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Sexual maturation of *Girella punctata* and *G. leonina* (Perciformes: Girellidae) in the neritic sea off the Pacific coast of Japan

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Abstract—The spawning ecology of the girellid fishes in Japanese waters has been reported almost only for *Girella punctata*, whereas little is known about *Girella leonina*. We examined sexual maturation of *G. punctata* and *G. leonina* collected from the coastal waters (neritic) of the Izu Peninsula and Tanegashima Island, based on gonadosomatic index (GSI) and gametogenesis, in order to clarify interspecific differences in spawning ecology. The GSI values of *G. punctata* in the Tanegashima Island region were markedly high in March, a month prior to the peak in April in the Izu Peninsula region. *Girella punctata* with elevated GSI had histologically mature ovaries and testes in spring in the both regions, suggesting spawning at this time of year. In contrast, the GSI values of *G. leonina* were much lower in the both regions, but the maximum GSI of both male and female *G. leonina* were slightly higher in the Tanegashima Island region than in the Izu Peninsula region. Furthermore, oogenesis in *G. leonina* was more advanced in the Tanegashima Island region. It is thus probable that Tanegashima Island is relatively near the spawning ground of *G. leonina*.

Key words: *Girella*, spawning, reproduction, Izu Peninsula, Tanegashima Island, Kuroshio, GSI, oogenesis, spermatogenesis

Introduction

Largescale blackfish *Girella punctata* and smallscale blackfish *G. leonina* (Perciformes, Girellidae) are commercially important fishes inhabiting the Japanese coastal rocky-bottom zone. It is generally supposed that both species are distributed from the coastal waters of the Boso Peninsula, central Honshu, Japan, to the coastal waters of the southern China in the northwestern Pacific Ocean (Nakabo 2000). On the other hand, the northern extremity of their distributions in the Sea of Japan is thought to slightly differ: the coastal waters of the Niigata Prefecture for *G. punctata* and the Tsushima Strait for *G. leonina* (Yagishita and Nakabo 2000).

It is probable that *G. punctata* and *G. leonina* also differ in the spawning season, but much more is known about the former species and very little of the latter. The spawning season of *G. punctata* is consistently regarded as February to June (Araga 1997, Konishi 2014a), whereas there are different opinions about the spawning season of *G. leonina*, November to December (Araga 1997) and October to February (Konishi 2014b). Both species spawn pelagic eggs and spend a planktonic life at the early life stage (Konishi 2014a, b), and juveniles of *G. punctata* are known to settle to the coastal rocky bottom after a one-month planktonic stage in the water column (Suzuki 2011). To properly manage these important fishery resources, more knowledge of their spawning ecology is needed and differences between the two species must be noted.

Past studies indicate that the gonadosomatic index (GSI) of *G. punctata* peaks in April in the coastal waters of the southern Kii Peninsula (Kushimoto) and the southern Izu Peninsula (Shimoda) and in May in the coastal waters of the northwestern part of the Kyushu District (Sasebo) (Mizue and Mikami 1960, Maeda et al. 2002, Nakai et al. 2015). Histological observation of the gonads by Mizue and Mikami (1960) found mature oocytes in *G. punctata* collected in May. Thus, the spawning ground of *G. punctata* is likely to form during spring in the broad region from the Izu Islands to the Kyushu District.

In contrast, mature *G. leonina* adults have not been reported anywhere around the Japanese Archipelago. In the Izu

Peninsula, Nakai et al. (2015) found the GSI of *G. punctata* was highly elevated (>15.0) in April, whereas the GSI of *G. leonina* were consistently low (<0.5). Further, a maturity analysis for *G. leonina* collected in the Kii Peninsula region by Maeda (2011) found that all *G. leonina* had immature gonads. Thus, the spawning ground of *G. leonina* is unlikely to form from the Izu Peninsula to the Kii Peninsula.

Nakai et al. (2015) reported that in the Izu Peninsula region juvenile *G. leonina* appeared from January to June, in contrast to a briefer period in *G. punctata*, mostly from May to July. If *G. leonina* has a one-month planktonic larval stage as well as *G. punctata* (Suzuki 2011), we infer that juveniles of *G. leonina* collected in the Izu Peninsula region were spawned during December to May. Since the Izu Peninsula is located along the lower reaches of the Kuroshio Current, we anticipate that *G. leonina* spawns in the upper reaches of the Kuroshio Current in winter and spring.

In this study, we examined the maturation process of *G. punctata* and *G. leonina* collected from waters adjacent to the Izu Peninsula and Tanegashima Island. Our goal was to clarify the interspecific differences in spawning ecology of these species in the neritic sea off the Pacific coast of Japan. While Nakai et al. (2015) showed seasonal variations in GSI of these species collected from the Izu Peninsula region, no histological observation of the gonads was completed. Here, we show gametogenesis of these species in the Izu Peninsula region for the same specimens as examined in Nakai et al. (2015).

Furthermore, we also show the seasonal variation in GSI and gametogenesis for these species collected from Tanegashima Island. This island is close to the Tokara Strait, which is a narrow path of the Kuroshio Current from the East China Sea to the Pacific Ocean. If *G. punctata* and *G. leonina* spawn in this region, their pelagic eggs, planktonic larvae and juveniles should be transported to the entire Pacific coast of Japan, thus influencing reproduction of girellid fishes off the Pacific coast. We therefore specifically examined the maturation process of these species in the Tanegashima Island region, with a view towards a better understanding of spawning ecology off the entire Pacific coast.

Materials and Methods

Fish sampling

A total of 185 girellid fish in the Izu Peninsula region were collected from a market of the Shimoda City Fisheries Cooperative every month from November 2011 to July 2012 (Table 1, Fig. 1), during the assumed spawning seasons of *G. punctata* (February to June) and *G. leonina* (November to December) (Araga 1997). The fish were captured by coastal

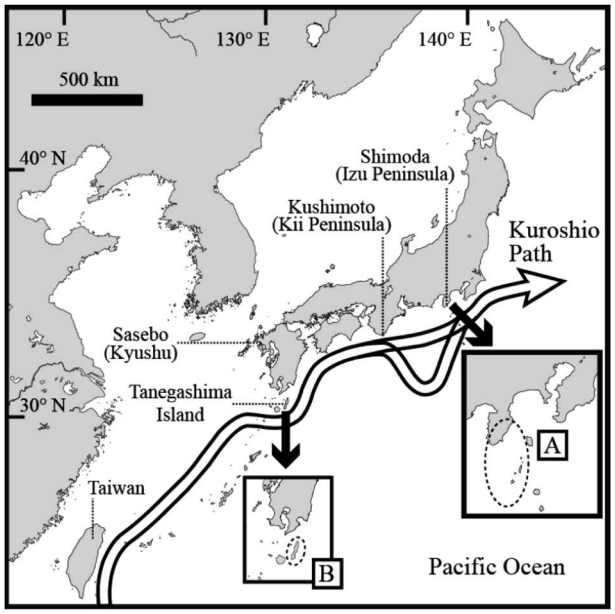

Fig. 1. Study areas (dotted circles). (A) The Izu Peninsula. (B) Tanegashima Island.

Table 1. The total number (N) of *G. punctata* and *G. leonina* collected from the Izu Peninsula during November 2011 to July 2012, and the number (n) and range of standard length (SL, mm) of each species.

Month		*G. punctata*							*G. leonina*						
		N	Females		Males		Unidentified		N	Females		Males		Unidentified	
			n	SL	n	SL	n	SL		n	SL	n	SL	n	SL
2011	Nov.	20	6	245.6–280.4	14	232.0–276.4	0	—	0	0	—	0	—	0	—
	Dec.	22	11	226.7–285.7	11	223.5–313.0	0	—	0	0	—	0	—	0	—
2012	Jan.	23	6	264.4–341.2	17	255.3–326.1	0	—	0	0	—	0	—	0	—
	Feb.	15	4	220.5–276.0	11	224.0–333.6	0	—	5	1	278.3	4	266.8–280.7	0	—
	Mar.	20	8	250.4–333.0	12	276.0–377.4	0	—	0	0	—	0	—	0	—
	Apr.	17	5	249.6–345.0	12	258.0–317.3	0	—	3	2	255.4–311.0	1	334.8	0	—
	May	9	4	279.8–359.3	5	258.4–343.9	0	—	11	8	250.0–330.6	2	298.0–322.2	1	276.4
	Jun.	17	6	234.9–328.8	11	256.4–353.9	0	—	3	0	—	2	293.8–299.0	1	326.5
	Jul.	18	6	234.5–299.5	11	245.1–321.2	1	291.1	2	0	—	1	340.4	1	310.5
The whole term		161	56	220.5–359.3	104	223.5–377.4	1	291.1	24	11	250.0–330.6	10	266.8–340.4	3	276.4–326.5

*We corrected the sexes identified in our previous report (Nakai et al. 2015) for six individuals, based on the results of the histological observation.

fisheries utilizing set nets, gill nets and angling. The Shimoda market does not distinguish between *G. punctata* and *G. leonina*, and lumps the two species together in the marketplace.

A total of 292 girellid fish in the Tanegashima Island region were collected from a market of the Tanegashima Fisheries Cooperative every month from December 2012 to July 2014 (Tables 2, 3). Collections were missing from April 2013 only. The Tanegashima market distinguishes between *G. punctata* (called "Jiguro") and *G. leonina* (called "Onaga"), and the total catch of *G. punctata* is generally smaller than that of *G. leonina* in the market (Tanegashima Fisheries Cooperative, personal communication). We could not collect sufficient specimens of *G. punctata* during 2012–2014. Therefore, additional 40 specimens of *G. punctata* were collected from January to April 2015. The fish were captured by gill nets and angling.

Species identification

Generally, morphological discrimination of *G. punctata* and *G. leonina* is mainly based on the shape of the caudal fin, number of scales, and pigmentation patterns (Araga 1997, Yagishita and Nakabo 2000), but these morphological criteria are unreliable in identifying juveniles (Fujita et al. 2000). Therefore, Itoi et al. (2007) developed a simple and highly sensitive method for separating these two species, using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA). In our previous study, we utilized this PCR-RFLP method on both juveniles and adults of the girellid fish collected from the Izu Peninsula, and clarified the species composition of *G. punctata* and *G. leonina* using these genetic criteria (Nakai et al. 2015). Thus, all the fish collected from the Izu Peninsula in the present study were genetically identified in the previous study.

We identified the species collected from Tanegashima Island on the basis of morphological criteria only, since we did not deal with juveniles in the present study. Our main criteria were the number of scales between the center of dorsal fin base and the lateral line, the number of pored lateral-line scales, and the color of the trailing edge of the opercle (Yagishita and Nakabo 2000).

Analysis of maturation stage

The specimens from the Izu Peninsula region were ana-

Table 2. The total number (N) of *G. punctata* collected from Tanegashima Island during December 2012 to April 2015, and the number (n) and range of standard length (SL, mm) of females and males.

Month		N	Females		Males		Unidentified	
			n	SL	n	SL	n	SL
2012	Dec.	3	1	328.8	2	324.2–324.8	0	—
2013	Jan.	5	1	289.9	4	250.9–269.0	0	—
	Feb.	1	1	267.0	0	—	0	—
	Mar.	4	1	299.2	3	243.7–318.4	0	—
	Apr.	0	0	—	0	—	0	—
	May	1	0	—	1	262.8	0	—
	Jun.	0	0	—	0	—	0	—
	Jul.	0	0	—	0	—	0	—
	Aug.	0	0	—	0	—	0	—
	Sep.	8	4	238.0–263.5	4	258.5–280.6	0	—
	Oct.	0	0	—	0	—	0	—
	Nov.	2	1	252.2	1	271.5	0	—
	Dec.	0	0	—	0	—	0	—
2014	Jan.	0	0	—	0	—	0	—
	Feb.	5	2	274.8–292.2	3	243.3–302.1	0	—
	Mar.	0	0	—	0	—	0	—
	Apr.	4	2	255.9–262.9	2	279.2–322.7	0	—
	May	6	4	243.4–268.8	1	297.6	1	259.2
	Jun.	2	0	—	2	298.0–328.3	0	—
	Jul.	0	0	—	0	—	0	—
2015*	Jan.	11	5	240.0–286.9	6	235.6–302.7	0	—
	Feb.	5	0	—	5	284.0–307.2	0	—
	Mar.	15	8	243.3–298.0	7	253.4–291.0	0	—
	Apr.	9	2	288.5–321.4	7	248.3–315.0	0	—
The whole term		81	32	238.0–347.2	48	235.6–334.0	1	259.2

* Additional 40 specimens were collected from January to April in 2015 as a supplementary sampling.

Table 3. The total number (N) of *G. leonina* collected from Tanegashima Island during December 2012 to July 2014, and the number (n) and range of standard length (SL, mm) of females and males.

Month		N	Females		Males		Unidentified	
			n	SL	n	SL	n	SL
2012	Dec.	4	2	334.4–366.2	2	328.3–336.3	0	—
2013	Jan.	17	6	252.4–311.2	11	249.8–297.5	0	—
	Feb.	16	5	255.0–277.2	11	238.0–272.7	0	—
	Mar.	14	9	243.2–283.7	5	233.0–267.2	0	—
	Apr.	0	0	—	0	—	0	—
	May	14	8	250.8–283.8	5	238.1–277.5	1	263.8
	Jun.	7	3	274.4–297.6	4	249.0–273.8	0	—
	Jul.	15	7	242.5–297.6	5	248.6–290.9	3	247.3–298.7
	Aug.	7	4	235.0–259.2	2	256.4–280.0	1	263.9
	Sep.	0	0	—	0	—	0	—
	Oct.	14	7	240.0–325.7	7	250.9–286.6	0	—
	Nov.	12	5	264.9–284.4	6	264.8–287.7	1	277.7
	Dec.	14	3	261.1–275.0	11	248.6–297.7	0	—
2014	Jan.	11	5	275.2–299.0	6	267.4–287.7	0	—
	Feb.	9	3	255.2–273.8	6	243.2–286.9	0	—
	Mar.	13	5	260.0–298.3	8	257.0–308.2	0	—
	Apr.	11	5	249.0–319.0	6	242.2–310.0	0	—
	May	15	7	243.2–295.2	8	246.5–274.7	0	—
	Jun.	12	7	235.4–268.9	3	230.1–259.6	2	241.6–250.8
	Jul.	6	4	269.6–319.7	2	295.8–326.3	0	—
The whole term		211	95	235.0–366.2	108	230.1–336.3	8	241.6–298.7

lyzed for gametogenesis, by using the same specimens as analyzed for GSI by Nakai et al. (2015). The specimens from the Tanegashima Island region were analyzed for both GSI and gametogenesis in the present study.

All fish collected from the market were measured for standard length (SL, mm) and body wet weight (BW, g). Gonads were extracted from the specimens and weighed to the nearest 0.1 g (GW, g wet weight), and fixed in 10% formalin. According to Maeda et al. (2002), the GSI was calculated for both sexes by the following formula: $GSI = (GW/BW) \cdot 10^2$.

Gonadal tissue was prepared using two methods: the paraffin sectioning method and the frozen section method. We used the paraffin sectioning method for all testes and the frozen section method for all ovaries. In the paraffin sectioning method, the fixed tissues were embedded in paraffin (mp 56 to 58°C), serially sectioned to 5 μm thickness, and stained with hematoxylin and eosin for microscopic observation. In the frozen section method, the fixed tissues were frozen at −80°C in hexane and dry ice, and serially sectioned to 4 μm thickness. The slices were placed on adhesive films (Kawamoto 2003) and stained with hematoxylin and eosin. We observed several parts of each gonad under light microscopy to determine state of maturation.

Statistical analysis

The GSI of the two species were compared using the Mann-Whitney *U* test. The statistical analysis was performed using Prism 6.0 h (GraphPad Software).

Results

GSI

Our previous study showed that the GSI of fish collected from the Izu Peninsula ranged from 0.13 to 15.45 in female *G. punctata* and 0.01 to 16.54 in males, and from 0.01 to 0.24 in female *G. leonina* and 0.01 to 0.41 in males (Nakai et al. 2015). Histological observation in the present study allowed correction of sexes identified in our previous report (Nakai et al. 2015), for six individuals that had small gonads (0.1–3.7 g) and were therefore attributed to the wrong sex in that report. However, the GSI ranges for both species are the same as previously reported, except for male *G. punctata* (0.04–16.54, this study). The GSI of *G. punctata* were significantly higher than in *G. leonina* in both females ($U = 8.0$, $P < 0.0001$) and males ($U = 149.0$, $P < 0.0001$).

The GSI of *G. punctata* collected from Tanegashima Island ranged from 0.35 to 11.31 in females and 0.13 to 14.35 in males (Fig. 2). The maximum values were found in March 2015 for both females and males. These maximum values are both lower than the maximum values (15.45–16.54) of *G. punctata* from the Izu Peninsula. The GSI of *G. leonina* collected from Tanegashima Island ranged from 0.02 to 4.02 in females and 0.01 to 4.85 in males (Fig. 3). Relatively high

GSI of 3.87–4.85 were found from fish collected in January and February. The maximum values of females (4.02) and males (4.85) in Tanegashima Island are both higher than the maximum values (0.24–0.41) of *G. leonina* from the Izu Peninsula. Additionally, in Tanegashima Island, the GSI of *G. punctata* were significantly higher than the values of *G. leonina* in both females ($U = 262.5$, $P < 0.0001$) and males ($U = 746.0$, $P < 0.0001$).

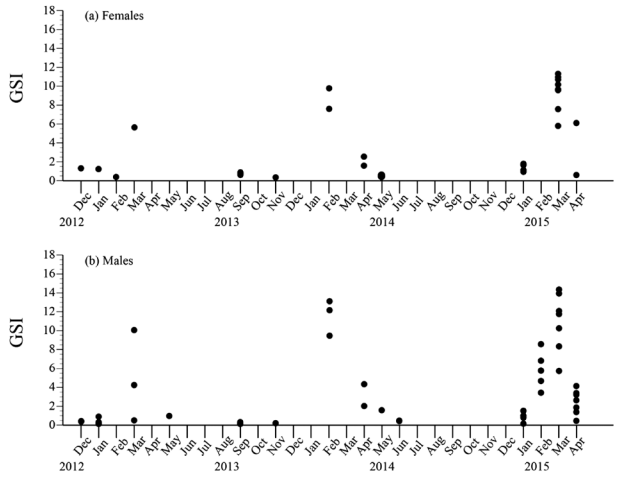

Fig. 2. Monthly changes in GSI of *G. punctata* collected from Tanegashima Island. (a) Females. (b) Males.

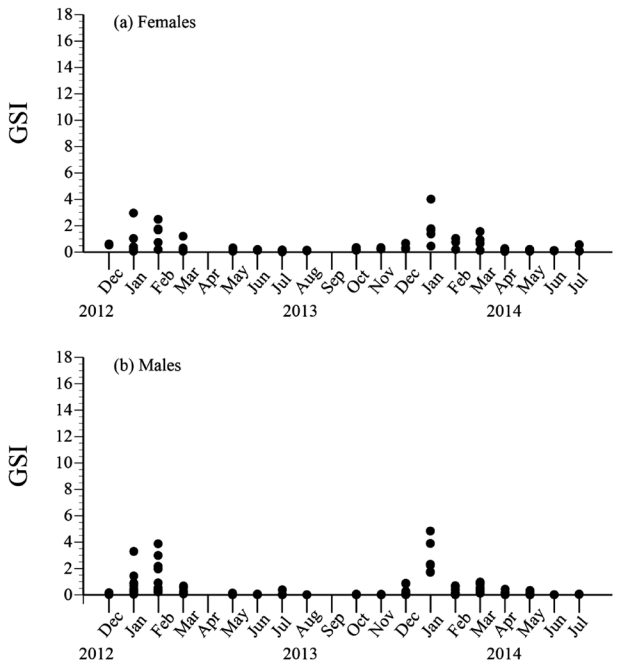

Fig. 3. Monthly changes in GSI of *G. leonina* collected from Tanegashima Island. (a) Females. (b) Males.

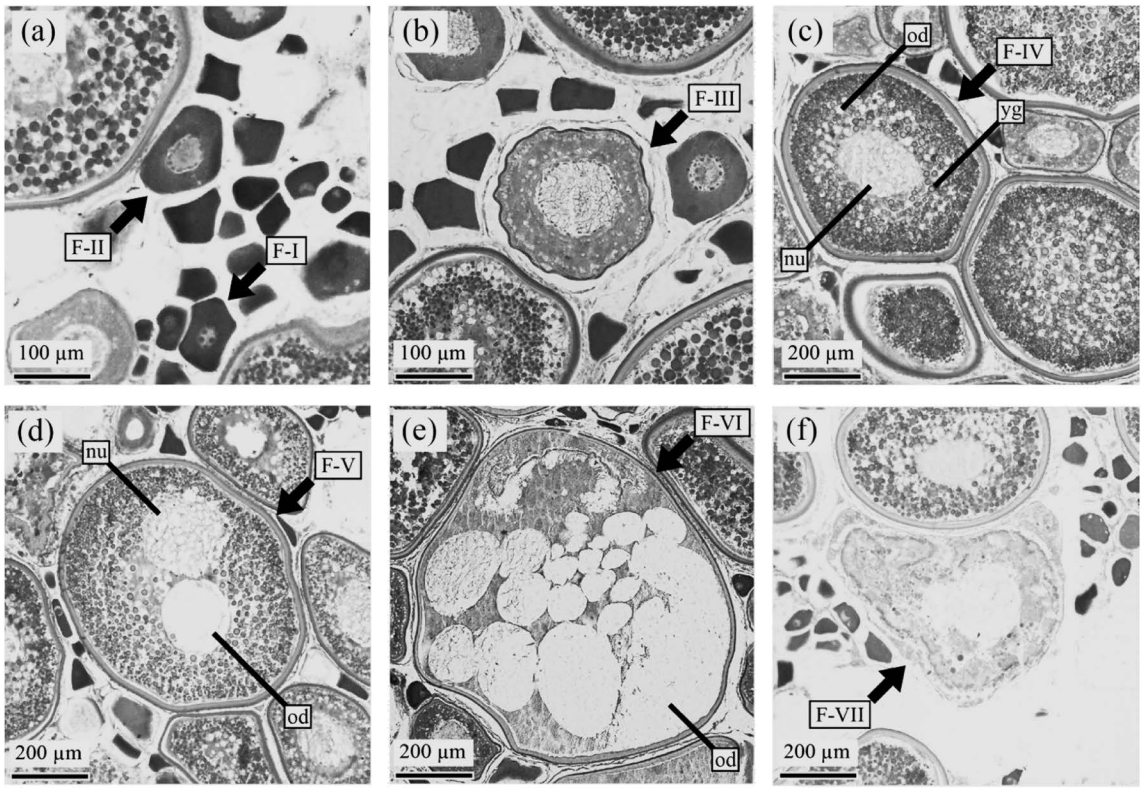

Fig. 4. Oogenesis maturation in female girellid fish. (a) Chromatin-nucleolus stage (F-I) and perinucleolus stage (F-II) (*G. punctata*, March). (b) Yolk vesicle stage (F-III, *G. punctata,* May). (c) Vitellogenic stage (F-IV, *G. punctata*, April). (d) Migratory nucleus stage (F-V, *G. punctata*, April). (e) Maturation stage (F-VI, *G. punctata*, April). (f) Regression stage (F-VII, *G. punctata*, June). Symbols indicate nucleus (nu), oil droplet (od), and yolk globule (yg).

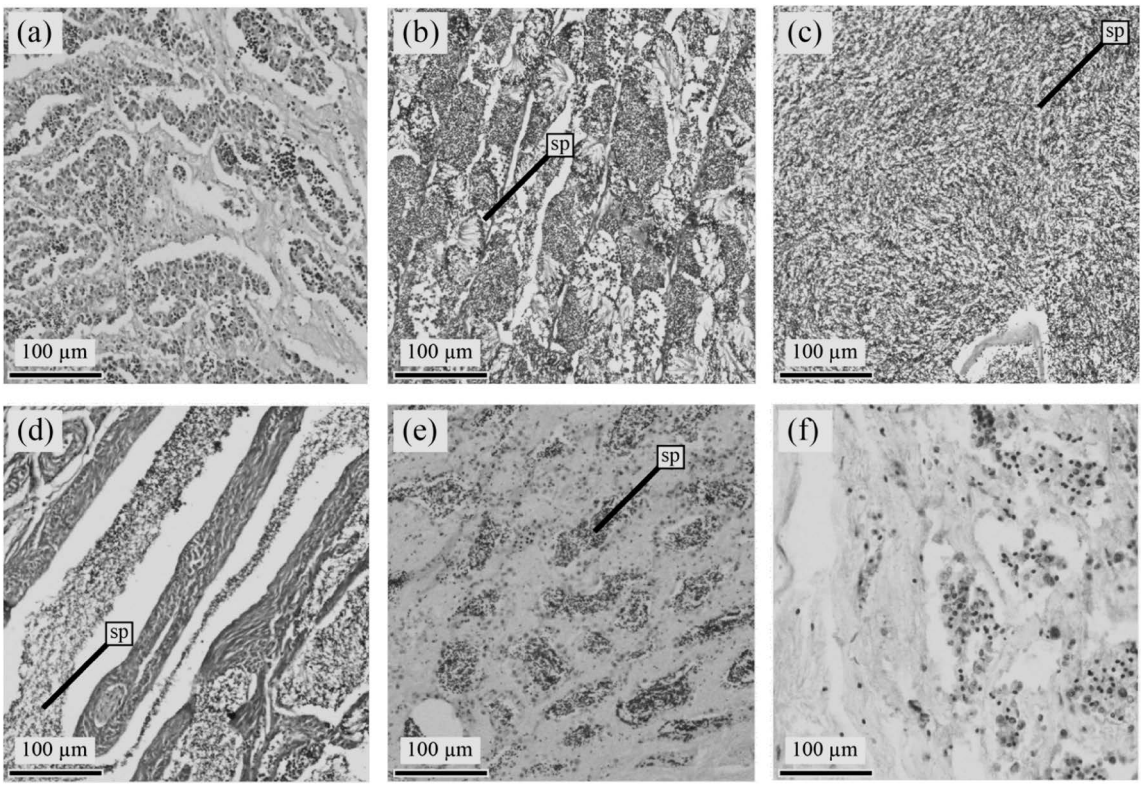

Fig. 5. Spermatogenesis maturation in male girellid fish. (a) Growth stage (M-I, *G. punctata*, January). (b) Growth–maturation stage (M-II, *G. punctata*, April). (c) Functional maturation stage (M-III, *G. punctata*, March). (d) Maturation–post-spawn stage (M-IV, *G. punctata*, April). (e) Post-spawn stage (M-V, *G. leonina*, July). (f) Testicular quiescent stage (M-VI, *G. leonina*, July). Sperm is indicated by the symbol (sp).

The *G. punctata* specimens collected from Tanegashima Island had maximum GSI at body lengths of 256.7 mm SL in females (11.31) and 253.8 mm SL in males (14.35). It is well consistent with the results for *G. punctata* from the Izu Peninsula (Nakai et al. 2015): relatively high GSI (>8.0) were found for females of 249.6–359.3 mm SL and males of 258.0–377.4 mm SL. In *G. leonina*, the relationship between GSI and body size was unclear, since *G. leonina* showed much lower GSI values relative to *G. punctata*.

Gametogenesis

Oocytes were found to vary in size and form in the ovaries of adult *G. punctata* collected from both regions. We classified oogenesis into 7 stages (F-I to F-VII) (Fig. 4), mainly referring to Takano (1989) and Takai et al. (2014): chromatin-nucleolus stage (F-I), perinucleolus stage (F-II), yolk vesicle stage (F-III), vitellogenic stage (F-IV), migratory nucleus stage (F-V), maturation stage (F-VI), and regression stage (F-VII).

At the beginning of oogenesis (Stage F-I), the size of the nucleoli in the nucleus increases slightly (Fig. 4a), and the nucleoli are located at the periphery of the nucleus at Stage F-II (Fig. 4a). In the next phase, yolk vesicles appear in the cytoplasm (F-III, Fig. 4b) and subsequently yolk globules appear in the cytoplasm (F-IV, Fig. 4c). Maturation of the oocyte leads to the migration of the nucleus toward the animal pole at Stage F-V (Fig. 4d), and finally to the disappearance of the nucleus, and the conglomeration of the oil droplets at Stage F-VI (Fig. 4e). At the end of oogenesis (Stage F-VII), several broken ova remain among many undeveloped oocytes (Fig. 4f).

Spermatogenesis was classified into 6 stages (M-I to M-VI), mainly referring to Takahashi (1989), Asahina et al. (1980), and Takai et al. (2014): growth stage (M-I), growth–maturation stage (M-II), functional maturation stage (M-III), maturation–post-spawn stage (M-IV), post-spawn stage (M-V), and testicular quiescent stage (M-VI) (Fig. 5).

During Stage M-I, seminal lobules are filled with spermatocytes by the growth of spermatogonia (Fig. 5a). In Stage M-II, the amount of spermatids and sperm increases (Fig. 5b), and the seminal lobules are filled with sperm at Stage M-III (Fig. 5c). The amount of sperm decreases during Stage M-IV, and empty spaces become conspicuous in the seminal lobules (Fig. 5d). Sperm still remains at Stage M-V, but the empty space increases and the walls bordering the cysts thicken (Fig. 5e). Eventually, the sperm almost completely disappears at Stage M-VI (Fig. 5f).

Maturation in the Izu Peninsula region

We identified the maturation levels of respective females

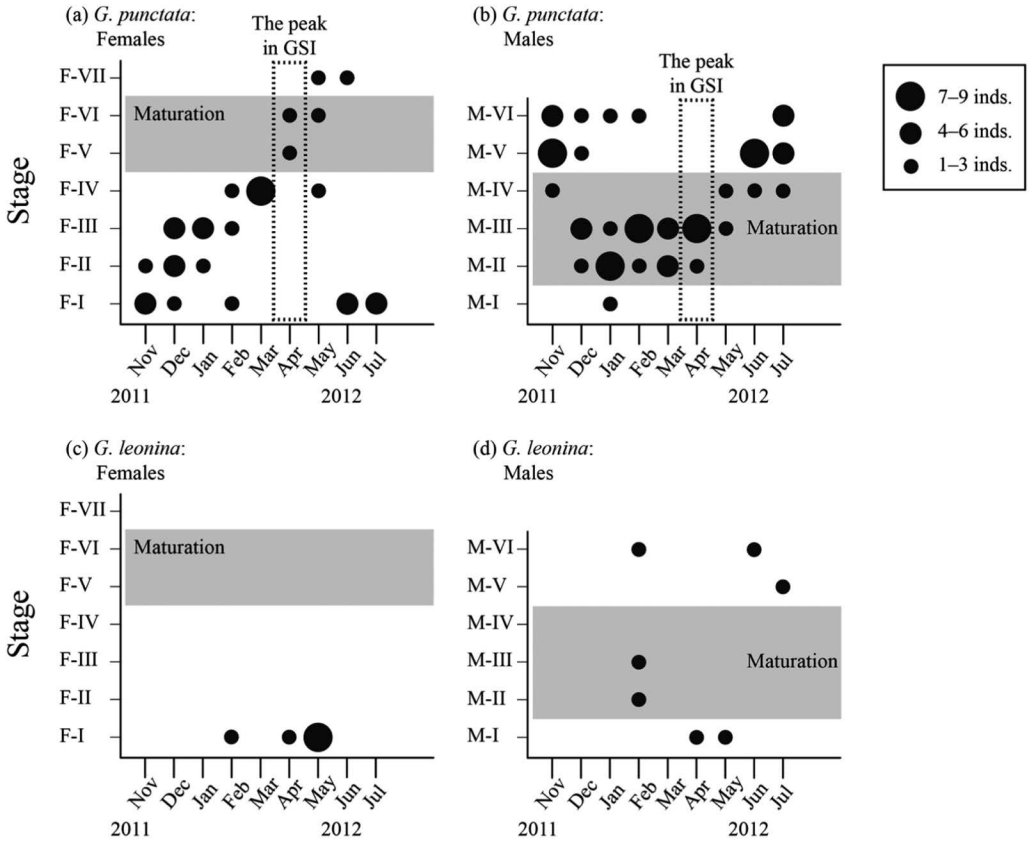

Fig. 6. Monthly changes in gametogenesis of *G. punctata* and *G. leonina* collected from the Izu Peninsula. (a) Oogenesis in *G. punctata*. (b) Spermatogenesis in *G. punctata*. (c) Oogenesis in *G. leonina*. (d) Spermatogenesis in *G. leonina*. The size of the solid circles reflects the number of individuals at each stage. The GSI peaks in *G. punctata* were found in April for the both sexes (dotted rectangles, Nakai et al. 2015).

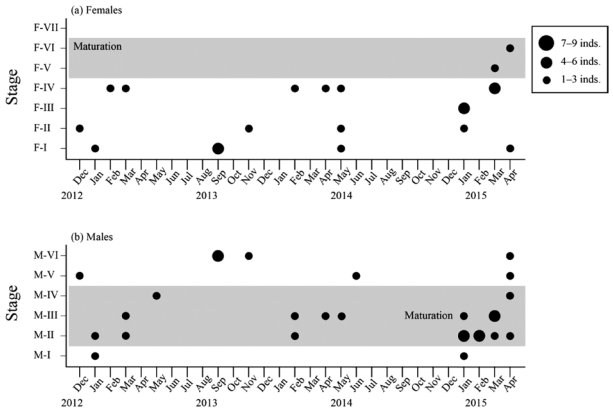

Fig. 7. Monthly changes in gametogenesis of *G. punctata* collected from Tanegashima Island. (a) Oogenesis. (b) Spermatogenesis. The size of the solid circles reflects the number of individuals at each stage.

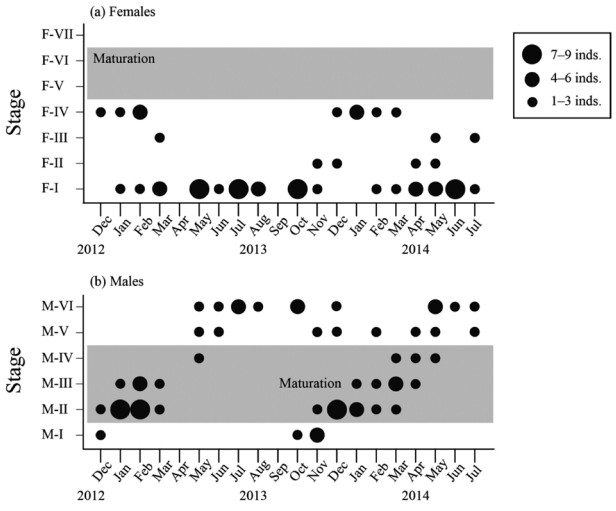

Fig. 8. Monthly changes in gametogenesis of *G. leonina* collected from Tanegashima Island. (a) Oogenesis. (b) Spermatogenesis. The size of the solid circles reflects the number of individuals at each stage.

on the basis of the oocytes at the most advanced stage, except for Stage F-VII. The ovaries of *G. punctata* collected from the Izu Peninsula included mature oocytes at stages F-V and F-VI in 7 of 56 females (Fig. 6a). These mature females were collected in April and May, consistent with the GSI peak in April. All the stages of spermatogenesis were found in males of *G. punctata* collected from the Izu Peninsula (Fig. 6b). A total of 32 males collected from December to May were identified as functionally mature (Stage M-III), the

most mature stage (Takahashi 1989). These mature testes were found not only in high-GSI males but also in low-GSI males (<4.0 in GSI).

Unlike *G. punctata*, all female *G. leonina* collected from the Izu Peninsula were at an early stage of maturity (F-I) (Fig. 6c). This result is consistent with the low GSI (0.01–0.24) observed. As seen in *G. punctata*, mature testes at Stage M-III were also found in two male *G. leonina*, irrespective of low GSI (0.03–0.07) (Fig. 6d).

Maturation in the Tanegashima Island region

We could not find mature oocytes in *G. punctata* collected from Tanegashima Island during 2012 to 2014, but found mature oocytes at Stages F-V and F-VI in four females additionally collected in 2015 (Fig. 7a). Stage F-V was found in three females in March and Stage F-VI was found in a single female collected in April. This result is consistent with the maximum GSI in March (11.31). In *G. punctata*, mature testes at Stage M-III were found from 11 males during January to May (Fig. 7b). These mature testes were found not only in high-GSI males (a maximum of 13.94) but also in low-GSI males (a minimum of 1.51).

Mature oocytes in *G. leonina* were not found in Tanegashima Island, similar to results from the Izu Peninsula (Fig. 8a). However, a total of 20 females during December to March had Stage IV oocytes, a relatively mature level. Mature *G. leonina* testes at Stage M-III were found from 20 males from January to April (Fig. 8b). These mature testes were found not only in high-GSI males (a maximum of 4.85) but also in low-GSI males (a minimum of 0.12).

Discussion

The maturation process in the Izu Peninsula region

Girella punctata ovaries collected from the Izu Peninsula were identified as mature (Stages F-V and F-VI) in 7 females in April and May (Fig. 6a). This result is consistent with the seasonal variation in GSI (a peak value of 15.45 in April) (Nakai et al. 2015), indicating that the females spawn in the region during April to May. Nakai et al. (2015) showed that the frequency of occurrence in *G. punctata* juveniles peaked in June in both tide pools and drifting seaweed, based on genetic identification of the species using the PCR-RFLP analysis of mtDNA. Generally, *G. punctata* juveniles settle near the coast after a one-month planktonic phase in the water column (Suzuki 2011). Therefore the spawning season, April to May, inferred from the gonad analysis is consistent with the observed peak in juvenile occurrence. A large number of juveniles spawned in the Izu Peninsula region are likely to settle in coastal benthic habitat around the spawning ground.

Girella punctata juveniles collected by Nakai et al. (2015) in the Izu Peninsula region consisted mostly of small individuals (<25 mm SL), and *G. punctata* of 25–150 mm SL have also been collected in abundance in the intertidal zone of the rocky shore in the region (Yoshihara et al. 1999, Mano and Itoi 2011). These results indicate that *G. punctata* inhabits this region at all life-stages: juvenile, young, and adult. A large number of *G. punctata* are therefore likely to spend their entire life history in waters adjacent to the Izu Peninsula.

The most mature *G. punctata* males (Stage M-III) occurred during a long period from December 2011 to May 2012 (Fig. 6b). The M-III testes from fish collected during December to February displayed a low GSI of 0.14–1.62, whereas the M-III testes during March to May had a high GSI of 3.49–16.54. It appears that the testes of *G. punctata* increased in size during the M-III stage.

All 11 females of *G. leonina* from the Izu Peninsula had immature ovaries (Stage F-I) (Fig. 6c), consistent with the observed low GSI of 0.01–0.24. The girellid adults collected from the Shimoda market consisted mostly of *G. punctata* (N = 161) and only 24 individuals of *G. leonina* from November 2011 to July 2012 (Table 1). This species composition seen in our specimens may reflect the population density of these species in the Izu Peninsula region during the sampling period. Given the immaturity of ovaries and rarity of adults, we infer that adults do not utilize this region as a spawning ground.

In the Izu Peninsula, *G. leonina* juveniles at <25 mm SL were collected from January to June (Nakai et al. 2015), and *G. leonina* at 10–40 mm SL were abundant during March to July (Mano and Itoi 2011). However, *G. leonina* was rarely collected during or after August (Mano and Itoi 2011). These results indicate that *G. leonina* utilizes this region as a nursery ground for a relatively short period. Juvenile *G. leonina* may start emigration to other regions in August.

Unlike female *G. leonina*, two *G. leonina* males from the Izu Peninsula had mature testes at Stage M-III, in spite of low GSI of 0.03–0.07, like the *G. punctata* males. This suggests that the testes of *G. leonina* also develop fully in the maturation process but do not increase in size. In any case, male *G. leonina* do not mate in the Izu Peninsula region, since mature females of *G. leonina* are likely absent from the region.

The maturation process in the Tanegashima Island region

Both sexes of *G. punctata* collected from Tanegashima Island had the highest GSI in March 2015 (11.31–14.35) (Fig. 2), and the gonads were mature during March to April in both ovaries (Stages F-V and F-VI) and testes (Stage M-III) (Fig. 7). Therefore, we infer that *G. punctata* spawns in the Tanegashima Island region as well as in the Izu Peninsula region. However, there are geographical differences in

the inferred spawning season. The season in Tanegashima Island (March to April) is a month earlier than the season (April to May) in the Izu Peninsula region and the coastal waters of Sasebo (Mizue and Mikami 1960). The earlier occurrence of spawning in Tanegashima Island may be related to its location further south.

Nakai et al. (2015) showed that *G. punctata* juveniles appeared in the Izu Peninsula region from April to July, with a peak from May to July. We expect that juveniles collected in April in the Izu Peninsula region partly originate from Tanegashima Island, due to rapid transport by the Kuroshio Current, sometimes exceeding 2 m/s (Teramoto 1987). Recent genetic studies have also suggested *G. punctata* larval dispersion by currents, based on genetic homogeneity among various sampling locations (Saito et al. 2008, Umino et al. 2009). Gene flow between the distant regions, such as Tanegashima Island and the Izu Peninsula, are likely to frequently occur off the Pacific coast of Japan.

Maeda (2011) reported that off the Kii Peninsula *G. punctata* females of about ≥280 mm fork length (FL) and males of about ≥250 mm FL possessed high GSI values and proposed that fish of these body sizes exceed three years old on the basis of age determination with scale rings. On the other hand, relatively high GSI (>8.0) in *G. punctata* collected from the Izu Peninsula region were found for females of ≥312.3 mm FL (≥249.6 mm SL) and males of ≥300.8 mm FL (≥258.0 mm SL) (Nakai et al. 2015), larger than the body sizes in the Kii Peninsula region. In *G. punctata* collected from Tanegashima Island, relatively high GSI (>8.0) were found for females of ≥308.6 mm FL (≥256.7 mm SL) and males of ≥310.5 mm FL (≥243.3 mm SL), like *G. punctata* in the Izu Peninsula region. The results in the regions of the Izu Peninsula and Tanegashima Island suggest that *G. punctata* possesses high GSI values at a body size of about ≥310 mm FL (about ≥250 mm SL), slightly larger than the body size inferred by Maeda (2011).

Girella leonina GSI had a reduced peak value (4.02–4.85) in both sexes, relative to *G. punctata* GSI, and histology indicated that all *G. leonina* ovaries were immature (Figs. 3, 8a). Therefore, we infer that *G. leonina* does not spawn in this region. However, here gonad maturity in *G. leonina* was more advanced than that of *G. leonina* in the Izu Peninsula region. The peak values of *G. leonina* in the Tanegashima Island region were much higher than the low GSI (≤0.41) in the Izu Peninsula region. Furthermore, several ovaries of *G. leonina* reached Stage F-IV, contrasting with females from the Izu Peninsula, with Stage F-I ovaries. It is thus likely that Tanegashima Island is closer to the *G. leonina* spawning ground.

As seen in Izu Peninsula males, males of both *G. punctata* and *G. leonina* had mature testes at Stage M-III. This suggests that the testes of both species in Tanegashima Island develop to full maturity, irrespective of testes size. Spermatogenesis of both species is likely characterized by the increase in testes size at maturity. Past studies have reported that there were significant intraspecific variations in the development of testis and sperm density in fish, probably related to sperm competition (Makiguchi et al. 2016). The seasonal size increase of the mature-stage testes in the girellid fish may also be related to the sperm competition within the species.

Spawning grounds and seasons

Markedly high GSI for *G. punctata* have been reported for the coastal waters of southern Japan in several areas, the northwestern Kyushu (Sasebo), the Kii peninsula (Kushimoto), and the Izu Peninsula (Shimoda) (Mizue and Mikami 1960, Maeda et al. 2002, Nakai et al. 2015). The present study showed that *G. punctata* GSI in Tanegashima Island were also notably elevated in February and March, and mature oocytes were found in both Shimoda (April and May) and Tanegashima Island (March and April). This suggests that *G. punctata* spawns during spring in various places around Kyushu and the southern part of Honshu (mainland).

The duration of the spawning season in *G. punctata* is likely to vary with spawning areas. In *G. punctata* collected from the Sasebo Bay, a sharp peak of the GSI was found in May, and the GSI values in April and June were much lower than the peak value in May (Mizue and Mikami 1960). Besides, mature oocytes were found in May only, and therefore Mizue and Mikami (1960) inferred that *G. punctata* spawns in May once a year. By contrast, *G. punctata* collected in the coastal waters of Kushimoto, Shimoda and Tanegashima showed high GSI values during 2–3 months, longer than the duration in the Sasebo Bay (Maeda et al. 2002, Nakai et al. 2015, Fig. 2). Mature oocytes were found in females from Shimoda and Tanegashima during two months (Figs. 6, 7), and the mature oocytes coexisted with stage-varied immature oocytes in the identical ovary (Fig. 4). These results suggest that the reproduction of *G. punctata* may be characterized by multiple spawning during a single spawning season. We infer that the difference in the results between the Sasebo Bay and the other areas would be related to the peculiar topography of the Sasebo Bay, which is a semi-closed area distant away from the Pacific Ocean and the Kuroshio Current. To clarify whether or not *G. punctata* spawns several times within a spawning season, it will be efficacious to examine the temporal change of sex hormones such as estradiol for *G. punctata* hereafter.

In contrast to *G. punctata*, mature *G. leonina* adults with high GSI were not found in either the Izu Peninsula or Tanegashima Island. Maeda (2011) hypothesized that *G. leonina* spawning ground might be located in the coastal waters of the Izu Islands or in the upstream portions of the Kuroshio Current south of Kyushu. Our results indicate that *G. leonina* spawns neither in the northern part of the Izu Islands, adjacent to the Izu Peninsula, nor in the Kuroshio Current around

Tanegashima Island. Accordingly, we should consider the possibility that *G. leonina* spawns in the southern part of the Izu Islands or further upstream in the Kuroshio Current. We predict that *G. leonina* spawns in the neritic zones of Taiwan, the southern-most region of the fish's distribution (Nakabo 2000). If, in fact, *G. leonina* spawns in the Taiwan region, the Kuroshio Current would transport eggs, larvae, and juveniles to coastal waters of the Japanese Archipelago and enable juvenile settlement along the entire coast of Japan. There is, therefore, an urgent need for data on the maturation of *G. leonina* from Taiwan. These data might clarify the entire life-history of this species.

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