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20	Abstract This study details the growth and maturation processes of Pacific saury <i>Cololabis saira</i> from
21	eggs to first spawning under laboratory conditions. They were reared in 20°C, and fed almost to
22	satiation every day. There was no significant difference in the knob length (KnL) between males and
23	females and therefore data were combined in the following Gompertz growth formula $KnL_t = 277.1$ exp
24	$(-\exp(-0.015 (t - 83.8)))$ . The first spawning was observed on 243 days after hatching (DAH). At the
25	beginning of spawning, when the mean KnL was about 250mm, only several hundred eggs were spawned
26	at most. The spawning continued, and the number of spawned eggs increased notably after 260 DAH.
27	Correlation and stepwise multiple regression analysis of gonad somatic index (GSI) of Pacific saury
28	versus KnL, CF, and DAH revealed that only DAH ( $R = 0.88$ , 0.72, male and female, respectively) was
29	significantly correlated with GSI of Pacific saury ( $P < 0.001$ ). This result suggests that DAH is one of
30	the most influential factors for maturation in this species.

32 Keywords Growth  $\cdot$  Maturation  $\cdot$  Pacific saury  $\cdot$  Spawning

33 Introduction

34

35 Currently many fisheries species are showing resource depletion and subsequently a reduction in 36 reproductive success [1]. These processes relate not only to global environmental change but also to 37 overfishing [2,3].

38Pacific saury Cololabis saira is an important pelagic commercial fish that is widely distributed in the 39northwestern Pacific [4]. However, because of its wide distribution area, the amount of total stock has 40 not yet been fully estimated and landings in Japan as well as body size distributions of the catch show 41 dramatic fluctuations from year by year [5,6]. Fortunately, resources of the species have not been 42depleted yet unlike many other fisheries species. Therefore we need to understand the causes of these 43stock fluctuations in order to protect the resource from excess exploitation. To determine the factors 44 causing fluctuations, first we need to obtain information on the life history, age and growth, and 45reproduction 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].

56Sampling for larvae and juveniles of Pacific saury which forms the basis of the stock analysis has 57continued [19,20]. Growth and survival in the early life history are well known particularly for the 58nearshore area of Japan [6,21,22]. However as the spawning continues for quite long and the area is 59widespread in the North Pacific [7,8], the biology of the early life history has not yet been clarified and 60 also their reproduction because the scarcity of samples from the spawning season as no commercial 61 fishing occurs during this season. In general, the initial body size of Pacific saury showing ovary 62maturation is over 250mm knob length (KnL) and the mode is 270mm KnL, however the minimum size 63 of individual that has already been observed in a spawning experiment was 253mm KnL [18]. Also about 64 200mm KnL individuals were considered to have taken part in spawning both in the Japan Sea [23] and 65 Suruga Bay [24]. Furthermore other specimens, even at 200mm KnL individuals were found to have 66 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.
- To 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 laboratoryconditions to obtain some parameters for the model of Ito et al. [25].

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78 Materials and Methods

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80 Fertilized eggs of the Pacific saury, attached to drifting brown algae, were collected by research vessel 81 'Asama' in Kumano-nada, Mie Prefecture (16-17 °C sea surface temperature), Pacific Ocean Japan on 82 21-23 April 2005. The eggs were kept in plastic bags containing 10 liters ambient seawater (17 °C 83 water temperature, 32-34 ppt salinity) with oxygen and then transported to National Fisheries Research 84 Institute, Fisheries Research Agency, Akkeshi by plane. The eggs were stocked and then incubated in a 8520 ton concrete tank at a temperature of about 17 °C (water exchange rate was 120 % / day). After 86 hatching, the larvae were reared in the same tank at a temperature of about 20°C, because the temperature 87 is the best for growth and survival for Pacific saury (for larvae and juveniles [32], from larvae to adults 88 [28–31]). At 116 days after hatching (DAH), 250 individuals of Pacific saury were transferred to a 40 89 ton concrete tank. The fish were fed to almost satiation on live food (rotifers Brachionus spp., and 90 Artemia nauplii): usually two times a day (08:00-09:00, 15:00-16:00), frozen copepods (Miyabi No. 1 91(300-700µm), and No. 2 (1000-1500µm): JCK Co. Ltd): three times a day (08:00-09:00, 11:00-12:00, 9215:00-16:00), artificial feed (Otohime A, B1, B2, and Hirame EP1: Nisshin Marubeni Co. Ltd,

93	thoroughly mixed): 10-20 times a day (depending on their appetite) using a self feeding machine during
94	06:00-17:00, mince (made of frozen mysid and squid): Monday, Wednesday, and Friday in the morning
95	10:00–11:00). Daily feed amount and details of the rearing conditions are shown in Table 1. Samples
96	of over 20 individuals were collected every 5 days from 0 to 40 DAH, from 50 to 140 DAH samples of
97	10-20 individuals every 10 days, from 160 to 260 DAH 10 individuals were collected every 20 days, and
98	when we could observe different or unusual behaviors related to spawning. The sampling was done at
99	feeding time because the fish could be readily caught without causing excess stress.
100	KnL and body weight in wet weight (BW) of Pacific saury were measured to the nearest 0.1mm and

101 0.01g after being anaesthetized with FA100 (Dainippon Pharma) or in seawater chilled with ice. KnL is

102 a unit of body length from the edge of the lower jaw to the edge of the meat of silver part which is inside

103 the caudal fin. This part has been shown to have the least measurement bias for Pacific saury [33]. In

104 addition, the condition factor (CF) was examined using the following formula.

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

106 Since after 140 DAH, we could make a distinction between males and females by observation of their

107 gonads under a stereomicroscope, all of them were subsequently distinguished for sex, and the samples

108 that were collected after 190 DAH were measured for the gonad weight (GW) and gonad somatic index

109 (GSI) calculated. GSI was calculated using the following formula.

110  $GSI = GW \ge 10^2 / BW$ 

111 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

113 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.

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

119 To estimate batch eggs of the first spawning, largest oocytes were counted at one side of the ovary 120 under the stereomicroscope.

121From after 200 DAH, 2 pieces of spawning beds (we cut 16mm diameter polyvinyl chloride pipe to a 122thickness of 2mm width and connected 20 pieces with fine line, like beads (Fig. 1a)) were set in the 123breeding tank to measure the wet weight of eggs spawned every day (one time a day: 08:00–09:00). The 124number of eggs laid was estimated after verification of whether any eggs had come off and sank or not 125(the eggs are heavier than seawater). That is, whether they attached only to the spawning materials or not, 126and whether cannibalism of eggs occurred (stomach contents of the broodstock were observed by using 127one part of the sample). The eggs laid on the spawning materials (Fig. 1b) were then removed, wet 128weight measured, and used as the number of eggs spawned. Eggs of Pacific saury are elliptical (length: 129 $1.8 \pm 0.04$ mm (mean  $\pm$  SD), width:  $1.6 \pm 0.06$ mm). Wet weight of one egg with attachment filament 130 was calculated as 0.0033g, and then one gram of the egg mass was estimated to be 300 eggs in this study. 131Three types of growth model (Logistic, von Bertalanffy, and Gompertz) of Akaike Information 132Criterion [35] calculated using the non-linear least-squares regression procedure (Microsoft Excel solver

133	routine), and the model which has the lowest Akaike Information Criterion value was judged as the best
134	model to data. Difference of growth between sexes was compared with ANCOVA using log transformed
135	data.
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137	Results
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139	Growth and feeding
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141	The relationships between DAH and KnL, and between DAH and BW under 20°C breeding conditions
142	(details shown in Table 1) are shown in Figs. 2, 3. Mean body size of 0 DAH larvae was $6.9 \pm 0.26$ mm
143	KnL (mean $\pm$ SD), and 5 DAH larvae was 7.7 $\pm$ 0.59mm KnL. Observations of Pacific saury larvae
144	immediately after hatching showed that their digestive tract had not been fully formed yet, although 1
145	DAH larvae already had a functioning digestive tract, therefore, feeding of rotifers started 1 DAH and
146	continued until 5 DAH. Feeding of Artemia nauplii started from 2 DAH, and continued until 40 DAH
147	(41.1 $\pm$ 4.73mm KnL). Frozen copepods and artificial feed (Otohime A, B1) started to be fed after 15
148	DAH (13.0 $\pm$ 1.87mm KnL). On 35 DAH (35.9 $\pm$ 3.99mm KnL), an auto feeding machine started to be
149	used. At this time, the main artificial feed was Otohime B1, B2. From after 140 DAH (186.9
150	± 13.66mm KnL), we fed not only artificial feed (Hirame EP1), but also fed raw mince.
151	

152 Growth process

- 154 After 140 DAH individuals that could be judged whether they were female or male by observation of the
  - 155 gonad under a stereomicroscope were used for estimation of the growth process separately for both male
  - 156 and female (Fig. 4). Their regressions lines are shown below:
  - 157 Male: y = 0.54x + 114.0 ( $n = 60, R^2 = 0.70$ )
  - 158 Female: y = 0.56x + 109.9 ( $n = 63, R^2 = 0.72$ )
  - 159 where x means DAH, and y means KnL. The regressions of the above two formulas were significant (P
  - 160 < 0.001) and there was no significant difference for both slope and intercept (P = 0.98, P = 0.77,
  - 161 respectively) and therefore, the growth formulas were combined for both sexes.
  - 162 Three types of growth regressions calculated (Logistic, Gompertz, von Bertalanffy), and then their
  - 163 equations were judged by Akaike Information Criterion. As a result, the Gompertz growth formula was
  - 164 adopted (Fig. 2). The formula is as follows:
  - 165  $KnL_t = 277.1 \exp(-\exp(-0.015(t 83.8)))$
  - 166 where *t* is DAH,  $KnL_t$  is KnL at t DAH.
  - 167
  - 168 Maturation and spawning

- 170 After 190 DAH, we observed and measured their gonads (Figs. 5, 6 and 7). According to Suyama et al.
- 171 [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

173	DAH (GSI =1.14, gonad weight = 1.0g). The minimum size of females that had a GSI over 1 was
174	234.7mm KnL (GSI = 2.80, gonad weight = 1.7g, 310 DAH). After 300 DAH, all female individuals
175	were over 1 GSI, except for 1 individual (GSI 0.91-4.20, gonad weight 0.9-5.5g). The male fish
176	exceeding 1.0 GSI also already had produced a sufficient quality of sperm in their testes to join the
177	spawning (Suyama Unpublished). The first individuals observed that had over 1 GSI was a male on 190
178	DAH (GSI = $1.14$ , gonad weight = $0.6g$ ). And also the male was a minimum size that had over 1 GSI
179	(223.6 mm KnL). After 300 DAH, all male individuals were over 1 GSI (GSI 2.18–3.43, gonad weight
180	2.5–5.5g).

181 Correlation and stepwise multiple regression analysis of GSI of Pacific saury versus KnL, CF, and 182 DAH revealed that only DAH (R = 0.88, 0.72, male and female, respectively) was significantly correlated 183 with GSI of Pacific saury (P < 0.001).

184 Oocyte diameters for 200 to 220 DAH individuals that had no experience of spawning were observed

185 (Fig. 8). Although 99% of oocyte diameter range showed overlap (oocyte in the early yolk vesicle stage,

186 e.g., 0.15–0.34mm oocyte diameter reported by Suyama et al. [18]), the remaining 1% were larger and

187 easy to distinguish from the other oocytes (oocyte size equivalent to the secondary yolk stage:

188 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.

190 The number of eggs and pattern of spawning is shown in Fig. 9. The first spawning (Fig. 1c) was 191 observed on 243 DAH (about 50 eggs attached to the spawning materials). After that, peaks were 192 observed every 3–4 days, and on these days, several hundred eggs were observed each day. About 60

193	days after first spawning, several thousand eggs were observed on the peak days which were observed in
194	the same cycle (every 3-4 days). At 94 days after first spawning, the total of spawned eggs from first
195	spawning was over 200,000 (number of breeding individuals at first spawning day: 89 individuals, 94
196	days after first spawning: 47 individuals). These spawned eggs that attached to the spawning materials
197	were almost 100% fertilized.
198	
199	Discussion
200	
201	Growth
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203	So far many studies have examined the growth and maturation of wild Pacific saury, however there has
204	not been a fully accepted theory yet. Some reports have discussed about the growth until 6 month old
205	fish. The body size estimates for 6 month old fish by Matsumiya and Tanaka [36] are 182 mm KnL,
206	Watanabe et al. [37] and Suyama [17] are about 200mm KnL respectively.
207	In this study, the water temperature was maintained at 20°C and the fish were fed to satiation as a
208	condition. Their growth until a half year after hatching (180DAH: 207.5 $\pm$ 19.6 mm KnL (mean
209	$\pm$ SD)) was similar to that in the wild, though the environmental conditions and prey items were quite
210	different and also includes a variety of stress. According to Odate [38], wild individuals of Pacific saury
211	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<sup>2</sup>), 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 20°C) with enough food at this temperature. Therefore, the growth process of this study would show similar to the growth process in the wild.

218From half a year after hatching, opinions on their growth vary among studies. Matsumiya and 219Tanaka [36] estimate that this species grows up 223mm KnL at one year based on size distribution. The 220Pacific saury caught from the western North Pacific were divided into three groups; the small size 221(200-240mm KnL), medium size (241-280mm KnL) and large size (> 280mm KnL) [39]. Watanabe et 222al. [37] estimate the period to grow from a small size (200–240mm KnL) to a large size (> 280mm KnL) 223to be half a year, and Suyama [17] estimates for the same period to be one year based on daily growth 224ring analysis. The reason for the difference of opinions is the existence of hyaline zones on the otolith. 225The zone does not have clear daily growth rings making the count of the number difficult. This study 226used reared individuals that were reared from eggs, therefore there is no doubt regarding the relationships 227between age and growth. Thus, we directly and firstly obtained evidence for the growth from half a year 228after hatching. The mean body size of 336 DAH individuals was 270mm KnL. This size is included in 229middle sized fish category (241-280mm KnL) used for grading commercially landed fish. And the 230 growth process (Fig. 2) expected that the growth speed would be quite slow after growth to the middle 231size (241–280mm KnL) and it would spend much time to grow to the large size (> 280mm KnL).

233 Maturation and spawning

235During the start of the spawning period, one spawner might spawn eggs a few at a time, or one or two 236spawners might spawn in turn. As their gonad weight was about 1g at most when the spawning began 237(GSI was about 1), the number of eggs released would total about 300 eggs. The number of peaks of 238the attached eggs on the spawning materials increased sharply after 60 days after first spawning (300 239DAH, estimated mean body size: 266.5mm KnL). At the time, the reason may be that not only most of 240the individuals started to spawn, but also the number of eggs per batch increased for each spawner. 241After 300 DAH, most of the females spawned. 242So far there are some reports about the number of eggs per batch for Pacific saury (Kubo [40]: 243120–1720 eggs, Hatanaka [41]: 300–4500 eggs, Kosaka [8]: 947–2547 eggs, and Suyama [17]: 500–4000 244eggs (mean: 2000)). In this study, we measured their oocyte diameter using 200 to 220 DAH individuals 245that had no experience of spawning. Although 99% of oocyte diameters showed overlap, the remaining 2461% of oocytes were quite large and easy to distinguish as a single batch. Their total number was 247estimated as 289-350 oocytes. The number of batch eggs at the first spawning was estimated to be 248about 300 eggs (gonad weight: 1g) at most. And after first spawning, the number of batch eggs 249increased during continued spawning. In the above reports which have differences of opinion probably 250due to the samples including a variety of spawners that have different spawning experience like first, 251second, and so on. In addition, the results of this study indicate that the number of DAH affects more 252their maturation than their body size. This may be one of the reasons for the difference of the reported

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biological minimum size of this species among sampling areas. Probably, they will be old enough to spawn, though the body size is small.



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

261 biological data on this species under a variety of environmental conditions.

262

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Days after hatching	Mean <sup>a</sup> KnL (mm)	Mean Body weight (wet: g)	<sup>b</sup> Rotifers (inds/ml)	<sup>b</sup> Artemia nauplii (inds/ml)	°Frozen copepods (%)	°Artificial feed (%)	°Mince (%)	Breeding individuals	Water temperature (°C)	Water volume of the tank (t)	Daily water exchange rate (%)
5	77		40	10				8320	20 0	12	300
10	10 2			15				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		30	33 0	83		4000	20 0	12	300
40	41 1	0 27		4 0		100		3858	20 0	12	300
50	53 6	0 57				100		2510	20 0	12	480
60	67 2	1 12				100		2422	20 0	12	480
80	99 8	3 82				80		2380	20 0	12	480
100	125 7	7 12				80		1225	20 0	12	480
120	159 4	15 60				80		250	20 0	12	300
140	186 9	28 88				70	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	53	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	57	76	20 0	20	0
327	288 5	135 67				33	4 1	27	20 1	20	0
340	287 0	121 74				27	4 2	23	19 9	20	0

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

<sup>a</sup> KnL means knob length
 <sup>b</sup>Feeding density at each feeding
 <sup>c</sup>Daily feeding amount per body wet weight of Pacific saury

- 378 Fig. 1 Spawning beds (using 16mm diameter polyvinyl chloride pipe (a)), the eggs (b) and spawning
- 379 behavior of Pacific saury *Cololabis saira* (c)
- 380 Fig. 2 Relationships between days after hatching (DAH) and knob length (KnL) of Pacific saury
- 381 Cololabis saira
- 382 Fig. 3 Relationships between days after hatching (DAH) and body weight in wet weight (BW) of
- 383 Pacific saury Cololabis saira
- 384 Fig. 4 Relationships between days after hatching (DAH) and knob length (KnL) of Pacific saury
- 385 Cololabis saira for each sex
- 386 Fig. 5 Relationships between knob length (KnL) and gonad somatic index (GSI) of Pacific saury
- 387 Cololabis saira
- 388 Fig. 6 Relationships between knob length (KnL) and gonad somatic index (GSI) of Pacific saury
- 389 Cololabis saira
- 390 Fig. 7 Relationships between days after hatching (DAH) and gonad somatic index (GSI) of Pacific
- 391 saury Cololabis saira
- 392 Fig. 8 Distribution of egg diameter of Pacific saury *Cololabis saira* before first spawning
- 393 Fig. 9 Relationships between days after first spawning and number of eggs released each day by the
- 394 Pacific saury Cololabis saira



Fig. 1 Nakaya et al.



Fig. 2 Nakaya et al.



Fig. 3 Nakaya et al.



Fig. 4 Nakaya et al.



KnL(mm)

Fig. 5 Nakaya et al.



CF

Fig. 6 Nakaya et al.



Fig. 7 Nakaya et al.



Fig. 8 Nakaya et al.



Fig. 9 Nakaya et al.