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Growth and maturation of Pacific saury *Cololabis saira* under laboratory conditions

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Abstract This study details the growth and maturation processes of Pacific saury *Cololabis saira* from eggs to first spawning under laboratory conditions. They were reared in 20ºC, and fed almost to satiation every day. There was no significant difference in the knob length (KnL) between males and females and therefore data were combined in the following Gompertz growth formula $KnL_t = 277.1 \exp(-\exp(-0.015\,(t - 83.8)))$. The first spawning was observed on 243 days after hatching (DAH). At the beginning of spawning, when the mean KnL was about 250mm, only several hundred eggs were spawned at most. The spawning continued, and the number of spawned eggs increased notably after 260 DAH. Correlation and stepwise multiple regression analysis of gonad somatic index (GSI) of Pacific saury versus KnL, CF, and DAH revealed that only DAH (R = 0.88, 0.72, male and female, respectively) was significantly correlated with GSI of Pacific saury ($P < 0.001$). This result suggests that DAH is one of the most influential factors for maturation in this species.

Keywords Growth · Maturation · Pacific saury · Spawning

Introduction

Currently many fisheries species are showing resource depletion and subsequently a reduction in reproductive success [1]. These processes relate not only to global environmental change but also to overfishing [2,3].

Pacific saury *Cololabis saira* is an important pelagic commercial fish that is widely distributed in the northwestern Pacific [4]. However, because of its wide distribution area, the amount of total stock has not yet been fully estimated and landings in Japan as well as body size distributions of the catch show dramatic fluctuations from year by year [5,6]. Fortunately, resources of the species have not been depleted yet unlike many other fisheries species. Therefore we need to understand the causes of these stock fluctuations in order to protect the resource from excess exploitation. To determine the factors causing fluctuations, first we need to obtain information on the life history, age and growth, and reproduction processes.

In previous studies on the life history, the general outline was deduced by Hotta [7] and Kosaka [8]. However after Pannella [9] reported the existence of daily growth rings on the otoliths of fish, it has enabled clarification of the growth processes of various kinds of fish species over the period of less than one year after hatching [10–14]. Also for Pacific saury, the microstructure has been observed for the otolith [15], and Watanabe and Kuji [16] showed that the growth rings are formed daily using a laboratory experiment and thus enabling aging of this species. For estimation of adult age, the first spawning would be expected to occur after the formation of the hyaline zone (there is no space between the rings and / or the rings can not be seen clearly), however for only 17–27% of individuals can the rings be counted during this period [17]. Therefore, true age of the first spawning individuals has not yet been obtained [18].

Sampling for larvae and juveniles of Pacific saury which forms the basis of the stock analysis has continued [19,20]. Growth and survival in the early life history are well known particularly for the nearshore area of Japan [6,21,22]. However as the spawning continues for quite long and the area is widespread in the North Pacific [7,8], the biology of the early life history has not yet been clarified and also their reproduction because the scarcity of samples from the spawning season as no commercial fishing occurs during this season. In general, the initial body size of Pacific saury showing ovary maturation is over 250mm knob length (KnL) and the mode is 270mm KnL, however the minimum size of individual that has already been observed in a spawning experiment was 253mm KnL [18]. Also about 200mm KnL individuals were considered to have taken part in spawning both in the Japan Sea [23] and Suruga Bay [24]. Furthermore other specimens, even at 200mm KnL individuals were found to have over 1g wet weight of ovary that are ripe eggs [24].

Thus, viewpoints regarding the growth and maturation of Pacific saury differ among reports, and the causes of the resource fluctuations remain undetermined. However based on the present sampling scale and methods, clarification of these missing links are unlikely to be resolved.

Ito et al. [25] suggested that modeling approaches are important tools to identify information and data gaps. To investigate the mechanisms affecting the variability in Pacific saury growth, abundance, and biomass, a model which is able to reveal the effect of environmental and feeding conditions is required.

However, experimental information on which to base such a model is still limited [26,27], because Pacific saury are easily damaged in rearing tanks [28–31].

In the present study, first we examined the growth and maturation of Pacific saury under laboratory conditions to obtain some parameters for the model of Ito et al. [25].

Materials and Methods

Fertilized eggs of the Pacific saury, attached to drifting brown algae, were collected by research vessel ‘Asama’ in Kumano-nada, Mie Prefecture (16–17 ºC sea surface temperature), Pacific Ocean Japan on 21–23 April 2005. The eggs were kept in plastic bags containing 10 liters ambient seawater (17 ºC water temperature, 32–34 ppt salinity) with oxygen and then transported to National Fisheries Research Institute, Fisheries Research Agency, Akkeshi by plane. The eggs were stocked and then incubated in a 20 ton concrete tank at a temperature of about 17 ºC (water exchange rate was 120 % / day). After hatching, the larvae were reared in the same tank at a temperature of about 20°C, because the temperature is the best for growth and survival for Pacific saury (for larvae and juveniles [32], from larvae to adults [28–31]). At 116 days after hatching (DAH), 250 individuals of Pacific saury were transferred to a 40 ton concrete tank. The fish were fed to almost satiation on live food (rotifers *Brachionus* spp., and *Artemia* nauplii): usually two times a day (08:00–09:00, 15:00–16:00), frozen copepods (Miyabi No. 1 (300–700μm), and No. 2 (1000–1500μm): JCK Co. Ltd): three times a day (08:00–09:00, 11:00–12:00, 15:00–16:00), artificial feed (Otohime A, B1, B2, and Hirame EP1: Nisshin Marubeni Co. Ltd, thoroughly mixed): 10–20 times a day (depending on their appetite) using a self feeding machine during 06:00–17:00, mince (made of frozen mysid and squid): Monday, Wednesday, and Friday in the morning 10:00–11:00). Daily feed amount and details of the rearing conditions are shown in Table 1. Samples of over 20 individuals were collected every 5 days from 0 to 40 DAH, from 50 to 140 DAH samples of 10–20 individuals every 10 days, from 160 to 260 DAH 10 individuals were collected every 20 days, and when we could observe different or unusual behaviors related to spawning. The sampling was done at feeding time because the fish could be readily caught without causing excess stress.

KnL and body weight in wet weight (BW) of Pacific saury were measured to the nearest 0.1mm and 0.01g after being anaesthetized with FA100 (Dainippon Pharma) or in seawater chilled with ice. KnL is a unit of body length from the edge of the lower jaw to the edge of the meat of silver part which is inside the caudal fin. This part has been shown to have the least measurement bias for Pacific saury [33]. In addition, the condition factor (CF) was examined using the following formula.

$$CF = (BW / KnL^3) \times 10^6$$

Since after 140 DAH, we could make a distinction between males and females by observation of their gonads under a stereomicroscope, all of them were subsequently distinguished for sex, and the samples that were collected after 190 DAH were measured for the gonad weight (*GW*) and gonad somatic index (*GSI*) calculated. *GSI* was calculated using the following formula.

$$GSI = GW \times 10^2 / BW$$

Ovary observations were done using females after 200 DAH that had yet to spawn. Multiple regression analysis [32] was performed (Mulvar95 Version 1.18, Kanda) to determine which factors (KnL, CF, and

DAH) significantly affected GSI of Pacific saury.

To estimate the distribution of their egg diameter, eggs were sampled from 100 randomly selected individuals in the middle part of gonad and then their size was measured to the nearest 0.01mm. Measurements of the egg diameter were based on the method of Suyama [17]. Eggs size was calculated using the equation below.

$$Radius = ((long\ radius^2 + short\ radius^2) / 2)^{1/2}$$

To estimate batch eggs of the first spawning, largest oocytes were counted at one side of the ovary under the stereomicroscope.

From after 200 DAH, 2 pieces of spawning beds (we cut 16mm diameter polyvinyl chloride pipe to a thickness of 2mm width and connected 20 pieces with fine line, like beads (Fig. 1a)) were set in the breeding tank to measure the wet weight of eggs spawned every day (one time a day: 08:00–09:00). The number of eggs laid was estimated after verification of whether any eggs had come off and sank or not (the eggs are heavier than seawater). That is, whether they attached only to the spawning materials or not, and whether cannibalism of eggs occurred (stomach contents of the broodstock were observed by using one part of the sample). The eggs laid on the spawning materials (Fig. 1b) were then removed, wet weight measured, and used as the number of eggs spawned. Eggs of Pacific saury are elliptical (length: 1.8 ± 0.04mm (mean ± SD), width: 1.6 ± 0.06mm). Wet weight of one egg with attachment filament was calculated as 0.0033g, and then one gram of the egg mass was estimated to be 300 eggs in this study.

Three types of growth model (Logistic, von Bertalanffy, and Gompertz) of Akaike Information Criterion [35] calculated using the non-linear least-squares regression procedure (Microsoft Excel solver routine), and the model which has the lowest Akaike Information Criterion value was judged as the best model to data. Difference of growth between sexes was compared with ANCOVA using log transformed data.

Results

Growth and feeding

The relationships between DAH and KnL, and between DAH and BW under 20°C breeding conditions (details shown in Table 1) are shown in Figs. 2, 3. Mean body size of 0 DAH larvae was 6.9 ± 0.26mm KnL (mean ± SD), and 5 DAH larvae was 7.7 ± 0.59mm KnL. Observations of Pacific saury larvae immediately after hatching showed that their digestive tract had not been fully formed yet, although 1 DAH larvae already had a functioning digestive tract, therefore, feeding of rotifers started 1 DAH and continued until 5 DAH. Feeding of *Artemia* nauplii started from 2 DAH, and continued until 40 DAH (41.1 ± 4.73mm KnL). Frozen copepods and artificial feed (Otohime A, B1) started to be fed after 15 DAH (13.0 ± 1.87mm KnL). On 35 DAH (35.9 ± 3.99mm KnL), an auto feeding machine started to be used. At this time, the main artificial feed was Otohime B1, B2. From after 140 DAH (186.9 ± 13.66mm KnL), we fed not only artificial feed (Hirame EP1), but also fed raw mince.

Growth process

After 140 DAH individuals that could be judged whether they were female or male by observation of the gonad under a stereomicroscope were used for estimation of the growth process separately for both male and female (Fig. 4). Their regressions lines are shown below:

Male: $y = 0.54x + 114.0$ ($n = 60$, $R^2 = 0.70$)

Female: $y = 0.56x + 109.9$ ($n = 63$, $R^2 = 0.72$)

where x means DAH, and y means KnL. The regressions of the above two formulas were significant ($P < 0.001$) and there was no significant difference for both slope and intercept ($P = 0.98$, $P = 0.77$, respectively) and therefore, the growth formulas were combined for both sexes.

Three types of growth regressions calculated (Logistic, Gompertz, von Bertalanffy), and then their equations were judged by Akaike Information Criterion. As a result, the Gompertz growth formula was adopted (Fig. 2). The formula is as follows:

$KnL_t = 277.1 \exp(-\exp(-0.015(t - 83.8)))$

where t is DAH, KnL_t is KnL at t DAH.

Maturation and spawning

After 190 DAH, we observed and measured their gonads (Figs. 5, 6 and 7). According to Suyama et al. [18] from the cortical alveoli stage to the primary yolk stage, GSI of Pacific saury notably increases and the GSI becomes more than 1. The first individuals observed that had over 1 GSI was a female on 240

DAH (GSI =1.14, gonad weight = 1.0g). The minimum size of females that had a GSI over 1 was 234.7mm KnL (GSI = 2.80, gonad weight = 1.7g, 310 DAH). After 300 DAH, all female individuals were over 1 GSI, except for 1 individual (GSI 0.91–4.20, gonad weight 0.9–5.5g). The male fish exceeding 1.0 GSI also already had produced a sufficient quality of sperm in their testes to join the spawning (Suyama Unpublished). The first individuals observed that had over 1 GSI was a male on 190 DAH (GSI = 1.14, gonad weight = 0.6g). And also the male was a minimum size that had over 1 GSI (223.6 mm KnL). After 300 DAH, all male individuals were over 1 GSI (GSI 2.18–3.43, gonad weight 2.5–5.5g).

Correlation and stepwise multiple regression analysis of GSI of Pacific saury versus KnL, CF, and DAH revealed that only DAH (R = 0.88, 0.72, male and female, respectively) was significantly correlated with GSI of Pacific saury (P < 0.001).

Oocyte diameters for 200 to 220 DAH individuals that had no experience of spawning were observed (Fig. 8). Although 99% of oocyte diameter range showed overlap (oocyte in the early yolk vesicle stage, e.g., 0.15–0.34mm oocyte diameter reported by Suyama et al. [18]), the remaining 1% were larger and easy to distinguish from the other oocytes (oocyte size equivalent to the secondary yolk stage: 0.52–0.85mm oocyte diameter [18]). These larger oocyte had diameters of 0.51–0.60mm, and their total number was estimated to be 289–350.

The number of eggs and pattern of spawning is shown in Fig. 9. The first spawning (Fig. 1c) was observed on 243 DAH (about 50 eggs attached to the spawning materials). After that, peaks were observed every 3–4 days, and on these days, several hundred eggs were observed each day. About 60 days after first spawning, several thousand eggs were observed on the peak days which were observed in the same cycle (every 3–4 days). At 94 days after first spawning, the total of spawned eggs from first spawning was over 200,000 (number of breeding individuals at first spawning day: 89 individuals, 94 days after first spawning: 47 individuals). These spawned eggs that attached to the spawning materials were almost 100% fertilized.

Discussion

Growth

So far many studies have examined the growth and maturation of wild Pacific saury, however there has not been a fully accepted theory yet. Some reports have discussed about the growth until 6 month old fish. The body size estimates for 6 month old fish by Matsumiya and Tanaka [36] are 182 mm KnL, Watanabe et al. [37] and Suyama [17] are about 200mm KnL respectively.

In this study, the water temperature was maintained at 20ºC and the fish were fed to satiation as a condition. Their growth until a half year after hatching (180DAH: 207.5 ± 19.6 mm KnL (mean ± SD)) was similar to that in the wild, though the environmental conditions and prey items were quite different and also includes a variety of stress. According to Odate [38], wild individuals of Pacific saury migrate from warm sea areas to the Oyashio area (cold sea: sea surface temperature is about 12°C) on their feeding migration. In the Oyashio area, there is much prey (wet mass of zooplankton, ca. 20–30 g /

m^2), though the temperature is low [38]. This migration enhances the growth rate and maturation of this species, although the metabolic mechanisms have not yet been fully clarified. This study was carried out under the optimum temperature condition for growth of Pacific saury (for larvae and juveniles [32], from larvae to adults [28–31]: that is 20ºC) with enough food at this temperature. Therefore, the growth process of this study would show similar to the growth process in the wild.

From half a year after hatching, opinions on their growth vary among studies. Matsumiya and Tanaka [36] estimate that this species grows up 223mm KnL at one year based on size distribution. The Pacific saury caught from the western North Pacific were divided into three groups; the small size (200–240mm KnL), medium size (241–280mm KnL) and large size (> 280mm KnL) [39]. Watanabe et al. [37] estimate the period to grow from a small size (200–240mm KnL) to a large size (> 280mm KnL) to be half a year, and Suyama [17] estimates for the same period to be one year based on daily growth ring analysis. The reason for the difference of opinions is the existence of hyaline zones on the otolith. The zone does not have clear daily growth rings making the count of the number difficult. This study used reared individuals that were reared from eggs, therefore there is no doubt regarding the relationships between age and growth. Thus, we directly and firstly obtained evidence for the growth from half a year after hatching. The mean body size of 336 DAH individuals was 270mm KnL. This size is included in middle sized fish category (241–280mm KnL) used for grading commercially landed fish. And the growth process (Fig. 2) expected that the growth speed would be quite slow after growth to the middle size (241–280mm KnL) and it would spend much time to grow to the large size (> 280mm KnL).

Maturation and spawning

During the start of the spawning period, one spawner might spawn eggs a few at a time, or one or two spawners might spawn in turn. As their gonad weight was about 1g at most when the spawning began (GSI was about 1), the number of eggs released would total about 300 eggs. The number of peaks of the attached eggs on the spawning materials increased sharply after 60 days after first spawning (300 DAH, estimated mean body size: 266.5mm KnL). At the time, the reason may be that not only most of the individuals started to spawn, but also the number of eggs per batch increased for each spawner. After 300 DAH, most of the females spawned.

So far there are some reports about the number of eggs per batch for Pacific saury (Kubo [40]: 120–1720 eggs, Hatanaka [41]: 300–4500 eggs, Kosaka [8]: 947–2547 eggs, and Suyama [17]: 500–4000 eggs (mean: 2000)). In this study, we measured their oocyte diameter using 200 to 220 DAH individuals that had no experience of spawning. Although 99% of oocyte diameters showed overlap, the remaining 1% of oocytes were quite large and easy to distinguish as a single batch. Their total number was estimated as 289–350 oocytes. The number of batch eggs at the first spawning was estimated to be about 300 eggs (gonad weight: 1g) at most. And after first spawning, the number of batch eggs increased during continued spawning. In the above reports which have differences of opinion probably due to the samples including a variety of spawners that have different spawning experience like first, second, and so on. In addition, the results of this study indicate that the number of DAH affects more their maturation than their body size. This may be one of the reasons for the difference of the reported biological minimum size of this species among sampling areas. Probably, they will be old enough to spawn, though the body size is small.

We need to continue rearing this batch to estimate the total number of spawned eggs in a lifetime. Total number of the spawned eggs in a single spawning season or spawning interval may change due to the feeding conditions during the spawning period. For example for northern anchovy [42] and Japanese anchovy [43], it is pointed out that the feeding conditions during the spawning period would effect the amount of eggs produced [44,45]. Feeding condition during spawning season also effect the growth. Further investigation is needed to compare the rearing data and the field data in order to obtain more biological data on this species under a variety of environmental conditions.

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Table 1 Details of the daily feeding amount and breeding conditions of Pacific saury *Cololabis saira* in this experiment

Days after hatching	Mean [a]KnL (mm)	Mean Body weight (wet: g)	[b]Rotifers (inds/ml)	[b]*Artemia* nauplii (inds/ml)	[c]Frozen copepods (%)	[c]Artificial feed (%)	[c]Mince (%)	Breeding individuals	Water temperature (℃)	Water volume of the tank (t)	Daily water exchange rate (%)
5	7 7		4 0	1 0				8320	20 0	12	300
10	10 2			1 5				6718	20 0	12	300
20	18 1	0 02		2 0	123 0	31 0		4864	20 0	12	300
30	28 3	0 09		3 0	33 0	8 3		4000	20 0	12	300
40	41 1	0 27		4 0		10 0		3858	20 0	12	300
50	53 6	0 57				10 0		2510	20 0	12	480
60	67 2	1 12				10 0		2422	20 0	12	480
80	99 8	3 82				8 0		2380	20 0	12	480
100	125 7	7 12				8 0		1225	20 0	12	480
120	159 4	15 60				8 0		250	20 0	12	300
140	186 9	28 88				7 0	4 5	229	20 0	20	300
160	197 2	33 42				3 7–4 0	3 0–3 9	200	20 0	20	0
180	207 5	38 13				3 7–4 0	5 3	149	20 0	20	0
200	226 7	53 46				3 7–4 0	4 2	132	19 6	20	0
250	248 4	76 78				3 7–4 0	5 7	76	20 0	20	0
327	288 5	135 67				3 3	4 1	27	20 1	20	0
340	287 0	121 74				2 7	4 2	23	19 9	20	0

[a] KnL means knob length
[b]Feeding density at each feeding
[c]Daily feeding amount per body wet weight of Pacific saury

Fig. 1 Spawning beds (using 16mm diameter polyvinyl chloride pipe **(a)**), the eggs **(b)** and spawning behavior of Pacific saury *Cololabis saira* **(c)**

Fig. 2 Relationships between days after hatching (DAH) and knob length (KnL) of Pacific saury *Cololabis saira*

Fig. 3 Relationships between days after hatching (DAH) and body weight in wet weight (BW) of Pacific saury *Cololabis saira*

Fig. 4 Relationships between days after hatching (DAH) and knob length (KnL) of Pacific saury *Cololabis saira* for each sex

Fig. 5 Relationships between knob length (KnL) and gonad somatic index (GSI) of Pacific saury *Cololabis saira*

Fig. 6 Relationships between knob length (KnL) and gonad somatic index (GSI) of Pacific saury *Cololabis saira*

Fig. 7 Relationships between days after hatching (DAH) and gonad somatic index (GSI) of Pacific saury *Cololabis saira*

Fig. 8 Distribution of egg diameter of Pacific saury *Cololabis saira* before first spawning

Fig. 9 Relationships between days after first spawning and number of eggs released each day by the Pacific saury *Cololabis saira*

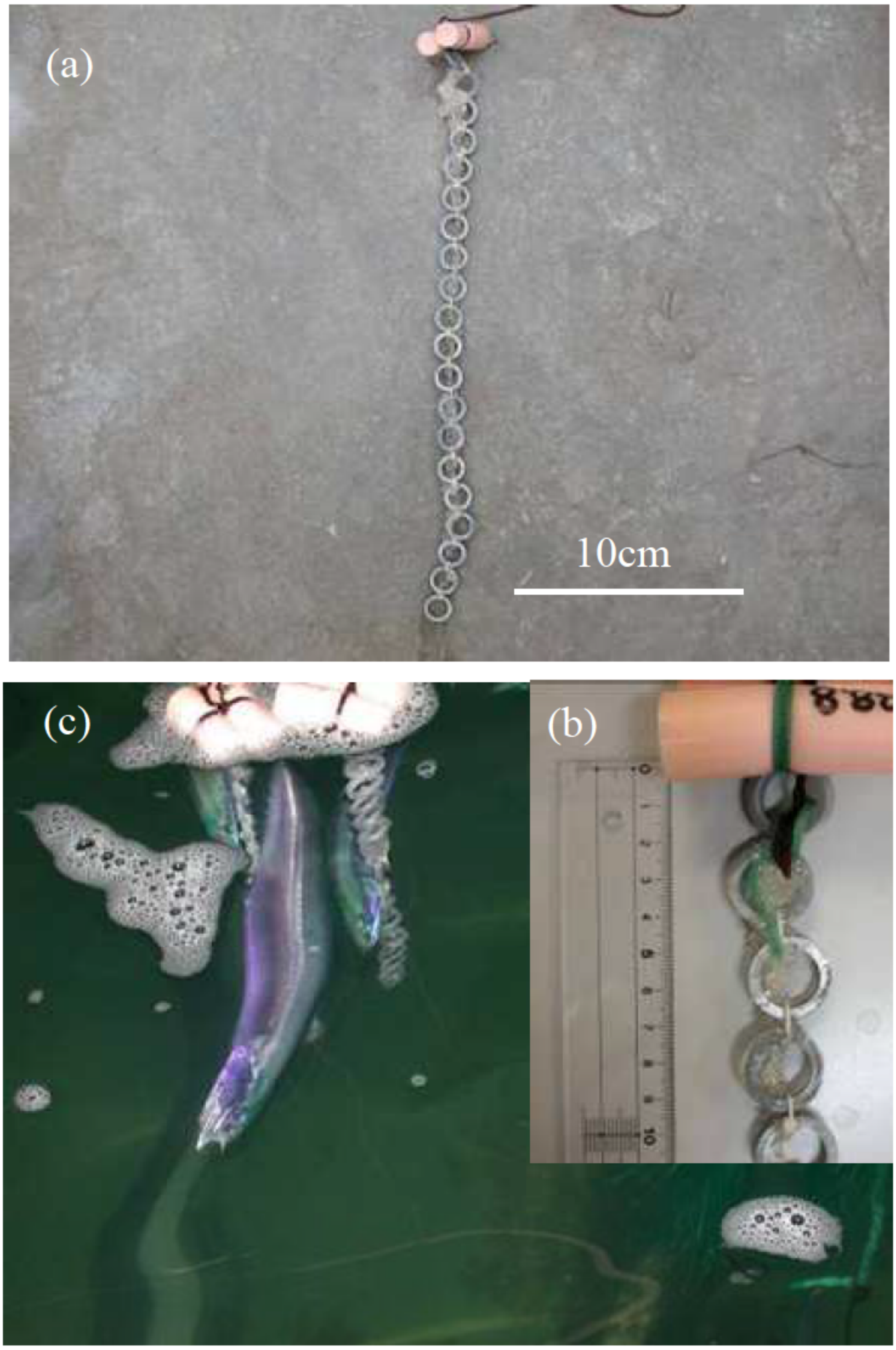


Fig. 1 Nakaya et al.

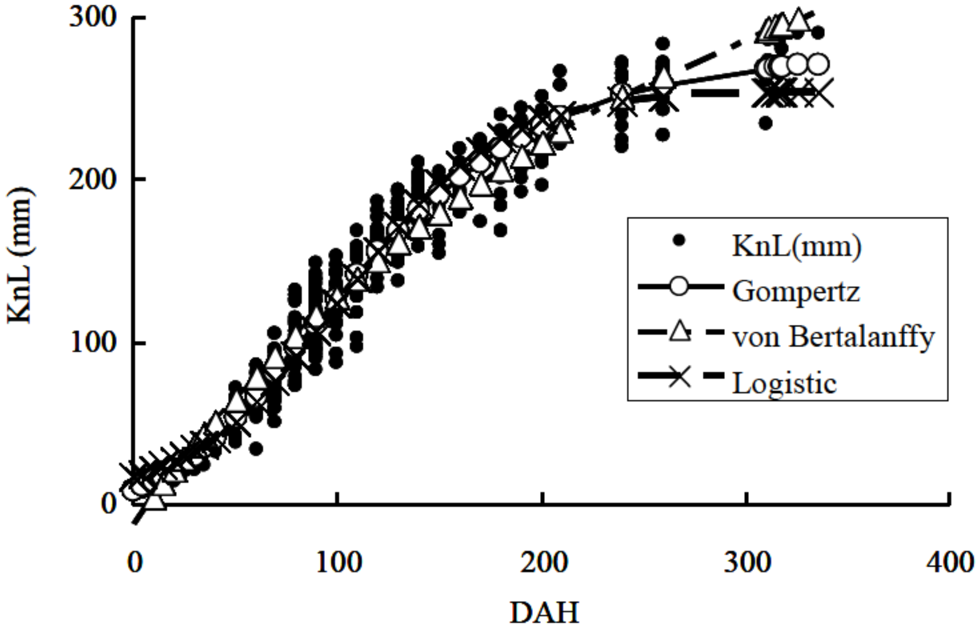
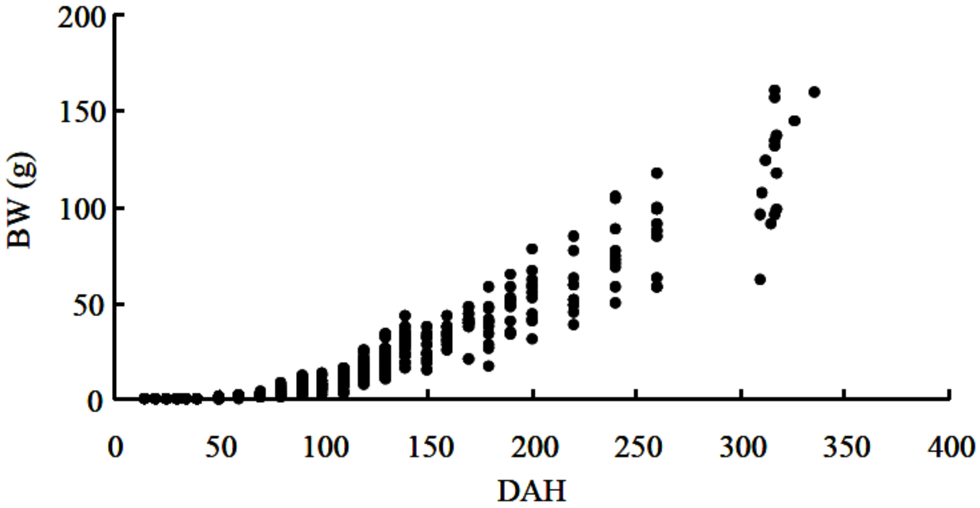


Fig. 2 Nakaya et al.



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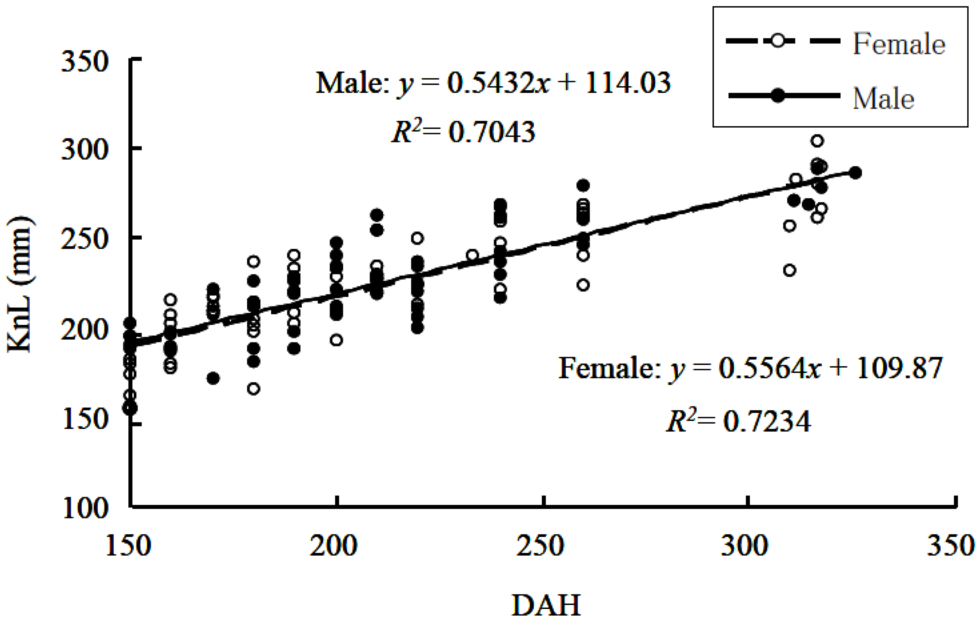

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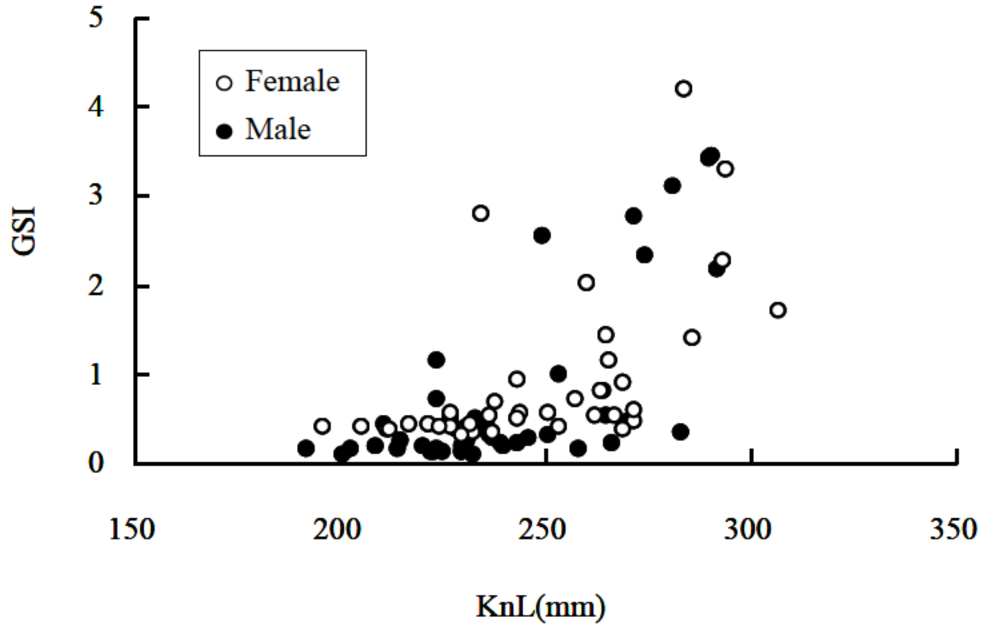

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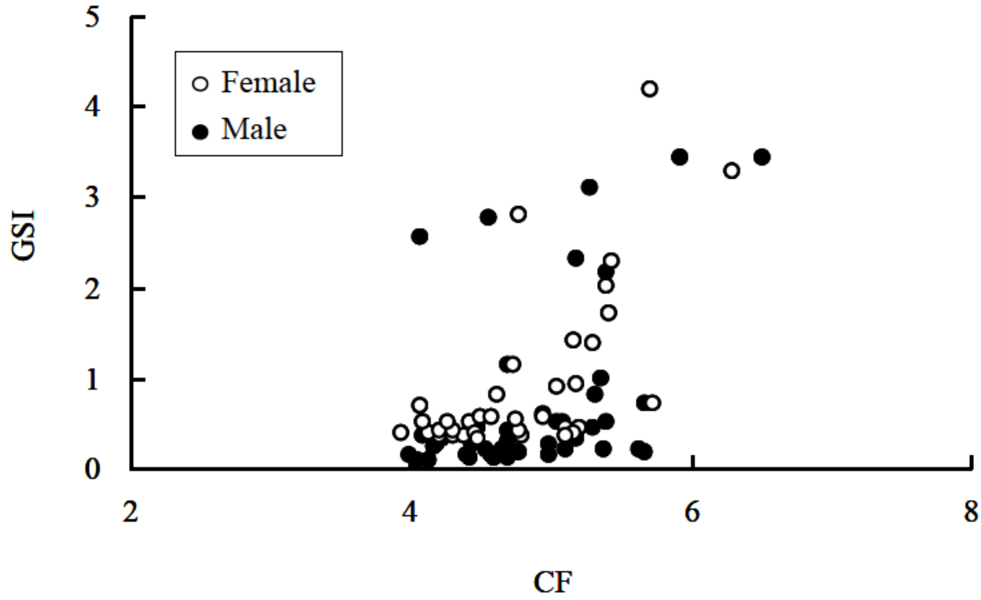

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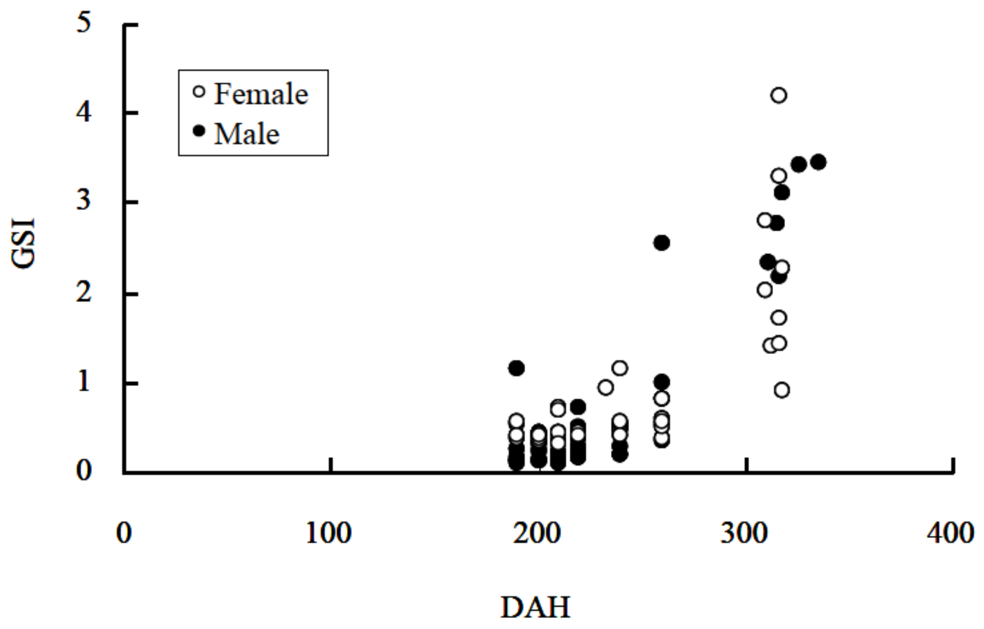

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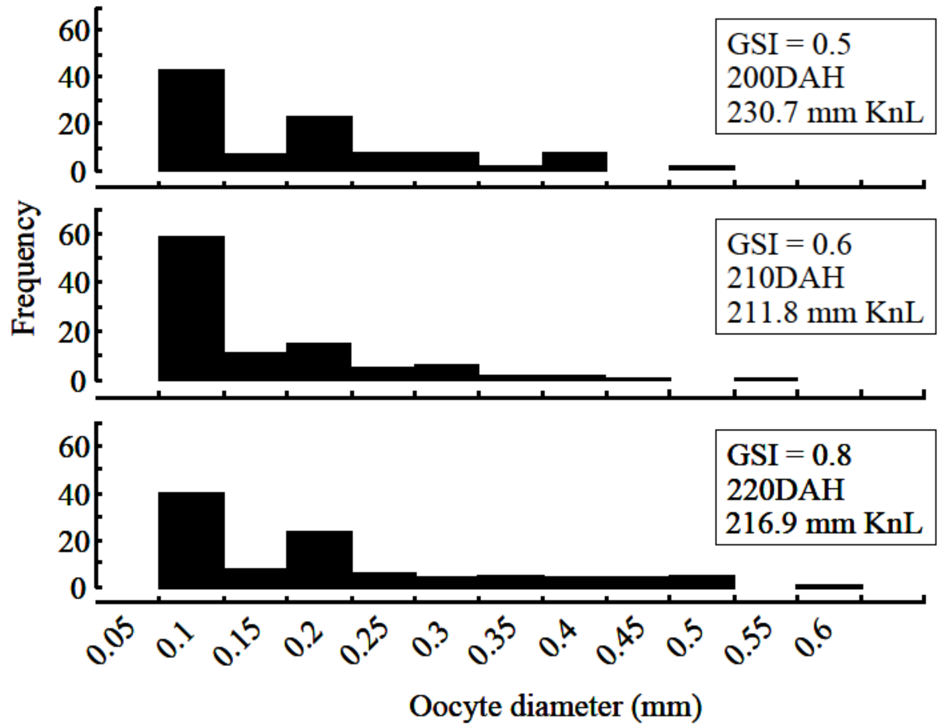

Fig. 8 Nakaya et al.

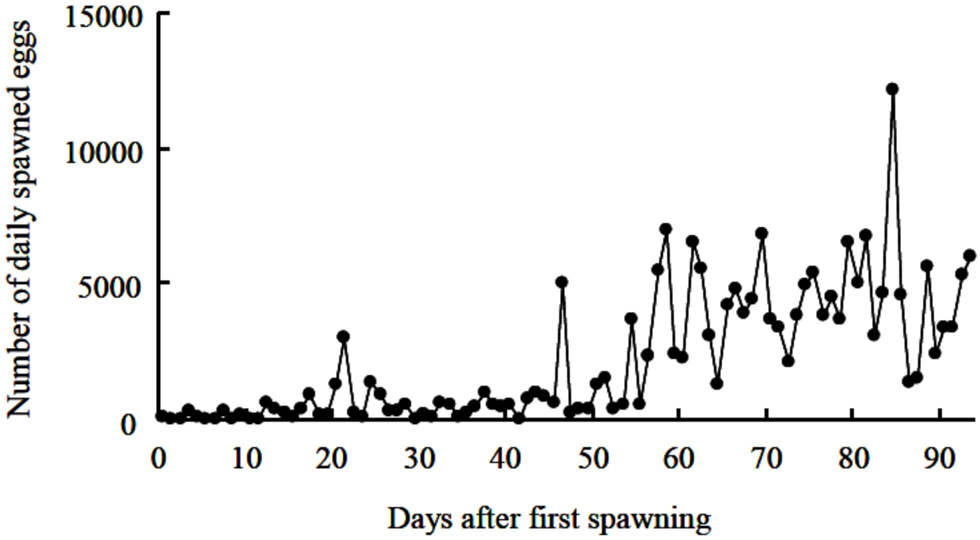

Fig. 9 Nakaya et al.