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| Title            | Validation of otolith daily increment formation and growth analysis of yellow goosfish <i>Lophius litulon</i>  |
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| Citation         | Fisheries Science, 87(4), 541-548<br><a href="https://doi.org/10.1007/s12562-021-01529-2">https://doi.org/10.1007/s12562-021-01529-2</a>                                     |
| Issue Date       | 2021-07  |
| Doc URL          | <a href="http://hdl.handle.net/2115/86189">http://hdl.handle.net/2115/86189</a>  |
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| Type             | article (author version)   |
| File Information | 210628 Validation Gao et al.pdf  |



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1 **Validation of otolith daily increment formation and growth analysis of yellow**  
2 **goosefish *Lophius litulon***

3  
4 Running head: Growth of goosefish in early life stages

5  
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33

34     **Abstract**

35     We validated the daily formation of increments in otoliths of yellow goosefish *Lophius litulon* using reared  
36     individuals to examine the growth in the field. Single and round shaped core structures were observed in  
37     41% of sagittae and in 73% of lapilli. Therefore, lapillar otoliths were used for further observations of daily  
38     increment analysis. Lapillus radius of newly hatched larvae was  $15.0 \pm 1.4 \mu\text{m}$  (mean  $\pm$  standard deviation)  
39     forming the hatch check (first check). At 6 days after hatching (DAH), the 2<sup>nd</sup> check was observed which  
40     may correspond to the energy transition from endogenous to exogenous nutrition, located at  $28.1 \pm 0.7 \mu\text{m}$   
41     from the core structures. Thereafter, clear daily increments formed outside the check. To estimate larval  
42     growth in the field, we examined the relationship between the notochord length (NL) and lapillus radius by  
43     an allometric equation. The mean growth rate was estimated as ca. 0.18 mm in NL/day during 10–40 DAH.  
44     The results of this study provide insights into the previously unknown early life history and will enable  
45     further understanding of the population dynamics of the genus *Lophius*.

46

47     **Keywords** Anglerfish · Goosefish · Growth · *Lophius litulon* · Monkfish · Otolith daily increment

48

## 49 **Introduction**

50 Various common names are used for species in the genus *Lophius* such as goosefish, anglerfish and  
51 monkfish, and these species are distributed worldwide and form important target fisheries. The yellow  
52 goosefish *Lophius litulon* is widely distributed in the northern Pacific Ocean and is an important demersal  
53 fish that is caught by bottom trawl net, gillnet and bottom setnet (Yamada et al. 2007). However, the catches  
54 of this species tend to widely fluctuate (Tominaga 1991; Suzuuchi 1993; Nihira 2004; Kawano 2010).  
55 Population dynamics of fish are particularly susceptible to fluctuations of survival rate during the early life  
56 stages (e.g. Houde 1997; 2002). To develop an effective stock management, biological information on early  
57 life stages is of pivotal importance. For this species, some information on egg collection and larval rearing  
58 is available (Mito 1963; Kim 1976; Hoshino et al. 2006; Nakaya et al. 2017), but information on the age  
59 and growth in the early life stages is still lacking.

60 The daily microstructure (daily increments) of otoliths enables us to assess the growth rate in relation to  
61 the early life survival of fish, information that is important to estimate recruitment (Sponaugle 2010). The  
62 early life history of *Lophius* spp. (*L. piscatorius* and *L. budegassa*) has been investigated by growth  
63 increment counts in the lapillar otoliths (Hislop et al. 2001; Wright et al. 2002; La Mesa and De Rossi 2008;  
64 Hernández et al. 2015; 2019). However, no evidence to validate the daily ring deposition has been reported  
65 for *Lophius* spp.

66 This study aimed to validate the daily increment formation and to detect the most suitable otolith  
67 microstructure for age and growth analysis of larval *L. litulon* using laboratory observations, and also to  
68 estimate the growth in the field.

69

## 70 **Materials and methods**

71

### 72 **Larval rearing**

73

74 An egg veil of yellow goosefish was collected from the sea surface (sea surface temperature (SST): 14.3°C)  
75 in the Tsugaru Strait off Kazamaura on 25 May 2016 (Fig. 1). The egg veil was incubated in a 100 L circular  
76 shaped tank at a temperature range of 17–18°C (water exchange rate was 30% / day). Newly hatched larvae  
77 were stocked in a circular shaped tank (water volume ca. 1 m<sup>3</sup>) at 18–20°C, and the light condition was the  
78 natural light period in the Tsugaru Strait of the spawning season (light and dark periods were 14 h and 10 h).  
79 We fed the larvae twice a day with rotifers *Brachionus plicatilis* (enriched with Super Chlorella V-12;  
80 Chlorella Industry Co., Ltd., feeding amount: 10–30 ind./mL) and nauplii of *Artemia* sp. (enriched with  
81 *Indeplius*; Sciencetech Co. Ltd.), feeding amount: 5–8 ind./mL) by 4 days after hatching (DAH). Every day  
82 from 0 to 10 DAH, and on 12, 15, 20 and 25 DAH, the larvae were sampled and anesthetized with MS-222  
83 and then preserved in 90% ethanol solution (Table 1).

84

#### 85 **Field sampling**

86

87 Wild fish larvae were collected using oblique tows from depth at 100 m (maximum) to the surface with a  
88 framed midwater trawl net (FMT: 2×2 m, 1.7 mm mesh) by the T/S Ushio Maru of Hokkaido University  
89 Faculty of Fisheries (179 t) on 26 July 2014 in Funka Bay, on 8 June 2015 in Tsugaru Strait (Fig. 1).  
90 Furthermore, the larvae were collected using horizontal tows at surface with a plankton net (PL-net: 80 cm  
91 diameter, 0.335 mm mesh) on 13 July 2016, 5 June and 23 July 2018 in Tsugaru Strait (Fig. 1; Table 2).  
92 The larvae were preserved in 90% ethanol solution. We inferred that released egg veils and larvae which  
93 were in the eastern part of Tsugaru Strait from April to June (Takeya et al. 2013) could be transported to  
94 this sampling area (from Tsugaru Strait to Funka Bay) by the Tsugaru Warm Current Water structure  
95 observed in early summer (Kobayashi et al. 2019).

96

97 **Age and growth analysis**

98

99 Notochord length (NL, recorded from snout tip to notochord tip) of larval gosefish was measured to the  
100 nearest 0.1 mm under a stereo-microscope. Lapillar and sagittal otoliths were extracted from individual  
101 larvae using dissecting needles and mounted on a glass slide with epoxy resin. The surface of the otoliths  
102 was polished with lapping film (grain size: 9.0  $\mu\text{m}$ ) and then etched with 0.1 N hydrochloric acid to enhance  
103 the contrast of increments. The morphological characteristics of otoliths (sagitta and lapillus) were observed  
104 under a binocular microscope. Thereafter the maximum radius of otoliths and growth increments were  
105 measured and counted using an otolith measurement system (ARP Ver. 5.27, Ratoc System Engineering Co.  
106 Ltd.). Because the otoliths of this species are bilaterally symmetrical, we did not distinguish between the  
107 left and right otoliths.

108 For reared samples, we examined using *t*-test whether the number of otolith growth increments and  
109 number of days since the formation of the ring pattern was observed had a significant difference. And also,  
110 *t*-test was used for comparison of otolith check radius between reared and wild individuals. The significance  
111 level was set at 0.05.

112 For field samples, NLs at each age were back-calculated using the biological intercept method (Campana  
113 1990) in order to obtain the growth trajectory and daily growth rate for each individual. The relationship  
114 between the *i* th lapillus radius and NL was described by the allometric function:  $NL_i = a \cdot OR_i^b$  where  $NL_i$   
115 is the NL (mm) at the *i* th day after first increment observed and  $OR_i$  is the otolith radius ( $\mu\text{m}$ ) at the *i* th  
116 increment of each individual fish. Parameters a and b were estimated using the Microsoft Excel solver  
117 routine.

118

119 **Results**

120

121 **Larval growth in rearing condition**

122

123 Notochord length of newly hatched larvae of *L. litulon* was  $4.5 \pm 0.2$  mm (mean  $\pm$  standard deviation (SD);  
124 Fig. 2). Most of the yolk sac was consumed by 5 DAH and then the larvae started feeding on rotifers from  
125 6 DAH onwards (NL:  $6.6 \pm 0.2$  mm). Also, some larvae started feeding on *Artemia* nauplii from 12 DAH  
126 (NL:  $7.8 \pm 0.1$  mm). Growth rate from hatching to 25 DAH showed a mean value of ca. 0.17 mm in NL/day  
127 and reached  $8.8 \pm 0.2$  mm at 25 DAH, although there was a temporary growth stagnation from 12 to 20  
128 DAH.

129

### 130 **Observation of otolith microstructure of reared larvae**

131

132 The microstructural features of otoliths (sagitta and lapillus) are shown in Fig. 3. In both the sagitta and  
133 lapillus, a check was observed in 6 DAH larvae, and clear and regular increments formed outside the check  
134 (Fig. 3). The relationship between *DAH* and the number of increments (*NI*) are for sagitta:  
135  $NI = 0.97DAH - 5.73$  ( $r^2 = 1.00$ ,  $P < 0.001$ ,  $n = 35$ ), lapillus:  $NI = 1.00DAH - 6.00$  ( $r^2 = 0.99$ ,  $P < 0.001$ ,  
136  $n = 35$ , Fig. 4). Slopes of both linear regressions were not significantly different from 1 (*t*-test,  $P = 0.11$  and  
137 0.29, respectively).

138 Single and round shaped core structures were observed in 41% of sagittae and 73% of lapilli (0–25 DAH  
139 samples). Sagittal otoliths of this species were generally characterized by having multiple core structures  
140 making it difficult to measure the radius (Fig. 3). Therefore, lapillar otoliths were used for further  
141 observations of daily increment analysis. Lapillus radius (LR) of newly hatched larvae was  $15.0 \pm 1.4$   $\mu$ m  
142 forming the hatch check (1<sup>st</sup> check). From hatch to 5 DAH, there was no clearly observable structure  
143 indicating the 2<sup>nd</sup> check. At 6 DAH, the 2<sup>nd</sup> check was observed which may correspond to the energy  
144 transition from endogenous to exogenous nutrition, located at  $28.1 \pm 0.7$   $\mu$ m (mean  $\pm$  SD) from the core  
145 structures. Thereafter the radius of the otolith increased and reached  $52.5 \pm 3.4$   $\mu$ m at 25 DAH (Fig. 5a).

146 From 7 DAH to 25 DAH, the range and mean ( $\pm$  SD) of increment widths were 1.2–1.6  $\mu\text{m}$  and  
147 1.4  $\pm$  0.1  $\mu\text{m}$ . The relationship between LR and NL was fitted by an allometric curve (Fig. 5b).

148

#### 149 **Age and growth of wild larvae**

150

151 The NL of wild larvae used for the otolith analysis ranged from 4.9–13.4 mm (Table 2). Range of 2<sup>nd</sup> check  
152 in lapillus radius of wild caught larvae was 24.1–30.2  $\mu\text{m}$  (26.6  $\pm$  1.9  $\mu\text{m}$  (mean  $\pm$  SD)) and the radius did  
153 not significantly differ from reared larvae at 6 DAH (*t*-test,  $P = 0.79$ ). We counted the otolith daily  
154 increments formed after the 2<sup>nd</sup> check and measured the increment widths (Fig. 6). As a result, 0–38 rings  
155 on the lapillar otoliths were counted. The range and mean ( $\pm$  SD) of increment widths were 1.4–2.1  $\mu\text{m}$  and  
156 1.7  $\pm$  0.1  $\mu\text{m}$  (Fig. 7a). For individuals with more than 1 ring, the number of rings plus 6 was used as the  
157 age in days. The hatch dates of the wild larvae occurred from early May to late July through the sampling  
158 years (Table 2).

159 The parameters (a, b) of the allometric relationship ( $NL = a \cdot LR^b$ ) were estimated using  $NL_0$  and  $LR_0$  at 6  
160 DAH determined as 6.6 mm and 28.1  $\mu\text{m}$  (from the reared samples),  $NL_i$  and  $LR_i$  for each individual at  
161 capture. For each sample, the relationship between LR and the NL was fitted by an allometric curve. The  
162 back-calculated NLs (mean  $\pm$  SD) with the biological intercept method were 7.1  $\pm$  0.3 mm at 10 DAH,  
163 8.8  $\pm$  0.5 mm at 20 DAH, 10.2  $\pm$  0.7 mm at 30 DAH and 12.4  $\pm$  0.7 mm at 40 DAH (Fig. 7b). The estimated  
164 mean growth rates during 10–20, 20–30 and 30–40 DAH were 0.17, 0.14 and 0.22 mm in NL/day,  
165 respectively (Fig. 7c). The mean growth rate of larvae was ca. 0.18 mm in NL/day during 10–40 DAH.

166

## 167 **Discussion**

168

### 169 **Otolith microstructure analysis**



170

171 In this rearing experiment, we analyzed the deposition timing of the checks, supporting the timing of check  
172 formation hypothesized by previous studies. The lapillus radius of newly hatched larvae was  $15.0 \pm 1.4 \mu\text{m}$   
173 (mean  $\pm$  SD) as the hatch check (1<sup>st</sup> check), though there were several not well-defined otolith daily  
174 increments observed after this check. At 18–20°C, the 2<sup>nd</sup> check (corresponding to the putative energy  
175 transition from endogenous to exogenous nutrition) formed on the lapillus 6 DAH (LR:  $28.1 \pm 0.7 \mu\text{m}$ ),  
176 and clear daily increments are formed after this check. The daily deposition of increments is regulated by  
177 circadian endocrine rhythms, which are synchronized at an early life stage with photoperiodicity (Tanaka  
178 et al. 1981; Radtke and Dean 1982). The cycle is based on a circadian rhythm (Nakaya et al. 2008), and if  
179 the circadian rhythm does not change, the daily deposition pattern is continued.

180 Previous studies reported the growth in early life stages of *Lophius* spp. using otolith microstructure  
181 analysis (*L. piscatorius*: Hislop et al. 2001; Wright et al. 2002; Hernández et al. 2019, *L. budegassa*: La  
182 Mesa and De Rossi 2008; Hernández et al. 2015). Hislop et al. (2001) and Wright et al. (2002) assumed  
183 that the first increment was formed on the day of hatching. La Mesa and De Rossi (2008) and Hernández  
184 et al. (2015; 2019) reported that there are two checks in the core area of lapilli of *Lophius* spp., and they  
185 assumed that the 1<sup>st</sup> (LR: ca. 15–17  $\mu\text{m}$ ) is the hatch check and the 2<sup>nd</sup> (LR: ca. 28–30  $\mu\text{m}$ ) is starting of  
186 exogenous feeding check. La Mesa and De Rossi (2008) found that after the less defined feeding check, the  
187 increments appeared more regular and defined. In this study, the timing of the formation of two  
188 characteristic rings (1<sup>st</sup> check: hatch and 2<sup>nd</sup> check: energy transition from endogenous to exogenous  
189 nutrition) on otolith which, based on the descriptions in the above cited literature, appear common to the  
190 genus *Lophius* was clarified. These results allow us to analyze the otolith microstructure for the genus  
191 *Lophius* with more certainty.

192

193 **Hatch date and growth of wild population**

194

195 No significant difference was found between the 2<sup>nd</sup> check in LR of wild and reared larvae. This result  
196 assures that increments after the 2<sup>nd</sup> check on the lapillus of wild larvae can be regarded as the starting point  
197 for reading. The hatch dates of the wild larvae were back-calculated to occur from early May to late July  
198 through the sampling years (2014–2018). Taking into account the spawning information for the coast of  
199 eastern Tsugaru Strait (mainly: April–June; Takeya et al. 2013), the present hatch dates estimates are  
200 consistent. However, the timing of the 2<sup>nd</sup> check formation may vary depending on the environment. Several  
201 studies have mentioned the effects of temperature on yolk-sac duration and onset of first feeding, pointing  
202 out that the rate of yolk disappearance and the start of feeding are faster at higher temperatures (Johns and  
203 Howell 1980; Fukuhara 1990; Dou et al. 2005; Nakaya et al. 2017). Further study is needed to understand  
204 the relationship between water temperature and age at 2<sup>nd</sup> check formation on the lapillus for greater  
205 accuracy of measurement.

206 The NL estimated for the field-collected specimens tended to be larger than larvae at 20 DAH obtained  
207 in this rearing (that is they did not experience the 12 to 20 DAH growth stagnation observed in the reared  
208 specimens). This may be the result of individuals collected in the field growing under more favorable  
209 conditions than those in the rearing conditions, or it may be the result of growth-selective survival (e.g.  
210 Meekan and Fortier 1996; Takahashi et al. 2012; Joh et al 2013). From 7 to 42 DAH, the daily increment  
211 widths of lapilli were within the range of 1.4–2.1  $\mu\text{m}$ , with a mean  $\pm$  SD value of  $1.7 \pm 0.1 \mu\text{m}$ . There is no  
212 information on the early growth of this species, so comparison can only be made with other species in the  
213 same genus, and this range was not remarkably different to other species (from 10 to 40 DAH: ca. 1–3  $\mu\text{m}$ )  
214 within the genus, such as *L. budegassa* in north-eastern Atlantic waters (Hernández et al. 2015) and *L.*  
215 *piscatorius* in the Bay of Biscay (Hernández et al. 2019). The mean growth rate of larval *L. litulon* was  
216 estimated ca. 0.18 mm /day during 10–40 DAH. Hislop et al. (2001) estimated the initial growth during the  
217 larval and juvenile stages of *L. piscatorius* using the Gompertz equation. The larval growth rate is much

218 higher (ca. 0.33 mm /day during 10–40 DAH) than that of *L. litulon*. However, the authors suggested that  
219 the accuracy of the estimate may not be high due to the limited number of samples of larvae and the data  
220 including the period of transition from the larval to juvenile stage. In our study, the daily increment analysis  
221 of otoliths enabled detailed assessment of growth during the larval stage. This method of estimation and  
222 analysis of the early growth of the genus *Lophius* potentially allows a clear understanding of early life  
223 history of this genus.

224

225 **Acknowledgments** We are grateful for the help of Mr. Yoshitada Kindaichi of Ikokuma Fisheries  
226 Cooperative Association for collection of the egg veils. We also thank to the staff of Kazamura Village  
227 Government, Laboratory of Marine Bioresources Ecology of Hokkaido University and also crew of the T/S  
228 *Ushio-maru* of Hokkaido University Faculty of Fisheries for their kind assistance in collection of specimens.  
229 We thank two reviewers who kindly read the manuscript and gave valuable suggestions. This manuscript  
230 has been proofread by the Japan Scientific Texts (JST).

231

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303

304 **Fig. 1** Location of study area. Arrows show the surface flow pattern detailed by Kobayashi et al. (2019).  
305 TW means Tsugaru Warm Current Water. Location of spawning area is quoted from Takeya et al. (2013;  
306 shaded area)

307 **Fig. 2** Relationship between days after hatching (DAH) and notochord length (NL) of yellow gosefish  
308 larvae reared in a tank

309 **Fig. 3** Otoliths from larval yellow gosefish. a and b: sagitta and lapillus of larvae 0 days after hatching,  
310 respectively. Arrows show the primordia. c and d: sagitta and lapillus of 25 DAH larva. Black and white  
311 arrows show the hatch check and 2<sup>nd</sup> check (energy transition check), respectively

312 **Fig. 4** Relationship between days after hatching (DAH) and number of increments (NI) for lapillus and  
313 sagitta of yellow gosefish reared in a tank. Numerals in the figure show the number of samples

314 **Fig. 5** Relationship between days after hatching (DAH) and lapillus radius (LR) a), and between LR and  
315 notochord length (NL) b) of yellow gosefish larvae reared in a tank. The curve shows allometric function  
316 assessed using regression analysis

317 **Fig. 6** Lapillar otolith from wild larvae of yellow gosefish (13.4 mm NL). a: microstructure of the core  
318 area (HC: Hatch check; 2C: 2<sup>nd</sup> check as energy transition check), b: whole image of the lapillus

319 **Fig. 7** Relationships between days after hatching (increment count of lapillus + 6; DAH) and a) increment  
320 width (IW), b) notochord length (NL) and c) growth rate (GR) (mm/day) of wild yellow gosefish larvae.  
321 Numerals in the figure show the number of samples. Circles and vertical bars indicate mean values and  
322 standard deviations, respectively

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**Table 1** Number of reared samples collected and used in the otolith analysis

| Days after hatching | No. of larvae collected | No. of larvae for otolith analysis |
|---------------------|-------------------------|------------------------------------|
| 0                   | 10                      | 10                                 |
| 1                   | 5                       | 5                                  |
| 2                   | 2                       | 2                                  |
| 3                   | 3                       | 3                                  |
| 4                   | 3                       | 3                                  |
| 5                   | 5                       | 4                                  |
| 6                   | 5                       | 5                                  |
| 7                   | 5                       | 5                                  |
| 8                   | 5                       | 5                                  |
| 9                   | 5                       | 5                                  |
| 10                  | 5                       | 5                                  |
| 12                  | 5                       | 5                                  |
| 15                  | 5                       | 4                                  |
| 20                  | 5                       | 2                                  |
| 25                  | 5                       | 4                                  |

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**Table 2** Sampling date, methods, number of samples, range of notochord length (NL) and hatch date in the field (see Fig. 1) during the summer of 2014–2018

| Year | Date    | Sampling |                      |   | Estimated        |                     |
|------|---------|----------|----------------------|---|------------------|---------------------|
|      |         | Gear     | Tows                 | n | Range of NL (mm) | Range of hatch date |
| 2014 | 26 July | FMT      | Oblique (0–max100m)  | 6 | 9.2–13.4         | 12 June–7 July      |
| 2015 | 8 June  |          |                      | 5 | 7.1–10.6         | 4–26 May            |
| 2016 | 13 July | PL-net   | Horizontal (surface) | 1 | 5.1              | 5 July              |
| 2018 | 5 June  |          |                      | 1 | 6.5              | 31 May              |
|      | 23 July |          |                      | 1 | 4.9              | 21 July             |

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327 \*FMT: framed midwater trawl net (2 x 2m, 1.7 mm mesh); PL-net: plankton net (80 cm diameter,  
328 0.335 mm mesh)

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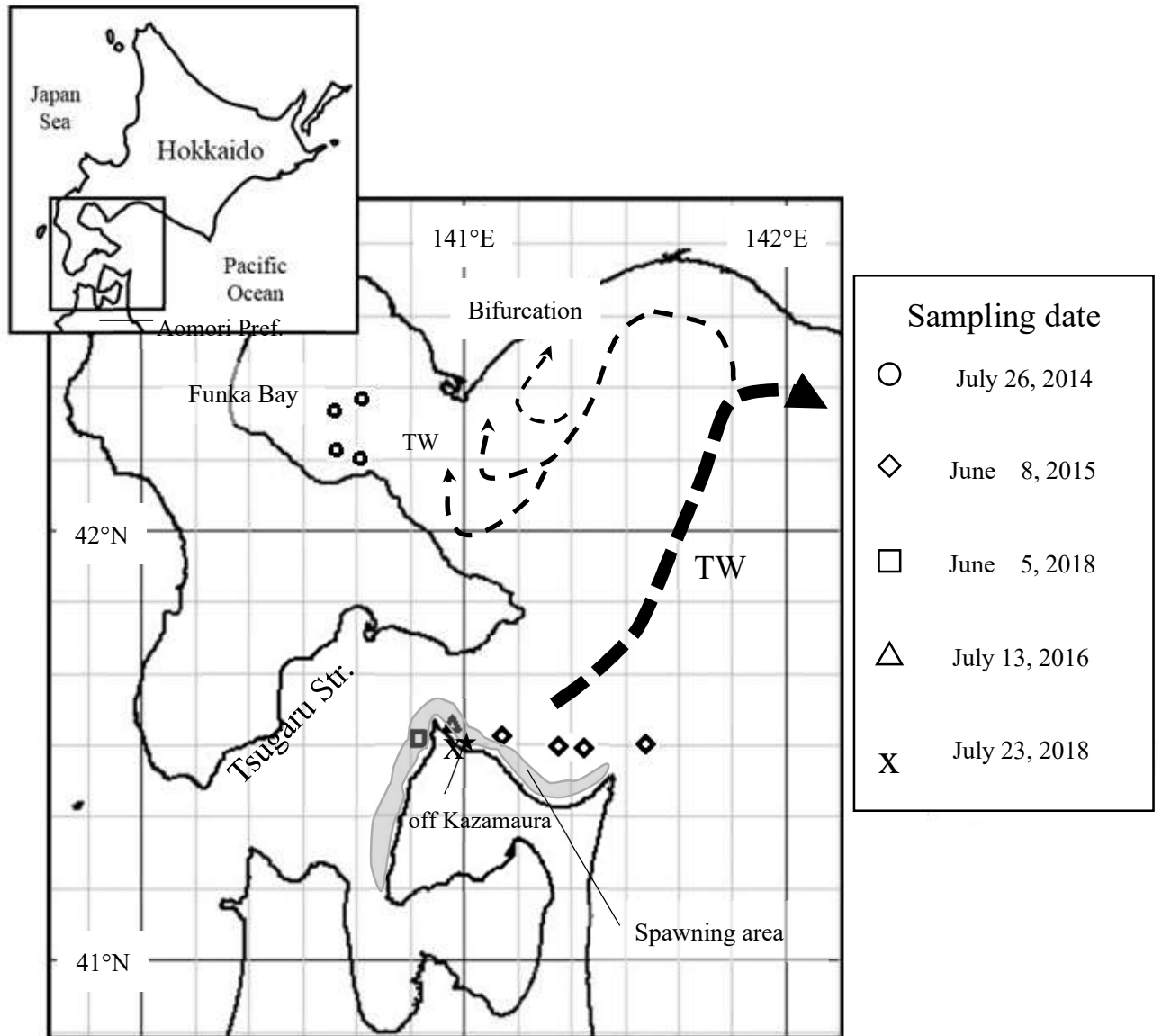


Fig. 1 Gao *et al.*

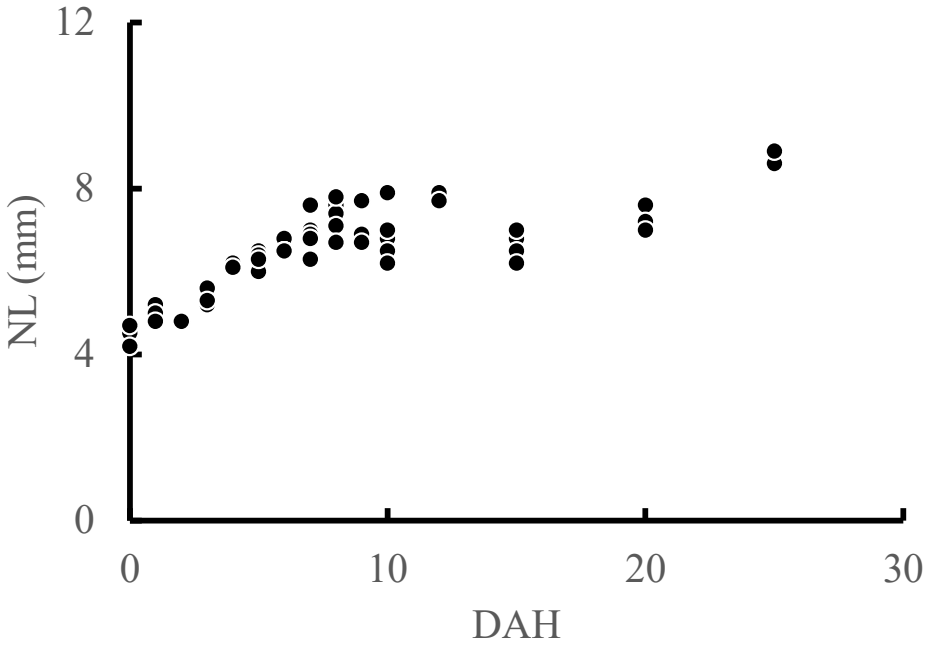


Fig. 2 Gao *et al.*

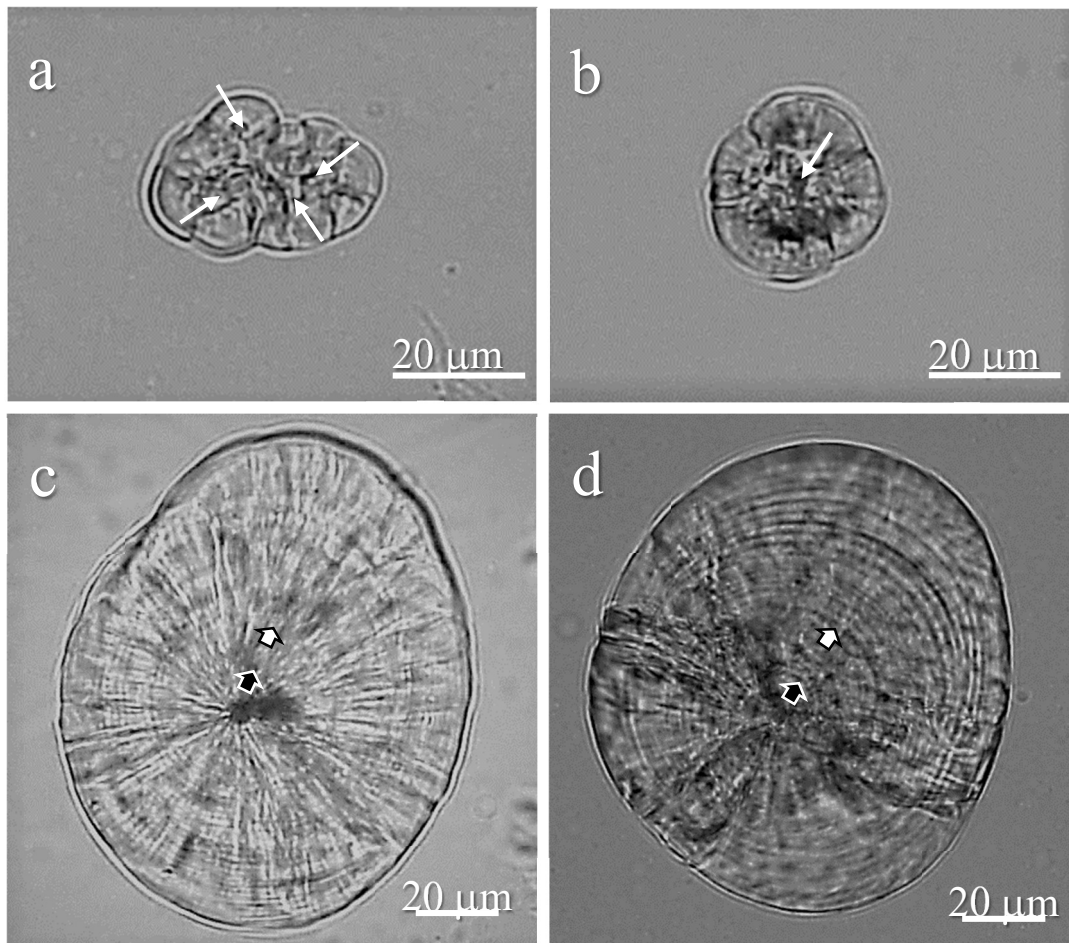


Fig. 3 Gao *et al.*

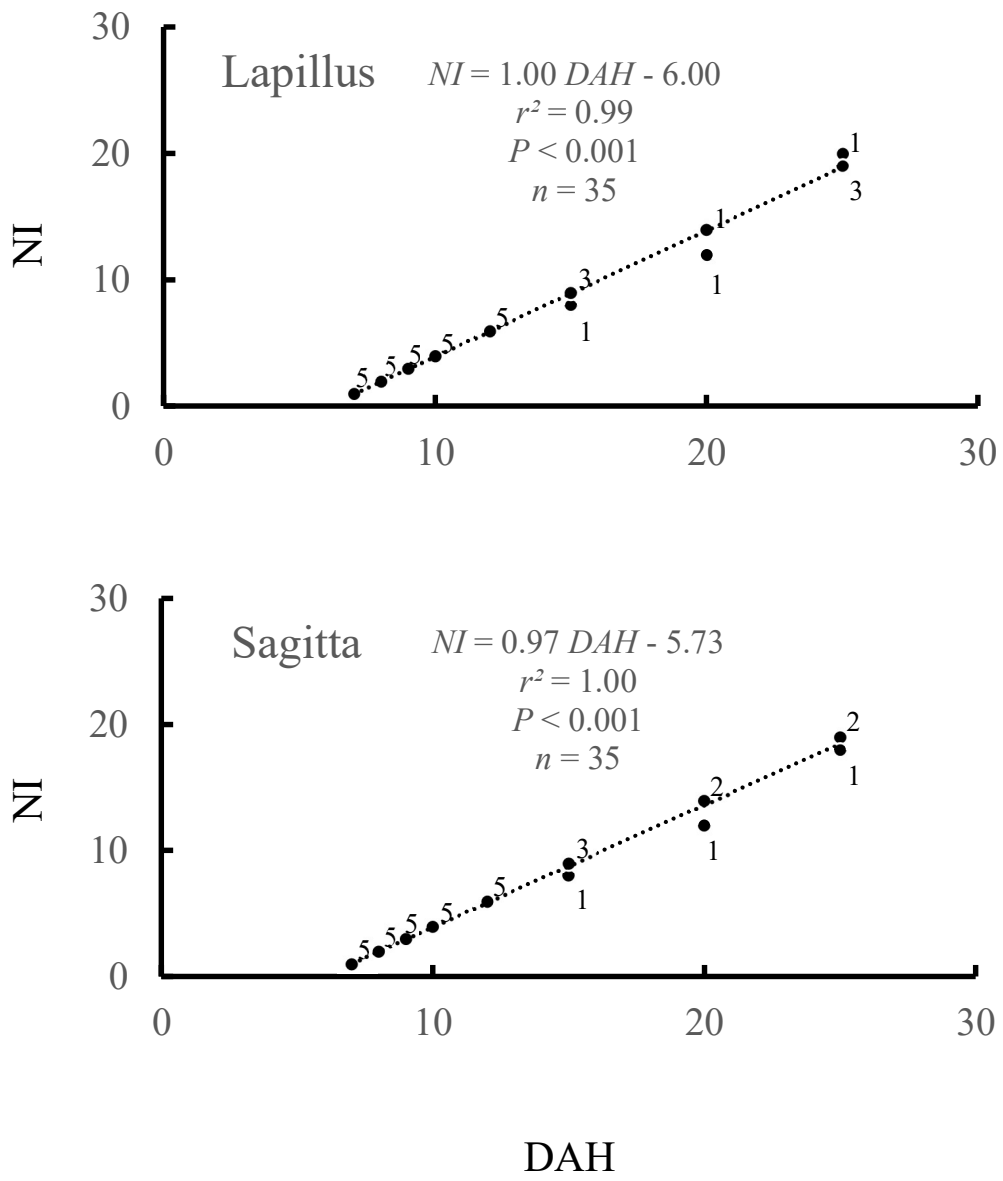


Fig. 4 Gao *et al.*

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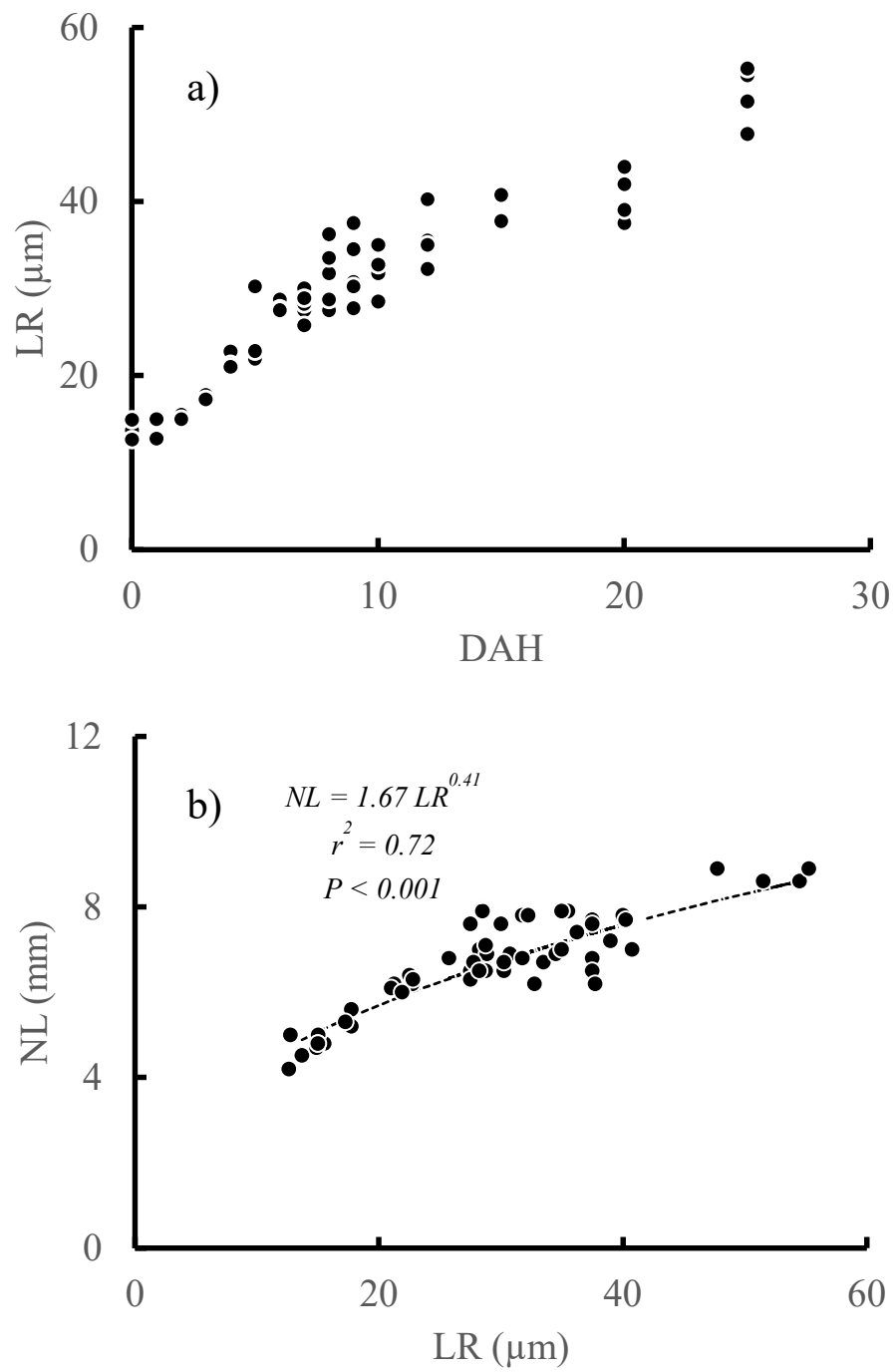


Fig. 5 Gao *et al.*

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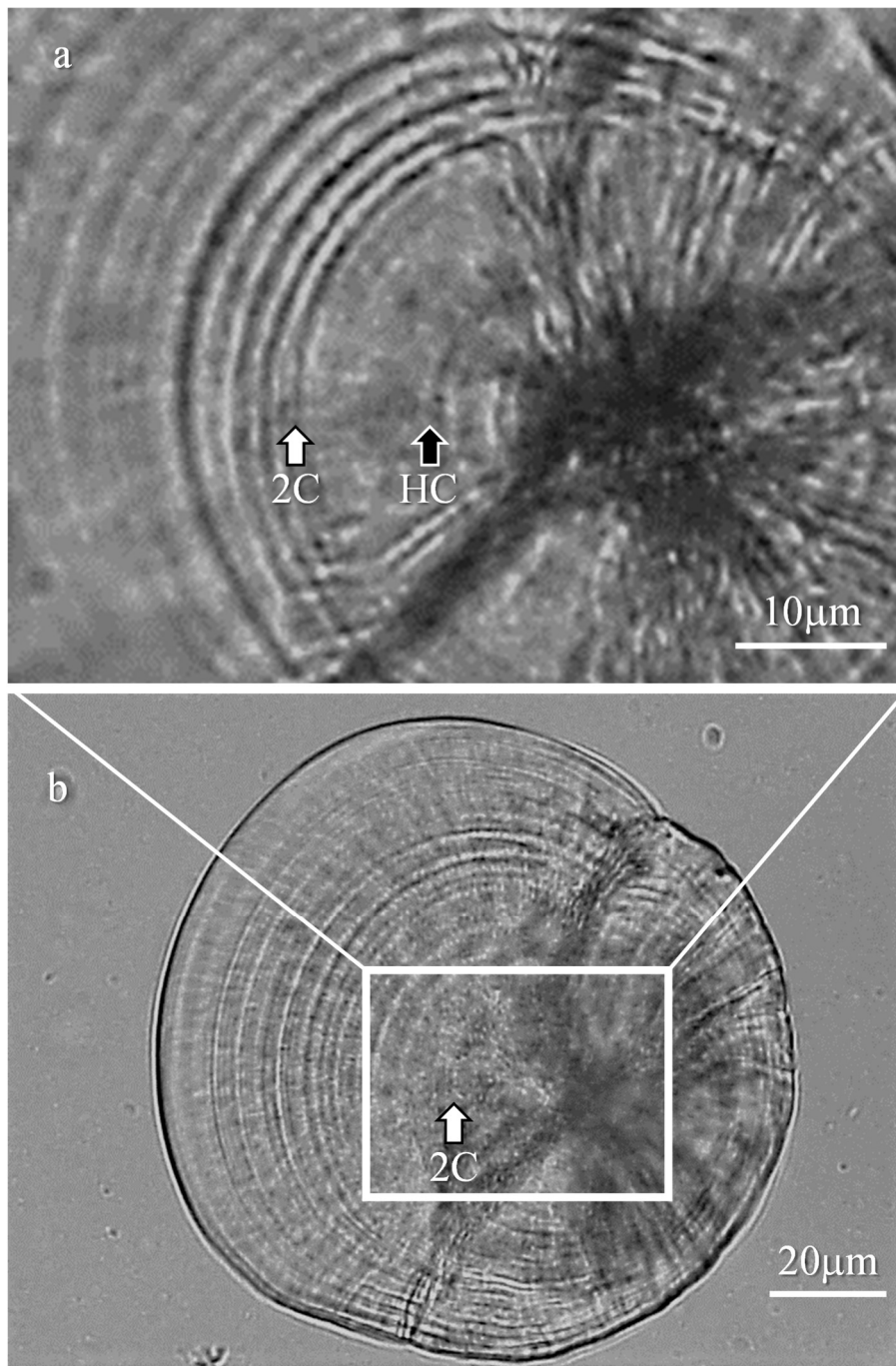


Fig. 6 Gao *et al.*

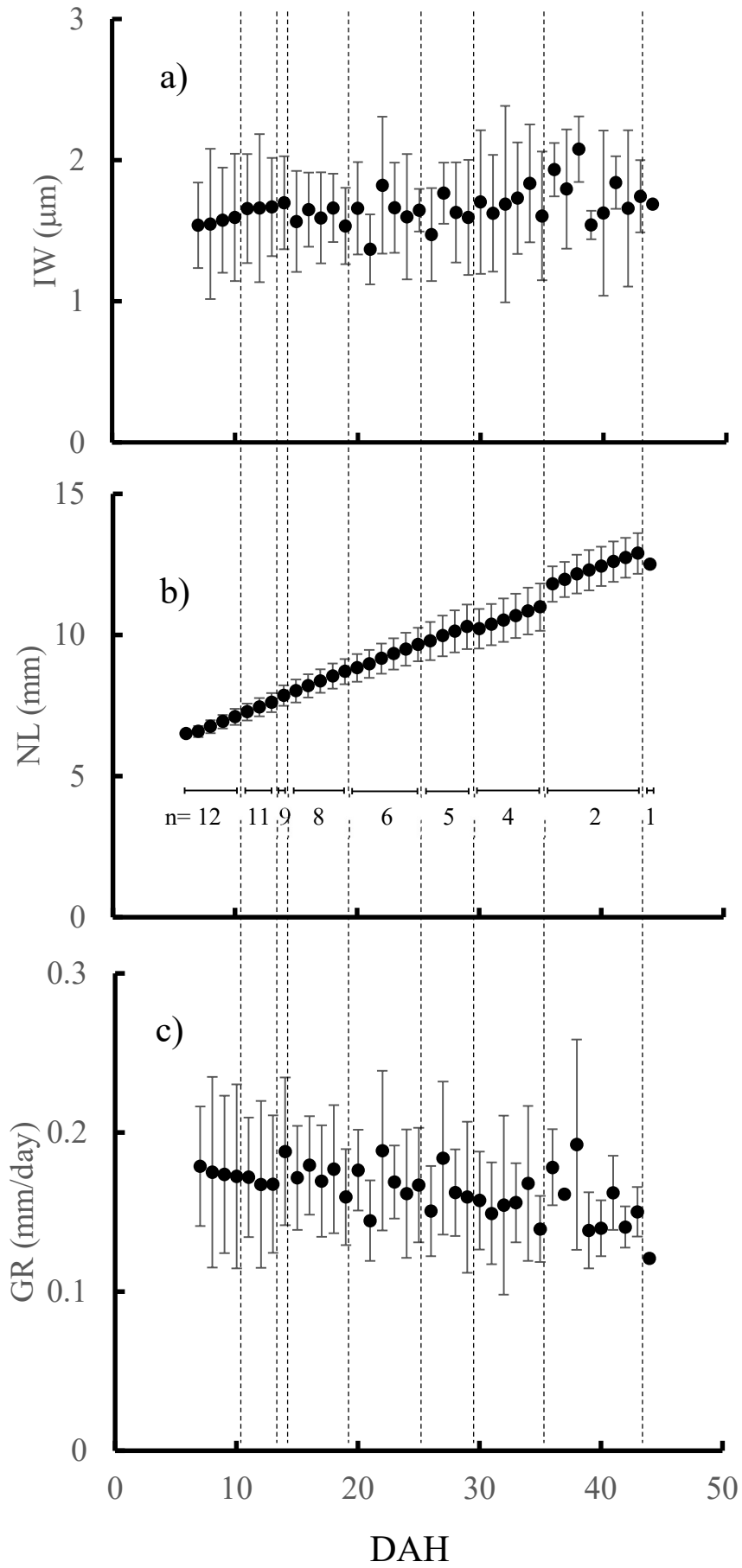


Fig. 7 Gao *et al.*