streams of the Ryukyu Islands. *Ecol. Stud. Nat. Cons. Ryukyu Isl*., 1: 113-143. [in Japanese]

OKIYAMA M., 1988. - An Atlas of the Early Stage Fishes in Japan. 1154 p. Tokyo: Tokai Univ. Press. [in Japanese]

THACKER C.E., 2003. - Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). *Mol. Phylgenet. Evol*., 26: 354-368.

VALADE P., LORD C., GRONDIN H., BOSC P., TAILLEBOIS L., IIDA M., TSUKAMOTO K. & KEITH P., 2009. - Early life history and description of larval stages of an amphidromous goby, *Sicyopterus lagocephalus* (Gobioidei: Sicydiinae). *Cybium*, 33: 309-319.

WATANABE S., IIDA M., FUKUI S., TAKINO S. & TSUKAMOTO K., 2007. - Seasonal changes in condition factor of an amphidromous goby *Sicyopterus japonicus* in three rivers in Nanki area, middle Japan. *Nanki-seibutsu*, 49: 125-130. [in Japanese]

WATSON R.E., 1991. - A provisional review of the genus *Stenogobius* with descriptions of a new subgenus and thirteen new species (Pisces: Teleostei: Gobiidae). *Rec. West. Aust. Mus*., 15: 571-654.

WATSON R.E., 1992. - A review of the gobiid fish genus *Awaous* from insular streams of the Pacific Plate. *Ichthyol. Explor. Freshw*., 3: 161-176.

WAY C.M., BURKY A.J., HARDING J.M., HAU S. & PULELOA W.K.L.C., 1998. - Reproductive biology of the endemic goby, *Lentipes concolor*, from Makamaka'ole Stream, Maui and Waikolu Stream, Moloka'i. *Environ. Biol. Fish*., 51: 53-65.

YAMASAKI N. & TACHIHARA K., 2006. - Reproductive biology and morphology of eggs and larvae of *Stiphodon percnopterygionus* (Gobiidae: Sicydiinae). *Ichthyol. Res*., 53: 13-18.

YAMASAKI N. & TACHIHARA K., 2007. - Eggs and larvae of *Awaous melanocephalus* (Teleostei: Gobiidae). *Ichthyol. Res*., 54: 89-91.

YAMASAKI N., MAEDA K. & TACHIHARA K., 2007. - Pelagic larval duration and morphology at recruitment of *Stiphodon percnopterygionus* (Gobiidae: Sicydiinae). *Raffles Bull. Zool. Suppl*., 14: 209-214.