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Spawning ecology of *Girella punctata* and *G. leonina* (Perciformes: Girellidae) in the coastal waters of the Izu Peninsula, Japan

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Abstract — The girellid fishes, *Girella punctata* and *G. leonina*, are commercially important fishes in Japan, but interspecific differences in their spawning ecology are unclear. In the present study, seasonal variations in the species composition and gonadosomatic index (GSI) were examined for *G. punctata* and *G. leonina* collected from the coastal waters of the Izu Peninsula, in order to clarify the spawning ecology of these two species. Both adults and juveniles were genetically identified by PCR-RFLP using mtDNA. The GSI of *G. punctata* showed markedly high values (>15.0) in April, whereas the GSI values of *G. leonina* were constantly low (<0.5). This result suggests that the coastal area of the Izu Peninsula is utilized as a spawning ground by *G. punctata* but not by *G. leonina*. Juveniles of *G. punctata* mostly appeared from May to July, whereas those of *G. leonina* appeared during the longer period from January to June. Juveniles of *G. punctata* born in the study area are inferred to settle to the sea bottom around the spawning ground after a one-month planktoninc life phase, whereas *G. leonina* juveniles are likely to be immigrants from another area.

Key words: *Girella*, spawning season, Izu Peninsula, GSI, juvenile, nursery, mtDNA, PCR-RFLP

Introduction

Largescale blackfish *Girella punctata* and smallscale blackfish *G. leonina* (Perciformes, Girellidae) are commercially important fishes inhabiting the coastal waters of the Japanese archipelago. 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). By contrast, the northern extremity of their distributions in the Sea of Japan is thought to differ, being the coastal waters of the Niigata Prefecture for *G. punctata* and the Tsushima Strait for *G. leonina* (Yagishita and Nakabo 2000).

Spawning ecology is also considered to differ between the species, with the spawning season being from February to June for *G. punctata* and from November to December for *G. leonina* (Araga 1997). However, little is known about any interspecific differences in their spawning grounds. A maturity analysis of gonad for girellid fish collected off the Kii Peninsula (central Honshu) by Maeda et al. (2002) showed that GSI peaked in April with a maximum value of>12.0 for *G. punctata*, and inferred that their main spawning season would be April in the area. By contrast, Maeda (2011) reported that all *G. leonina* individuals collected in the same area exhibited undeveloped gonads, suggesting that it does not spawn around the Kii Peninsula. Maeda (2011) hypothesized that the spawning ground for *G. leonina* might be located in the Izu-Islands region or in the upstream of the Kuroshio Current to the south of Kyusyu.

In the Izu-Islands region, no precise maturity analysis has yet been performed for the girellid fish species, but the frequency of appearance of girellid fish was examined for juveniles and young of 10–80 mm standard length (SL) by Mano and Itoi (2011). In this research, both *G. punctata* and *G. leonina* appeared in abundance in the coastal waters of Shimoda from March to June (*G. leonina*) and from May to October (*G. punctata*). Mano and Itoi (2011) inferred that the main spawning season of *G. leonina* might be approximately two months prior to that of *G. punctata* based on these seasonal changes in relative abundance. However, this inference can only be verified by understanding the adult maturation process in the area.

In the present study, seasonal variations in the species composition and the GSI for *G. punctata* and *G. leonina* collected from the coastal waters of the Izu Peninsula were examined to clarify their spawning ecology. Species composition was examined for size-classed individuals from juveniles to adults based on genetic criteria. Morphological discrimination of these species is mainly based on the shape of the caudal fin, the number of scales and pigmentation pat-

terns on the body surface (Yagishita and Nakabo 2000). These morphological criteria are readily applicable to adult fish, but are extremely difficult to use for identifying juveniles (Fujita et al. 2000). Itoi et al. (2007) developed a simple and highly sensitive method for the identification of these species based on polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA). We utilized this method to identify girellid fish species and furthermore analyzed the GSI of the both species collected during the formerly assumed spawning season. We present speculation on the relationship between adult maturity and the juvenile nursery.

Materials and Methods

Fish sampling

We collected 185 girellid fish from a market of the Shimoda City Fisheries Cooperative every month from November 2011 to July 2012, during the assumed spawning seasons of *G. punctata* (February to June) and *G. leonina* (November to December). The fish were captured in the coastal waters of the southeastern Izu Peninsula (the coastal waters of the northwestern Izu-Islands) by coastal fisheries of set nets, gill nets and angling (Fig. 1). The market does not distinguish between *G. punctata* and *G. leonina*, and lumps the two species together.

Juveniles of the girellid fish were collected every month from tide pools during February 2011 to October 2012 and from drifting seaweed during April 2011 to November 2011. Juveniles settling on the littoral floor were collected from pools at low tide on the rocky shore of the Tanoura Bay, Suzaki Peninsula, the southeastern part of the Izu Peninsula, using hand nets. We searched for drifting seaweed for about 1–2 hours around the Suzaki Peninsula on the research vessel Suzaki II (9 t) and collected seaweed and associated gathered juveniles with hand nets and a larva net (1.3 m mouth diameter, 0.3 mm mesh for the aperture). When we found several seaweed patches, we collected the patches as many as possible.

All the fish collected from the market were measured for standard length (SL, mm) and body wet weight (BW, g). Muscle was excised from the lateral side of the body for species identification using DNA analysis. All the juveniles collected from tide pools and drifting seaweed were measured for SL. Although all individuals were subjected to DNA analysis, approximately 30 individuals of tide pools and 80 individuals of drifting seaweed were randomly chosen every month when samples were abundant. For the purposes of this study, small individuals of<25 mm SL were regarded as juveniles that had recently settled on the sea floor.

DNA extraction, PCR amplification, and PCR-RFLP analysis

Total genomic DNA was extracted from skeletal muscle using the method of Sezaki et al. (1999). DNA fragments corresponding to the control region and the 16S ribosomal RNA (rRNA) gene in mitochondrial DNA (mtDNA) were

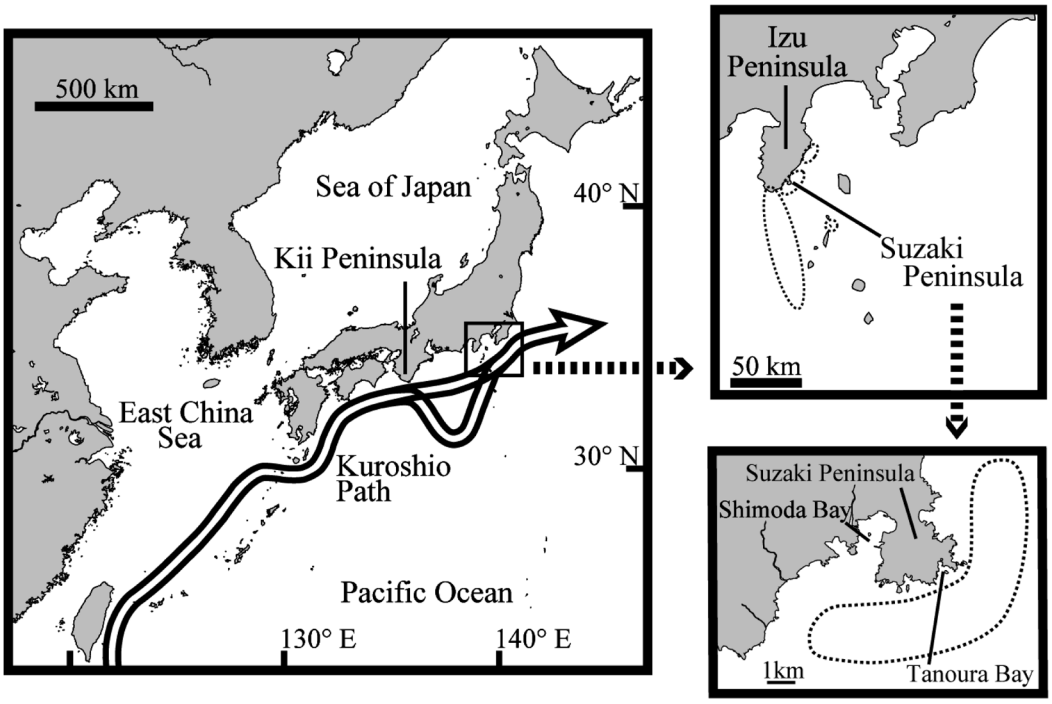

Fig. 1. The study area. Size-varied individuals of girellid fish were captured in the coastal waters of the southeastern Izu Peninsula (dotted circle) by coastal fisheries. Juveniles were collected from tide pools and drifting seaweed (dotted circle) around the Suzaki Peninsula.

amplified by PCR. Primers 16SAR-L (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SBR-H (5′-CCGGTCTGAACTCAGATCACGT-3′) from Palumbi et al. (1991) were used to amplify the partial 16S rRNA gene fragment. Primers fDloop_F (5′-TTCCTGGCATTTGGTTCCTACTTCAG-3′) and ftRPhe_R (5′-CCATCTTAACATCTTCAGTGTTATGC-3′) from Itoi et al. (2007) were used to amplify the partial control region flanked by part of a transfer RNA (tRNA) gene. PCR amplification was performed using a reaction mixture containing genomic DNA as a template, 1 unit of GoTaq Flexi DNA polymerase (Promega, USA), 4 μl of 5×Green GoTaq Flexi Buffer (Promega), 2.6 μl of 5 μM primers, 1.6 μl of 2.5 mM dNTP Mix and 2 μl of 25 mM $MgCl_2$, and the total volume was brought to 20 μl with sterile water. The thermal cycling profile of PCR consisted of an initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 55°C for 30 s and extension at 72°C for 45 s.

RFLP analysis of the gene products was performed by digesting 5 μl of each amplified product with 10 units of restriction enzyme: *Hin*fI (Nippongene, Japan) for 16S rRNA and *Xba*I (Nippongene) for the control region. The reaction was carried out at 37°C for 1h in a reaction mixture containing buffer supplied with the kit. The digested samples were subjected to electrophoresis on a 3% agarose gel and stained with Midori Green DNA Stain (NIPPON Genetics, Japan).

Analysis of maturation process

Gonads were extracted from the specimens by dissection, weighed to the nearest 0.1 g (GW, g wet weight), and fixed in 10% formalin. According to Maeda et al. (2002), the gonadosomatic index (GSI) was calculated for both sexes by means of the following formula: $GSI=(GW/BW)\cdot 10^2$.

Statistical analysis

The frequency of occurrence of *G. punctata* and *G. leonina* collected by the coastal fisheries and their sex ratios were compared using paired *t*-test. The frequency of occurrence of the two species in the juveniles collected from the tide pools was compared using a chi squared test. The SL of juveniles was compared between *G. punctata* and *G. leonina* using Mann–Whitney *U* test. Statistical analyses were performed using Ekuseru-Toukei 2012 (Social Survey Research Information Co., Ltd., Japan).

Results

The species composition of girellid fish captured by the coastal fisheries

The mtDNA PCR-RFLP analysis showed that the girellid fish captured by coastal fisheries consisted of 161 individuals of *G. punctata* and 24 individuals of *G. leonina* (Table

Table 1. Species composition of girellid fish collected from a market of Shimoda during November 2011 to July 2012.

Month		N	*G. punctata*				*G. leonina*					
			Female		Male		Female		Male		unidentified	
			n	SL (Average±SD)	n	SL (Average±SD)	n	SL (Average±SD)	n	SL (Average±SD)	n	SL (Average±SD)
2011	Nov.	20	6	245.6–280.4 (261.5±12.5)	14	232.0–276.4 (257.4±13.1)	0	—	0	—	0	—
	Dec.	22	11	226.7–285.7 (254.9±18.5)	11	223.5–313.0 (265.0±29.7)	0	—	0	—	0	—
2012	Jan.	23	6	264.4–341.2 (297.2±27.1)	17	255.3–326.1 (283.4±21.9)	0	—	0	—	0	—
	Feb.	20	4	220.5–276.0 (252.9±23.4)	11	224.0–333.6 (270.5±31.6)	0	—	5	266.8–280.7 (274.3±6.0)	0	—
	Mar.	20	8	250.4–333.0 (287.7±25.7)	12	276.0–377.4 (310.8±29.5)	0	—	0	—	0	—
	Apr.	20	5	249.6–345.0 (288.6±36.3)	12	258.0–317.3 (294.5±18.7)	1	311.0	2	255.4–334.8 (295.1±56.1)	0	—
	May	20	4	279.8–359.3 (304.2±37.4)	5	258.4–343.9 (302.0±38.8)	7	250.0–330.6 (292.5±27.4)	3	266.9–322.2 (295.7±27.7)	1	276.4
	Jun.	20	5	250.9–328.8 (285.1±28.6)	12	234.9–353.9 (298.5±35.4)	0	—	2	293.8–299.0 (296.4±3.7)	1	326.5
	Jul.	20	6	234.5–299.5 (275.5±25.6)	12	245.1–321.2 (281.9±28.2)	0	—	2	310.5–340.4 (325.5±21.1)	0	—
The whole term		185	55	220.5–359.3 (276.5±29.4)	106	223.5–377.4 (283.5±31.0)	8	250.0–330.6 (294.8±26.2)	14	255.4–340.4 (292.3±26.6)	2	276.4–326.5 (301.5±35.4)

Species identification was performed using PCR-RFLP of mt DNA. N: the total number of girellid fish; n: the number of species-identified individual; SL: standard length (Min.–Max., mm).

1). The occurrence of *G. punctata* was higher than that of *G. leonina* in every month except for May (paired *t*-test, $P<0.001$). Body length ranged from 220.5–377.4 mm SL for *G. punctata* and 250.0–340.4 mm SL for *G. leonina*. Both sexes of *G. punctata* were collected every month during the research period, with significantly more males than females (paired *t*-test, $P<0.001$). Similarly, *G. leonina* males appeared more frequently than the females except for May 2012.

GSI

The GSI of *G. punctata* ranged from 0.13–15.45 in females and 0.01–16.54 in males for the girellid fish captured by the coastal fisheries. The maximum value occurred in April for both females (15.45) and males (16.54) (Fig. 2). By contrast, the GSI of *G. leonina* was constantly low, with ranges of 0.01–0.24 in females and 0.01–0.41 in males.

The body length of females and males ranged from 220.5–359.3 mm SL and 223.5–377.4 mm SL in *G. Punctata* respectively, and ranged from 250.0–330.6 mm SL and 255.4–340.4 mm SL in *G. leonina* respectively (Table 1, Fig. 3). The GSI of *G. punctata* showed low values of<2.0 at smaller body sizes of 220.5–246.5 mm SL in females and 223.5–256.8 mm SL in males (Fig. 3a). On the other hand, relatively high GSI values of>8.0 were found for larger individuals of 249.6–359.3 mm SL in females and 258.0–377.4 mm SL in males. A particularly high value of 11.34 was found for a single female of 249.6 mm SL collected in April. A similarly sized male (258.0 mm SL) also showed a relatively high value of 8.57. As for *G. leonina*, there is no clear relationship between GSI and body size (Fig. 3b).

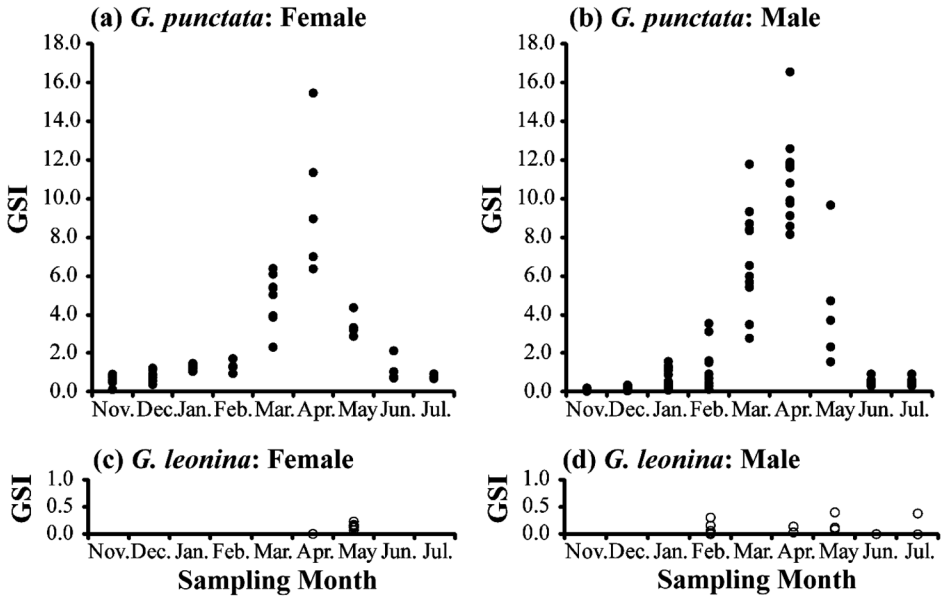

Fig. 2. Monthly changes in GSI of *G. punctata* and *G. leonina*. (a) Female of *G. punctata*. (b) Male of *G. punctata*. (c) Female of *G. leonina*. (d) Male of *G. leonina*.

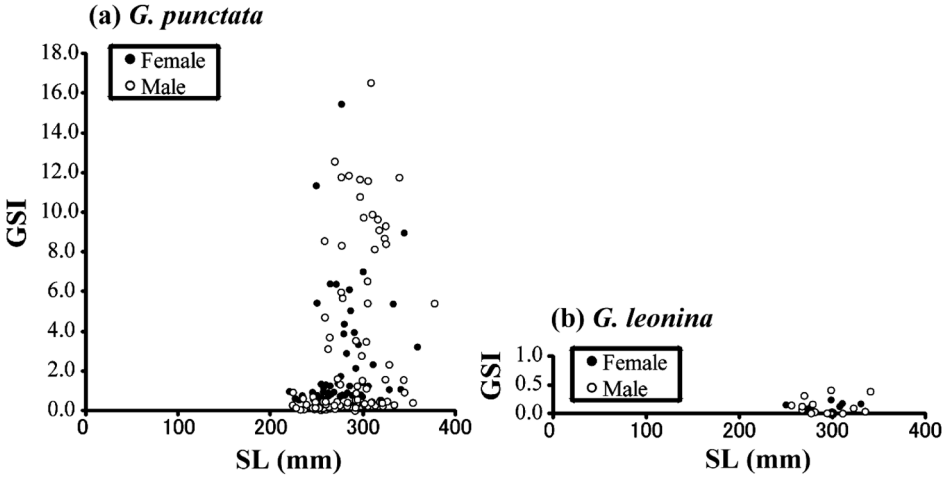

Fig. 3. The relationships between GSI and standard length (SL, mm) in female and male girellid fishes. (a) *G. punctata*. (b) *G. leonina*.

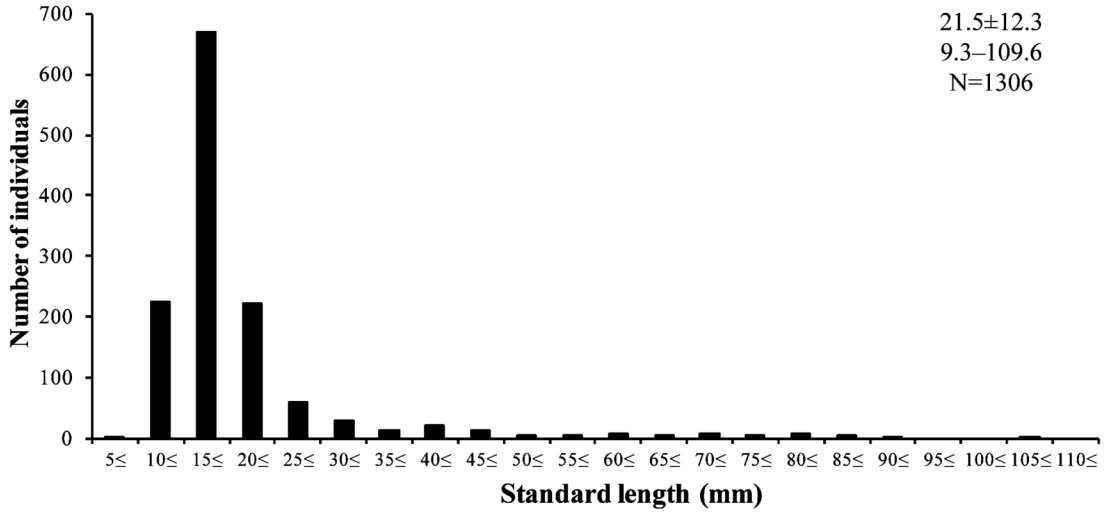

Fig. 4. Distribution of standard length (SL, mm) in all the juvenile girellid fish collected from tide pools and drifting seaweed around the Suzaki Peninsula (including individuals of ≥25 mm SL). Average±SD and range of SL, and number of individuals are shown.

Table 2. The total number (N) of juveniles of girellid fish collected from tide pools from February 2011 to October 2012.

Month		N	SL (mm)		DNA
			Average±SD	Min.–Max.	analysis (n)
2011	Feb.	9	21.2±1.8	18.5–24.0	9
	Mar.	ns	—	—	ns
	Apr.	34	17.6±2.3	12.3–22.9	29
	May	114	19.6±2.1	15.2–25.0	32
	Jun.	239	18.5±1.9	11.1–24.8	29
	Jul.	23	22.5±1.8	18.0–24.8	23
	Aug.	0	—	—	0
	Sep.	0	—	—	0
	Oct.	0	—	—	0
	Nov.	0	—	—	0
	Dec.	0	—	—	0
2012	Jan.	34	16.9±1.1	14.5–18.4	34
	Feb.	32	19.5±2.4	14.7–23.3	32
	Mar.	9	20.3±1.9	18.3–24.2	9
	Apr.	11	21.5±2.7	17.8–24.9	11
	May	42	19.5±2.0	14.2–23.4	30
	Jun.	135	18.8±2.9	13.0–24.9	30
	Jul.	15	19.7±3.0	15.4–24.7	14*
	Aug.	0	—	—	0
	Sep.	0	—	—	0
	Oct.	0	—	—	0
The whole term		697	19.0±2.4	11.1–25.0	282

The standard length (mm) and the number (n) of samples for DNA analysis are shown. ns: no sampling. *: Species of one individual could not be identified because of degradation of DNA.

Table 3. The total number (N) of juveniles of girellid fish collected from drifting seaweeds from April to November 2011.

Month		N	SL (mm)		DNA
			Average±SD	Min.–Max.	analysis (n)
2011	Apr.	5	18.1±1.7	17.0–21.1	5
	May	75	13.7±1.3	10.1–16.8	74*
	Jun.	340	15.6±2.2	9.3–21.1	82
	Jul.	0	—	—	0
	Aug.	0	—	—	0
	Sep.	ns	—	—	ns
	Oct.	ns	—	—	ns
	Nov.	0	—	—	0
The whole term		420	15.3±2.2	9.3–21.1	161

The standard length (mm) and the number (n) of samples for DNA analysis are shown. ns: no sampling. *: Species of one individual could not be identified because of degradation of DNA.

The species composition of juveniles

A total of 1306 individuals were collected from the coastal waters of the Suzaki Peninsula (Fig. 4). The juveniles consisted mostly of small individuals of<25 mm SL (85% of the total). Accordingly, we focused on 1117 individuals of<25 mm SL (697 from tide pools and 420 from drifting seaweed) as settled juveniles (Tables 2, 3).

The mtDNA PCR-RFLP analysis showed that both species were abundantly present in the tide pools (Fig. 5). On the other hand, genetically identified juveniles from drifting seaweed consisted mostly of *G. punctata* (n=151), and included only ten individuals of *G. leonina* (Fig. 6). In both 2011 and 2012, the frequency of occurrence of *G. punctata* and *G. leonina* in the tide pools was significantly different among the sampling months when we could collect girellid juveniles (chi-squared test, $P<0.0001$ in 2011 and $P<0.0001$ in 2012). In both years, *G. leonina* mostly appeared in winter and spring (January to May), and did not appear in summer and autumn except for a single juvenile collected in June 2011. Juveniles of *G. leonina* from the drifting seaweed also appeared in the spring (April and May). By contrast, the juveniles of *G. punctata* appeared in later seasons. Juvenile *G. punctata* appeared in the tide pools in spring and summer (April to July), with the greatest abundance in both years occurring in June. Juveniles of *G. punctata* from the drifting

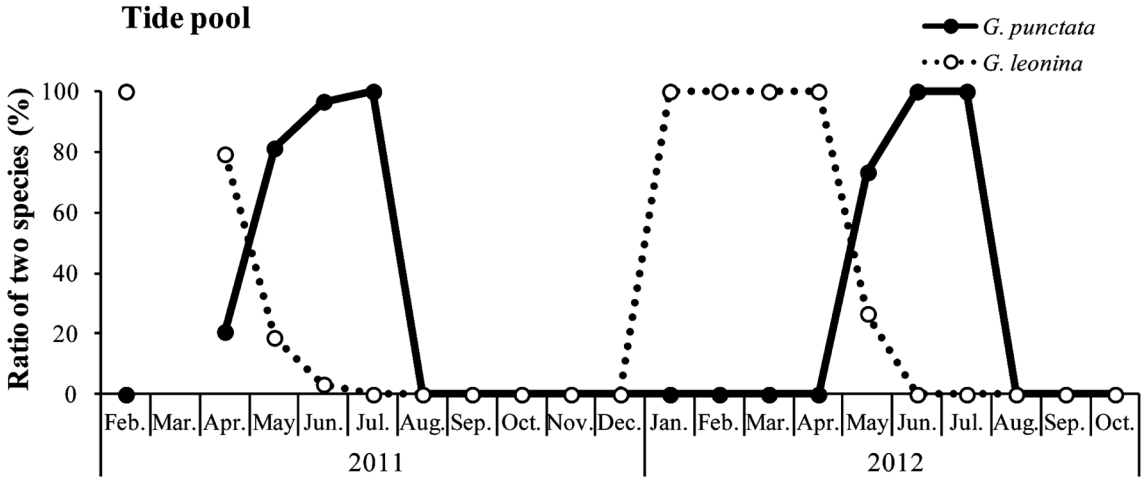

Fig. 5. Monthly changes in the species composition of *G. punctata* and *G. leonina* juveniles collected from tide pools of the Suzaki Peninsula, based on species identification by PCR-RFLP of mtDNA.

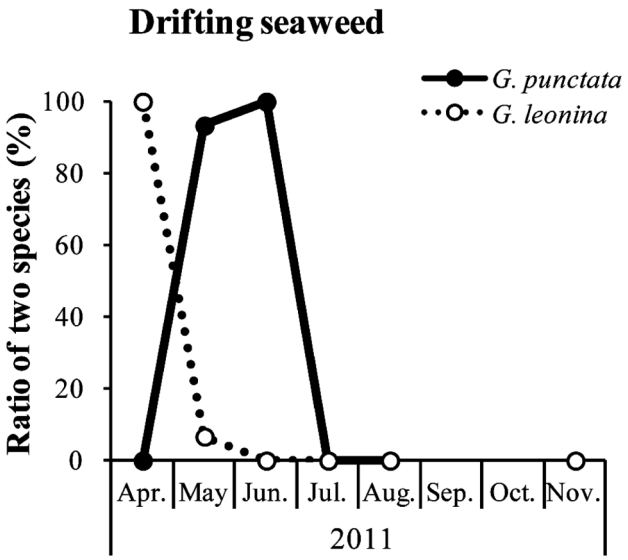

Fig. 6. Monthly changes in the species compositions of *G. punctata* and *G. leonina* juveniles collected from drifting seaweed off the Suzaki Peninsula, based on species identification by PCR-RFLP of mtDNA.

seaweed also appeared within spring and early summer (May and June).

The SL of juveniles of<25 mm was significantly larger in *G. leonina* than in *G. punctata* (Mann–Whitney *U* test, $P<0.0001$) (Table 4, Fig. 7). Minimum SL of *G. leonina* was 14.4 mm, whereas minimum SL of *G. punctata* was 12.3 mm for the juveniles from the tide pools and 9.3 mm for the juveniles from the drifting seaweed.

Discussion

Seasonal variations in the GSI differed markedly between *G. punctata* and *G. leonina*. The GSI of *G. punctata* showed peak values of 15.45 for females and 16.54 for males in April, in contrast to extremely low GSI values of<0.5 for both sexes of *G. leonina*. This interspecific difference was consistent with the GSI variations reported for the girellid fish collected off the Kii Peninsula by Maeda (2011): in that study high GSI values exceeding 10 were found for *G. punctata* only. It was thus inferred that the coastal area of the Izu Peninsula is utilized as a spawning ground by *G. punctata* but not by *G. leonina*, similarly to the inference regarding the coastal area of the Kii Peninsula made by Maeda (2011). The concurrent GSI peak in April for *G. punctata* collected in both areas suggests that *G. punctata* mainly spawns around April along the Pacific side of the central Honshu.

The smallest individuals exceeding 8.5 in GSI were 249.6 mm SL for a female and 258.0 mm SL for a male in *G. punctata*. Thus, the minimum body size at maturity for both males and females is likely about 250 mm SL in the coastal area of the Izu Peninsula. Maeda (2011) reported that off the Kii Peninsula *G. punctata* females of about≥280 mm in fork length (FL) and males of about≥250 mm in 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. In this study, the fish with the highest GSIs, 11.34 for females and 8.57 for males, had FLs of 312.3 mm (249.6 mm SL) and 300.8 mm (258.0 mm SL) respectively, larger than the individuals collected off the Kii Peninsula. It is therefore necessary to clarify the regional differences in the age and minimum body size at maturity for *G. punctata*.

The frequency of occurrence of *G. punctata* juveniles peaked in June for both tide pools and drifting seaweed. In the tide pools, there were clear differences in the occurrence times of *G. leonina* and *G. punctata* with the former appearing in winter and spring and the latter in spring and summer. Mano and Itoi (2011) showed a similar time lag in the appearance of these two species with 10–80 mm SL around the

Table 4. Species composition and body size of juveniles of girellid fish identified by DNA analysis.

Month		N	*G.punctata*			*G.leonina*		
			n	SL(mm)		n	SL(mm)	
				Average±SD	Min.-Max		Average±SD	Min.-Max
Tide pool					—			
2011	Feb.	9	0	—		9	21.2±1.8	18.5–24.0
	Mar.	ns	—	—	—	—	—	—
	Apr.	29	6	14.9±1.4	12.3–16.1	23	18.2±2.1	14.5–22.9
	May	32	26	18.0±1.6	15.2–22.0	6	19.8±3.1	16.1–24.9
	Jun.	29	28	17.9±2.4	13.4–23.7	1	20.2	—
	Jul.	23	23	22.5±1.8	18.0–24.8	0	—	—
	Aug.	0	—	—	—	—	—	—
	Sep.	0	—	—	—	—	—	—
	Oct.	0	—	—	—	—	—	—
	Nov.	0	—	—	—	—	—	—
	Dec.	0	—	—	—	—	—	—
2012	Jan.	34	0	—	—	34	16.9±1.1	14.5–18.4
	Feb.	32	0	—	—	32	19.5±2.4	14.7–23.3
	Mar.	9	0	—	—	9	20.3±1.9	18.3–24.2
	Apr.	11	0	—	—	11	21.5±2.7	17.8–24.9
	May	30	22	18.4±1.5	14.2–21.1	8	21.2±1.5	19.2–23.4
	Jun.	30	30	18.2±2.8	13.0–24.6	0	—	—
	Jul.	14	14	20.0±2.9	15.4–24.7	0	—	—
	Aug.	0	—	—	—	—	—	—
	Sep.	0	—	—	—	—	—	—
	Oct.	0	—	—	—	—	—	—
Drifting seaweed								
2011	Apr.	5	0	—	—	5	18.1±1.7	17.0–21.1
	May	74	69	13.6±1.2	11.1–16.8	5	15.2±0.7	14.4–16.1
	Jun.	82	82	14.7±2.4	9.3–21.1	0	—	—
	Jul.	0	0	—	—	0	—	—
	Aug.	0	0	—	—	0	—	—
	Sep.	ns	—	—	—	—	—	—
	Oct.	ns	—	—	—	—	—	—
	Nov.	0	0	—	—	0	—	—
Total		443	300	16.5±3.4	9.3–24.8	143	18.9±2.6	14.4–24.9

Species identification was performed using PCR-RFLP of mt DNA. N: the total number of species-identified individual; n: the number of samples; ns: no sampling.

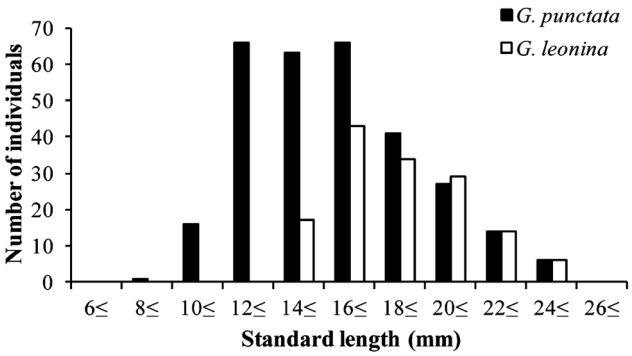

Fig. 7. Distributions of standard length (SL, mm) in juvenile *G. punctata* and *G. leonina* collected from tide pools and drifting seaweed around the Suzaki Peninsula, based on species identification by PCR-RFLP of mtDNA.

coastal area of the Izu Peninsula, suggesting interspecific differences between their spawning seasons. The present study focused on the juveniles of<25 mm SL, and clearly showed the juveniles' periods of appearance. *G. punctata* juveniles were frequently collected during May to July but not during August to March. It seems likely that juvenile *G. punctata* moved from the tide pools to another nursery ground in late summer, as suggested by Mano and Itoi (2011).

Interspecific differences in the seasonal occurrence of juveniles probably correspond to their differing spawning seasons. Our inferred *G. punctata* spawning season (April–May, based on GSI peak) was consistent with the main period of occurrence (May–July) of settled juveniles. Juvenile girellid fish generally settle to the coastal bottom after a one-month planktonic phase in the water column (Suzuki 2011).

Accordingly, juveniles born in this study area in April and May are inferred to settle to the bottom in the littoral and sublittoral zones around the spawning ground in May and June. Here it is necessary to consider the possibility that the settled juveniles include immigrants from other areas. In particular, we expect that a portion of juveniles born around the Kii Peninsula are transported to the coastal waters of the Izu Peninsula, due to the rapid velocity of Kuroshio Current, sometimes exceeding 2 m/s (Teramoto 1987). Recent genetic studies have also suggested larval dispersion of *G. punctata* by currents, based on shallow genetic differentiation among various sampling locations (Saito et al. 2008, Umino et al. 2009). Juvenile *G. punctata* born in distant regions of the Kii Peninsula and the Izu Peninsula are likely to coexist in the Izu Peninsula region during the same period. We also expect that the juveniles collected in July, the last month of the main period of occurrence, included abundant immigrants from the Kii Peninsula region.

In contrast to *G. punctata*, juvenile *G. leonina* appeared over a longer period, from January to June (Fig. 5). Considering that no mature adult fish was captured by the coastal fisheries, it was inferred that all the juveniles collected in this study area were immigrants from another region. As shown in Fig. 7, the SL of juveniles *G. leonina* was significantly larger than the SL of *G. punctata*. Smaller juvenile *G. leonina* of<14 mm SL likely grow up in the transport process from the spawning ground.

If *G. leonina* also has a one-month planktonic larval stage (Suzuki, 2011), we infer that juveniles collected in the study area from January to June were spawned during December to May. Mano and Itoi (2011) estimated that the spawning season of *G. leonina* is two months earlier than that of *G. punctata*, based on the interspecific differences between the appearance of juveniles and young with 10–80 mm SL. However, the time lag in the appearance of the settled juveniles measuring 10–25 mm SL cannot be identified as consistently two months (Fig. 5). The results of our study demonstrate that *G. leonina* spawns in winter and spring prior to *G. punctata*, but the spawning ground for *G. leonina* must be discovered to more precisely clarify the spawning season.

We demonstrated that the reproductive traits of *G. punctata* and *G. leonina* differ markedly in the coastal waters of the Izu Peninsula. Mature *G. punctata* have been found in several areas of the Japanese Archipelago; e.g., off Sasebo, the northwestern Kyushu in May (Mizue and Mikami 1960) and off Kushimoto, the Kii Peninsula during March to April (Maeda et al. 2002). We also found mature *G. punctata* in the coastal waters of the Izu Peninsula, suggesting the species spawns throughout the southern part of the Japanese Archipelago. By contrast, mature *G. leonina* have not been reported previously, and were also not found in the present study. Maeda (2011) hypothesized that *G. leonina* might spawn in the Izu-Islands region or to the south of Kyushu, but our results indicate that it does not spawn in the northern part of the Izu-Islands region. We predict that *G. leonina* spawns in the upstream of the Kuroshio Current or around the southern part of the Izu-Islands region.

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