Annual reproductive cycle and sexual dimorphism in the dragonet, *Repomucenus valenciennei*, in Tokyo Bay, Japan

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Abstract The annual reproductive cycle of the dragonet, *Repomucenus valenciennei*, from Tokyo Bay, Japan, was studied by analysis of seasonal trends in gonadosomatic indices and histological observations of gonads. Sexual dimorphism in the growth of several body parts relative to standard length (SL) and changes in color pattern of the first dorsal fin with growth were also investigated. The spawning season lasted from spring (April [1991] or February [1992]) to autumn (October) with two spawning peaks, in spring and autumn. In spring, only one-year-old (age 1+) fish spawned, age 0+ females not spawning until autumn, at which time they had reached age 1+. Likewise, histological observations of males indicated that testes had reached full maturity by 80 mm SL (age 1+). The minimum mature size of females was estimated as 60 mm SL. In males 45–80 mm SL, the first spine of the first dorsal fin, last ray of the second dorsal fin, last ray of the anal fin, and caudal fin ray showed strongly positive allometry, indicating rapid growth of these structures relative to SL. Subsequently, their growth returned to an isometric pattern in males >80 mm SL. In females, on the other hand, the same body parts showed slightly positive allometry throughout their growth. The color pattern on the first dorsal fin also changed in males 45–80 mm SL.

Key words. — *Repomucenus valenciennei*; annual reproductive cycle; sexual dimorphism; Tokyo Bay.

The dragonet, *Repomucenus valenciennei* (Callionymidae) (Japanese name hatatatenumeri), inhabits coastal areas of Japan and adjacent waters (Nakabo, 1983). In recent years, *R. valenciennei* has become a dominant species in the demersal fish fauna of Tokyo Bay (Shimizu, 1990). Growth, life span and seasonal changes in distribution of the species have been reported in previous papers (Ikejima and Shimizu, 1996, 1997). Takita and Okamoto (1979) reported spawning behavior of *R. valenciennei* in an aquarium. However, to date there have been no reports on the reproductive cycle in a naturally occurring population.

Callionymid fishes are known to be characterized by conspicuous sexual dimorphism, adult males having longer fin spines and rays and being much more colorful than females (Nakabo, 1983). The morphological development of the sexual characters has been reported for two species, *Synchiropus altivelis* (Akazaki, 1957) and *Callionymus maculatus* (Gibson and Ezzi, 1979).

The purpose of this study was to elucidate the annual reproductive cycle, size and age at maturity, and development of sexually dimorphic characters in *R. valenciennei* in Tokyo Bay.

Materials and Methods

Fish were collected from the inner part of Tokyo bay (35°20′–30′N, 139°40′–55′E) in depths of 20–50 m from July 1990 to June 1992, using a commercial fishing trawl (beam width 5.5 m; stretched mesh size ca. 3 cm at cod end). Sampling was conducted monthly where possible. Because bad weather prevented sampling in November 1990, two collections were made in December 1990. No male fish were captured in February 1991. A total of 1380 specimens (730 females, 650 males) was collected and returned to the laboratory on ice, whereupon, the standard

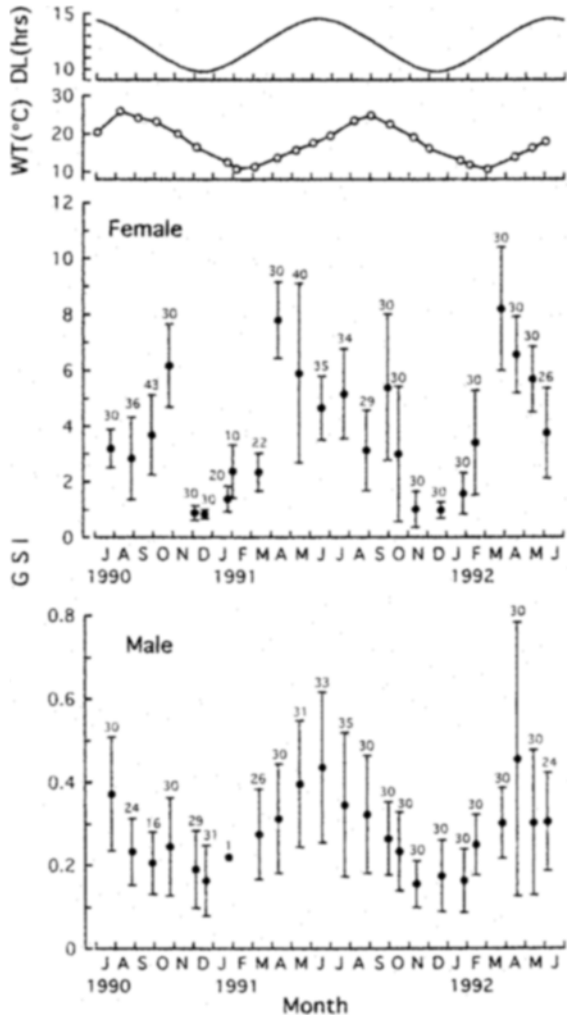

Fig. 1. Changes in daylength, water temperature and gonadosomatic index (GSI) of *Repomucenus valenciennei* in Tokyo Bay from July 1990 to June 1992. Closed circles and vertical bars show means and standard deviations, respectively. Numbers above the bars indicate sample sizes.

length (SL, 0.1 mm), body weight (BW, 0.01 g) and sex of each fish were immediately recorded, and gonads weighed (GW) to the nearest 0.01 g. Gonadosomatic index (GSI) was calculated as: GSI$=10^2$ (GW/BW).

From the specimens collected from May to October 1991 and February to June 1992, when the average GSI value was high (see Results), 128 females and 41 males were subsampled for histological observations. Subsamples were taken from several size classes in order to cover the overall range of specimens. The gonads were fixed with Bouin's solution for 24–48 h and then transferred to 70% ethanol for storage. They were embedded in paraffin wax, sectioned at 5 μm, and stained with hematoxylin and eosin. The stages of the most developed oocytes and presence of atretic oocytes were used to describe the ovary maturity phase. Testes were examined for the presence of sperm.

Water temperature at 15 m, at 35°22′N, 139°40′E, was taken from reports on water quality monitoring by Kanagawa Prefectural agency.

To examine the development of sexual dimorphism, additional samples were obtained using two different beam trawls (mesh sizes 20 and 1.6 mm at cod end, respectively) at fixed stations in Tokyo Bay (see Ikejima and Shimizu, 1996). In a total of 264 specimens (110 females, 154 males) collected, SL, the lengths of the snout (SN), pelvic fin (PF) (measured from anterior of fin base to tip of longest ray), first spine of the first dorsal fin (FSDF), last ray of the second dorsal fin (LRDF), last ray of the anal fin (LRAF) and longest caudal fin ray (CFR) were measured to the nearest 0.1 mm. To compare the relative growth of these body parts to SL, regression lines were calculated with log transformed variables. If the slope did not differ significantly from one (t-test, $p>0.05$), the growth of that part was classified as isometric. If the slope was significantly greater or smaller than one, the relationship was classified as positive or negative allometry, respectively (Oliveira and Almada, 1995). The color pattern of the first dorsal fin was also examined.

Results

Annual reproductive cycle

Figure 1 shows the seasonal changes in daylength, water temperature and mean GSI values. There was a highly significant difference between months in both sexes (Kruskal-Wallis test; $p<0.001$, Mann-Whitney U-test; $p<0.001$, for both sexes) GSI means in females peaked in October 1990, April 1991, September 1991 and March 1992. The lowest means following autumn peaks (October 1990 and September 1991) occurred in December 1990 and November and December 1991. The mean GSI in males peaked in June 1991 and April 1992. The lowest means occurred in December 1990 and from November 1991 to February 1992.

Histological observations indicated three main phases of ovarian maturity: phase I, immature (most advanced oocytes at yolk vesicle stage) (Fig. 2A); phase II , developing (most advanced oocytes at yolk globule stage); phase III, spawning (most advanced oocytes at migratory nucleus or mature stage, or with

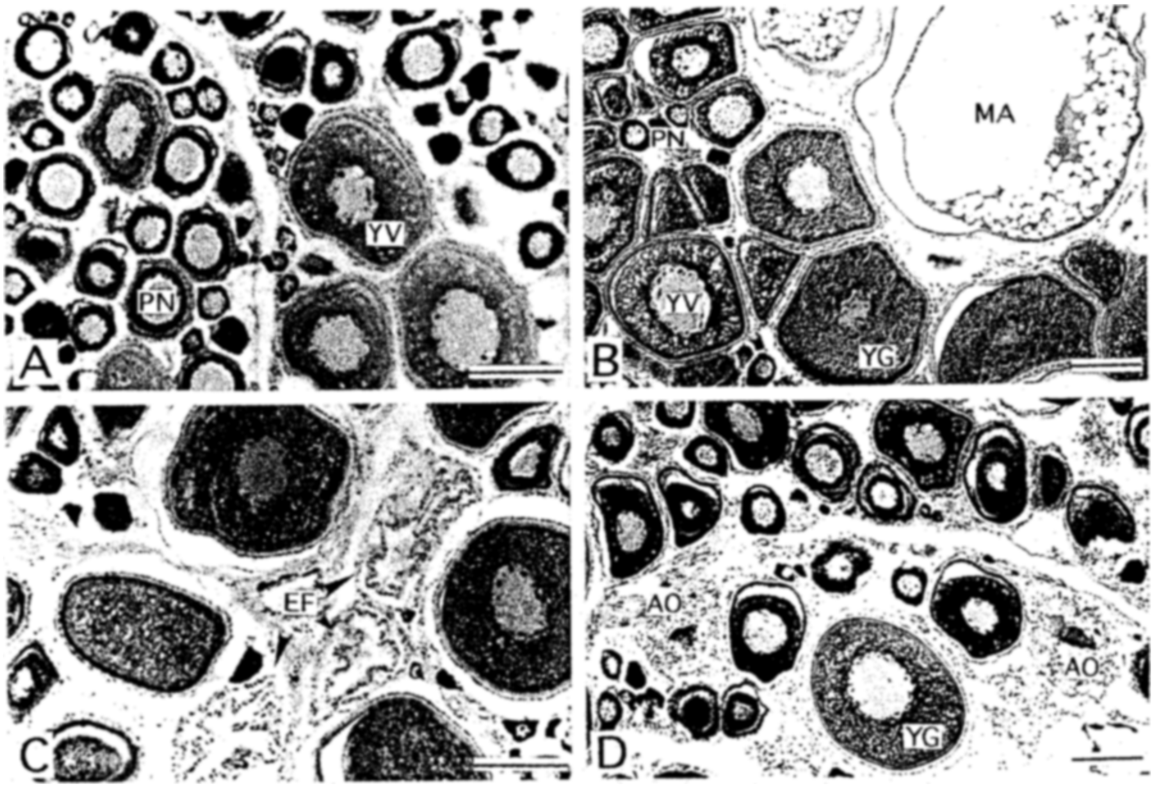

Fig. 2. Histological sections of ovary of *Repomucenus valenciennei*. A) Phase I (immature); B) phase IIIa (spawning); C) phase IIIa (spawning), empty follicles (*EF*) apparent; D) phase IIb (developing), atretic oocyte (*AO*) apparent. *PN*—peri-nucleolus stage; *YV*—yolk vesicle stage; *YG*—yolk globle stage; *MA*—mature stage. Bars indicate 0.1 mm.

empty follicles) (Fig. 2B, C). In addition, phases II and III were divided into two subphases according to the absence (a) or presence (b) of atretic oocytes (Fig. 2D). Females with phases III(a) or III(b) appeared from April to October 1991 and after February 1992, when mean GSI values were high (Fig. 3). Various oocyte stages from peri-nucleolus to ripe were found in phase III ovaries (Fig. 2B). Ovaries with atretic oocytes, II(b) or III(b), occurred from June to September (peaking in August) 1991 and in May and June 1992. Females with GSI >5 had phase III in April and May 1991 and from February to May 1992; those with GSI >3 had phase III from June to September 1991 and in June 1992.

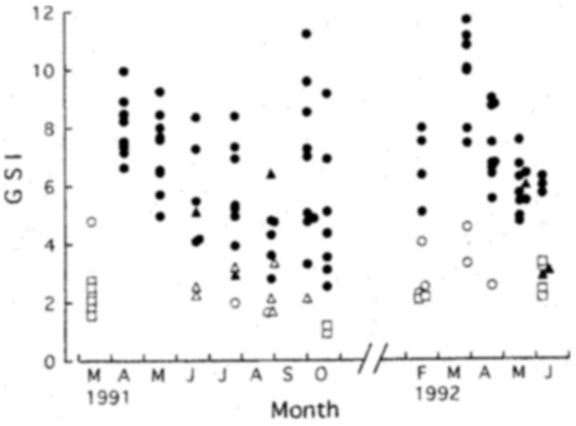

Fig. 3. Changes in gonadosomatic index (GSI) and maturity phase of female *Repomucenus valenciennei*. ☐, phase I (immature); ○, phase IIa (developing); △, phase IIb (developing, atretic oocytes); ●, phase IIIa (spawning); ▲, phase IIIb (spawning, atretic oocytes).

Figure 4 shows changes in the relationship between GSI and female SL. Most females >80 mm SL showed sufficiently high GSI values (April and May, >5; June to September, >3) to enable spawning from April to July in 1991 and March to June in 1992. High GSI values were also recorded from 70–80 mm SL in June and July 1991, whereas females <70 mm SL caught in May 1991 and June 1992 had lower GSI values (May, <5; June, <3). Because over half of the females had lower GSI values (<3), the smallest size of spawning females was unclear in July and August 1990 and August 1991. Most females >60 mm SL

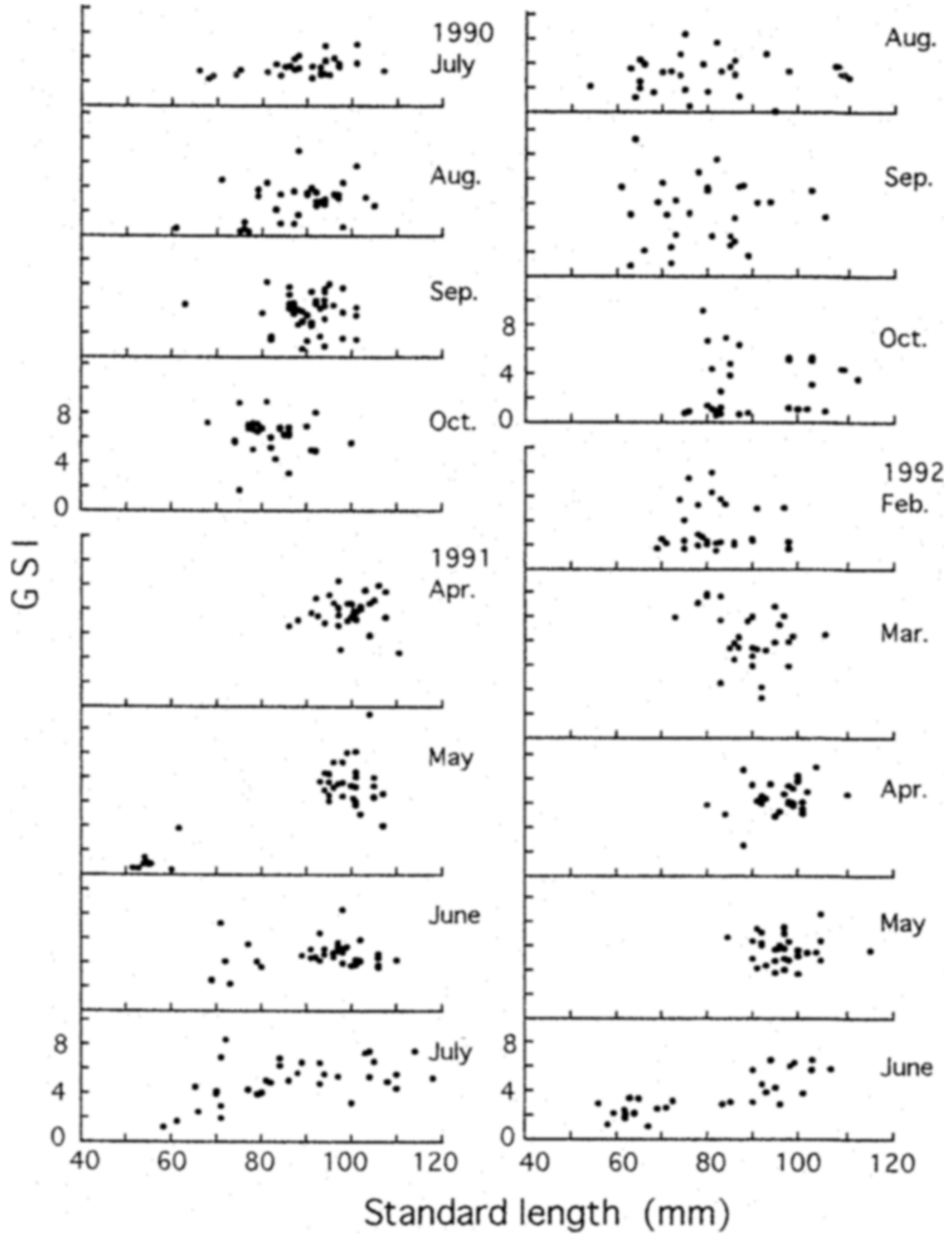

Fig. 4. Female gonadosomatic index (*GSI*) of *Repomucenus valenciennei* plotted against standard length from July 1990 to June 1992.

captured in September 1991 had high GSI values (>3).

Histological observations of the testis of 41 males ranging from 68 to 131 mm SL, showed only 2 individuals over 80 mm SL as not containing sperm. Since only two individuals <80 mm SL were examined and the smaller of these (68 mm SL) contained sperm, the minimum mature size of males was not clear.

Sexual dimorphism

All relationships between SL and lengths of body parts in females are shown by single regression lines for the entire range of SL (Fig. 5). In males, on the other hand, while the SN and PF relationships with SL are shown as single regression lines, as judged by eye, FSDF, LRDF, LRAF and CFR relationships were divided into three phases: <45 mm SL, relationships

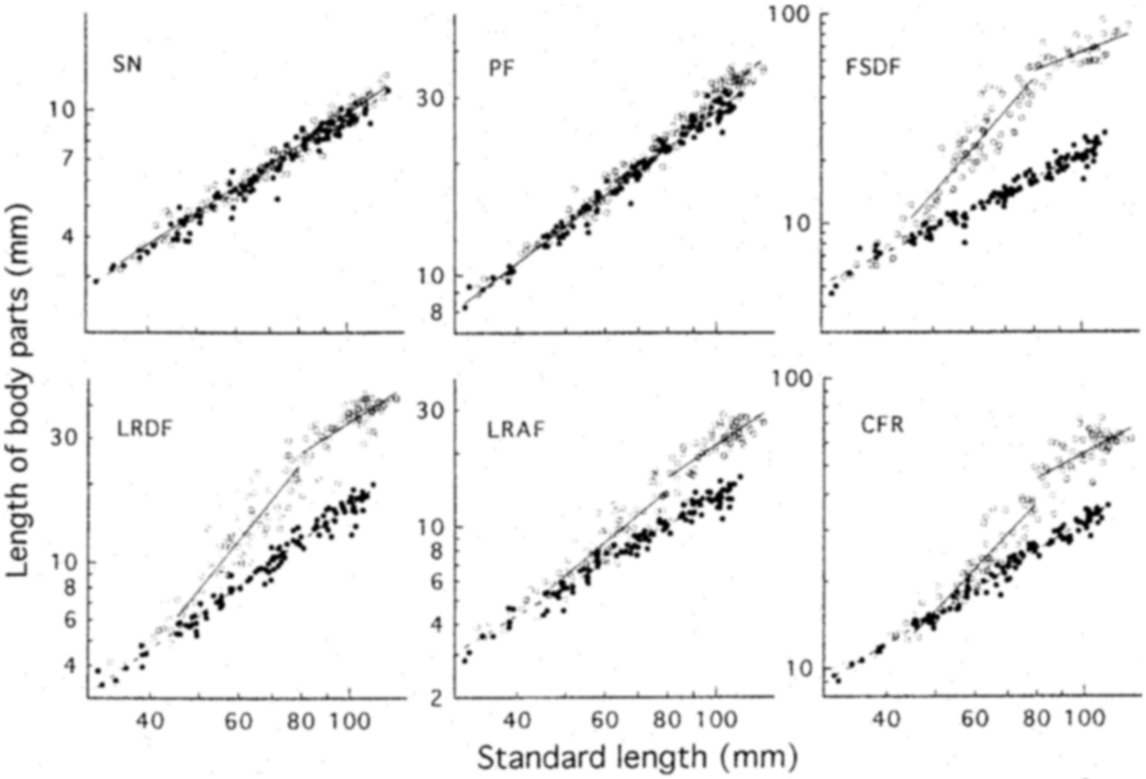

Fig. 5. Relationships between selected body parts and standard length of *Repomucenus valenciennei*. ●: female; ○: male. *SN*—snout; *PF*—pelvic fin; *FSDF*—first spine of first dorsal fin; *LRDF*—last ray of second dorsal fin; *LRAF*—last ray of anal fin; *CFR*—longest caudal fin ray.

between SL and body parts almost identical to those in females; 45–80 mm SL, slopes greater than corresponding slopes in females, indicating that body parts became proportionally much larger in males; ≧80 mm SL, males with proportionally greater length of above body features, but with decreased slopes.

In females, SN and PF showed isometric growth, whereas FSDF, LRDF, LRAF and CFR showed slightly positive allometry at a highly significant level ($p<0.001$) (Table 1). In males, SN and PF showed positive allometry over the entire range of SL, but they differed little from an isometric growth pattern (SNL: $b=1.04$, PFL: $b=1.12$). Strongly positive allometry was found in FSDF, LRDF, LRAF and CFR in males from 45 to 80 mm SL, indicating a rapid increase of each part relative to SL. In males ≧80 mm SL, only LRAF continued to show positive allometry, the other dimorphic elements being characterized by isometry or negative allometry. Males <45 mm SL were omitted from the analysis because of the low number of specimens.

Three patterns of black spots were observed on the first dorsal fin in males: A) a single large well-defined black spot between the 3rd and 4th spines, as in adult females; B) a large poorly-defined black spot and several small black spots; C) several small black spots only (Fig. 6). All males <40 mm SL had color pattern A. Pattern B appeared in the 40 to 80 mm SL class, the smallest male with B measuring 44.5 mm SL. All males over 80 mm SL had pattern C (Fig. 7).

Discussion

Annual reproductive cycle

Repomucenus valenciennei spawned from April (1991) or February (1992) to October (i.e. from spring to autumn), the proportion of spawning females being low in July (1990) and August (1990 and 1991). Two spawning peaks (spring and autumn) were apparent.

Repomucenus huguenini in Tosa Bay, Japan (Gonzales et al., 1997) and *R. beniteguri* in Lake Hamana, Japan (Zhu et al., 1989b) are characterized by two spawning seasons, spring and autumn. Zhu et al. (1989b) suggested that *R. beniteguri* may undergo ovarian regression, induced by high summer tempera-

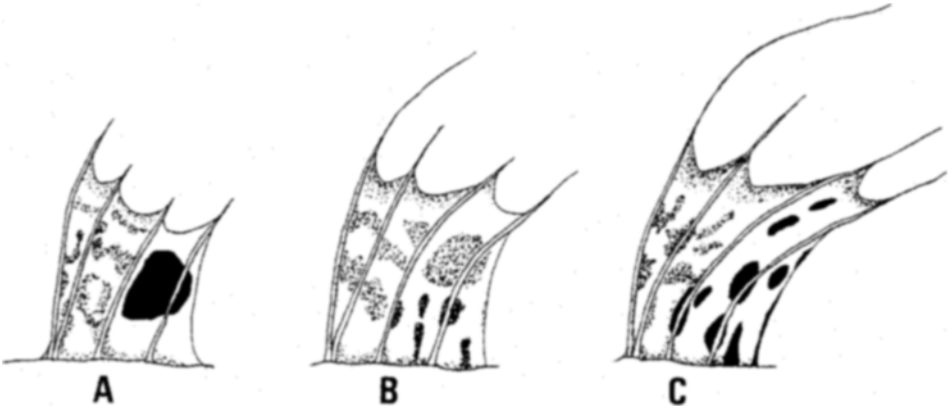

Fig. 6. Diagrammatic illustrations of three first dorsal fin color patterns in male *Repomucenus valenciennei*.

tures, and possibly spawned during summer months in the open sea, where water temperatures were lower. According to this view, *R. valenciennei* might be expected to have two separate spawning seasons in areas of maximum water temperature higher than that in Tokyo Bay (e.g. southern part of geographic distribution of the species). In this study, increased occurrence of atretic oocytes in the summer months coincided both with decreased mean GSI values and proportions of spawning females, and rising water temperatures, as observed in *R. beniteguri* (Zhu et al., 1989b).

Table 1. Regression equations, $\log y = a + b \log x$, for the relative growth of six body parts to standard length in *Repomucenus valenciennei*

Variables	*n*	a	b	r^2	*t*
Female: Total					
SN	110	−1.07	1.02	0.963	1.074
PF	110	−0.607	1.02	0.976	1.546
FSDF	110	−1.05	1.19	0.953	7.450***
LRDF	110	−1.56	1.39	0.973	17.34***
LRAF	110	−1.33	1.23	0.965	10.10***
CFR	110	−0.63	1.07	0.977	4.440***
Male: Total					
SN	154	−1.09	1.04	0.963	2.329*
PF	154	−0.756	1.12	0.977	3.354***
Male: 45≦SL<80mm					
FSDF	68	−3.45	2.70	0.786	9.715***
LRDF	70	−3.15	2.38	0.791	9.229***
LRAF	70	−2.08	1.69	0.766	6.051***
CFR	65	−1.89	1.82	0.802	7.124***
Male: 80 mm≦SL					
FSDF	39	3.36	0.619	0.450	3.342**
LRDF	72	−0.905	1.22	0.552	1.676
LRAF	72	−1.44	1.39	0.634	3.069**
CFR	71	−0.078	0.910	0.359	0.615

Males <45 mm SL omitted from analysis owing to low number of specimens. *n*, sample size; *t*, *t* value of *t*-test for null-hypothesis of slope, b=1. $^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$. Where b=1, growth classified as isometry. Where b>1 or b<1, growth classified as positive or negative allometry, respectively.

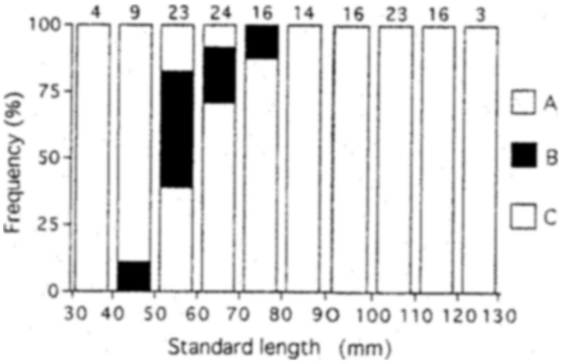

Fig. 7. Length-frequency distributions of first dorsal fin color patterns in male *Repomucenus valenciennei* (see Fig. 6). Sample numbers above each column.

The spawning seasons of three Australian dragonets of genus *Repomucenus* (after Nakabo, 1982) have been reported. *R. sublaevis* and *R. calcaratus* have spring and autumn spawning seasons (Johnson, 1973), whereas *R. belcheri* has only an autumn spawning season (Johnson, 1972). Two spawning seasons or spawning peaks may therefore be the norm for most *Repomucenus* species.

Most callionymid species other than *Repomucenus* have been reported as having longer spawning periods. *Diplogrammus xenicus* (Fricke and Zaiser, 1982) and *Synchiropus moyeri* (Zaiser and Fricke, 1985) spawn from June to October and from May to October in Miyake-Jima, Japan, respectively, and *Calliurichthys japonicus* spawns from June to September in Tosa Bay, Japan (Gonzales et al., 1997). *Callionymus maculatus* spawns from April to September in Scotland (Gibson and Ezzi, 1979) and *C. lyra* from January to August in Galway Bay, Ireland (King et al., 1994).

Repomucenus valenciennei ovaries were found to include oocytes of various stages, from peri-nucleolus to ripe (Fig. 2B), suggesting serial spawning. Takita and Okamoto (1979) found that aquarium-held *R. valenciennei* spawned daily. Similarly, captive *R. beniteguri* spawned every day during the spawning season (Zhu et al., 1991), a group of oocytes developing daily from the vitellogenic stage to the final maturation stage, followed by ovulation (Zhu et al., 1989a). Accordingly, we defined the final stage of ovarian maturation and subsequent ovulation as the 'spawning' phase.

The size range of spawning females changed during the spawning periods, only larger females (>80 mm SL in 1991, or >70 mm in 1992) spawning in spring, whereas most females>60 mm SL spawned in September. Ikejima and Shimizu (1996) showed that *R. valenciennei* lives up to two years, year class juveniles being recruited during autumn (September to November) and dying by the autumn of the second year following. Two age classes were distinguishable by size. Females ≧80 mm and <80 mm SL caught between April and July 1991 were recognized as age 1+ (1989 year class) and 0+ (1990 year class), respectively. Therefore, only age 1+ females spawned in spring, with fast growing individuals of age 0+ (>70 mm SL) spawning from June. The younger age class (0+) reached 72 mm (mean SL) (exceeding the minimum mature size-60 mm SL) in autumn (September and October), at that time attaining age 1+. Accordingly, both year classes spawned in autumn. Males attained full sexual maturity by 80 mm SL (age 1+ according to Ikejima and Shimizu [1996]).

Most females of the older age class (1+ in spring) spawned in spring and autumn, suggesting that the same individuals spawned in both seasons. Zhu et al. (1991) reported that the same individuals of *R. beniteguri* spawned in both spring and autumn of the same year.

Sexual dimorphism

From the growth of fin spines and rays, and changes in dorsal fin color, it was concluded that secondary sexual characters of males started to develop at about 45 mm SL, being completed by about 80 mm SL. FSDF, LRDF and CFR length in males rapidly increased relative to SL during this period, the rate of increase thereafter slowing. The first spine of the first dorsal fin (FSDF) and first ray of the second dorsal fin showed a similar growth pattern in *Callionymus maculatus*, in which the critical SLs at which changes in the growth rates of dorsal fin spines and rays occurred closely corresponded to the onset and completion of sexual maturity (Gibson and Ezzi, 1979). In this study, only a few small specimens were used for the examination of testes, most males appearing to be fully sexually mature by 80 mm SL, coincident with the completed development of their secondary sexual characteristics.

Many other dragonets also show sexual dimorphism in spines, rays, color and size, males growing faster and larger than females (Chang, 1951; Johnson, 1972, 1973; Gibson and Ezzi, 1979; King et al., 1994; Ikejima and Shimizu, 1996). Common reproductive behavior has been reported in the family (Takita and Okamoto, 1979; Takita et al., 1983; Thresher, 1984; Moyer, 1994; Gonzales et al., 1996). A male begins courtship with display behavior, holding all fins erect in front of a female, and ends with pair spawning. Fighting has been observed only between males, larger males being dominant. Erect fins were also displayed during fighting. Altogether, these point to the larger body, elongated fin spines and rays, and fin and body color as playing important roles to dominate in reproduction.

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