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Genetic Differentiation in Populations of the Ryukyu-ayu *Plecoglossus altivelis ryukyuensis* on Amami-oshima Island

Yasumasa Sawashi[1,2] and Mutsumi Nishida[1,3]

[1] Department of Marine Sciences, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903–01, Japan
[2] Present address: Japan Wildlife Research Center, 2–29–3 Yushima, Bunkyo-ku, Tokyo 113, Japan
[3] Present address: Department of Marine Bioscience, Fukui Prefectural University, 1–1 Gakuen-cho, Obama, Fukui 917, Japan

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Abstract The Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, currently occurs only on Amami-oshima Island, and is represented by geographically separated, amphidromous populations in the eastern and western parts of this island (Sumiyo Bay and Yakeuchi Bay areas, respectively). To examine the level of isolation between the populations in the two areas, ecological and genetic investigations were made. The occurrence of young fish ascending streams from the sea was determined by a survey throughout the island, there being no evidence of occurrence in streams in the region separating the above areas of distribution. Genetic examination of 28 loci using allozyme electrophoresis revealed considerable allele frequency differences ($p<0.001$) between the eastern and western populations at two loci. These results suggest that the two populations are substantially independent from each other with little gene flow via larval migration along the coast. The considerable genetic heterogeneity in *P. a. ryukyuensis* on Amami-oshima Island should be taken into account in future conservation procedures involving this endangered subspecies.

The Ayu in the Ryukyu Islands, known as the Ryukyu-ayu, is regarded as a distinct subspecies, *Plecoglossus altivelis ryukyuensis*. Although the subspecies has been recorded from both Okinawa and Amami-oshima Islands, in the central Ryukyus (Nishida, 1988), recent intensive surveys failed to find the fish on Okinawa Island, leading to the conclusion that the Okinawa Island population had become extinct (Nishida et al., 1992). The only remaining populations of the Ryukyu-ayu, on Amami-oshima Island, are currently threatened by habitat destruction resulting from human activities. Clearly, a basic understanding of the population structure of the subspecies is essential for its future protection and management.

Recent surveys of the distribution and abundance of *P. a. ryukyuensis* on Amami-oshima Island revealed the subspecies to be distributed within two isolated areas, the Sumiyo-Isu Bay area in the eastern part of the island and the Yakeuchi Bay area in the western part, suggesting that the populations in the two areas were relatively isolated from each other (Nishida et al., 1992; Sawashi et al., 1992). The present study was undertaken to examine the level of isolation between the two populations.

The most important issue in the present study is the level of gene flow between the two areas. Two approaches, ecological and genetic, were taken for assessment. Initially, a detailed survey of the occurrence of young ascending streams was made throughout the island. The Ryukyu-ayu is an amphidromous fish, with larvae and juveniles occupying coastal waters and subadults and adults occupying rivers after ascended as young in the spring season. Considering such a life cycle, it was expected that young would be observed ascending streams in the region between Sumiyo-Isu Bay and Yakeuchi Bay in the spring season, if the populations in the two bay areas were continuous. Because the population size of the fish may fluctuate considerably (Kawanabe, 1970), population size in the year surveyed was evaluated by comparison with those of recent years. Secondly, allozyme electrophoresis was performed to examine the level of genetic differentiation between the east-

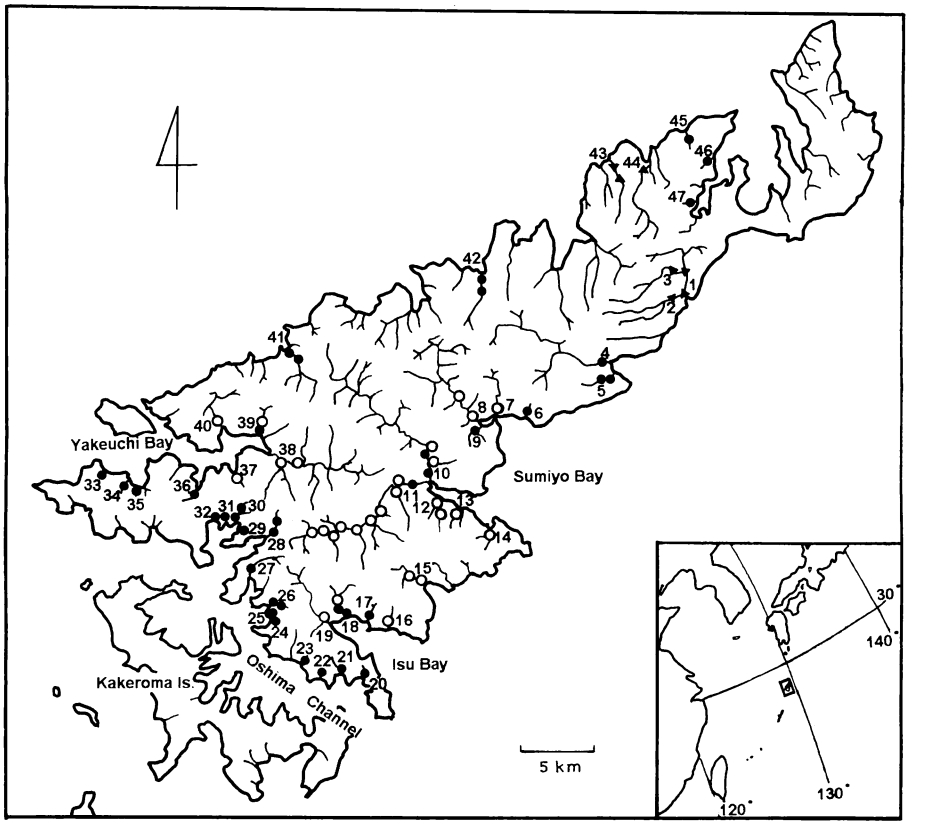

Fig. 1. Occurrence of young *Plecoglossus altivelis ryukyuensis* in 47 rivers throughout Amami-oshima Island in May 1992. See Table 2 for names of rivers. ○: stations where the fish were observed; ●: fish not observed; ▲: water clarity not sufficient for observations.

ern and western populations.

Materials and Methods

Annual changes in population size

Detailed surveys of the size of populations of the Ryukyu-ayu on Amami-oshima Island were made in 1986 and 1990 (Nishida et al., 1992; Sawashi et al., 1992). Because the overall population size on the island depends primarily on population sizes in a few major rivers, the Sumiyo, Yakugachi, and Kawauchi-W Rivers (see Table 2 for the suffix W) in particular (Nishida et al., 1992; Sawashi et al., 1992), annual changes in overall population size on the island can be estimated, as a first approximation, on the basis of comparisons of population density in the above rivers, examined using the same survey method. For the present study, population densities of the fish in the three rivers were surveyed in 1991 and 1992, being conducted in the summer and late autumn of each year (see Table 1 for details). The observation stations in each river were established in the same places as in the previous studies (Nishida et al., 1992; Sawashi et al., 1992). Censuses of the Ryukyu-ayu were conducted at those stations in the same manner, mainly by underwater observation, as previously. Fish density at each station was recorded using the following six categories; 0 individuals per $10\,m^2$ (0), less than 0.05 (1), 0.05 to 0.5 (2), 0.5 to 5 (3), 5 to 50 (4), and no data due to low water clarity (x).

Distribution of young in the upstream migration season

The distribution and abundance of young in the upstream migration season was surveyed in 47 rivers in Amami-oshima Island (Fig. 1) from May 10–14,

1992. At least one observation station was established in each river, with underwater observation as the main recording technique. Fish densities were recorded in the same manner as described above.

Allozyme electrophoresis

Horizontal starch-gel electrophoresis was used to analyze allozymes and to assess genetic differences between the eastern and western populations of *P. a. ryukyuensis*. The former population was sampled in the Yakugachi River, flowing into Sumiyo Bay on the eastern part of the island in December 1992 ($n=38$), and the latter, in the Kawauchi-W River in the Yakeuchi Bay area on the western part, in May ($n=24$) and July ($n=20$), 1992 ($n=44$ in total). A few individuals of the nominotypical subspecies, *P. a. altivelis*, were used as mobility standards in each electrophoretic run.

Sample preparation and electrophoretic procedures followed Nishida (1985), who used standard procedures described by Shaw and Prasad (1970) and Harris and Hopkinson (1976), with slight modifications. A starch mixture composed of STARCH-art starch (80%) and Connaught starch (20%) was used at a concentration of 13% (w/v). Enzymes examined and buffer systems used were basically the same as those in Sawashi et al. (1993), except as noted below. Loci and allele nomenclature followed Nishida (1985) and Sawashi et al. (1993), and also Shaklee et al. (1990). Of the 28 loci studied by Sawashi et al. (1993), the *GDH* locus was not included in the present analysis, owing to its non-appearance on the gels. On PGM gels, an additional system, with clear bands migrating faster in the anodal direction than those of the previous PGM system, was observed and interpreted as being controlled by a newly-found locus. The new locus was termed *PGM-1* and the previous *PGM* locus, *PGM-2*. The *ACP* locus, which was reported as polymorphic in the Ryukyu-ayu (Seki et al., 1988), was also resolved, but its banding patterns proved too com-

Table 1. Results of the present and previous surveys on the density of *Plecoglossus altivelis ryukyuensis* in three major rivers in Amami-oshima Island

No.*1	River	Station number	Density*2 (Water clarity in parentheses)*3					
			Growing season			Spawning season		
			2–5*4 Aug. 1986	26–29 Jul. 1991	29–31 Jul. 1992	5–10*5 Dec. 1990	29 Nov.– 1 Dec. 1991	4–6 Dec. 1992
10	Sumiyo R.	1	4 (A)	2 (B)	3 (B)	x (D)	3 (B)	1 (C)
		2	2 (B)	3 (A)	—	0 (C)	0 (B)	2 (B)
		3	4 (A)	4 (A)	4 (A)	4 (A)	4 (A)	3 (B)
		4	—	x (D)	—	3 (C)	0 (C)	0 (C)
11	Yakugachi R.	1	3 (A)	—	3 (A)	0 (A)	—	2 (A)
		1′	—	4 (A)	3 (A)	0 (B)	3 (B)	3 (A)
		2	2 (A)	0 (A)	3 (A)	0 (A)	0 (A)	1 (A)
		3	3 (A)	2 (A)	3 (B)	1 (A)	2 (B)	3 (A)
		4	2 (A)	3 (B)	3 (A)	1 (A)	2 (B)	1 (A)
		5	3 (A)	3 (B)	3 (B)	1 (A)	1 (C)	3 (B)
		6	4 (A)	3 (C)	3 (B)	2 (C)	x (D)	2 (B)
		7	4 (A)	x (D)	2 (B)	x (D)	2 (C)	3 (B)
		8	3 (B)	x (D)	0 (C)	x (D)	1 (C)	0 (B)
38	Kawauchi-W R.*6	2	4 (B)	—	—	0 (B)	0 (C)	—
		2′	—	—	3 (B)	3 (B)	4 (B)	4 (B)
		3	—	—	x (D)	—	3 (B)	x (D)
Density grade average			3.2	2.7	2.7	1.3	1.8	2.0

*1 See Figure 1; *2 0: 0 (inds./10 m^2), 1: <0.05, 2: 0.05–0.5, 3: 0.5–5, 4: 5–50, x: no data due to the low water clarity; *3 A: observed clearly more than 3m, B: observed less than 3m, C: observed less than 1m, D: not observed due to low water clarity, —: no data; *4 data from Nishida et al. (1992); *5 data from Sawashi et al. (1992); *6 Kawauchi River located in the western part of the island.

Table 2. Occurrence of young *Plecoglossus altivelis ryukyuensis* in 47 streams throughout Amami-oshima Island in May 1992

No.*1	River	Station number	Distance from the mouth (km)	Reach type*2	Density*3
1	Omi R.	1	2.0	Bb	0
2	Toguchi R.	1	1.0	Bb	0
		2	0.3	Bb–Bc	0
3	Nakagachi R.	1	3.1	Bb	0
4	Okawa R.	1	1.3	Bb	0
5	Kominato R.	1	2.0	Bb	0
		2	0.3	Bb–Bc	0
6	Wase R.	1	0.2	Bb	0
7	Kanekuda R.	1	0.1	Bb	2
8	Kawauchi-E R.*4	2	3.6	Bb	3
		3	2.7	Bb	3
9	Mizato R.	1	0.1	Bb	0
10	Sumiyo R.	1	4.8	Aa	3
		2	3.4	Bb	0
		3	2.6	Bb	4
		4	1.9	Bb–Bc	0
11	Yakugachi R.	1	13.7	Aa	4
		1′	13.0	Bb	3
		2	11.2	Aa	3
		3	9.9	Aa	3
		4	8.9	Bb	3
		5	7.5	Bb	2
		6	6.3	Bb	3
		7	5.2	Bb	1
		8	2.8	Bb	0
12	Yanma R.	1	0.9	Aa	3
		2	0.1	Bb	1
13	Todama R.	1	0.3	Aa	3
14	Okawa R.	1	0.9	Bb	3
15	Katoku R.	1	1.5	Bb	1
		2	0.5	Bb	2
16	Sekko R.	1	0.3	Bb	1
17	Aminoko R.	1	0.3	Bb	0
18	Kachiura R.	1	1.7	Bb	2
		2	1.5	Bb	0
		3	0.1	Bb–Bc	0
19	Agina R.	1	2.3	Bb	1
20	Sokaru R.	1	0.1	Bb–Bc	0
21	Katetsu R.	1	0.1	Bb–Bc	0
22	Seisui R.	1	0.1	Bc*5	0
23	Sekui R.	1	0.2	Bb	0
24	Kunetsu R.*6	1	0.1	Bb	0
25	Yui R.*6	1	0.5	Aa	0
		2	0.1	Bb	0
26	Atetsu R.	1	0.1	Bb	0
27	Konase R.	1	0.1	Aa–Bb	0
28	Shinokawa R.	1	1.1	Aa–Bb	0
		2	0.7	Bb	0
29	Koshiji R.*6	1	0.1	Bb	0
30	Kaneku R.	1	0.1	Aa	0
31	Ura R.*6	1	0.1	Aa	0
32	Ime R.	1	0.1	Aa	0

Table 2. (Continued)

No.	River	Station number	Distance from the mouth (km)	Reach type*2	Density*3
33	Yadon R.	1	0.1	Bb–Bc	0
34	Amuro R.	1	0.1	Bb	0
35	Heda R.	1	0.1	Bb–Bc	0
36	Okawa R.	1	0.7	Bb	0
37	Buren R.	1	0.2	Bb	3
38	Kawauchi-W R.*4	2′	3.0	Bb	4
		3	2.8	Aa	0
39	Taken R.	1	0.6	Bb	2
40	Ora R.	1	0.8	Bb	2
41	Naon R.	1	1.2	Aa	0
		2	0.6	Bb	0
42	Chinase R.	1	1.5	Bb	0
		2	0.1	Bb	0
43	Ashikebu R.	1	2.6	Aa	—
		2	0.6	Bb	—
44	Akina R.	1	1.7	Bb	—
45	Moto R.	1	0.2	Bb	0
46	Tatsugo R.	1	0.2	Bb	0
47	Sedome R.	1	0.1	Bb	0

*1 Rivers numbered 1–5 are in the northeastern area of the island; Nos. 6–14 in the eastern (Sumiyo Bay) area; Nos. 15–19 in the southeastern (Isu Bay) area; Nos. 20–32 in the southern (Oshima Channel) area; Nos. 33–40 in the western (Yakeuchi Bay) area; and Nos. 41–47 in the northwestern area. See also Figure 1; *2 followed Kani (1944) and Mizuno and Kawanabe (1981); *3 0: 0 (inds./10 m^2), 1: <0.05, 2: 0.05–0.5, 3: 0.5–5, 4: 5–50, —: no data; *4 two 'Kawauchi' Rivers on Amami-oshima Island are discriminated by the suffix E (eastern) or W (western) following the name; *5 this station was located in the brackish water zone; *6 rivers too small to appear on a 1/25,000 map.

plex to be scored consistently. A total of 28 loci were examined in this study.

Results

Population densities in 1992

The results of the population density surveys are presented in Table 1, together with those from previous studies. On the basis of the average values of density categories at all sites, fish density in summer, 1992, was similar to that in 1991, being somewhat lower than in 1986. In the late autumn, the reproductive season of the subspecies, however, fish density was rather higher in 1992 than in 1990 and 1991. The survival rate of Ayu in subadult and adult stages after upstream migration is known to be relatively stable, unlike in larval and juvenile stages in the sea (Kawanabe, 1970). Accordingly, the overall fish density on Amami-oshima Island during the upstream migration period in 1992 appears to have been at the same level at least as in the other three years recorded.

Distribution of young during the upstream migration season in 1992

The results of the survey on the occurrence of young in the upstream migration season in 1992 are presented in Table 2. Young of *P. a. ryukyuensis* occurred in 11 rivers in the Sumiyo-Isu Bay area and in 4 rivers in the Yakeuchi Bay area, but were not recorded in 13 rivers flowing into the Oshima Channel, which is flanked by the above two bays (Fig. 1, Table 2). No evidence was found for the occurrence of young in 12 rivers in the northern region of the island.

Genetic differentiation

Allozyme electrophoresis revealed considerable genetic differentiation between the eastern and western populations. Electrophoretic results for the eastern population were consistent with those in previous genetic studies of *P. a. ryukyuensis*, based on specimens from the Sumiyo Bay area (Nishida, 1985; Seki et al., 1988; Sawashi et al., 1993), almost all loci being monomorphic and the average heterozygosity

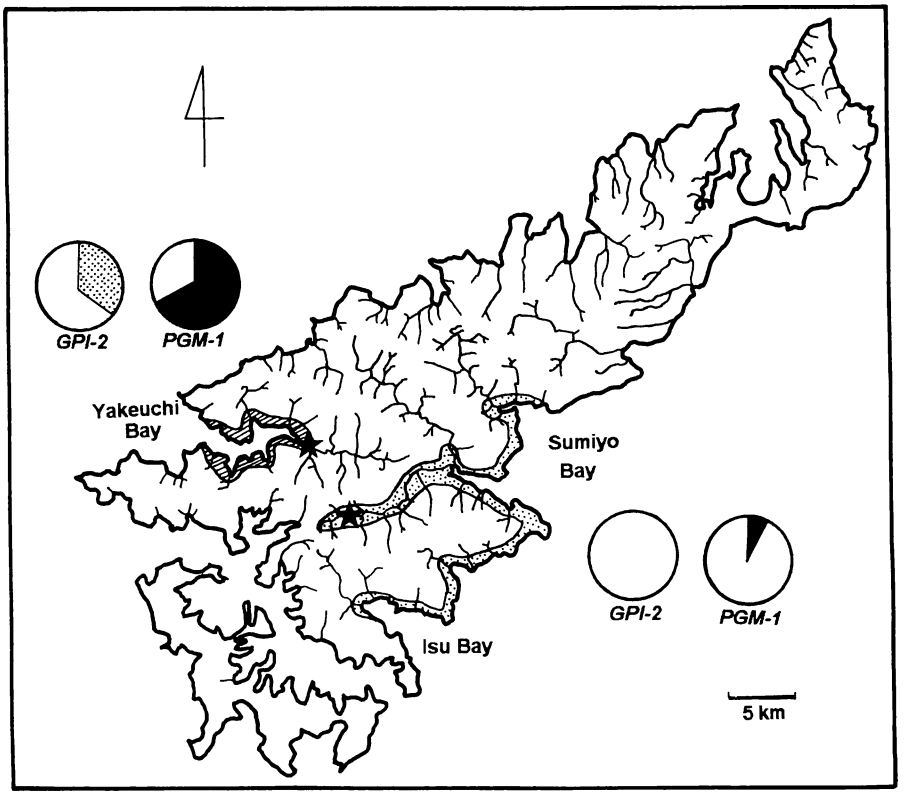

Fig. 2. Two principal distribution areas of *Plecoglossus altivelis ryukyuensis* on Amami-oshima Island, concluded from results of the present and previous studies, and comparison of allele frequencies at two polymorphic loci (*GPI-2* and *PGM-1*) in populations in the two areas. Stars indicate sampling sites. Allele frequencies in the Yakugachi River fish from the Sumiyo Bay area: *GPI-2*a* = 0.000, *GPI-2*b* = 1.000, *PGM-1*a* = 0.068, *PGM-1*b* = 0.932; those in the Kawauchi-W River fish from the Yakeuchi Bay area: *GPI-2*a* = 0.337, *GPI-2*b* = 0.663, *PGM-1*a* = 0.667, *PGM-1*b* = 0.333. The other 26 loci examined in the present study were fixed in both populations for the same allele, as found in previous studies (Nishida, 1985; Seki et al., 1988; Sawashi et al., 1993).

(Nei, 1978) therefore very low (0.5%). However, genetic characteristics in the western population were found to differ considerably from the former, two loci, *GPI-2* and *PGM-1*, being highly polymorphic and the average heterozygosity moderate (3.1%). Allele frequencies of the above two loci were significantly different ($p < 0.001$, Fisher's exact probability test) from those of respective loci in the eastern population (Fig. 2). The genetic distance (Nei, 1978) between the two populations was 0.017.

Discussion

The investigation of annual changes in population size of Ryukyu-ayu on Amami-oshima Island indicated that fish density in the upstream migration season in 1992 was at approximately the same level as in recent years. There was no evidence of young occurring in streams draining into the Oshima Channel region or the northern region of the island, suggesting that larval migration does not occur along the coast between the eastern and western populations, at least under current conditions.

The present genetic investigation using allozyme electrophoresis showed considerable differences in allele frequency at two loci between the eastern and western populations. Significant genetic differentiation between the two populations was also shown by a recent DNA fingerprinting study (Takagi and Taniguchi, 1994). These findings indicate their substantial isolation from each other, and supports Nishida et al. (1992), who suggested a more or less isolated nature for the populations existing in the

Sumiyo Bay and Yakeuchi Bay areas. In fact, the isolation has apparently been sufficient to allow considerable genetic differentiation to occur between the two populations.

Such genetic differentiation might be due simply to a bottleneck effect owing to the small population sizes. However, the western population, by far the smaller of the two (Nishida et al., 1992; Table 1), has the higher genetic variability (average heterozygosity 3.1% compared with 0.5%), suggesting that some other factors may be at least partially responsible.

The lack of suitable bays associated with large rivers, other than the Sumiyo and Yakeuchi Bays, may be primarily attributable to the isolation of the eastern and western populations. In particular, streams draining into the Oshima Channel region are very small, most being less than 1.5 km long. Even if juveniles migrate into such streams, those streams could be unable to support a viable population and could not thereby function so as to assist gene flow. Another factor influencing the current level of isolation may be the limited sizes of the populations (Nishida et al., 1992; Sawashi et al., 1993), leading to reduced chances of direct, long-distance dispersal of free-swimming larvae.

The considerable genetic differentiation between the populations of *P. a. ryukyuensis* on Amami-oshima Island has an important bearing on the conservation of this endangered subspecies on the island. Recently, being part of the efforts made to prevent further population decrease, young ascending streams lacking suitable habitat have been transplanted into other nearby rivers. Although such transplantations have been done within the Sumiyo Bay area up to now, caution should be taken in order to preserve the genetic heterogeneity of the subspecies. The most important approach may be to protect the natural habitat of this fish.

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奄美大島におけるリュウキュウアユ集団の遺伝的分化

澤志泰正・西田　睦

現在の唯一の生息地である奄美大島において，リュウキュウアユは不連続な東西2地域（住用・伊須湾域と焼内湾域）に分布する．この両地域の集団がどの程度独立であるかを明らかにするため，生態学的側面および遺伝学的側面の両面から検討を試みた．まず，遡上期における稚魚の分布を全島的に調査したところ，東西2地域では従来通り本亜種の生息が確認できたが，それらを隔てる南部や北部地域の河川では本亜種は全く出現せず，沿岸海域を通じての東西間の仔稚魚の移動の証拠は得られなかった．次に，両地域の集団から得た標本について電気泳動分析を行ったところ，調べた28遺伝子座のうち多型的な2遺伝子座で，遺伝子頻度の顕著な差異（それぞれ $p<0.001$）が認められた．以上の結果は，東西両集団は，相互の間でほとんど交流のない，相対的に独立したものであることを示唆する．本亜種の保護を考える際には，こうした奄美大島における遺伝的多様性を慎重に考慮することが重要であると考えられた．

（〒903-01 沖縄県西原町千原1　琉球大学理学部海洋学科；　澤志　現住所：　〒113 東京都文京区湯島2-29-3（財）自然環境研究センター；西田　現住所：〒917 小浜市学園町1-1　福井県立大学生物資源学部海洋生物資源学科）