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

Running head: Growth of goosefish in early life stages

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Abstract

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

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

Introduction

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

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

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

Materials and methods

Larval rearing

An egg veil of yellow goosfish was collected from the sea surface (sea surface temperature (SST): 14.3°C) in the Tsugaru Strait off Kazamaura on 25 May 2016 (Fig. 1). The egg veil was incubated in a 100 L circular shaped tank at a temperature range of 17–18°C (water exchange rate was 30% / day). Newly hatched larvae were stocked in a circular shaped tank (water volume ca. 1 m³) at 18–20°C, and the light condition was the natural light period in the Tsugaru Strait of the spawning season (light and dark periods were 14 h and 10 h). We fed the larvae twice a day with rotifers *Brachionus plicatilis* (enriched with Super Chlorella V-12; Chlorella Industry Co., Ltd., feeding amount: 10–30 ind./mL) and nauplii of *Artemia* sp. (enriched with Indeplus; Sciencetech Co. Ltd.), feeding amount: 5–8 ind./mL) by 4 days after hatching (DAH). Every day from 0 to 10 DAH, and on 12, 15, 20 and 25 DAH, the larvae were sampled and anesthetized with MS-222 and then preserved in 90% ethanol solution (Table 1).

Field sampling

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

Age and growth analysis

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

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

For field samples, NLs at each age were back-calculated using the biological intercept method (Campana 1990) in order to obtain the growth trajectory and daily growth rate for each individual. The relationship between the *i* th lapillus radius and NL was described by the allometric function: $NL_i = a \cdot OR_i^b$ where NL_i 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 increment of each individual fish. Parameters *a* and *b* were estimated using the Microsoft Excel solver routine.

Results

Larval growth in rearing condition

Notochord length of newly hatched larvae of *L. litulon* was 4.5 ± 0.2 mm (mean $\pm$ standard deviation (SD); Fig. 2). Most of the yolk sac was consumed by 5 DAH and then the larvae started feeding on rotifers from 6 DAH onwards (NL: 6.6 ± 0.2 mm). Also, some larvae started feeding on *Artemia* nauplii from 12 DAH (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 and reached 8.8 ± 0.2 mm at 25 DAH, although there was a temporary growth stagnation from 12 to 20 DAH.

Observation of otolith microstructure of reared larvae

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

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

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

Age and growth of wild larvae

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

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

Discussion

Otolith microstructure analysis

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

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

Hatch date and growth of wild population

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

The NL estimated for the field-collected specimens tended to be larger than larvae at 20 DAH obtained in this rearing (that is they did not experience the 12 to 20 DAH growth stagnation observed in the reared specimens). This may be the result of individuals collected in the field growing under more favorable conditions than those in the rearing conditions, or it may be the result of growth-selective survival (e.g. Meekan and Fortier 1996; Takahashi et al. 2012; Joh et al 2013). From 7 to 42 DAH, the daily increment 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 information on the early growth of this species, so comparison can only be made with other species in the same genus, and this range was not remarkably different to other species (from 10 to 40 DAH: ca. 1–3 μm) within the genus, such as *L. budegassa* in north-eastern Atlantic waters (Hernández et al. 2015) and *L. piscatorius* in the Bay of Biscay (Hernández et al. 2019). The mean growth rate of larval *L. litulon* was estimated ca. 0.18 mm /day during 10–40 DAH. Hislop et al. (2001) estimated the initial growth during the larval and juvenile stages of *L. piscatorius* using the Gompertz equation. The larval growth rate is much

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

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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 goosfish
308 larvae reared in a tank

309 **Fig. 3** Otoliths from larval yellow goosfish. 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 goosfish 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 goosfish 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 goosfish (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 goosfish 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

Sampling				Estimated		
Year	Date	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

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

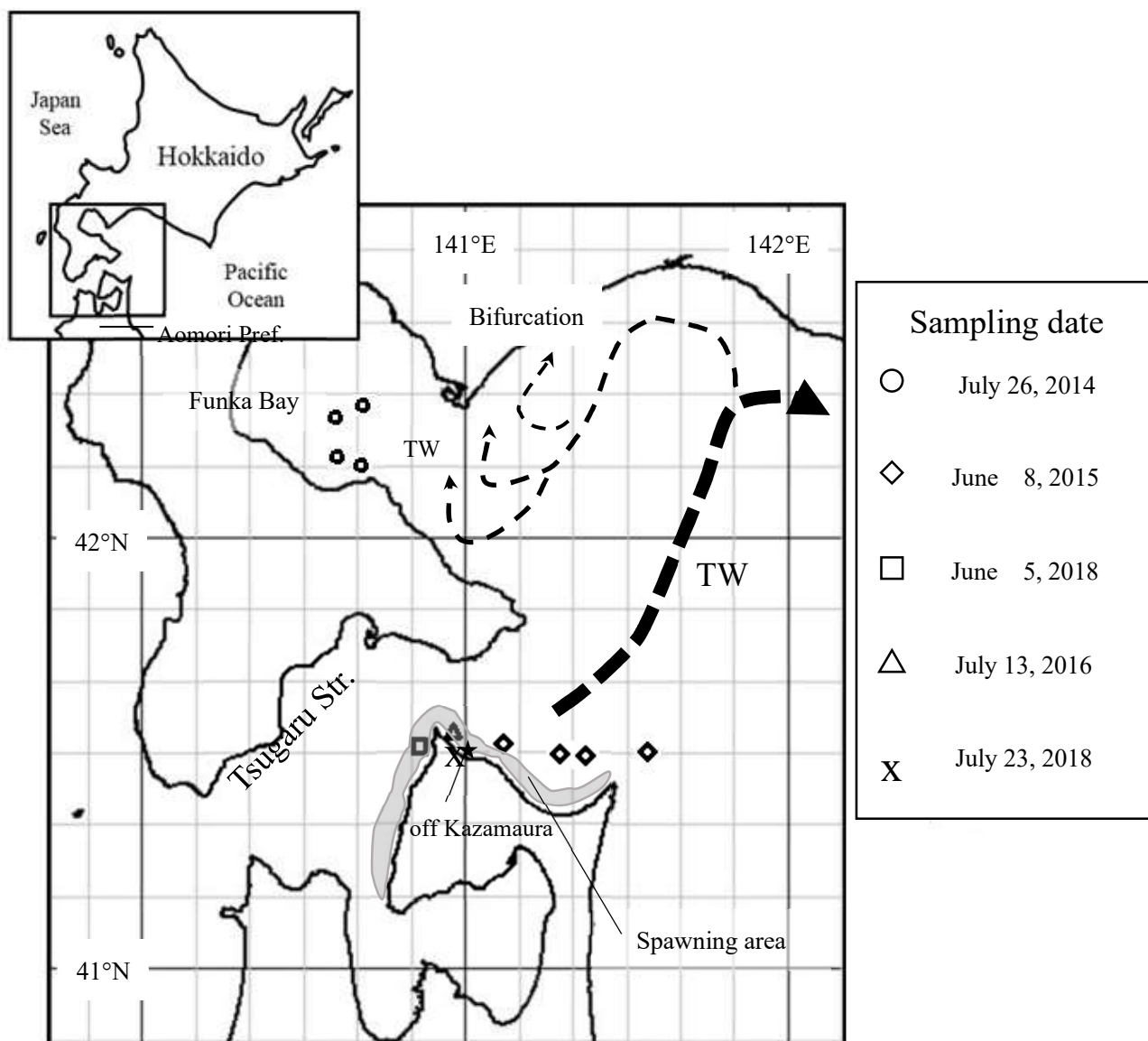


Fig. 1 Gao *et al.*

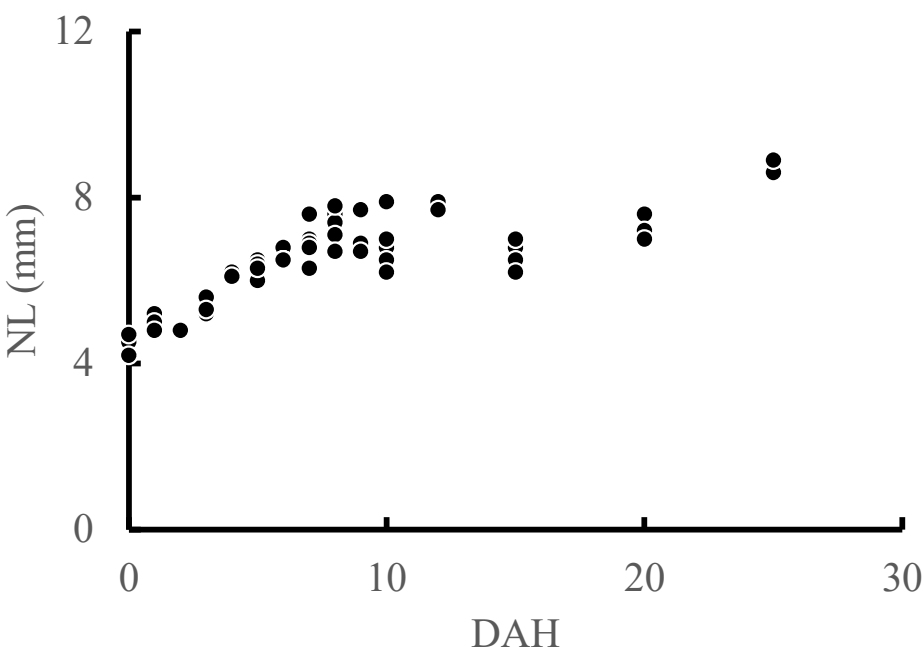


Fig. 2 Gao *et al.*

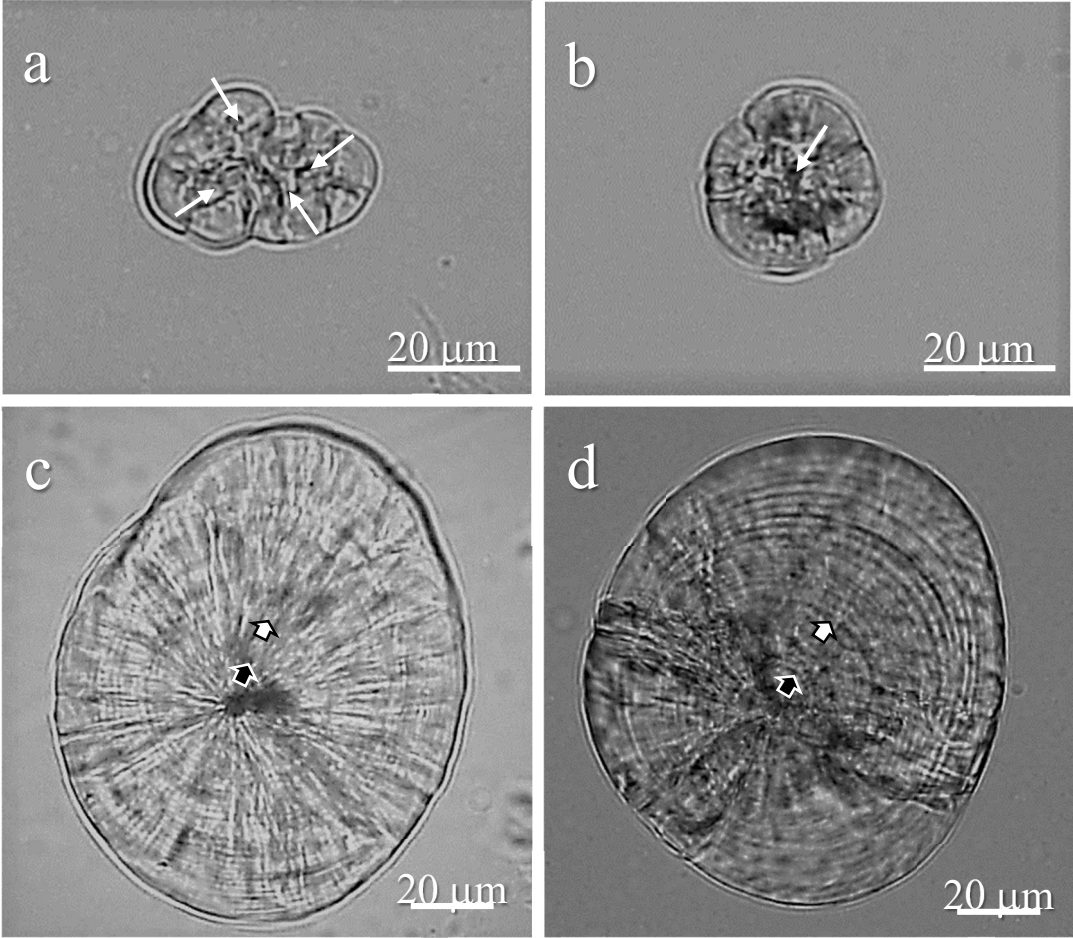


Fig. 3 Gao *et al.*

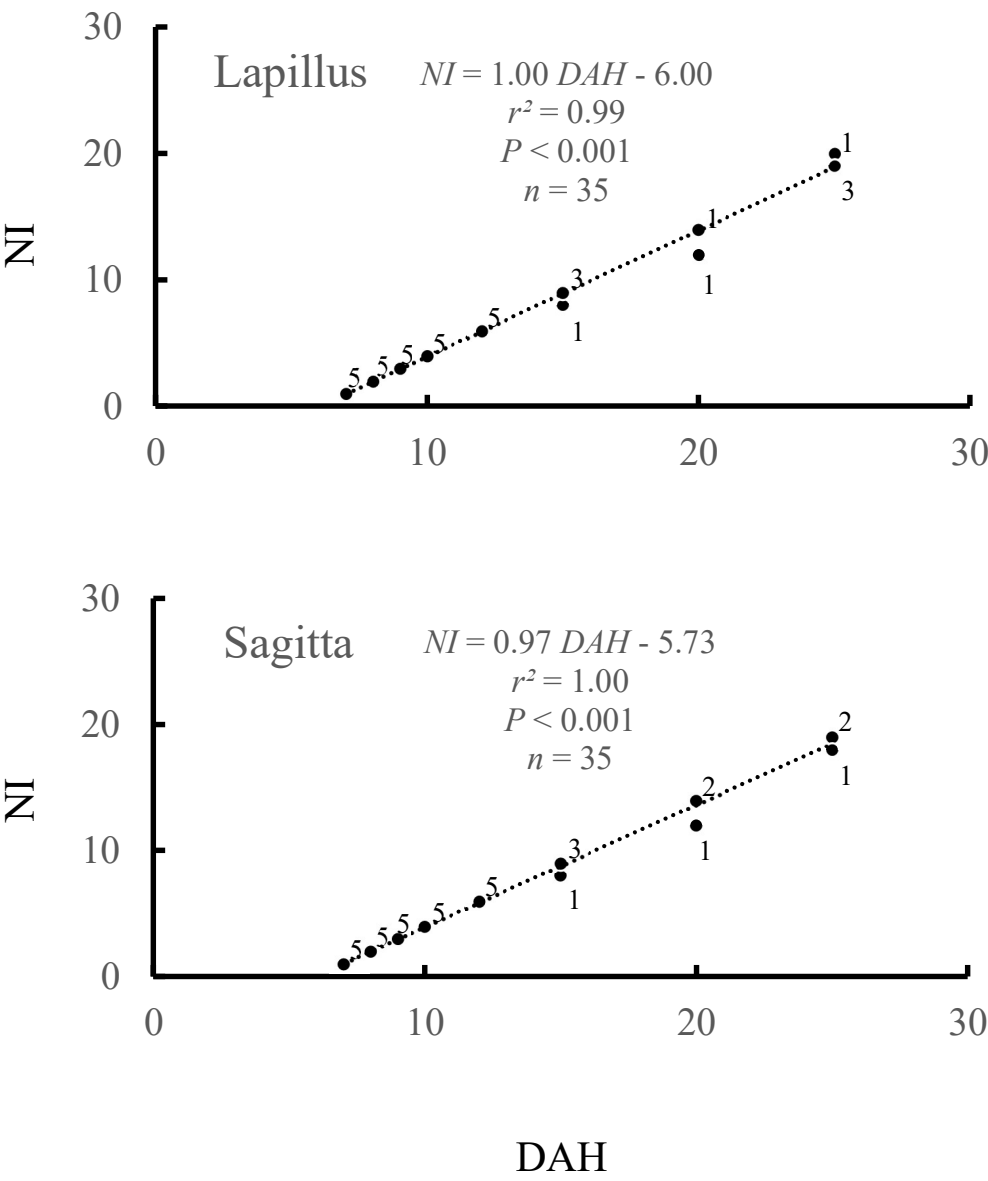


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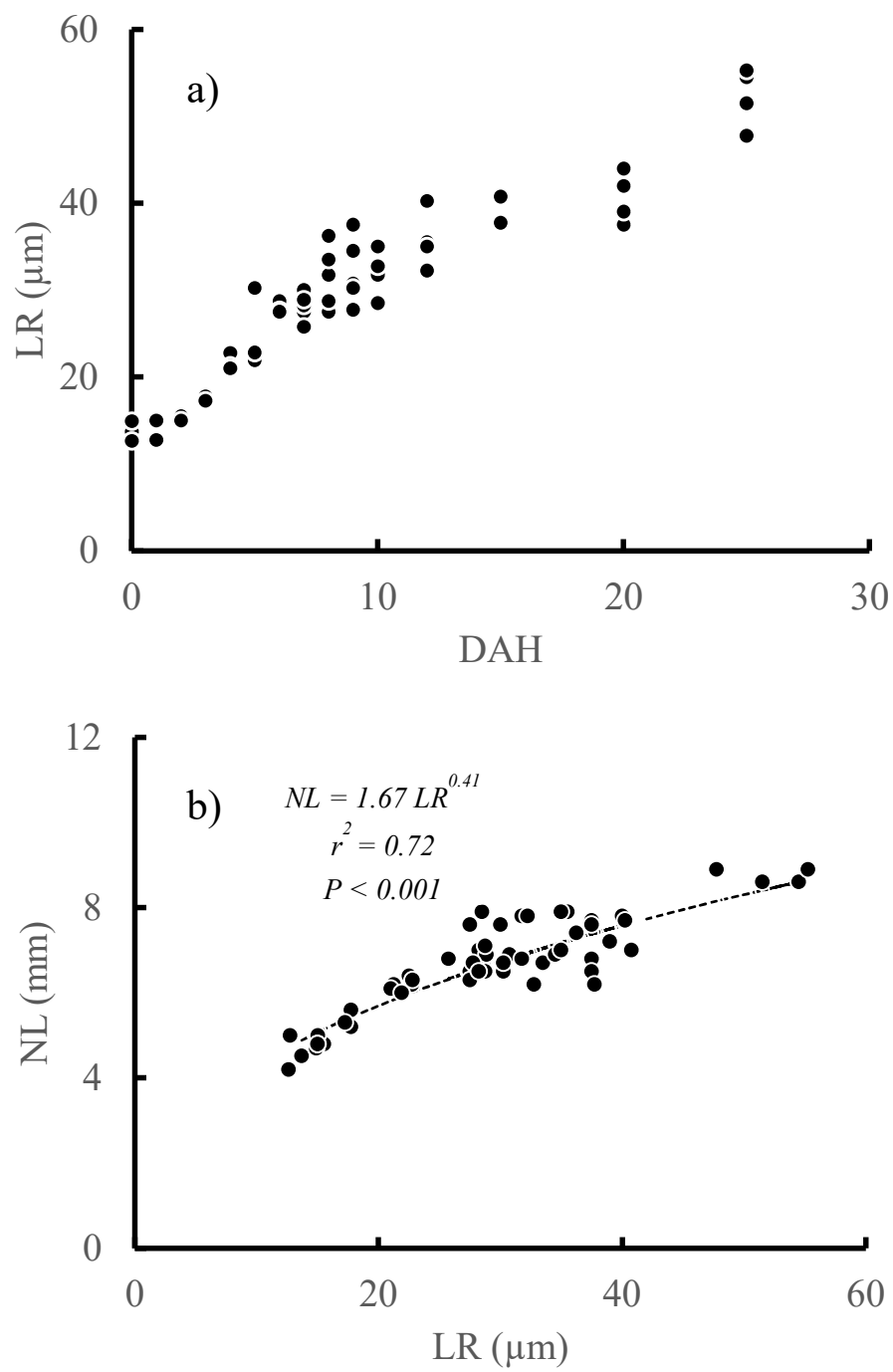


Fig. 5 Gao *et al.*

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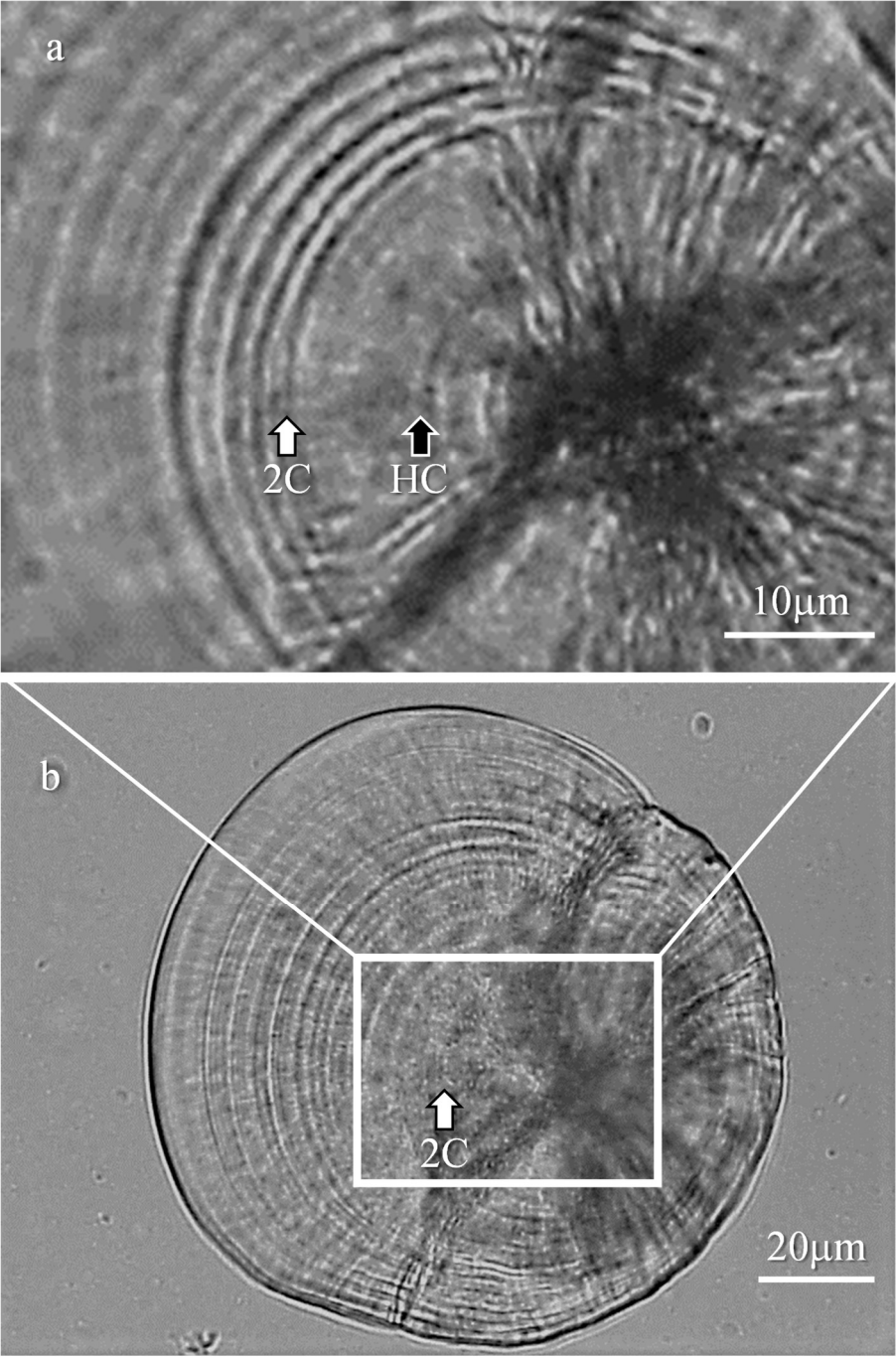


Fig. 6 Gao *et al.*

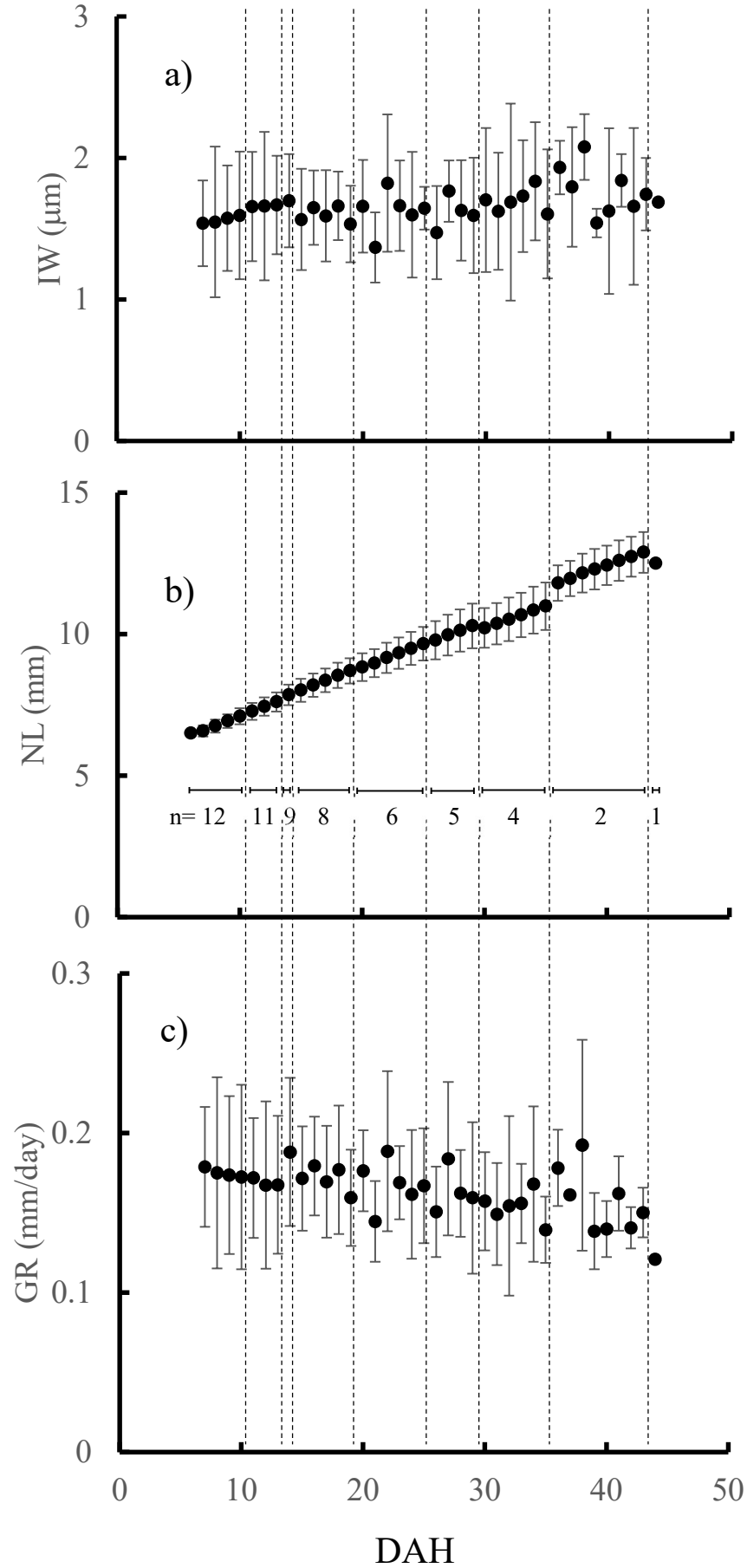


Fig. 7 Gao *et al.*