



Title	Validation of otolith daily increment formation and growth analysis of yellow goosfish <i>Lophius litulon</i>
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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 2nd 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 goosfish 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 m³) 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 Indeplus; 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 μm) 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 (μm) 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 ± 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 ± 0.2 mm). Also, some larvae started feeding on *Artemia* nauplii from 12 DAH
126 (NL: 7.8 ± 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 ± 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 ± 1.4 μ m
142 forming the hatch check (1st check). From hatch to 5 DAH, there was no clearly observable structure
143 indicating the 2nd check. At 6 DAH, the 2nd check was observed which may correspond to the energy
144 transition from endogenous to exogenous nutrition, located at 28.1 ± 0.7 μ m (mean \pm SD) from the core
145 structures. Thereafter the radius of the otolith increased and reached 52.5 ± 3.4 μ 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 μm and
147 1.4 \pm 0.1 μm . 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 2nd check
152 in lapillus radius of wild caught larvae was 24.1–30.2 μm (26.6 \pm 1.9 μm (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 2nd 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 μm and
156 1.7 \pm 0.1 μm (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 μm (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 (1st check), though there were several not well-defined otolith daily
174 increments observed after this check. At 18–20°C, the 2nd 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 1st (LR: ca. 15–17 μm) is the hatch check and the 2nd (LR: ca. 28–30 μm) 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 (1st check: hatch and 2nd 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 2nd check in LR of wild and reared larvae. This result
196 assures that increments after the 2nd 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 2nd 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 2nd 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 μm , 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 μm)
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

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231

232 **References**

233

- 234 Campana SE (1990) How reliable are growth back-calculations based on otoliths? *Can J Fish Aquat Sci*
235 47: 2219–2227
- 236 Dou SZ, Masuda R, Tanaka M, Tsukamoto K (2005) Effects of temperature and delayed initial feeding on
237 the survival and growth of Japanese flounder larvae. *J Fish Biol* 66: 362–377
- 238 Fukuhara O (1990) Effects of temperature on yolk utilization, initial growth, and behavior of unfed marine
239 fish-larvae. *Mar Biol* 106: 169–174
- 240 Hernández C, Landa J, Barrado J, Antolínez A, Santos MB (2015) First estimates of age and growth of
241 juvenile black anglerfish (*Lophius budegassa*), in north-eastern Atlantic waters. *Fish Res* 161:

242 269–272

243 Hernández C, Landa J, Antolínez A, Barrado J, Villamor B (2019) Estimation of age and growth of juvenile
244 angler *Lophius piscatorius* in the Bay of Biscay from otolith microstructure analysis. Reg Stud Mar
245 Sci 27: 100508. <https://doi.org/10.1016/j.rsma.2019.100508>

246 Hislop JRG, Gallego A, Heath MR, Kennedy FM, Reeves SA, Wright PJ (2001) A synthesis of the early
247 life history of the anglerfish, *Lophius piscatorius* (Linnaeus, 1758) in northern British waters. ICES J
248 Mar Sci 58: 70–86

249 Hoshino N, Ichige S, Suzuki M, Yamaguchi Y, Toyoshima S (2006) Breeding experiment of anglerfish,
250 *Lophius litulon* and examination as an object species farming. Bull Ibaraki Pref Fish Exp Stn 40: 11–28
251 (in Japanese with English abstract)

252 Houde ED (1997) Mortality. In Chambers CR & Trippel E (eds) Early life history and recruitment in fish
253 populations. Chapman and Hall Publications, London, pp 173–196

254 Houde ED (2002) Mortality. In Fuiman LA & Werner RG (eds) Fishery Science: The unique contributions
255 of early life stages. Blackwell Science Publications, Oxford, pp 64–87

256 Johns DM, Howell WH (1980) Yolk utilization in summer flounder (*Paralichthys dentatus*) embryos and
257 larvae reared at two temperatures. Mar Ecol Prog Ser 2: 1–8

258 Joh M, Nakaya M, Yoshida N, Takatsu T (2013) Interannual growth differences and growth-selective
259 survival in larvae and juveniles of marbled sole *Pseudopleuronectes yokohamae*. Mar Ecol Prog Ser 494:
260 267–279

261 Kawano M (2010) Study on management of anglerfish, *Lophius litulon* in the southwestern Japan Sea-II A
262 trial of population analysis of anglerfish. Bull Yamaguchi Pref Fish Res Cen 8: 37–43 (in Japanese
263 with English abstract)

264 Kim YU (1976) On the morphology of larval stages of *Lophius litulon* (Jordan). Bull Korean Fish Soc 9:
265 273–280 (in Korean with English abstract)

266 Kobayashi N, Isoda Y, Horio Y (2019) Numerical experiments of surface clockwise circulation in Funka
267 Bay during early summer. *Oceanogr Jpn* 28: 51–74 (in Japanese with English abstract)

268 La Mesa M, De Rossi F (2008) Early life history of the black anglerfish *Lophius budegassa* Spinola, 1807
269 in the Mediterranean Sea using otolith microstructure. *Fish Res* 93: 234–239

270 Meekan MG and Fortier L (1996) Selection for fast growth during the larval life of Atlantic cod *Gadus*
271 *morhua* on the Scotian Shelf. *Mar Ecol Prog Ser* 137:25–37

272 Mito S (1963) Pelagic fish eggs from Japanese waters—X Gadida and Lophiida. *Jpn J Ichthyol* 11: 103–113
273 (in Japanese with English abstract)

274 Nakaya M, Morioka T, Fukunaga K, Sekiya S, Jinbo T, Nagashima H, Ueno Y (2008) Validation of otolith
275 daily increments for larval and juvenile Japanese halfbeak *Hyporhamphus sajori*. *Fish Res* 93:
276 186–189

277 Nakaya M, Takeya Y, Suzuki R, Noro K, Gao W, Takatsu T (2017) The effect of temperature on the early
278 development and starvation tolerance of yellow goosfish *Lophius litulon*. *Aquacult Sci* 65: 251–254

279 Nihira A (2004) Climate change and population dynamics of demersal fishes. *J North Jpn Fish Eco* 32:
280 42–50 (in Japanese)

281 Radtke RL, Dean JM (1982) Increment formation in the otoliths of embryos, larvae and juveniles of the
282 mummichog, *Fundulus heteroclitus*. *Fish Bull* 80: 201–215

283 Sponaugle S (2010) Otolith microstructure reveals ecological and oceanographic processes important to
284 ecosystem-based management. *Environ Biol Fish* 89: 221–238

285 Suzuuchi T (1993) Yellow goosfish. *Hokusuishi Dayori, Hokkaido Chuo Fish Exp Stn* 22: 29–31 (in
286 Japanese)

287 Takahashi M, Sassa C, Tsukamoto Y (2012) Growth-selective survival of young jack mackerel *Trachurus*
288 *japonicus* during transition from pelagic to demersal habitats in the East China Sea. *Mar Biol* 159:
289 2675–2685

- 290 Takeya Y, Nara K, Kosaka Y (2013) Behavioral analysis of the yellow gosefish *Lophius litulon* using bio-
291 logging techniques. J Fish Technol 6: 1–15 (in Japanese with English abstract)
- 292 Tanaka K, Mugiya Y, Yamada J (1981) Effect of photoperiod and feeding on daily growth patterns in
293 otoliths of juvenile *Tilapia nilotica*. Fish Bull 79: 459–466
- 294 Tominaga O (1991) Drastic increase in catch of yellow gosefish, *Lophius litulon*, in the coastal waters of
295 western Hokkaido in 1989. Sci Rep Hokkaido Fish Exp Stn 36: 13–17 (in Japanese with English
296 abstract)
- 297 Wright PJ, Woodroffe DA, Gibb FM, Gordon JDM (2002) Verification of first annulus formation in the
298 illicia and otoliths of white anglerfish, *Lophius piscatorius* using otolith microstructure. ICES J Mar
299 Sci 59: 587–593
- 300 Yamada U, Tokimura M, Horikawa H, Nakabo T (2007) Fishes and Fisheries of the East China and Yellow
301 Seas. Tokai Univ. Press, Hadano, Japan. (in Japanese)
- 302

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 goosefish
308 larvae reared in a tank

309 **Fig. 3** Otoliths from larval yellow goosefish. 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 2nd 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 goosefish 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 goosefish 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 goosefish (13.4 mm NL). a: microstructure of the core
318 area (HC: Hatch check; 2C: 2nd 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 goosefish larvae.
321 Numerals in the figure show the number of samples. Circles and vertical bars indicate mean values and
322 standard deviations, respectively

323

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

324

325

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		n	Range of NL (mm)	Estimated
		Gear	Tows			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

326

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)

329

330

331

332

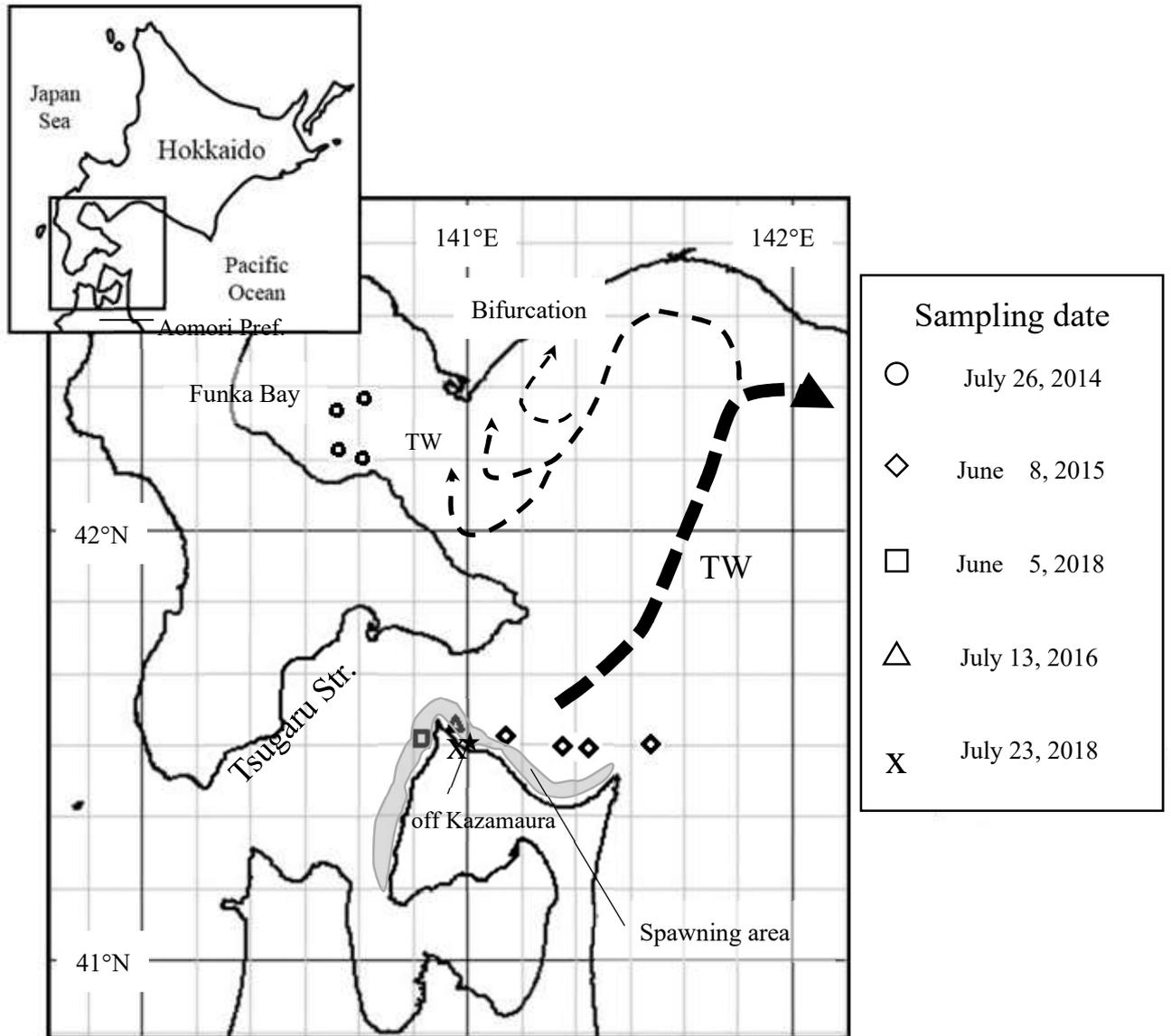


Fig. 1 Gao *et al.*

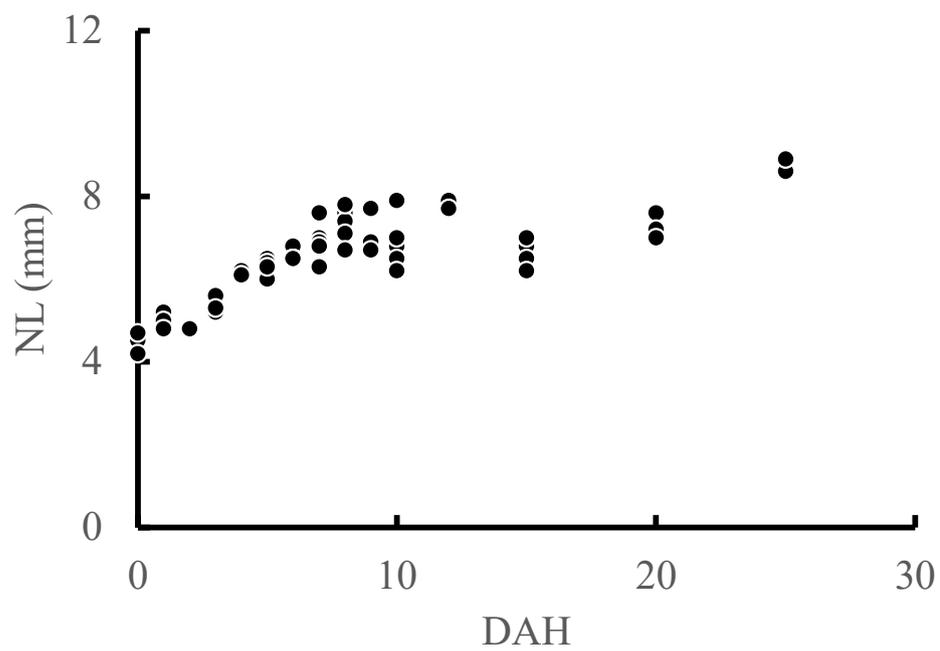


Fig. 2 Gao *et al.*

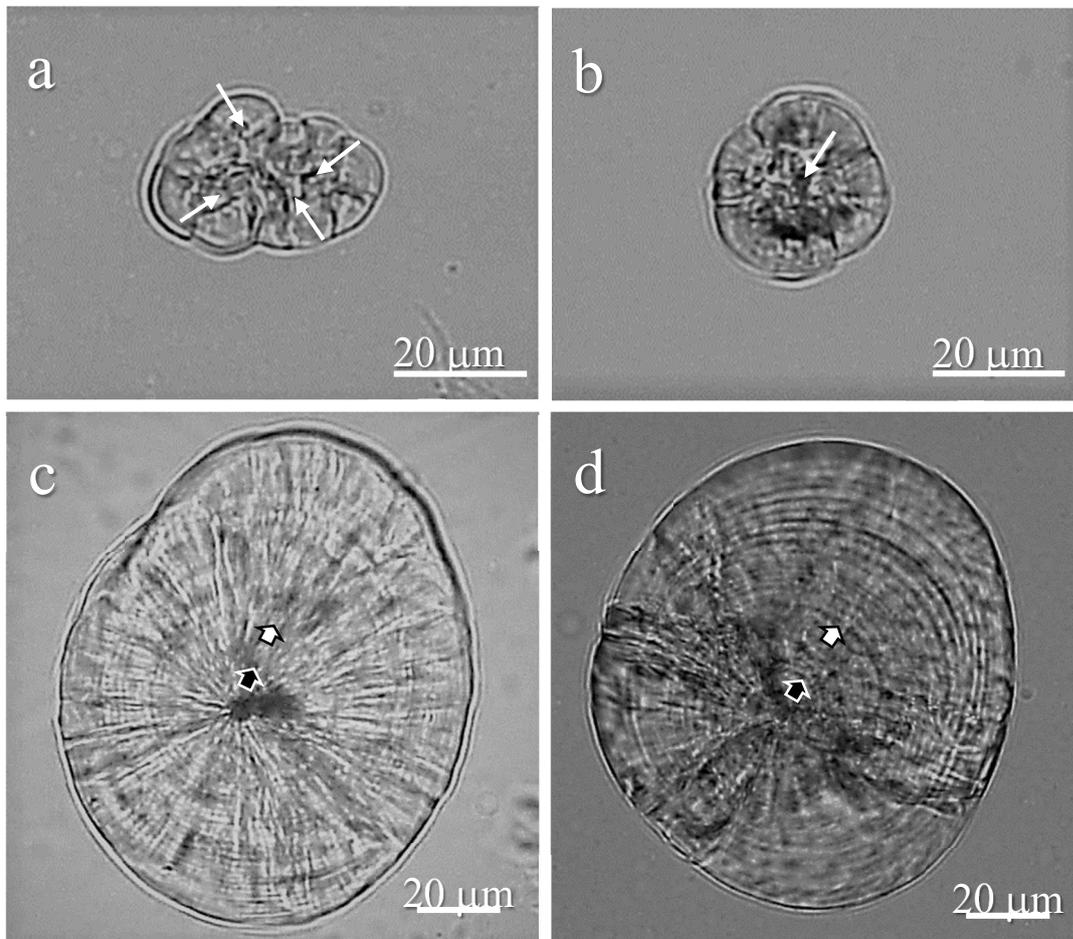


Fig. 3 Gao *et al.*

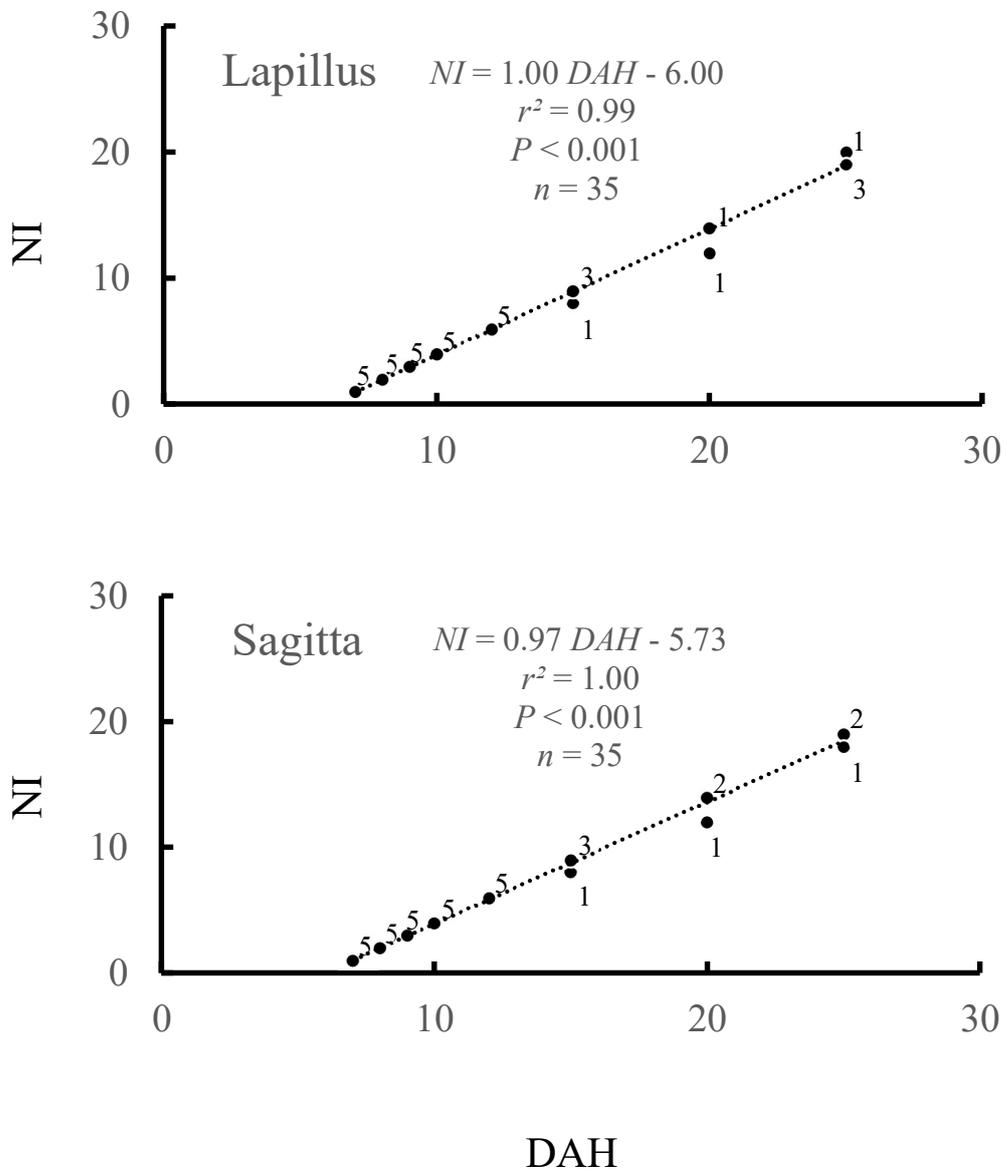


Fig. 4 Gao *et al.*

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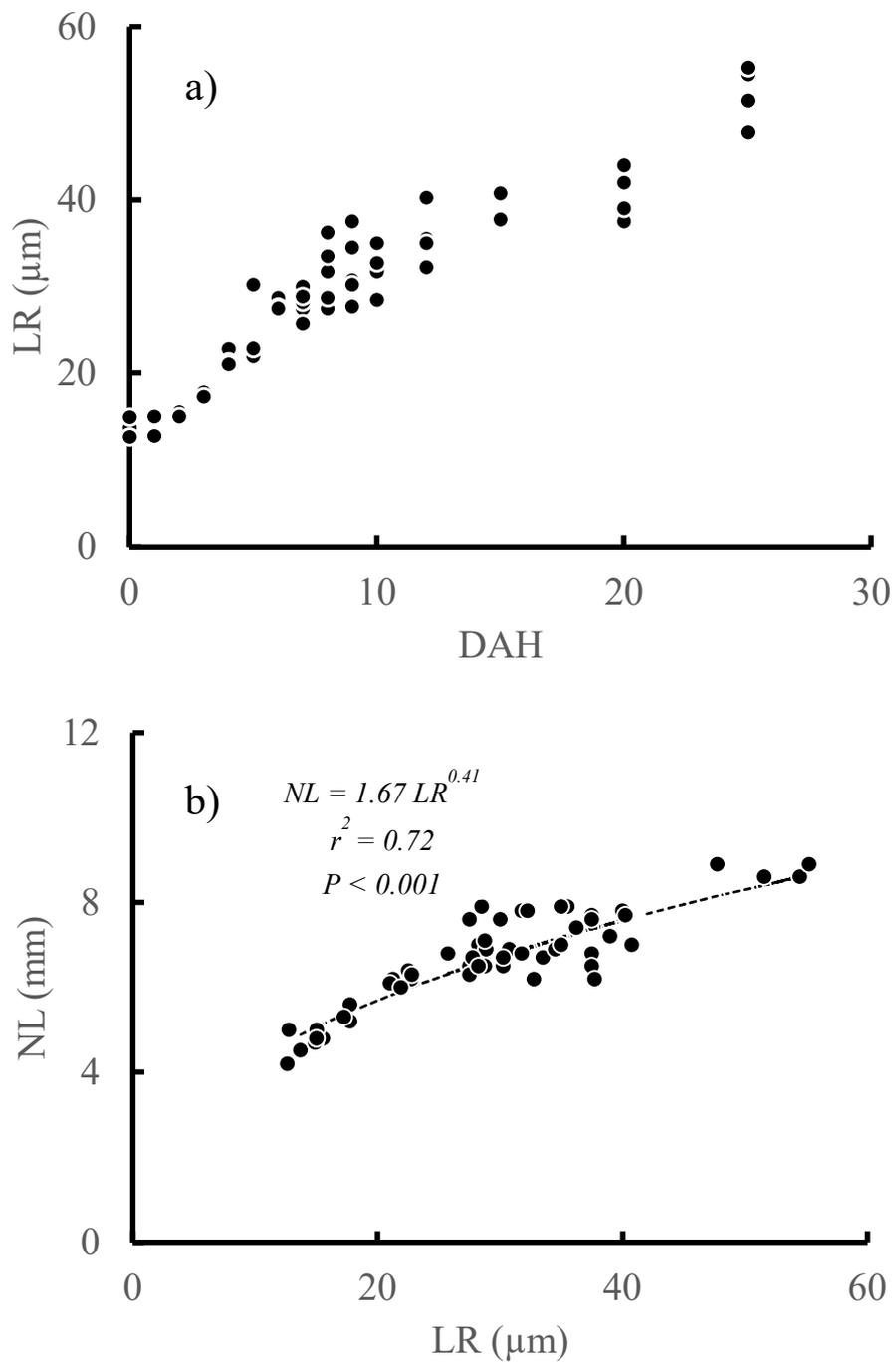


Fig. 5 Gao *et al.*

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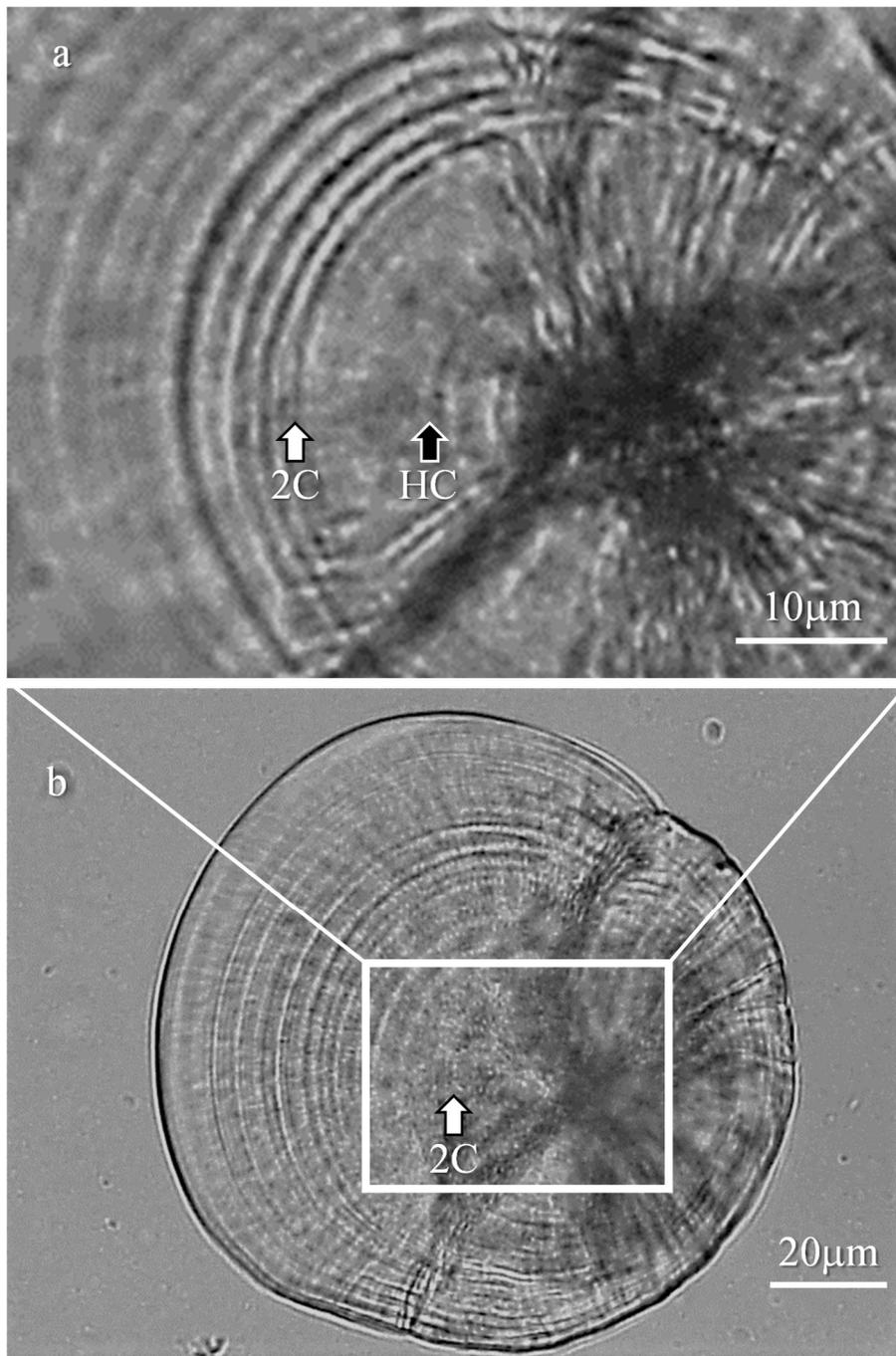


Fig. 6 Gao *et al.*

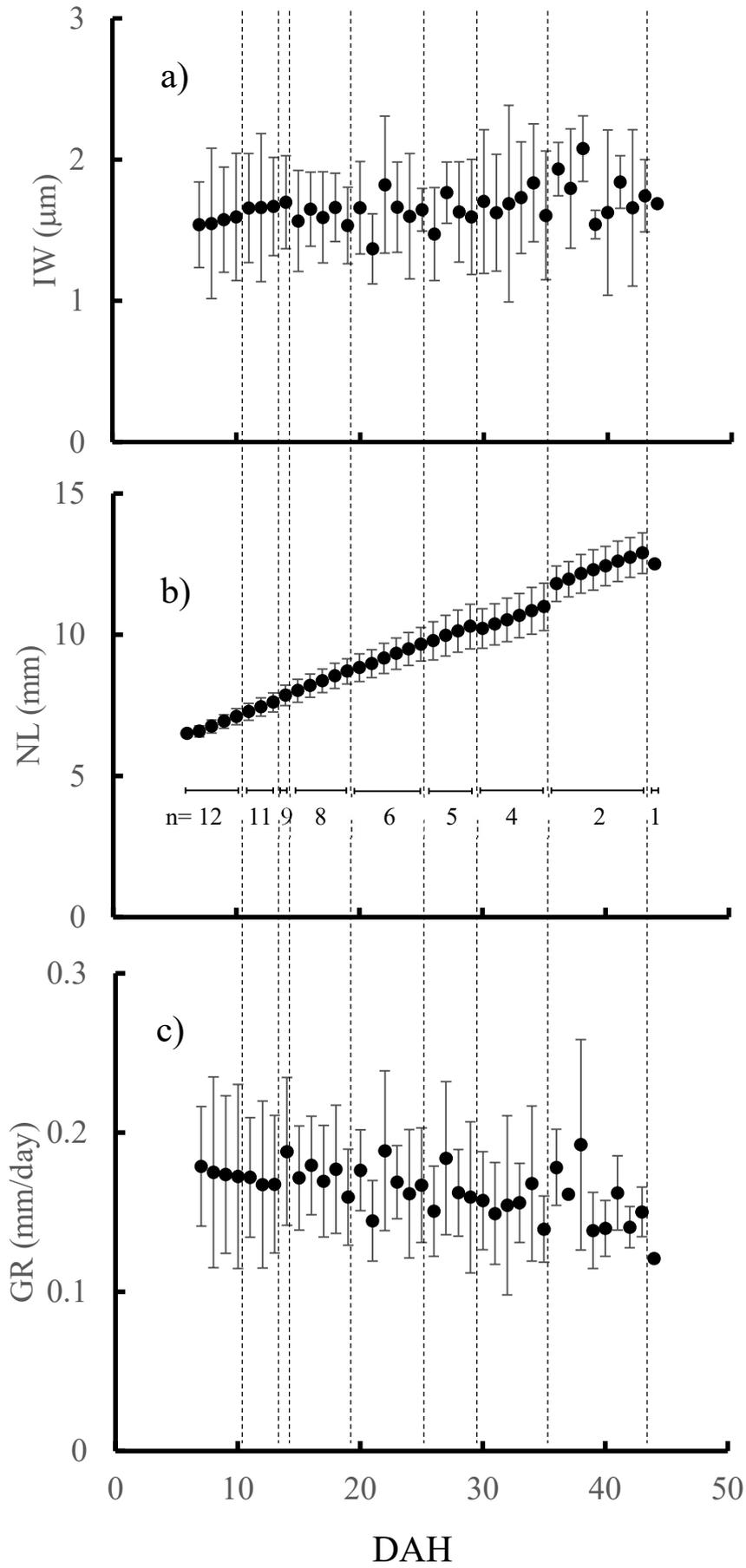


Fig. 7 Gao *et al.*