Reproductive biology of female Rikuzen sole (*Dexistes rikuzenius*)*

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Abstract—The annual ovarian cycle, mode of maturation, age at maturity, and potential fecundity of female Rikuzen sole (*Dexistes rikuzenius*) from the North Pacific Ocean off the coast of Japan were studied by 1) histological examination of the gonads, 2) measurement and observation of the oocytes, and 3) by otolith aging. The results indicated that ovulation occurs from September to December and peaks between September and October. Vitellogenesis began again soon after the end of the current season. Maturity was divided into eight phases on the basis of oocyte developmental stages. Mature ovaries contained developing oocytes and postovulatory follicles but no recruiting oocytes, indicating that this species has group-synchronous ovaries and is a multiple spawner. Almost all females matured first at an age of 1+ year and spawned every year until at least age 8+ years. Potential fecundity increased exponentially with body length and the most fecund fish had 15 times as many oocytes as the least fecund fish. Potential fecundity and relative fecundity were both positively correlated with age from 1 to 6+ years, but were negatively correlated, probably because of senescence, in fish over 7 years. These results emphasize that the total productivity of a *D. rikuzenius* population depends not only on the biomass of females older than 1+ but also on the age structure of the population.

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To understand fish population dynamics, reproductive information, such as the maturation of oocytes, the size and age at first maturity, and fecundity, is indispensable. Gonadal maturation is determined from the external appearance of the gonads, the gonadosomatic index, and oocyte size, or from observations of histologically prepared gonads (West, 1990). With the former two methods it is possible to measure samples in the field and to record data on numerous samples in a short period of time; however, the mode of oocyte development can only be clarified by using observations of histologically prepared gonads (Wallace and Selman, 1981). The methods used to determine if an individual has spawned and to measure the number of eggs spawned in the current reproductive season differ with the mode of oocyte development (West, 1990).

In fishery models, reproductive potentials are conventionally represented by spawning stock biomass (Ricker, 1954; Beverton and Holt, 1957; Trippel et al., 1997). However, at the population level spawning stock biomass does not always correlate with egg productivity. Length at first maturation, the frequency of occurrence of degenerated oocytes, and fecundity (that is, the total number of offspring produced in a reproductive season by an individual female) are closely related to the age and energetic conditions of an individual (Hunter and Macewicz, 1985a; Horwood et al., 1986, 1989; Trippel et al., 1997; Sampson and Al-Jufaily, 1999; Kurita et al., 2003). Therefore, examination of age and body size in relation to fecundity is useful in determining the abundance of eggs laid in a population.

Oocyte development can be divided into three types (Wallace and Selman, 1981). In determinate fecundity, fecundity is fixed before spawning starts, such as in species which have synchronous or group-synchronous ovaries. In indeterminate fecundity (i.e., for those species whose ovaries develop asynchronously), unyolked oocytes grow to maturity after the onset of spawning (Hunter and Macewicz, 1985b; Hunter et al., 1992). In addition, the development of oocytes can vary even among populations of a single species (Sampson and Al-Jufaily, 1999) and some females classified as maturing or mature by external observation are often actually immature, and vice versa (Hunter et al., 1992; Zimmermann, 1997). Hence, with a species or a population for

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which little information is available, it is important to determine specific reproductive traits by using the most accurate methods and to compare the results with those of simpler methods.

Rikuzen sole (*Dexistes rikuzenius*) (also known as Rikuzen flounder, FAO) is a coastal flatfish that lives at depths of 100 to 360 m in the waters off the south coast of southern Hokkaido, Japan, and the southern Korean Peninsula (Sakamoto, 1984). It inhabits sandy bottoms and preys mainly on benthic invertebrates (Fujita et al., 1995). It is relatively abundant in the North Pacific off the coast of Japan and is an important fishery resource for bottom trawlers (Ishito, 1964; Ogasawara and Kawasaki, 1980). The commercial catch of flatfish such as the Rikuzen sole has fluctuated widely in this area over the past few decades (Anonymous, 2002), and therefore fisheries management is needed to maintain stable and appropriate fish-density levels.

In addition to fisheries, various internal and external conditions may affect the fluctuations in abundance of fish populations. Understanding reproductive traits, or survival in the early life stages, is a step toward revealing population dynamics. Although both sexes have indeterminate growth trajectories, conspicuous sexual dimorphism occurs during the growth and life span of Rikuzen sole. Females are larger at any given time after age 1+ and live longer than males (Ishito, 1964). The spawning period of the Sendai Bay population occurs from late October to late January and peaks from November to December (Ogasawara and Kawasaki, 1980). Using measurements of oocyte diameter and the appearance of the whole ovary, Ogasawara and Kawasaki (1980) revealed that females spawn several batches of eggs during one spawning season. However, because histological observations of the gonads have not been conducted, details of the reproductive biology, such as annual cycle of oocyte development, and body size and age at maturity, have not been determined. In addition, no information about fecundity has been reported.

We examined the oogenesis of Rikuzen sole caught in the North Pacific Ocean off the coast of Japan over a period of one year. The aim was to determine the mode of maturation, annual reproductive cycle, and age at first maturity based on histological examinations, age determinations from otolith growth increments, and gonadosomatic indices (GSIs). Using these results, we were able to estimate body size and age-related potential fecundity and were able to develop a simpler method for determining potential fecundity.

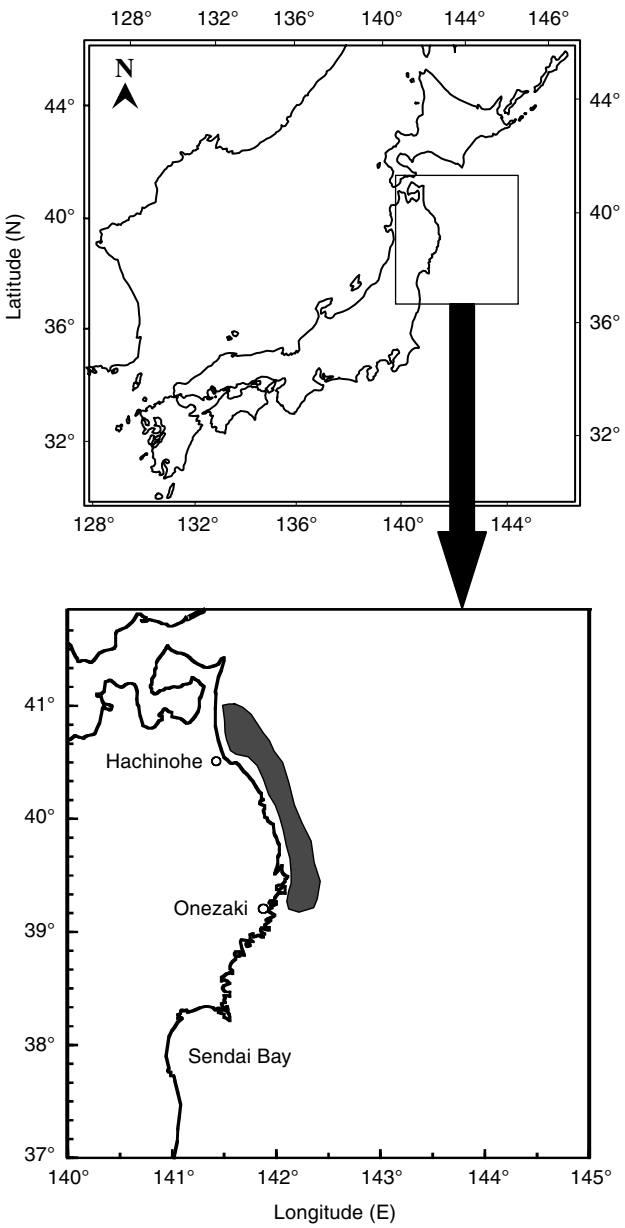

Figure 1

Catch area for Rikuzen sole (*Dexistes rikuzenius*) in the Northern Pacific Ocean off the northeast coast of Japan, 2000–2001.

Materials and methods

From May 2000 to April 2001, except for July and August when commercial bottom trawl fishing was prohibited, Rikuzen sole samples were collected once or twice a month from the fisheries market in Hachinohe, Aomori Prefecture, Japan. All samples were caught by bottom trawl nets in the coastal waters off Shitsukari (41°22′, 141°33′E) and Hachinohe (40°43′N, 144°44′E), from depths of 70–300 m (Fig. 1). During July and August, samples were collected with bottom long lines off the coast at Onezaki (39°12′N, 141°56′E) from a depth of 85–109 m.

A total of 1031 females were collected and their standard lengths (SL) to the nearest mm, total body weights, eviscerated body weights, and ovary weights to the nearest 0.1 g were measured. The GSI and body condition (BC) of each specimen were calculated with the following formulas: GSI = (*gonad weight/eviscerated body weight*)×100, and BC = (*eviscerated body weight*/SL^3)×100. Ovaries and sagittal otoliths were removed

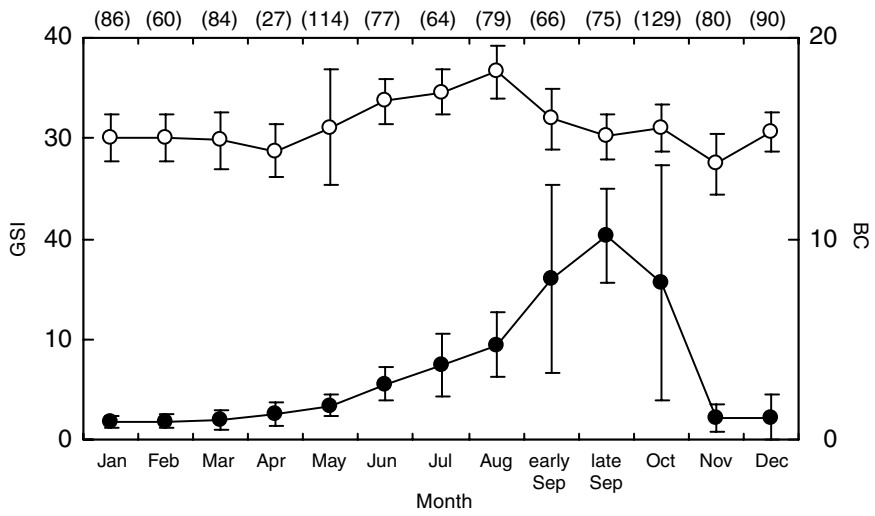

Figure 2

Annual changes in the gonadsomatic index (GSI) and body condition (BC) values of female Rikuzen sole (*Dexistes rikuzenius*). Solid and open circles show the mean values of GSI and BC, respectively. Vertical bars represent the standard deviations of these means. Sample numbers are shown in brackets.

within a day after each catch for histological observations and age determination, respectively. The otoliths were washed with distilled water and left to dry until preparation for age determination. Ovaries were fixed in 10% buffered formalin for 24 hours. The middle portions of eyed-side ovaries of 309 specimens were extracted, dehydrated, embedded in paraffin, sectioned at 8 μm, and stained with Mayer's hematoxylin and eosin (HE) and periodic acid Schiff (PAS).

Prepared sections were examined under a light microscope. The oocytes were then divided into eight stages according to the guidelines of Yamamoto (1956). Postovulatory follicles (POFs), which indicate spawning experience, were also examined. New POFs are easily identifiable, but those that have degenerated are difficult to distinguish from atretic follicles. In our study, only those that could be easily identified were defined as POFs. Atretic oocytes, namely advanced yolked oocytes that have been resorbed into the ovaries, were also determined; similarly, only those easily identifiable were defined as atretic oocytes. The percentage of advanced oocytes that were atretic was determined monthly for 10 randomly selected 2–7+ year-old fish (body size range: 143–210 mm SL).

Maturity was classified by the stage of the most advanced oocyte and the presence of POFs. By observing maturity and advanced oocyte diameter, we tested 15 ovaries for possible differences in oocyte development between anterior, middle, and posterior positions in the eyed-side ovary lobe, and between eyed-side and blind-side ovary lobes.

Oocyte diameter distributions in the late vitellogenic maturity phase were examined; the reason this maturity phase was selected is described in the "Results" section. The diameters of 50 randomly selected oocytes, extracted from the middle portions of the ovaries, were measured under a dissecting microscope to the nearest 20 μm. Potential fecundity was estimated with the gravimetric method by using ovaries in the late vitellogenic maturity phase. Extracted ovaries were rinsed and then weighed to the nearest 0.0001 g, and only developing oocytes, whose size is also described in the "Results" section, were counted.

Age was determined for all fish samples. Blind-side otoliths were used for the analyses according to the methods of Ishito (1964). The lateral surfaces of the otoliths were polished with 1500-grit sand paper until the transparent zones were visible. Ishito (1964) revealed that one transparent zone is formed at the edge of the otolith each winter and suggested that this may be regarded as an annual mark. However, the most interior ring appears when fish are aged 0+ (Ishito, 1964); therefore the number of transparent zones minus the 0-year-old zone was the formula used for aging, and the relationship between age and potential fecundity was analyzed.

Results

Annual changes in gonadosomatic index and body condition

The annual changes in gonadosomatic index (GSI) and body condition (BC) are shown in Figure 2. The GSI was

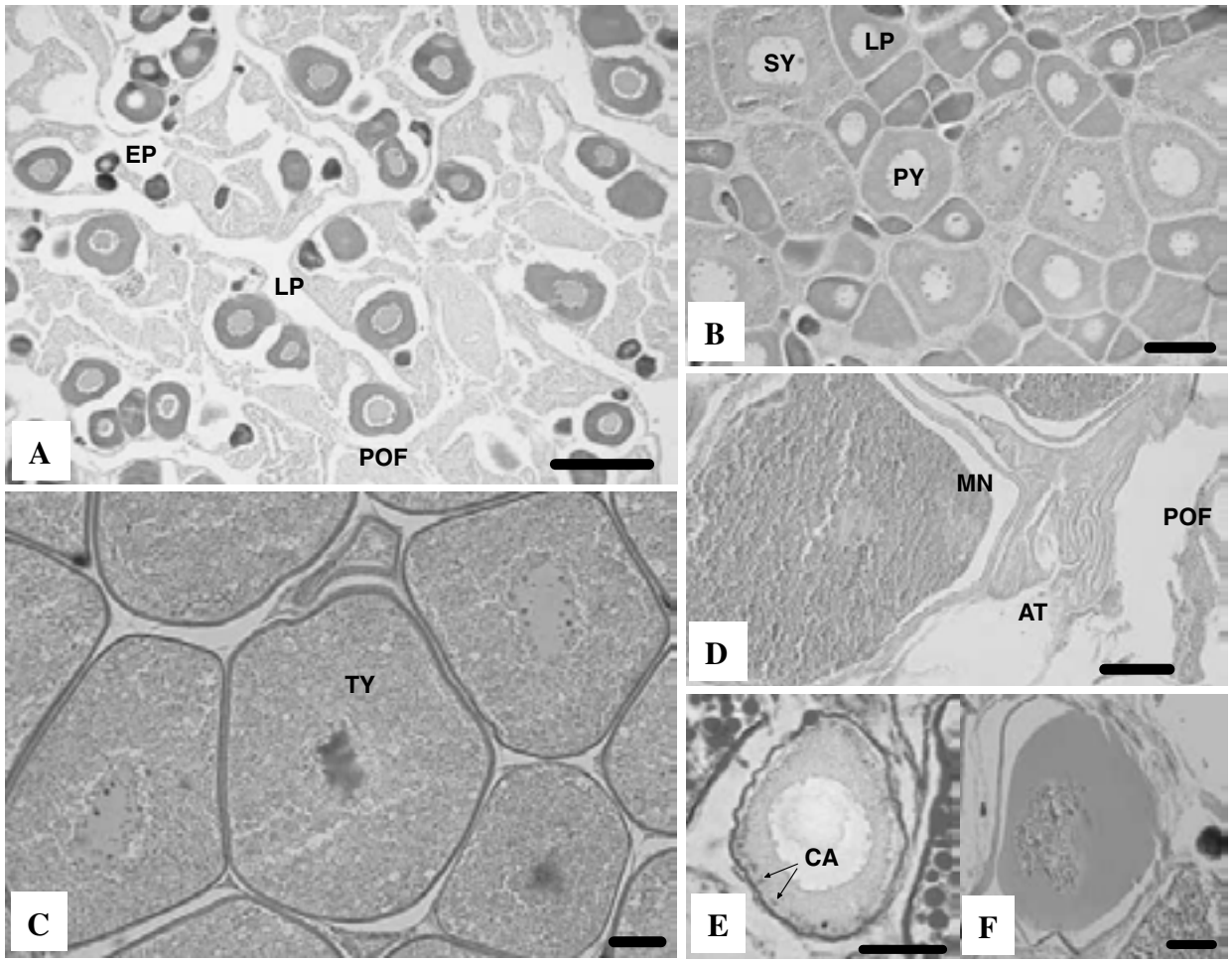

Figure 3

Histology of the ovarian maturity and oocyte developmental stages of Rikuzen sole (*Dexistes rikuzenius*). (**A**) Spent phase. Bar: 200 μm. (**B**) Middle vitellogenic phase. Bar: 200 μm. (**C**) Late vitellogenic phase. Bar: 200 μm. (**D**) Maturity phase. Bar: 200 μm. (**E**) Oocyte at the cortical alveolus stage. Bar: 50 μm. (**F**) Oocyte at the premature stage. Bar: 50 μm. EP=early perinucleolus stage, LP=late perinucleolus stage, CA=cortical alveolus, PY=primary yolk stage, SY=secondary yolk stage, TY=tertiary yolk stage, MN=migratory nucleus stage, AT=atretic oocyte, POF=postovulatory follicle.

relatively low, less than 2.0, from January to March, increased steeply from April to August, and progressed to more than 15.0 during September to October. Values then rapidly decreased from October to November. The BC was low from January to April, increased to a maximum value of 18.3 in August, and then decreased to 13.7 in November.

Histological observations of oocyte development

Although oogenesis is continuous, in order to explain the developmental process, oocyte development was divided into eight stages, basically according to Yamamoto (1956) (Fig. 3). The characteristics of oocytes, cell and nuclear diameters, and time of occurrence of each stage of oocyte and POF are shown in Table 1.

Maturity

Ovary maturity did not differ among positions in the ovarian lobe or between eyed-side and blind-side lobes. In addition, the diameters of the largest oocytes did not vary significantly among positions (ANOVA, $F_{2, 42}$= 0.354, P=0.704) or between lobes (paired t-test, f=14, t=0.058, P=0.955). Therefore, maturity was determined from observations of the middle portions of eyed-side ovaries.

Maturity was classified into eight phases, the characteristics of which are shown in Table 2. Because oocytes younger than the late perinucleolus stage occurred throughout the year, maturity was determined as occurring from this phase onwards. GSI values significantly varied among maturity phases (ANOVA, $F_{4,205}$,

Table 1

Characteristics, cell and nuclear diameters, and occurrence of oocytes and postovulatory follicles at each developmental stage. Developmental stage and measurements were determined by histological observations (EP=early perinucleolus, LP=late perinucleolus, CA=cortical alveoli, PY=primary yolk, SY=secondary yolk, TY=tertiary yolk, MN=migratory nucleus, PM=prematuration, POF=postovulatory follicle). HE=hematoxylin and eosin; PAS=periodic acid Schiff.

Developmental stage	Characteristics	Cell diameter (μm)	Nuclear diameter (μm)	Occurrence
EP	The ooplasm is strongly stained by haematoxylin. Several basophilic nucleoli stained by hematoxylin are present inside the nuclear membrane.	20–70	10–35	year round
LP	The ooplasm increases in volume with growth of the oocyte and becomes less basophilic than that of the previous EP stage.	70–150	35–80	year round
CA	Cortical alveoli, which appear in the ooplasm, are seen as a small empty spherical structure with conventional HE preparations, and are stained reddish by PAS reagents.	160–200	80–120	Feb, Jun, Aug, Oct
PY	The yolk granule occurs at the periphery of the oocytes.	180–220	80–120	year round
SY	The yolk granule increases in number and occurs towards the nuclear membrane, and the ooplasm occurs slightly at the periphery of the nuclear membrane.	260–440	90–130	Jan to Oct
TY	The oocyte is characterized by occupancy of the total volume of the oocyte by a yolk granule. The nucleus of the oocyte is still located at the center of the oocyte.	420–680	120–170	May to Dec
MN	The germinal vesicle migrates to the periphery of the oocyte and becomes elongated and globular in shape.	600–740	130–190	Sep to Dec
PM	The germinal vesicle has broken down. Yolk granules fuse with each other, and are stained light pink by eosin.	620–800	—	Sep to Nov
POF	The POF, containing granular, is a convoluted folded shape.	—	—	Sep to Jan

F=124.1, P<0.0001) and became significantly higher in each successive stage of maturity (Fisher's PLSD test, P<0.05), except for the first two phases (P=0.687). The mature- and spent-phase ovaries were excluded from the test because their values fluctuated depending on spawning times or the degree of POF absorption.

Ovaries in the late vitellogenic maturity phase, which occurred from May to September, contained oocytes in the tertiary yolk stage, secondary yolk stage, cortical alveoli stage, and late and early perinucleolus stages, but not in the primary yolk stage (Table 3). Ovaries in the premature phase, which occurred from September to October, also revealed two peaks and a hiatus in oocyte developmental composition. As described before, ovaries with POFs also contained maturing oocytes. These results show that this species is a multiple-spawner and has group-synchronous ovaries (Wallace and Selman, 1981; Takano, 1989); therefore, fecundity is fixed before spawning starts.

On the other hand, ovaries in the mid-vitellogenic phase were observed from January to September and contained oocytes in the secondary and primary yolk stages, and in the late perinucleolus stage. Cortical alveoli are very small and were present in only 10 of the 309 ovaries observed in our study. It is possible that the duration of this stage is very short. Therefore, in the ovaries oocytes do not divide into two groups, those that spawn in the next reproductive season and those that do not, until they have progressed to the late vitellogenic maturity phase.

Oocyte composition

Table 3 shows the annual changes in oocyte composition. One ovary observed in January contained POFs and perinucleolus stage oocytes, whereas the others contained oocytes in the primary and secondary yolk stages. Of those observed from February to April, none contained POFs. Frequency of occurence of ovaries with secondary yolk-stage oocytes increased during the season. From May to August the most advanced oocyte observed was in the tertiary-yolk vitellogenic stage, and the frequency of this stage also increased in number throughout this season. Migratory-nucleus–stage and

Table 2

The characteristics, occurrence and gondadosomatic index values of each maturity phase. Developmental oocyte stages were abbreviated as follows: EP=early perinucleolus, LP=late perinucleolus, CA=cortical alveoli, PY=primary yolk, SY=secondary yolk, TY=tertiary yolk, MN=migratory nucleus, PM=prematuration, POF=postovulatory follicle. *n*=number of samples. PAS=periodic acid Schiff.

Maturity phase	Characteristics	The most advanced oocyte observed	Occurrence	GSI (mean±SD)	*n*
Immature	Ovaries contain only EPs and LPs, but not POFs.	LP	Jan to Apr	1.57 ±0.34	13
Previtellogenic	This phase can be discriminated by PAS staining. Specimens in this phase were scarce.	CA	Feb	1.67	1
Early vitellogenic	Ovaries consist of PY and unyolked oocytes, and occur prevalently from March to April.	PY	Jan to Oct	2.09 ±0.61	41
Mid-vitellogenic	Ovaries contain SY and all stages of oocytes younger than the SY stage.	SY	Jan to Oct	4.40 ±1.72	61
Late vitellogenic	Ovaries contain TY and all stage oocytes younger than TY, but not SY.	TY	May to Dec	12.98 ±5.74	67
Premature	Ovaries lack PY and SY, occurs prevalently during September.	MN or PM	Sep to Dec	17.56 ±5.00	28
Mature	Ovaries contain both empty follicles and oocytes that have advanced beyond the secondary yolk stage.	advanced more than SY	Sep to Dec	16.21 ±8.34	25
Spent	Ovaries contain empty follicles but oocytes that have advanced beyond the secondary yolk stage are absent.	LP	Sep to Jan	2.71 ±2.41	73

premature-stage oocytes and POFs began to occur in September. The composition of oocytes observed during this month was divided into three groups: premature, maturing, and postmature oocytes. In October, almost all ovaries (96.2%) contained POFs. Of these, 28.0% also contained oocytes at the tertiary yolk stage or migratory-nuclear stage (or at both stages) and the remaining 72.0% contained primary-yolk stage or less advanced stage oocytes (or both of these stages). From November to December, all ovaries contained POFs and only a few (3.7% in November and 5.3% in December) also contained vitellogenic oocytes. Therefore, almost all individuals had finished spawning by October, although a few continued to spawn until December.

Atretic oocytes were found in samples throughout the year, except February, in ovaries of various maturity phases. Frequency of occurrence was highest in May, and gradually decreased until the spawning season (Table 3). Oocytes that ovulated but remained in the ovigenous folds and were resorbed later were treated as atretic oocytes because it was difficult to distinguish between them and atretic oocytes if they were somewhat absorbed. Atretic oocytes did not always correspond to the most advanced oocytes in the ovaries. They occupied 0.3–1.8% (mean ±SD=1.0 ±0.5) of the yolked, advanced oocytes observed in the ovaries in May (the number of oocytes counted in 10 ovaries ranged from 117 to 615), and from 0.4 to 1.8% (1.0 ±0.4) of those observed in August (range: 108–383 oocytes in 10 ovaries).

Body length and age at first maturity

The relationship between SL or age and maturity of the fish caught between the prespawning month (August) and the late-spawning month (December) was examined. Otolith growth increments were counted for all specimens. Because the spawning season occurs from September to December, the birth dates of all fish were conveniently defined as 1 January; age was then determined accordingly. SL ranged from 114 to 237 mm (*n*=189, 170.6 ±25.3) and age, from 1 to 8+ years (2.8 ±1.4). Individuals grew steeply until 2 years and moderately until 6 years, after which time their growth was slow (Fig. 4). All females whose ages were estimated at more than 2+ years (*n*=152) were identified as maturing or spent-stage females. Only one 1+ year-old specimen (131 mm SL) was classified as immature, whereas the other 1+ specimens (*n*=36, 140.0 ±11.8) were classified as maturing or postmaturation females (Fig. 4).

Potential fecundity

The diameters of oocytes in late vitellogenic maturity phase ovaries were measured because potential fecundity was determined as occurring before this maturity phase. Oocytes ranged in diameter from less than 100 to 950 μm and were separated into a small (less than 200 μm) or large group (larger than 300 μm, Fig. 5).

Table 3

Annual changes in the composition of female Rikuzen sole oocytes in each maturity phase. Some maturing and spent ovaries contained ovulated but not spawned oocytes. Such ovaries were included under "Number of samples with atretic oocytes." Developmental oocyte stages were abbreviated as follows: EP=early perinucleolus, LP=late perinucleolus, CA=cortical alveoli, PY=primary yolk, SY=secondary yolk, TY=tertiary yolk, MN=migratory nucleus, PM=prematuration, POF=postovulatory follicle.

Year	Month	EP	LP	CA	PY	SY	TY	MN	PM	POF	*n*	Maturity phase	Number of samples with atretic oocytes
2000	May	+	+		+						4	Early vitellogenic	4
		+	+		+	+					19	Mid vitellogenic	15
		+	+			+	+				1	Late vitellogenic	1
	Jun	+	+		+	+					18	Mid vitellogenic	10
		+	+	+	+	+					1	Mid vitellogenic	0
		+	+			+	+				3	Late vitellogenic	2
	Jul	+	+		+	+					7	Mid vitellogenic	2
		+	+			+	+				4	Late vitellogenic	1
	Aug	+	+		+						1	Early vitellogenic	0
		+	+	+	+	+					1	Mid vitellogenic	0
		+	+		+	+					4	Mid vitellogenic	1
		+	+			+	+				33	Late vitellogenic	9
		+	+				+				1	Late vitellogenic	0
	early Sep	+	+		+	+					2	Mid vitellogenic	1
		+	+			+	+				4	Late vitellogenic	0
		+	+				+				6	Late vitellogenic	2
		+	+	+			+	+			1	Premature	0
		+	+				+	+			8	Premature	1
		+	+				+	+	+		2	Premature	1
		+	+							+	4	Spent	1
		+	+	+						+	3	Spent	1
		+	+				+	+		+	11	Mature	3
		+	+				+	+	+	+	2	Mature	0
	late Sep	+	+			+	+				3	Late vitellogenic	0
		+	+	+			+				1	Late vitellogenic	0
		+	+				+				12	Late vitellogenic	2
		+	+	+			+	+			1	Premature	0
		+	+				+	+			12	Premature	1
		+	+				+	+	+		2	Premature	1
		+	+				+	+		+	3	Mature	2
	Oct	+	+				+	+			1	Premature	0
		+	+							+	10	Spent	4
		+	+	+						+	1	Spent	0
		+	+		+					+	7	Spent	4
		+	+				+			+	2	Mature	1
		+	+				+	+		+	5	Mature	3
	Nov	+	+							+	24	Spent	15
		+	+		+					+	2	Spent	1
		+	+				+	+		+	1	Mature	1
	Dec	+	+							+	13	Spent	5
		+	+		+					+	5	Spent	1
		+	+				+	+		+	1	Mature	0

continued

Those in the large-diameter group were regarded as advanced yolked oocytes that would be spawned in the next reproductive season and were used for estimations of potential fecundity. Potential fecundity varied widely among individuals from 24,765 (114 mm SL) to 393,212 (204 mm SL) eggs (an average of 161,340 ±90,688 eggs (165 ±25 mm SL)). Potential fecundity (PF) was positively correlated with body size and the relationship was expressed by the following equation:

$$PF = 0.000235SL^{3.96} \quad \text{(Fig. 6)}.$$

Potential fecundity and relative fecundity (potential fecundity/eviscerated body weight) increased with

Table 3 (continued)

Year	Month	EP	LP	CA	PY	SY	TY	MN	PM	POF	*n*	Maturity phase	Number of samples with atretic oocytes
2001	Jan	+	+								2	Immature	1
		+	+		+						8	Early vitellogenic	0
		+	+		+	+					2	Mid vitellogenic	0
		+	+							+	1	Spent	0
	Feb	+	+								5	Immature	0
		+	+	+							1	Previtellogenic	0
		+	+		+						5	Early vitellogenic	0
		+	+		+	+					1	Middle vitellogenic	0
	Mar	+	+								3	Immature	0
		+	+		+						15	Early vitellogenic	2
		+	+		+	+					1	Mid vitellogenic	1
	Apr	+	+								3	Immature	0
		+	+		+						10	Early vitellogenic	3
		+	+		+	+					6	Mid vitellogenic	1
	Total										309		104

growth at age ≤6+ years and decreased at ≥7+ years (Fig. 7). Comparisons of the relative fecundity among age groups (1–2+, 3–4+, 5–6+, and 7–8+) revealed significant differences with age (ANOVA, $F_{3,38}$=7.431, P<0.0005). In addition, *post hoc* tests (Fisher's PLSD, P<0.05) revealed significant differences between the following age groups: 1–2+ and 3–4+, 1–2+ and 5–6+, and 5–6+ and 7–8+. The GSI and BC values of individuals aged ≥7+ years were also lower than those of individuals aged 5+ and 6+ years, but the differences were not significant (*aq*-test, P>0.05); however, the sample size was very small; therefore the tests have little power.

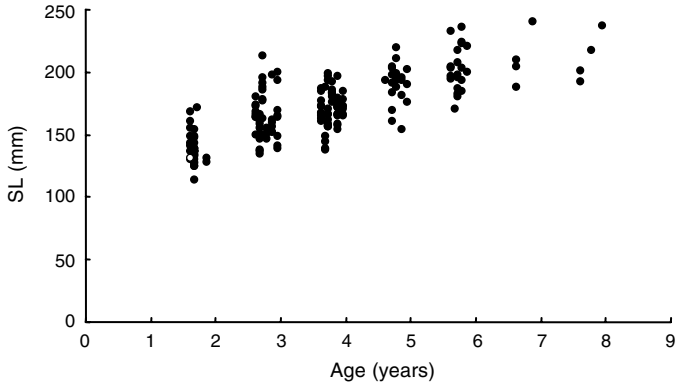

Figure 4

Relationship between age, including maturity, and the standard length of Rikuzen sole (*Dexistes rikuzenius*) caught between August and December. Solid circles represent maturing or spent individuals and the open circle at age 1+ represents an immature individual.

Discussion

Gonadal maturation

GSI and histological examinations showed that oocytes develop rapidly from May to August and that the reproductive season lasts from September to December; mainly from September to October in the study area. Mature females in the Sendai Bay area were also observed for four months, but the reproductive season in this area occurs from October to January and peaks in November (Ogasawara and Kawasaki, 1980), which was later than the peak documented in the present study for the area off the Hachinohe coast. The Sendai Bay catch area was located at a lower latitude (37°00′N–38°05′N; Ogasawara and Kawasaki, 1980) than that of the Hachinohe study area (Fig. 1); this difference is relevant because gonadal maturation is usually dependent on water temperature (Kruse and Tyler, 1983; Asahina and Hanyu, 1983; Conover, 1990). In 2000, the water temperature in the Hachinohe study area decreased faster than that of Sendai Bay in 1977 and 1978 when studied by Ogasawara and Kawasaki (TNFRI[1]). These results indicate that gonadal maturation in Rikuzen sole also depends on water temperature.

[1] TNFRI (Tohoku National Fisheries Research Institute). 2004. Unpubl. data. Water temperature data. Tohoku National Fisheries Research Institute, Fisheries Research Agency of Japan. Shiogama City, Miyagi Prefecture 985-0001 Japan.

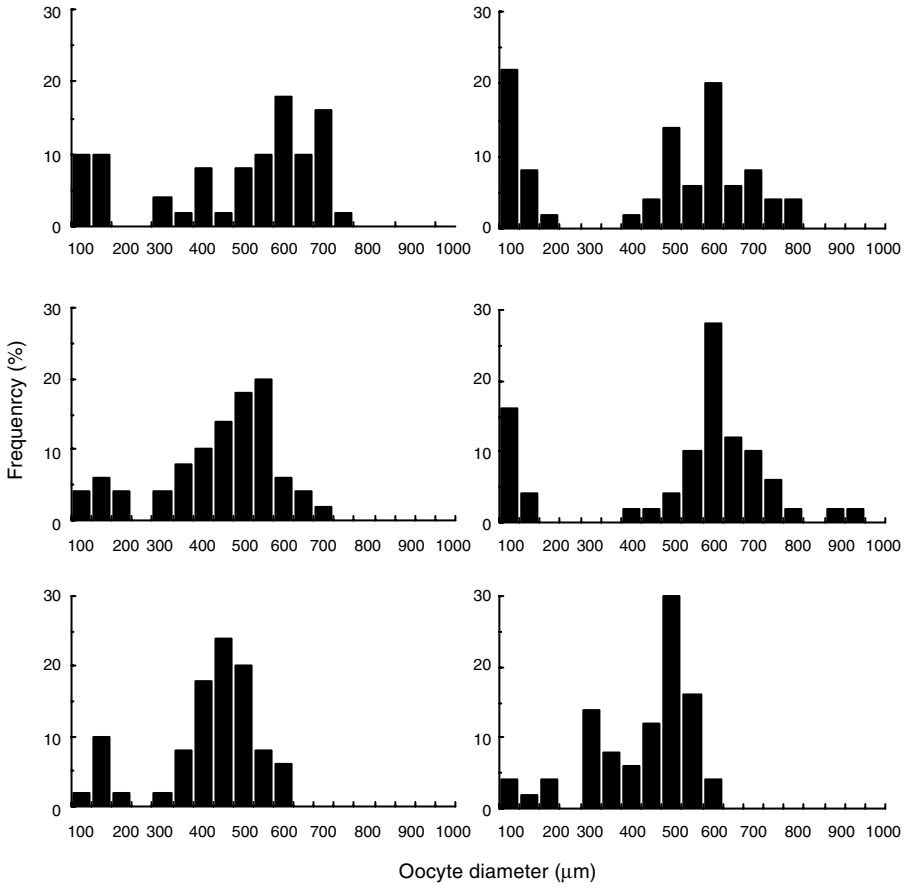

Figure 5

Oocyte diameter distributions just before the spawning season of Rikuzen sole (*Dexistes rikuzenius*). Oocyte diameter was divided into small-scale (less than 200 μm) and large-scale groups (more than 300 μm).

Rikuzen sole require a long period of time for vitellogenesis and therefore the reproductive cycle may differ among areas.

In some flatfishes, it has also been reported that oocytes in the cortical alveoli stage appear for only a short period of time because they develop rapidly into the primary yolk stage (Yamamoto, 1956; Janssen et al., 1995). In the present study, only a small percentage of individuals contained this stage of oocytes; however, cortical alveoli were present throughout various months from June to October and in February. These results are similar to results for other flatfish and may indicate that the absence of cortical alveoli oocytes in some ovaries does not represent an incontinuity of oocyte composition.

From October to December some females possessed primary yolk-stage oocytes,

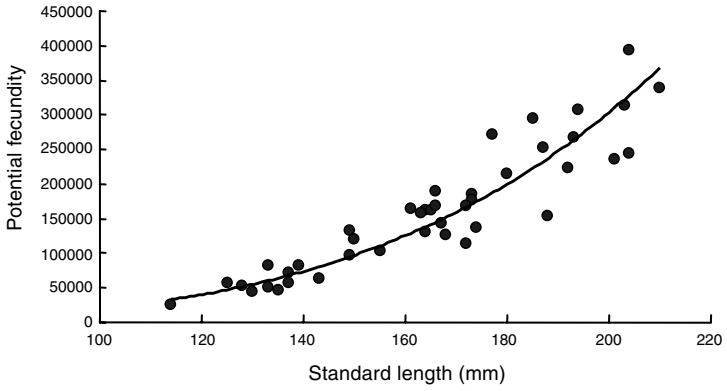

Figure 6

Relationship between the standard length and potential fecundity of Rikuzen sole (*Dexistes rikuzenius*). Potential fecundity was measured only for advanced yolk oocytes in late vitellogenic maturity phase ovaries. The equation of the regression curve is shown in the text.

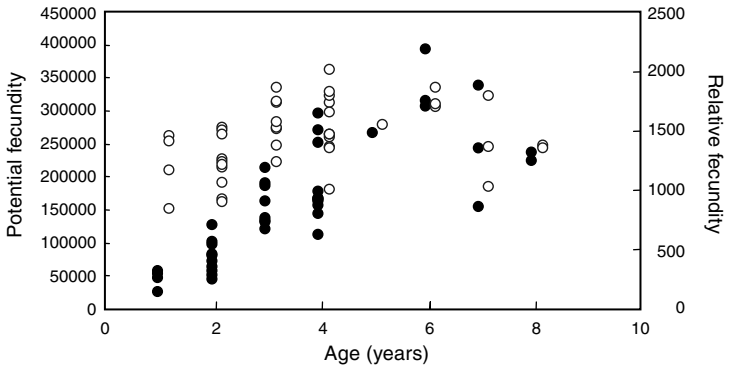

Figure 7

Relationship between age (years) and potential fecundity (solid circle, oocyte number/female) and relative fecundity (open circle, oocyte number/female per g) of Rikuzen sole (*Dexistes rikuzenius*) in the late vitellogenic maturity phase.

although they had no other vitellogenic oocytes. There are three potential hypotheses to explain the fate of these primary yolk oocytes. One explanation is that the oocytes are spawned in the current reproductive season. Maddock and Burton (1999) showed that in American plaice (*Hippoglossoides platessoides*), a group-synchronous spawner, the size frequency of oocytes during the prereproductive season was not continuous, whereas during the reproductive season the size frequency was continuous. The reason for this difference was that during the reproductive season cortical alveoli stage oocytes are larger than those during the prereproductive season. It is unclear, however, whether these cortical alveoli oocytes will be spawned during the reproductive season (Maddock and Burton, 1999). Although similar to those of the American plaice, all Rikuzen sole ovaries with primary yolk-stage oocytes contained no secondary or more advanced stage oocytes. In addition, oocytes that would be spawned in the current reproductive season developed beyond the secondary yolk stage before the beginning of the reproductive season. Therefore, primary yolk-stage oocytes occurring late in the reproductive season might not be spawned that season.

Primary yolk-stage oocytes were found from October to August (the late reproductive to vitellogenic season) (Table 3). From October to December only a small percentage of individuals possessed oocytes in this stage, whereas their ratio increased from January to April. These results indicate that females begin vitellogenesis for the next reproductive season shortly after spawning. This hypothesis is supported by reports that the vitellogenesis of flatfishes takes a long time (Yamamoto, 1954, 1956; Ishida and Kitakata, 1982; Zamarro, 1992; Harmin et al., 1995).

Atretic oocytes were present in low proportions from March to April and in high proportions in May. The mature phase of ovaries with atretic oocytes did not differ from that of ovaries without atretic oocytes. In addition, developmental stage did not differ between atretic and normal oocytes in any ovary. Therefore, it seems that the primary yolk-stage oocytes observed late in the reproductive season will not selectively degenerate, rather they will be spawned.

Decisions regarding maturity and age at maturity

POFs were present from September to January and all specimens caught during this period had either oocytes in the advanced yolk stage or POFs in their ovaries. All specimens caught between November and December contained ovaries with POFs, whereas they were observed only in a small percentage of the specimens caught in January. The spawning season lasted from September to December, but almost all spawning had finished by October. These results indicate that the duration until resorption of the POFs ranges from a few weeks to two months. For a few weeks immediately following spawning, the presence of POFs can be used as a criterion for the differences between post- and prespawning individuals. This feature is consistent with that of other flatfish in which POFs degenerate within one or two months (Barr, 1963; Janssen et al., 1995).

By noting the presence of POFs and advanced yolked oocytes, we were able to classify individuals as mature or immature. All but one individual caught during the reproductive period were maturing or had spawned. The body size of the mature females ranged from 114 to 237 mm SL, which corresponded to an age from 1 to 8+ years, respectively, whereas the immature female (131 mm SL) was age 1+. These results indicated that most female Rikuzen sole in this population mature at 2 years old, or at the latest at 3 years old, and spawn every year after maturation. Almost all (99.5%) fish caught commercially are adult individuals.

Fecundity

The potential fecundity of group-synchronous spawning fish can be determined prior to the spawning season (Takano, 1989). In Rikuzen sole, oocyte-stage composition became discontinuous beyond the late vitellogenic maturity phase, when a gap was found between secondary or tertiary yolk stages and the late perinucleolus stage. Oocyte diameter distributions in late vitellogenic maturity phase ovaries revealed that oocytes could be divided into small (less than 200 μm) and large (more than 300 μm) scale groups. Taking into account the oocyte diameters observed in the histological sections, small-scale group oocytes corresponded to cortical alveoli or less advanced stage oocytes, whereas larger oocytes corresponded to secondary yolk or more advanced stage oocytes.

The occurrence of atretic oocytes was highest in May and became lower as the season progressed until the end of the spawning season. These phenomena may correlate with both annual feeding cycles and maturation. Ogasawara and Kawasaki (1980) showed that in the Sendai Bay population, Rikuzen sole feed actively for a few months after spawning and then feed passively for the next few months. Gut-content weight began to increase again in June. In our study area, BC increased from about May, corresponding to the time when the oocytes begin to mature rapidly. As described before, vitellogenesis in this species takes a long time. Because oocytes are metabolically active in the season when the energetic condition of Rizuzen sole is still recovering, a higher proportion of atretic oocytes occur during this period.

Potential fecundity may not correspond to annual fecundity because of the presence of atretic and residual oocytes (Witthames and Greer Walker, 1995; Kurita et al., 2003). Therefore, we examined the potential fecundity of fish in the late vitellogenic maturity phase just before the spawning season. The frequency of occurrence of atretic oocytes may be underestimated because these oocytes have shrunk and are smaller than the maturing yolked oocytes. In addition, atretic oocytes may occur in the ovaries during the premature maturity phase. However, in our samples a low percentage of atretic oocytes were observed. Only a small percentage of premature ovaries were found on or before the reproductive season; this finding seems to indicate that the oocytes of this species take a short time to develop from the tertiary vitellogenic stage to maturation. These results make clear that potential fecundity differs from annual fecundity, but the extent of this difference was nevertheless relatively small in the samples. Moreover, ovulated, but not spawned oocytes were observed in the maturing and spent ovaries; these oocytes have the potential to cause an overestimation of annual fecundity. However, the frequency of ovaries with residual ovulated oocytes was small; therefore, such oocytes may not seriously influence annual fecundity, as with the case of Dover sole (*Microstomus pacificus*) (Hunter et al., 1992).

Vitellogenesis in American plaice was seen to begin soon after spawning (Zamarro, 1992), as with Rikuzen sole. Separation of oocyte diameter in this species occurs approximately three months before the start of the spawning season. In Rikuzen sole, potential fecundity was determined as being much closer to the reproductive season. Reproduction occurred from early September, but occurrence of the maturity phase in August varied largely among individuals. The potential fecundity of almost all fish (85%) could be determined until August. These results indicate that certain conditions and measurements are necessary when examining potential fecundity without histological methods.

Potential fecundity became determinate for the first time at maturity during the late vitellogenic phase. Some of the maturity phase ovaries contained secondary yolk-stage oocytes and all contained tertiary yolk-stage oocytes. The secondary yolk-stage oocytes ranged in diameter from 260 to 440 μm—a range that does not overlap with the diameter range of primary yolk-stage oocytes (180–220 μm). Therefore, to measure potential fecundity without histological observations, it is first necessary to clarify the division of oocyte diameter into large- and small-scale groups in order to identify determinate fecundity. In ovaries that contain large- and small-scale oocytes, only oocytes greater than 260 μm in diameter but that do not experience ovulation between May and August are targets for potential fecundity measurements. This method will make it easier to measure the potential fecundity of this population in the future.

Potential fecundity increased curvilinearly with SL. The body size of the females continued to grow even after maturation; the most fecund individual had 15 times more maturing oocytes than the least fecund one. Potential fecundity also increased until age ≤6+ years but decreased in individuals at ≥7+ years. One reason that older fish have less potential fecundity is a lesser energetic condition with senescence. Fecundity has been also reported as declining with age in other fish. As American plaice in the tail of the Grand Bank of Newfoundland become older, the number of eggs produced by females decreased (Horwood et al., 1986). Orange roughy, mature first at 25 years old and live for more than 100 years; their fecundity increases from an age of 25 to 60 years old, then decreases in individuals aged over 60 years old (Koslow et al., 1995). Fecundity is positively correlated with BC in the orange roughy. The oldest Rikuzen sole to appear in that study area was 10+ years (Ishito, 1964) and body growth almost finished by age 6+ years (Fig. 6). In addition, both the BC and relative fecundity of fish over 7+ years were lower than those of fish from 4 to 6+ years.

Spawning stock biomass (SSB) has been used to examine the relationship of spawning fish and recruitment; however, recent studies have indicated that SSB is not always linked to reproductive potential, mainly because age composition and nutrient conditions also affect fecundity (Hunter et al., 1985a; Trippel et al., 1997; Marshall et al., 1998). Our study shows that relative fecundity is positively correlated with body length. In addition, both relative and potential fecundity increase

with age, but decrease again in later years. These results support previous studies and emphasize the importance of understanding the demographic structure and reproductive biology of a population for the management of fish resources.

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