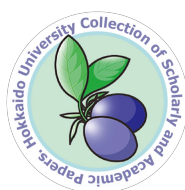




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Title	Growth of the brittle star <i>Ophiura sarsii sarsii</i> in Funka Bay, Hokkaido, Japan
Author(s)	Orino, Kazuki; Ishigane, Kosuke; Suzuki, Kota et al.
Citation	Fisheries Science, 85, 705-716 https://doi.org/10.1007/s12562-019-01323-1
Issue Date	2019-06-11
Doc URL	https://hdl.handle.net/2115/78529
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Type	journal article
File Information	article.pdf



1 Growth of the brittle star *Ophiura sarsii sarsii* in Funka Bay, Hokkaido, Japan

2 Growth of a brittle star

3

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27 **Abstract** The brittle star *Ophiura sarsii* of < 9 mm in disc diameter (DD) is an important prey item for flathead
28 flounder *Hippoglossoides dubius* in Funka Bay. To establish an age-determination technique and estimate the
29 growth equation for *Ophiura sarsii sarsii* (dominant sub-species of *O. sarsii* in Funka Bay), we observed ridges
30 on the surface of arm ossicles. Specimens were collected using a sledge net, their disc diameter measured, and
31 first arm ossicles examined on images taken by SEM. Because the outermost ridges were formed from November
32 to March, ridges were identified as yearly rings (annuli). The maximum age was 16.83 years (16.46 mm DD), and
33 the largest sized individual was 22.19 mm DD (13.75 years). The best growth equation between *DD* and age (*t*)
34 was determined as the Richard's model using minimum Akaike's Information Criterion and growth rate
35 comparison: $DD_t = 24.02 \cdot \{1 - 1.70 \cdot \exp[-0.067 \cdot (t + 8.25)]\}^{-1/(-1.70)}$. Small-sized (< 9 mm DD) and large-sized
36 (around 15 mm DD) cohorts were composed from multi-year classes, and estimated growth rates from cohort
37 progressions were 2.25 mm/year and 0.98 mm/year, respectively. We presumed that the large-sized cohort was
38 formed by high survival rate and slow growth rate after age 5 or 6.

39
40 **Key words** age validation, Funka Bay, growth equation, growth rate, Ophiuroidea

41

Introduction

The brittle star *Ophiura sarsii* lives in cold-water areas of the northern hemisphere and occurs over a wide bathymetrical range (3–3,000 m; Djakonov 1954). *O. sarsii* is one of the prey items for carnivorous fishes such as American plaice *Hippoglossoides platessoides* (Packer et al. 1994) and kichiji rockfish *Sebastolobus macrochir* (Ohmura 2007). In Funka Bay, located in the southern part of Hokkaido Island, Japan, flathead flounder *Hippoglossoides dubius* is the dominant species in the demersal fish assemblage and feeds on *O. sarsii* (Yokoyama 1992). *O. sarsii* has a low caloric value as prey for demersal fishes, but low mobility, so fishes can catch *O. sarsii* easily (Packer et al. 1994; Iwakawa et al. 2013). In Funka Bay in 1995–1997 many benthic species including *O. sarsii* decreased because of severe hypoxia (0.6–1.1 ml/l of dissolved oxygen) on the seafloor from summer to autumn (Maita 1998). Almost all *O. sarsii* disappeared from the seafloor within a depth range of 80–98 m in the central basin of Funka Bay, and the mean densities of shrimp species and small crustaceans (e.g. cumaceans and benthic amphipods) decreased to 35% and 2–10% of the level in 1985–1986, respectively (Yokoyama 1992; Inagaki et al. 2012; Izumiura Y, unpubl. data 2004). Because of the prey decrease, the 1995 year-class of *H. dubius* showed slower growth trajectories and somatic condition than the other year-classes (Kimura et al. 2004; Iwakawa et al. 2013). After 2007, small *O. sarsii* with < 9 mm in disc diameter (DD) have reappeared and increased in the basin, and the year-classes of 2003, 2004, 2006 and 2007 of *H. dubius* returned to former high growth rates and somatic condition (Inagaki et al. 2014).

The annual growth rates of *O. sarsii* were estimated through seasonal variations of modes in size-frequency distributions off Otsuchi (Fujita and Ohta 1990), in the Gulf of Maine (Packer et al. 1994). Although the age-determination using ridges on the surface of arm ossicles has been performed in some ophiuroid species (Gage 1990; 2003; Dahm 1993; Dahm and Brey 1998; Gage et al. 2004; Ravelo et al. 2017), formation of annual ridges on the surface of arm ossicles of *O. sarsii*, *O. sarsii sarsii* in the strict sense (see Materials and Methods) remains to be validated. The aim of this study is to establish an age-determination technique using ridges and reveal the growth pattern of *O. s. sarsii* in order to clarify its biological features in Funka Bay.

Materials and Methods

Field survey

Sampling was conducted aboard the T/S *Ushio-maru* (179 t) of Faculty of Fisheries, Hokkaido University at a sampling station (77 m depth; Fig. 1) where various sized *O. s. sarsii* have previously been found to be distributed (Izumiura Y, unpubl. data, 2004). Ophiuroids were collected using a Hirota's sledge net (40 cm height×60 cm width

frame; Hirota et al. 1989) with the modified mesh aperture from 0.76 mm to 0.50 mm, every month from January in 2014 to May in 2016 except for April, August, September and December in 2014, July and September in 2015. The sledge net was towed at a speed of 1.0 m/s for 5 min on the seafloor. On board the benthos samples were separated using a 1-mm mesh sieve. Ophiuroids were immediately picked out, frozen, and brought to the laboratory. Water temperature and salinity at 2 m above the seafloor were measured using a conductivity-temperature-depth profiler (CTD, SBE-19plus; Sea-Bird Scientific, Bellevue, WA, USA) from February in 2015 to February in 2016 except for July and September in 2015.

Laboratory procedures

In the laboratory, the ophiuroids were thawed and identified, and individuals of *Ophiura sarsii sarsii* were picked out. *O. sarsii* is composed of two sub-species as *O. sarsii sarsii* and *Ophiura sarsii vadicola* (Djakonov 1954), *O. s. sarsii* is a cold-water species and distributed < 200 m depth in the Sea of Japan (Kogure and Hayashi 1998), and *O. s. vadicola* occurs on shallower bottoms than *O. s. sarsii* (Kogure 1999). Many researchers have not differentiated them to the sub-species level. In Funka Bay, *O. s. vadicola* which has a similar shape to *O. s. sarsii* occurs, so we identified *O. sarsii* to the sub-species level using the shape of arm combs following Djakonov (1954) and Kogure (2002; Fig. 2a-b). It has been found that *O. s. sarsii* accounted for more than 80% in *Ophiura* spp. and the other ophiuroid is *O. s. vadicola* on the seafloor in Funka Bay (Izumiura Y, unpubl. data 2004). The disc diameter (DD) from the base of one arm to the opposite interradius on the aboral side of *O. s. sarsii* was recorded to the nearest 0.01 mm using an electronic slide caliper or an eyepiece micrometer under a stereoscopic microscope (Fig. 2c). *O. s. sarsii* of < 9 mm DD and ≥ 9 mm DD were defined as small-sized *O. s. sarsii* and large-sized ones, respectively, because the maximum DD of *Ophiura* spp. in stomachs of *H. dubius* has previously been found to be 8.9 mm (Inagaki et al. 2014). Disc-diameter-frequency distributions were described monthly from individuals collected from February in 2015 to May in 2016 and separated by fitting normal (Gaussian) distributions using the least-squares method (Aizawa and Takiguchi 1999). The growth rate of each cohort was estimated by a linear equation and multiple linear equations separated by season using the least-squares method. The best model was determined from the minimum value of Akaike's Information Criterion (AIC). All analyses were conducted by Microsoft Excel solver.

Estimation of settlement period

Tyler (1980) reported that *O. sarsii* has a pelagic larval and juvenile stages and settled on the seafloor after metamorphosis. To estimate the settlement period, pelagic juveniles of Ophiuroidea sp. with a similar shape to adults

106 but fewer arm nodes (Fig. 2d) were collected by vertical hauls from the 3 m layer above the seafloor using a NORPAC
107 net (0.45 m diameter and 0.10 mm mesh aperture) with a flowmeter from November in 2015 to October in 2016 at the
108 same station where the sledge net was towed. Specimens of plankton were immediately fixed in 5% buffered formalin
109 solution. In the laboratory, pelagic juveniles of Ophiuroidea sp. were counted under a stereoscopic microscope and DD
110 was measured in some individuals. Because the identification key to genus level for Ophiuroidea (inclusive of
111 *Ophiura* spp.) is unclear for the pelagic juvenile stage, these juveniles are represented as Ophiuroidea sp. in this study.

112

113 **Age-determination and estimation of growth equation**

114

115 A total of 154 individuals of *O. s. sarsii* were randomly picked out from the sledge net samples and boiled for a few
116 minutes to remove the first arm ossicles beside their mouths (Fig. 2c). Removed arm ossicles were dried, washed in
117 96% ethanol, dried again, mounted horizontally on stubs, and plated with platinum. Images of arm ossicles were taken
118 by a scanning electron microscopy (SEM: JEOL Ltd., JSM-6010LA, Tokyo, Japan).

119 Gage (1990) and Dahm (1993) reported that ridges on vertebrae were periodically formed in some ophiuroids.
120 Marginal ridge rate (*MR*) on the first arm ossicle in *O. s. sarsii* was estimated using data from January 2014 to May
121 2016 to validate the annual formation as follows:

$$122 \quad MR = (R - r_n) / (r_n - r_{n-1})$$

123 where *R* is the radius with an upper slant of 45 degrees from the center to the outermost margin of the arm ossicle
124 (Fig. 2e), *r_n* is the radius from the center to the outermost ridge in the arm ossicle, and *r_{n-1}* is the radius from the center
125 to the next outermost ridge in the arm ossicle. If *MR* is small (nearly 0) or large (nearly 1 and more), it means that the
126 outermost ridges has been formed recently or a more extensive period of time has elapsed since formation, respectively.
127 Data obtained from unclear margins were excluded from this *MR* estimation. The monthly variation of *MR* was also
128 estimated by Microsoft Excel solver and fitted to linear and logistic models using the least-square regressions. Because
129 wide *MR* values co-occurred from November to June, early (nearly 0) and late (nearly 1 and more) ridge formed
130 individuals were exploratorily divided by minimum AIC of models.

131 The age-determination was conducted using images taken by a SEM and performed independently by three of the
132 authors (reader A, B, and C). When we performed the age-determination, we observed the top of ridges on arm ossicles.
133 The following four types of ridges were not counted: the ridge within 268.9 μm from the center of arm ossicle
134 corresponding to the radius of arm ossicle in the minimum-sized individual which settled in the major settlement period
135 (see result) in 2015 and did not have any ridges, the short arc with < 33% to the longest arc in the upper left or right
136 sphere (e.g. the second or first quadrant with 90 degree at ossicle center; Fig. 2e), the width with < 50% between ridges
137 and the nearest inner ridge, and the intermittent ridges.

138 Readability for ridges of *O. s. sarsii* was evaluated using 154 individuals by a coefficient of variation (CV; Chang
139 1982) and an average percent error (APE; Beamish and Fournier 1981) as follows:

$$147 \quad CV_j [\%] = \sqrt{\frac{\sum_{i=1}^S \frac{(X_{ij} - \bar{X}_j)^2}{S-1}}{X_j}} \cdot 100$$

$$148 \quad APE_j [\%] = \sum_{i=1}^S \frac{|X_{ij} - \bar{X}_j|}{\bar{X}_j} \cdot 100$$

140 where S is the repeat number of ridge-readings for an *O. s. sarsii* individual (3 in this study), X_{ij} is i th time of ridge-
141 reading of the j th *O. s. sarsii*, $\bar{X}_j$ is the mean number of ridges of the j th *O. s. sarsii*. The growth equation models were
142 estimated from data only with $CV \leq 10\%$ and $APE \leq 7.2\%$. These threshold levels were based on the validation values
143 of fish vertebrae (Campana 2001) which have similar morphological features with arm ossicles of *O. s. sarsii*. Accuracy
144 of readers was evaluated using 154 individuals by index of average percent error (IAPE; Beamish and Fournier 1981)
145 and ICV among three readers and between two readers (A vs. B, B vs. C, and C vs. A) by the method detailed in
146 Campana et al. (1995) as follows:

$$150 \quad IAPE [\%] = \frac{1}{N} \sum_{j=1}^N APE_j$$

$$151 \quad ICV [\%] = \frac{1}{N} \sum_{j=1}^N CV_j$$

149 where N is the number of *O. s. sarsii* used for the age-determination.

152 Dahm (1993) and Dahm and Brey (1998) reported that the articulating area (Fig. 2) increased with the growth of
153 ossicles and covered more and more of innermost ridges, so the innermost ridges could not be observed. In this study, to
154 compensate for the ridges that become hidden by overgrowth of the ossicle, the total number of ridges (T) was
155 estimated and expressed as $T=V+C$, where the total number of visible and covered ridges are V and C , respectively
156 (Fig. 3). The m th visible ridge radius is r_m , the n th estimated ridge radius is e_n , and the radius of articulating area is r_a .

Fig. 3

157 Procedure for T estimation is as follows:

- 158 1. We estimate the mean e_1 ($\bar{e}_1$) from individuals satisfied with $r_a < 268.9 \mu\text{m}$. Individuals satisfied with $r_a < \bar{e}_1$
159 are $T=V$ (Fig. 3a).
- 160 2. We estimate the mean e_2 ($\bar{e}_2$) from individuals satisfied with $r_a < \bar{e}_1$. Individuals satisfied $\bar{e}_1 \leq r_a < \bar{e}_2$ are
161 $T=V+1$ (Fig. 3b).
- 162 3. We estimate the mean e_3 ($\bar{e}_3$) from individuals satisfied with $r_a < \bar{e}_2$. Individuals satisfied $\bar{e}_2 \leq r_a < \bar{e}_3$ are
163 $T=V+2$ (Fig. 3c).
- 164 4. The procedure of 3 is repeated until no covered ridge appears in an arm ossicle.

165 In Funka Bay, the range of $(\overline{r_m} - \overline{r_{m-1}})/(\overline{r_{m-1}} - \overline{r_{m-2}})$ ($3 \leq m \leq 8$) was 0.65–1.44. If the width ratio between the
166 outermost covered ridge and the first visible ridge exceeds 1.44, it is judged that there is (are) invisible ridge(s) because
167 of image problems (i.e. Fig. 3d).

168 To determine the best growth equation, von Bertalanffy, Gompertz, Logistic, and Richard's models were calculated
169 using the least-squares method and minimum AIC by Microsoft Excel solver.

170

171 **Simulation of size-frequency distributions adopted with the growth model to the virtual population**

172

173 In order to reveal how survival rate affects the size-frequency distributions in May, major settlement period of pelagic
174 juveniles in Funka Bay (see Results), we attempted to produce size-frequency distributions using the growth equation
175 estimated in this study by the boot-strap simulation (1000 times repetition) using virtual 1000 individuals after
176 settlement in five conditions of survival rate (scenarios a–e, shown below). Variability of mean DD estimated from the
177 growth model is presumed to follow the Gaussian distribution and randomness is 5% of mean DD. If we increase the
178 randomness (like 7% and 9%), DD overlaps between age groups increase. Boot-strap simulation was conducted by
179 Microsoft Excel macro.

180 a: 0.1/year from settlement to age 1, 0.9/year from age 1.01 to 25

181 b: 0.1/year from settlement to age 1, 0.5/year from age 1.01 to 2, 0.9/year from age 2.01 to 25

182 c: 0.1/year from settlement to age 1, 0.5/year from 1.01 to 3, 0.9/year from 3.01 to 25

183 d: 0.1/year from settlement to age 1, 0.5/year from 1.01 to 4, 0.9/year from 4.01 to 25

184 e: 0.1/year from settlement to age 1, 0.5/year from age 1.01 to 5, 0.9/year from 5.01 to 25

185 Similarities of size-frequency distributions between simulation and outdoor samples were evaluated by percent
186 similarity index (*PS*).

187
$$PS[\%] = (1 - 0.5 \cdot \sum |X_i - Y_i|) \cdot 100$$

188 where X_i means the frequency of every 1 mm DD estimated by the boot-strap simulation, Y_i means the frequency of
189 every 1 mm DD at the sampling station in May as the peak settlement period of pelagic juveniles and the start month of
190 age counting.

191

192

193 **Results**

194

Seasonal variation of water temperature and salinity

At 2 m above the seafloor of the sampling station, water temperature was 5.0°C on February 7, 2015, and the minimum temperature of 3.4°C was recorded on March 6, 2015. From March 18 (4.9°C) to August 3 (3.6°C), a relative low temperature was maintained, and water temperature rose from 5.9°C on August 30 to the maximum temperature of 9.9°C on December 15, 2015. Temperature dropped to 6.8°C on January 13 and 4.5°C on February 25, 2016. Maximum salinity of 33.78 was recorded on March 18, 2015 and minimum salinity of 32.72 on August 30, 2015. Low salinity with ≤ 33.3 was recorded from June 15 (33.29) to August 30, 2015. No Oyashio Coastal Current water ($\leq 3^\circ\text{C}$ and $\leq 33.0\text{--}33.3$ in salinity) was observed at 2 m above the seafloor at the sampling station.

Size-frequency distributions and cohort progressions

The disc-diameter range of *O. s. sarsii* collected by the sledge net from February in 2015 to May in 2016 was 0.50–23.58 mm DD, and small-sized individuals (< 9 mm DD) accounted for 83% of all individuals (Fig. 4). In the monthly size-frequency distributions separated by fitting normal distributions, cohorts of small-sized individuals were found in all sampling months and cohorts of large-sized individuals were observed except for March, April, May, June and December in 2015. A few large-sized individuals were found when large-sized cohorts could not be observed. Very small-sized cohorts (≤ 4.56 mm DD), which were smaller than small-sized cohorts, were observed in June, August and November 2015, and an intermediate-sized cohort was observed in April 2016.

Fig. 4

The relationship between disc diameter and sampling months of small-sized individuals showed triple linear equations with significant positive slopes separated at 0.4 year (May in the first year) and 1.0 year (December) ($0 \leq \text{year} \leq 0.4$: $DD = 2.87 \text{ year} + 4.67$, $r^2 = 0.099$, $p < 0.001$, $n = 744$; $0.4 < \text{year} \leq 1$: $DD = 0.99 \text{ year} + 6.01$, $r^2 = 0.052$, $p < 0.001$, $n = 513$; $1 < \text{year} \leq 1.4$: $DD = 2.55 \text{ year} + 4.25$, $r^2 = 0.059$, $p < 0.001$, $n = 349$; Fig. 5), which showed minimum AIC. Growth rate through the period was estimated at 2.25 mm/year. On the other hand, the relationship in large-sized cohorts showed a linear equation with a significant positive slope ($DD = 0.98 \text{ year} + 14.74$, $r^2 = 0.18$, $p < 0.001$, $n = 146$; Fig. 5), and the estimated growth rate was 0.98 mm/year.

Fig. 5

Settlement period of Ophiuroidea sp. pelagic juveniles

Pelagic juveniles of Ophiuroidea sp. in the plankton net samples occurred from March to October except for the no-data months, and the highest density was observed in April (422 ind./100 m³; Fig. 6). Relatively high densities were also observed in May (206 ind./100 m³) and October (142 ind./100 m³). In this study, the settlement period of *O. s. sarsii*

Fig. 6

227 was determined as from April to October, and the high frequency of settlement period was defined from April to May in
228 Funka Bay, and the start date of age-determination was set at May 1. The maximum size in disc diameter of
229 Ophiuroidea sp. pelagic juvenile was 0.50 mm DD which was the same as the minimum size (0.50 mm DD) collected
230 by the sledge net. In large pelagic juveniles, arm nodes were formed, but arm ossicles were not completely formed
231 (Fig. 2d).

232

233 **Monthly change in marginal ridge rate**

234

235 Monthly changes in the marginal ridge rate (*MR*) from November to March were divided into two groups by
236 minimum AIC (Fig. 7). Individual *MRs* were fitted to Logistic and linear models, and the AIC of Logistic model was
237 1.05 lower than that of the linear model as follows:

$$238 \quad MR = 0.995 / \{1 + \exp[-0.237 (month - 5.48)]\} \quad (n=143, r^2=0.61, p < 0.001)$$

239 where *month* ranged from 0 to 17 representing November 1 and March 31, 17 months later, respectively. The marginal
240 ridge with low *MR* from November to March showed that the annulus was already formed before May 1, while the
241 marginal ridge of high *MR* showed that the annulus was not formed in April and May.

242

243 **Precision of age-determination**

244

245 The three readers had an *IAPE* of 17.9% and an *ICV* of 23.8% for the 154 individual samples examined for the age-
246 determination. The number of individuals for which the age assigned by one reader corresponded to the age assigned by
247 another reader was 87 individuals between A and B (*IAPE* =11.0%, *ICV* =15.6%), 84 individuals between B and C
248 (*IAPE* =11.3%, *ICV* =16.0%), and 88 individuals between A and C (*IAPE* =10.8%, *ICV* =19.8%). In small-sized
249 individuals (< 9 mm DD), the maximum difference between two readers was 2. In large-sized individuals (≥ 9 mm DD),
250 the maximum difference was 10 both for between A and B and between B and C, and 8 between A and C.

251

252 **Best growth model**

253

254 The number of individuals within 7.2% in *APE* and 10% in *CV* was 69 individuals of 154 individuals (45%). In
255 69 individuals, individuals which had no ridge (Fig. 3a), one ridge (Fig. 3b), two ridges (Fig. 3c), and three or more
256 ridges hidden by overgrowth accounted for 29%, 36%, 10% and 25%, and individuals which had an invisible ridge(s)
257 because of image problems (Fig. 3d) accounted for 3%. The maximum age was 16.83 years (16.46 mm DD collected on
258 March 5, 2015), and the largest sized individual was 22.19 mm DD (13.75 years collected on February 7, 2015).

Fig. 7

259 The minimum AIC was obtained from the von Bertalanffy model estimated from 69 individuals and differences of
 260 AICs between the von Bertalanffy model and others ranged from 0.33 to 1.91 (Table 1). Sakamoto et al. (1983)
 261 reported that AIC was not sufficient to determine the best model in case of an AIC difference of ca. 1–2 and another
 262 criterion was needed. From estimated growth rates in size-frequency distributions, mean DDs of small-sized and large-
 263 sized cohorts on January 1, 2015 (Julian day= 0) were estimated from regression lines as 4.67 mm and 14.74 mm DD
 264 (corresponding to intercepts) and growth rates were 2.25 mm/year and 0.98 mm/year through the year, respectively
 265 (Fig. 5). Richard's model showed the nearest growth rates of those as follows (Fig. 8):

Table 1

$$DD_t \text{ (mm)} = 24.02 \cdot \{1 - 1.70 \cdot \exp[-0.067 \cdot (t + 8.25)]\}^{-1/(-1.70)}$$

Fig. 8

267 where DD_t means disc diameter (mm) at age t (year), t means age starting from May 1. This Richard's model indicated
 268 $m = -1.70$, and the von Bertalanffy model ($m = -1$) showed the nearest m value among three equations (Logistic model:
 269 $m = 1$ and Gompertz model: m is near 0).

270

271 **Estimated size-frequency distributions from the growth model**

272

273 Modes of 5 mm DD (age 1), 7 mm DD (age 2) and 9 mm DD (age 3: 90.8–94.6%, age 4: 5.2–9.1%) appeared in
 274 size-frequency distributions estimated by the the boot-strap simulation in May in five conditions, and wide cohorts
 275 appeared around 15 mm DD (Fig. 9). The highest similarity index between size-frequency distributions estimated by
 276 simulation and at the sampling station were scenario-e ($PS=63.6\%$) in May 2015 and scenario-d (66.2%) in May 2016
 277 (Table 2).

Fig. 9

Table 2

278

279

280 **Discussion**

281

282 Relative low water temperature condition may be a necessary factor for the high growth rate of *O. s. sarsii*. Growth of
 283 some ophiuroid species changes by season and the ridges on vertebrae are formed in slow or no growth seasons (Gage
 284 1990; Dahm 1993). The progression of small-sized individuals (Fig. 5) and the monthly change of *MR* (Fig. 7) account
 285 for the seasonal change in growth rate in *O. s. sarsii* in Funka Bay, too. The reason why the growth rate of *O. s. sarsii* in
 286 Funka Bay changes by season is that the relatively low water temperature in spring is suitable for the growth of
 287 *O. s. sarsii*, as a cold-water species (Djakonov 1954; Kogure and Hayashi 1998). In Funka Bay, the Tsugaru Water
 288 Current flows into the bay between August and December, and the water temperature rises (Inagaki et al. 2012). Vertical
 289 mixing starts because cooling from the sea surface occurs from November, and the water mass becomes uniform
 290 vertically from the sea surface to the seafloor in January, Oyashio Coastal Current water starts to enter between January

291 and March from the sea surface, and OCC prevents the temperature from rising from the sea surface until TWC water
292 enters (Ohtani and Kido 1980). In fact, water temperature was low (5.0°C) in early February, the minimum temperature
293 of 3.4°C was recorded in March and remained low (3.6–4.9°C) at the sampling station to early August in 2015. The
294 seasonal change in the marginal ridge rate showed that ridges on the first arm ossicle in *O. s. sarsii* were mainly formed
295 from November to March (Fig. 7). The early ridge formation occurred in relatively high-water temperature (growth
296 stagnation period; Gage 1990; Dahm 1993) between November in 2015 and January in 2016. On the other hand, the late
297 ridge formation occurred by March after the growth stagnation period because we can distinguish the presence of a low
298 growth ridge with an outer edge that is a non-ridge region formed near the marginal edge in the fast growth period in
299 spring. Kogure and Hayashi (1998) and Kogure (1999) have reported that *O. s. sarsii* is a cold-water species and
300 distributed on the sea-floor of < 200 m depth and *O. s. vadicola* occurs on shallower bottoms than *O. s. sarsii* in the Sea
301 of Japan. Because cold Oyashio Coastal Current intrudes into the shallow bay area of Funka Bay (maximum depth is
302 98 m) and inhibits the water temperature rising from spring to summer, *O. s. sarsii* may maintain being the dominant
303 species in the benthos assemblage.

304 Campana (2001) reported that various studies on ageing can be carried out with a CV (ICV in this study) of less than
305 7.6%, corresponding to an APE (IAPE in this study) of 5.5%. Because this study is carried out with an IAPE of 17.9%
306 and an ICV of 23.8%, the accuracy of age-determination in this study is lower than in general for past studies. It was
307 suggested that the age-determination of *O. s. sarsii* should be performed with a reader-independent protocol for reliable
308 age-determination.

309 The smallest individual collected by the sledge net was 0.50 mm DD and the largest individual of Ophiuroidea sp.
310 pelagic juvenile collected by a NORPAC net was 0.50 mm DD in Funka Bay. It was reported that *O. s. sarsii* accounted
311 for more than 80% of the *Ophiura* spp. on the sea floor in Funka Bay (Izumiura Y, unpubl. data 2004). It can be
312 inferred that many individuals of Ophiuroidea sp. pelagic juveniles were *O. s. sarsii* and they settled at a size of ca.
313 0.50 mm DD. The disc diameter at age 0 estimated by the Richard's model was 2.81 mm DD and bigger than 0.50 mm
314 DD. This might be because the ossicle is formed after settlement and/or growth might be faster just after settlement.
315 Pelagic juveniles occurred from March to October in Funka Bay (Fig. 6), while very small-sized cohorts (may be age 0)
316 were observed on June, August and November.

317 Small-sized individuals (< 9 mm DD) accounted for 83% of all individuals, while large-sized individuals ($\geq$ 9 mm
318 DD) accounted for only 17% (Fig. 4). This dominance of small-sized individuals might be because of the recruitment
319 success in the environment recovering from hypoxia in recent years.

320 In virtual size-frequency distributions by boot-strap simulation with the growth model, scenarios-d and -e showed
321 higher similarities than others to those at the sampling station (Table 2). This indicates that the survival rate of this
322 species in Funka Bay is relatively low until age 4 or 5 (mean DDs are 10.7 mm and 11.8 mm, respectively), and high

323 after age 5 or 6. Also, the phenomenon of low survival rate until age 4 or 5 might be the reason that *H. dubius* which is
324 the dominant and abundant demersal fish species mainly preyed on small-sized individuals of *Ophiura* spp. (< 9 mm
325 DD; Yokoyama 1992; Inagaki et al. 2014). From the estimated growth model, *O. s. sarsii* required a mean period of
326 2.72 years after settlement until they grew to ≥ 9 mm DD, so the density of large-sized individuals might be low. It was
327 reported that *H. platessoides* predation could influence the population size-structure of the green sea urchin,
328 *Strongylocentrotus droebachiensis* (Bernstein et al. 1981; Keats 1991). In this study, predation by *H. dubius* might also
329 influence the population size structure of *O. s. sarsii*.

330 Modes around 15 mm DD were produced by the boot-strap simulation with the growth equation in this study, but low
331 occurrence of 11–13 mm DD was not explained. We did not consider annual variations in the survival rate in this
332 simulation and presumed that size-frequency distributions of *O. s. sarsii* in Funka Bay might be influenced by a variable
333 survival rate year by year.

334 We validated the age-determination technique using ridges on the surface of first arm ossicles and revealed the growth
335 pattern of *O. s. sarsii* in Funka Bay. This knowledge is useful to reveal the biological features of this species, the growth
336 variation of predators (ex. *H. dubius*), and changes in abiotic environment like hypoxia and ecosystem structure. In
337 future studies, the relationship between the growth rate and the food consumption of *O. s. sarsii* should be examined.
338 *Ophiura* species typically are deposit feeders, consuming organic detritus, benthic microalgae, and benthic invertebrates
339 (Warner 1982). In Funka Bay, the downward particle flux is most abundant from April to June because detrital
340 phytoplankton settle after the decay of the spring bloom (Maita et al. 1999), but seasonal changes in food availability
341 and consumption of *O. s. sarsii* remain unclear. Also, new etching and/or staining techniques that are suitable for
342 observation of ossicles under the optical microscope should be developed because SEM images for the age
343 determination require high costs and more time than techniques for the optical-microscope.

344

345

346 **Acknowledgements**

347

348 We would like to thank Associate Professor T. Nakatani for his meaningful comments and Associate Professor H.
349 Kudo for supporting our usage of a SEM. We also thank Captain K. Kamei and crew of the T/S *Ushio-maru*, students of
350 Graduate School of Fisheries Sciences and Faculty of Fisheries, Hokkaido University. We thank two anonymous
351 reviewers and an editor for their insightful suggestions. This study was supported by Hokusui Society Foundation,
352 Sapporo, and JSPS KAKENHI Grant Numbers 25450269, 16K07834.

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423 offshore waters, Hokkaido. PhD dissertation, Hokkaido University, Hakodate (in Japanese)

424 **Fig. 1** Locations of the sampling station (open star) off Mori Town in Funka Bay. Dashed lines indicate bottom isobaths

425 **Fig. 2** Arm comb plates (ACP) and arm comb spines (ACS) of *Ophiura sarsii sarsii* (22.19 mm DD) collected in Funka

426 Bay in aboral (dorsal) view (a). ACP and arm-comb bulged needles (ACBN) of *Ophiura sarsii vadicola* (12.73 mm

427 DD) collected in Funka Bay in aboral view (b). Adoral (ventral) view of *O. s. sarsii* and measured part (a double-

428 headed arrow) indicating the disc diameter (DD) but measurement was carried out from aboral side (c). A white

429 rhombus shows the location of the first arm ossicle which is part of the endoskeleton and cannot be observed from

430 the surface (c). A pelagic Ophiuroidea sp. juvenile (0.50 mm DD) collected by vertical haul with a NORPAC net

431 (0.10 mm mesh aperture) at the sampling station (d). The SEM photograph of a first arm ossicle of *O. s. sarsii*

432 (18.21 mm DD with 9 ridges; e). Reverse white triangles show ridges on the fossae. The black line with an arrow

433 with an upper slant of 45 degrees shows the positions of the ossicle center and direction of growth of the ossicle

434 diameter. The white line with an arrow shows the hidden-area (articulating area) diameter by overgrowth. The

435 broken line shows the edge of articulating area.

436 **Fig. 3** One-year old specimen, no overgrowth ridge (a); 3-years old specimen, the first ridge overgrowth by the

437 articulating area (b); 5-years old specimen, first and second ridges overgrowth by the articulating area (c); 5-years

438 old specimen, first and second ridges overgrowth by the articulating area with a missing inner ridge (d). Shading

439 area: articulating area; V1, V2 and V3 (solid line): each visible ridge; C1 and C2 (broken line): each covered ridge;

440 I1 (short dashed line): invisible inner ridge in old specimens; T1, T2, T3, T4, and T5 (solid line + broken line + short

441 dashed line): each ridge estimated; R: ossicle radius; r_1 , r_2 , and r_3 : each visible ridge radius; r_a : articulating-area

442 radius; C_O: the central arm ossicle

443 **Fig. 4** Size-frequency distributions of *O. s. sarsii* collected at the sampling station off Mori Town in Funka Bay from

444 February 2015 to May 2016. Each position of an inverted triangle indicates the mean value of each cohort

445 **Fig. 5** Progressions in disc-diameter-frequency distributions of *O. s. sarsii* collected at the sampling station off Mori

446 Town

447 **Fig. 6** The monthly change in density by the number of individuals per 100 m³ of Ophiuroidea sp. pelagic juveniles

448 collected with a NORPAC net at the sampling station in Funka Bay from November 2015 to October 2016. " 0"

449 and " Nd" indicate absence and no data, respectively

450 **Fig. 7** The monthly change in marginal ridge rate (MR) of arm ossicles in *O. s. sarsii*. The solid curve divided in two

451 parts represents the fitted logistic model. Month=0 and 17 represent November 1 and March 31 in two years later,

452 respectively. Data from November to March were divided by minimum AIC exploratorily

453 **Fig. 8** Relationship between age and disc diameter and two growth curves estimated. von Bertalanffy growth curve

454 (solid line) and Richard growth curve (dashed line)

455 **Fig. 9** Size-frequency distributions estimated by the boot-strap simulation with the Richard’s growth model. Survival
456 situations from a to e in Table 2 correspond to a to e

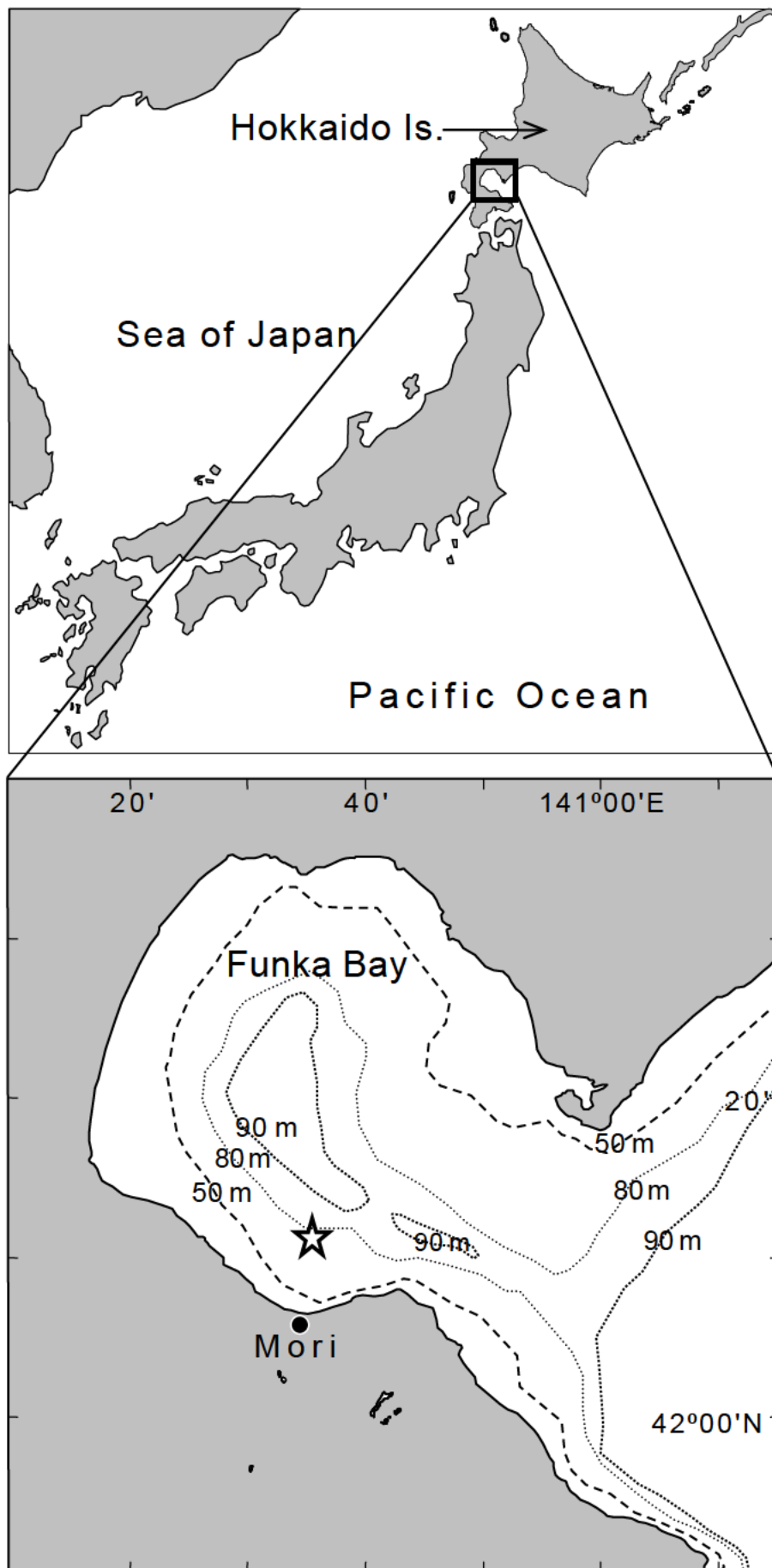
1 **Table 1** Estimated growth parameters and differences between AIC and minimum AIC (MAIC) of *Ophiura sarsii sarsii*
2 estimated from 69 individuals with $\leq 7.2\%$ of *APE* and $\leq 10\%$ of *CV* from three ridge readers

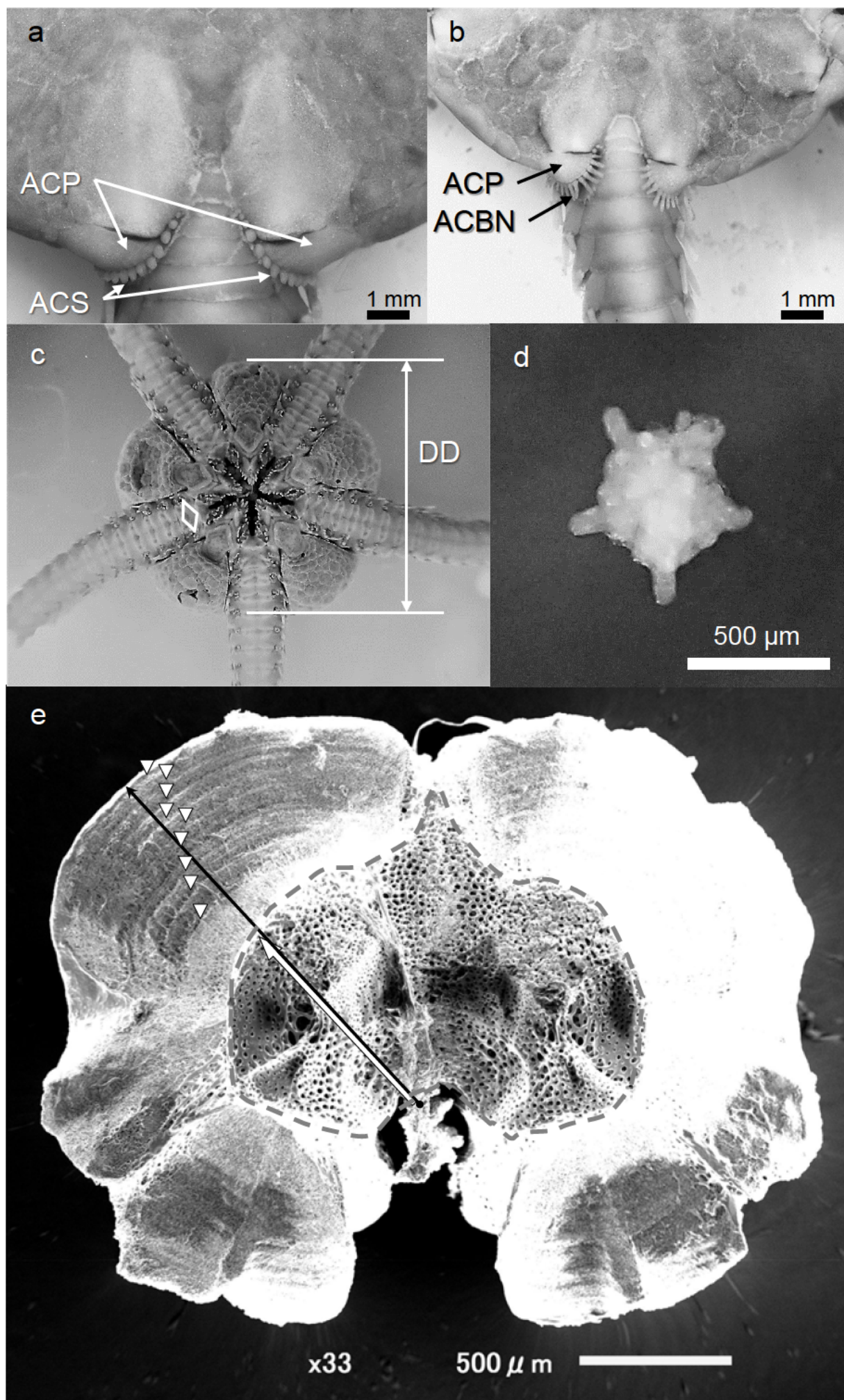
Growth formula	DD_{∞}	K	t_0	m	r^2	AIC–MAIC
von Bertalanffy	20.64	0.13	–1.49	–	0.92	0
Gompertz	19.02	0.23	1.62	–	0.90	0.33
Logistic	18.34	0.32	3.11	–	0.89	0.75
Richard	24.02	0.067	–8.25	–1.70	0.92	1.91

3 DD_{∞} , K , t_0 and m mean asymptotic length, growth rate, an inflexion point and a fixed number, respectively

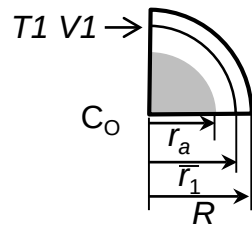
5 **Table 2** The comparison of similarity index in each survival rate between size-frequency distributions estimated with
6 the Richard's model and those at the sampling station in May 2015 and in May 2016

Survival rate (/year)	Total number of individuals per trial	Similarity index to May 2015	Similarity index to May 2016
a: 0.10 to age-1 and 0.90 later	930	31.5%	56.2%
b: 0.10 to age-1, 0.50 to age-2, and 0.90 later	560	37.7%	61.3%
c: 0.10 to age-1, 0.50 to age-3, and 0.90 later	378	50.3%	64.7%
d: 0.10 to age-1, 0.50 to age-4, and 0.90 later	288	61.7%	66.2%
e: 0.10 to age-1, 0.50 to age-5, and 0.90 later	244	63.6%	65.1%



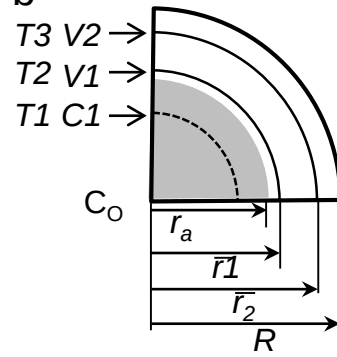


a



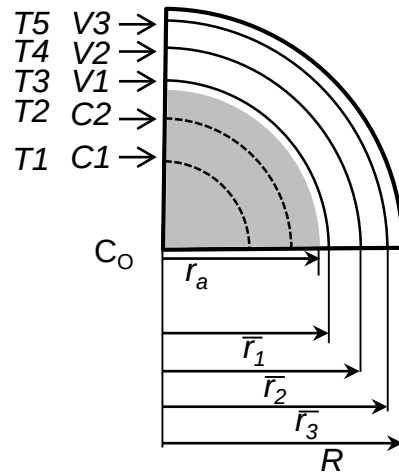
$$T=V+C=1+0=1$$

b



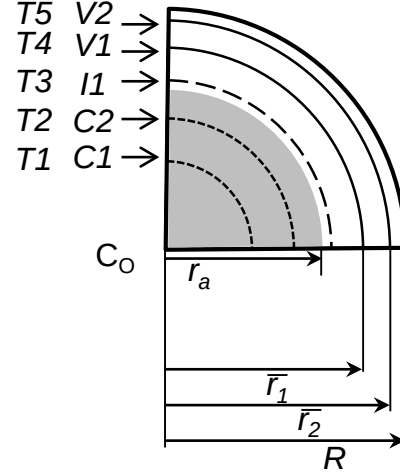
$$T=V+C=2+1=3$$

c



$$T=V+C=3+2=5$$

d



$$T=V+I+C=2+1+2=5$$

