Seasonal variability in the development of Japanese anchovy during the transition from larval to juvenile stages

Naotaka Yasue[1,*], Shigeo Harada[2], Akinori Takasuka[3]

[1]Agriculture, Forestry, and Fisheries General Affairs Division, Wakayama Prefectural Government, 1-1 Komatsubara-dori, Wakayama 640-8585, Japan

[2]Wakayama Prefectural Fisheries Experimental Station, 1557-20 Kushimoto, Kushimoto, Higashimuro, Wakayama 649-3503, Japan

[3]National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa 236-8648, Japan

ABSTRACT: Seasonal (inter-cohort) variability in the development of Japanese anchovy *Engraulis japonicus* during the transition from larval to juvenile stages was examined based on changes in head size, degree of guanine deposition and ossification. Seasonal variability was observed in the relationships between head length and standard length (SL) at 20, 25, and 30 mm SL, which were all positively related to sea temperature. In addition, SL immediately before and immediately after the start of guanine deposition was negatively related to temperature. Moreover, the development of the caudal fin supports was faster in the order August (warmest month), June (intermediate), and January (coldest); the full complement of pectoral fin rays ($\geq$14 rays) was observed at 26.5 mm SL in August and 32.7 mm SL in June, but was not observed in January. Thus, morphology at length differed among seasons. Furthermore, there was parallel development of external (head length and pectoral fin rays) and internal (degree of guanine deposition and caudal fin supports) characteristics. The energy requirement for development of some characteristics did not result in delayed development of other characteristics. Overall, larvae at the same somatic size would have a higher potential for predator avoidance and prey capture under higher temperatures, although additional factors will influence potential survival.

KEY WORDS: Development · Head length · Guanine deposition · Ossification · Japanese anchovy · Sea temperature

INTRODUCTION

Processes impacting the survival of early life stages have been considered critical for understanding the mechanism of population dynamics (Houde 1987, 2008). Anderson (1988) concluded that the 'growth–mortality' hypothesis, which posits that survival is a direct function of growth, provides a rational theoretical framework for recruitment research. Previous field studies have shown that during the early life period, survival and subsequent recruitment are coupled with the growth rate (Meekan & Fortier 1996,

Takahashi & Watanabe 2004a, Robert et al. 2007) such that faster-growing larvae have a higher probability of survival than slower-growing conspecifics. The 'growth–mortality' hypothesis comprises 3 non-exclusive functional mechanisms (Takasuka et al. 2004, Leggett & Frank 2008): the 'bigger is better' (Miller et al. 1988), 'stage duration' (Chambers & Leggett 1987, Houde 1987), and 'growth-selective predation' (Takasuka et al. 2003, 2007a) mechanisms. The 'stage duration' mechanism predicts that faster-growing larvae should experience lower cumulative mortality during the larval stage, during

which mortality rates are known to be very high (Leggett & Frank 2008). This mechanism has received support from field studies examining growth and survival of fish larvae (Fontes et al. 2011, Grorud-Colvert & Sponaugle 2011, Rankin & Sponaugle 2011, Takasuka et al. 2016). The 'stage duration' mechanism enables a theoretical link between the growth rate and the timing of the transition from larval to juvenile stages. In a more general context, the larval stage duration could be regulated by developmental processes that are influenced by environmental factors. Seasonal variability has been observed in the development of fish (Noichi et al. 1997, Takahashi et al. 2000, Radtke et al. 2001). As such, the seasonal variability in morphological (external and internal) development with somatic size would provide useful information on vital parameters regulating the population dynamics of fish.

Japanese anchovy *Engraulis japonicus* is a small pelagic fish species that is widely distributed from inshore to offshore waters in the western North Pacific (Zenitani & Kimura 1997, Wang & Tzeng 1999, Takahashi et al. 2001, Takasuka et al. 2008). The anchovy larvae are characterized by their elongated and transparent bodies. As anchovy larvae grow, the shape of the snout tip changes from pointed to rounded (Fukuhara 1983), and the degree of guanine deposition increases (Takahashi & Watanabe 2004b). This species has a protracted spawning season that lasts almost throughout the year, although peak spawning occurs mainly from March to August off the Pacific coast of Japan (Oozeki et al. 2007). Therefore, larvae occur all year round in the coastal areas and their abundance fluctuates seasonally (Yasue & Takasuka 2009, Yasue et al. 2011). Seasonal variability in growth rate has been reported for Japanese anchovy larvae (Chiu & Chen 2001, Yasue & Takasuka 2009) and other *Engraulis* species (Castello & Castello 2003, Bacha & Amara 2012). Nonetheless, seasonal variability in development remains largely unexamined but may be important in explaining seasonal differences in potential survival.

The Kii Channel is a spawning, nursery, and feeding ground for Japanese anchovy (Yasue et al. 2014). The environmental conditions in the channel show large seasonal fluctuations, including sea surface temperature, which ranges from approximately 12.5°C in winter to 29.1°C in summer. Anchovy larvae are caught in the channel throughout the year despite such large temperature variations. The anchovy larvae are utilized as a food source (called 'shirasu') and thus are commercially important in Japan. As such, the Kii Channel provides an excel-lent location to collect early life stages of anchovy throughout the year.

The objective of the present study was to examine the seasonal (inter-cohort) variability in the development of Japanese anchovy during the transition from larval to juvenile stages. Developmental differences were quantified based on changes in head size and the degrees of guanine deposition and ossification in anchovy collected throughout the year from the Kii Channel. The seasonal variability in these characteristics relative to body size (standard length) are examined with respect to seasonal changes in temperature and discussed in terms of potential survival via predator avoidance and prey capture ability.

MATERIALS AND METHODS

Field sampling

Samples of anchovy during the early life period were collected from commercial catches in the Kii Channel, Japan, during 18 July 2008 to 29 January 2013 (Table 1, Fig. 1). The fish were caught by a commercial 'shirasu' trawl with a cod end mesh size of 1.5–2.0 mm (Yasue & Takasuka 2009). The 'shirasu' trawl usually targets shoals of fish with a standard length (SL) in the range of ca. 13–30 mm. Fish samples were preserved in 10% formalin for the growth analysis of head length (HL) relative to SL and for observations of osteological elements, and in chilled sea water for observations of the degree of guanine deposition.

Sample measurement and analysis

Standard length and head length of each larva were measured to the nearest 0.1 mm using digital calipers to examine the growth of HL relative to SL. SL was measured directly, and HL was measured under a stereo microscope at a magnification of 5×–10×.

The degree of guanine deposition (Takahashi & Watanabe 2004b) was examined using a stereo microscope at a magnification of 10×–63×. Fish were assigned to 2 developmental stages, as follows. GD-0 stage: no guanine deposition observed on the peritoneal surface (lateral part of the abdominal cavity near the pelvic fin base); GD-1 stage: guanine deposition observed on the peritoneal surface.

Skeletal specimens were prepared based on the double staining method (Kawamura & Hosoya 1991), which enables the muscle to appear almost transpar-

Table 1. Sample profiles of Japanese anchovy *Engraulis japonicus* in the Kii Channel. Sampling date, sampling area, sea surface temperature, and standard length and sample size of larvae and juveniles are indicated. A, B and C indicate sampling areas in the Kii Channel (Fig. 1). SST: sea surface temperature; SD: standard deviation; n: sample size; GD: guanine deposition; OS: ossification; RG: relative growth of head length to standard length

Date	Sampling area	SST (°C)	Standard length (mm) Mean ± SD	Range	n	Sample analysis
18 Jul 2008	B	23.5	33.6 ± 5.1	25.2–48.5	39	GD
4 Sep 2008	C	28.4	29.9 ± 4.0	23.9–39.1	27	GD
7 Oct 2008	B	23.0	33.2 ± 3.9	27.6–40.2	38	GD
10 Nov 2008	B	22.1	34.6 ± 5.0	25.8–45.4	37	GD
17 Jan 2012	A	14.1	29.0 ± 3.4	21.4–36.7	110	RG
13 Feb 2012	A	12.5	27.4 ± 3.0	21.6–33.7	110	RG
19 Apr 2012	A	14.0	18.7 ± 3.3	11.1–29.7	110	RG
10 May 2012	A	17.3	18.7 ± 3.5	12.7–28.8	110	RG
24 May 2012	A	17.3	21.6 ± 2.4	16.5–28.1	110	RG
7 Jun 2012	A	20.7	23.1 ± 5.1	13.6–32.4	30	OS
8 Jun 2012	A	20.7	20.6 ± 3.6	13.1–32.3	110	RG
21 Jun 2012	A	20.7	25.4 ± 4.6	19.3–32.7	10	OS
19 Jul 2012	A	24.9	21.4 ± 2.5	14.6–26.9	110	RG
10 Aug 2012	C	29.1	23.2 ± 5.0	13.2–30.6	20	OS
	C	29.1	21.9 ± 3.2	15.3–29.7	110	RG
20 Aug 2012	C	29.1	23.1 ± 5.6	11.9–30.3	20	OS
	C	29.1	23.5 ± 3.9	14.2–34.9	110	RG
23 Aug 2012	A	27.7	21.6 ± 3.0	14.8–28.0	110	RG
13 Sep 2012	A	27.6	22.9 ± 2.4	15.4–30.8	110	RG
30 Oct 2012	A	25.2	23.1 ± 3.3	13.8–29.9	109	RG
28 Nov 2012	A	21.1	24.4 ± 3.4	16.1–31.6	110	RG
3 Dec 2012	A	16.5	26.7 ± 4.4	18.7–43.1	110	RG
7 Dec 2012	B	16.5	34.9 ± 4.9	27.5–48.4	44	GD
11 Dec 2012	C	16.3	33.7 ± 4.7	24.4–40.7	43	GD
	C	16.3	28.0 ± 3.3	19.1–38.5	110	RG
28 Dec 2012	C	16.3	36.8 ± 5.2	28.3–49.8	38	GD
	C	16.3	29.4 ± 3.3	22.3–39.7	110	RG
11 Jan 2013	A	14.1	39.9 ± 4.6	32.6–51.7	31	GD
29 Jan 2013	C	17.8	29.6 ± 2.5	25.3–35.1	20	OS

ent while allowing clear differentiation between cartilage and bone. For this, Alcian blue was used to stain cartilage in blue; digestion of the muscle was carried out in a potassium hydroxide aqueous solution. Alizarin red was then used to stain calcified structures in red, and the specimens were preserved in glycerin. For each skeletal specimen, SL was measured to the nearest 0.1 mm using digital calipers in the stained state. A possible effect of SL change during the sample processing (Potthoff & Tellock 1993) was ignored. The osteological elements were observed under a stereo microscope. Bone terminology follows Balart (1995). For caudal fin supports, neural arch and spine 1 (NA1, cartilage bone), neural arch and spine 2–4 (NA2–4, cartilage bone), epural (EP, cartilage bone), hypural 1–3 (HY1–3, cartilage bone), parhypural (including hypurapophysis, PH, cartilage bone), haemal arch and spine 2 (HA2, cartilage bone), haemal arch and spine 3 (HA3, cartilage bone) and radial cartilage 5 (RC5, cartilage) were observed (Fig. 2). Moreover, pectoral fin rays (either right or left side) were counted at the base of the fin. The observations were not made in some skeletal specimens (June: pectoral fin rays, n = 2; January: NA1, n = 2).

Data analysis

Sea surface temperature (SST, °C) data were obtained from records of the sea temperature survey of Wakayama Prefectural Fisheries Experimental Station. In the survey, SST was measured at 15 stations on the northern side of
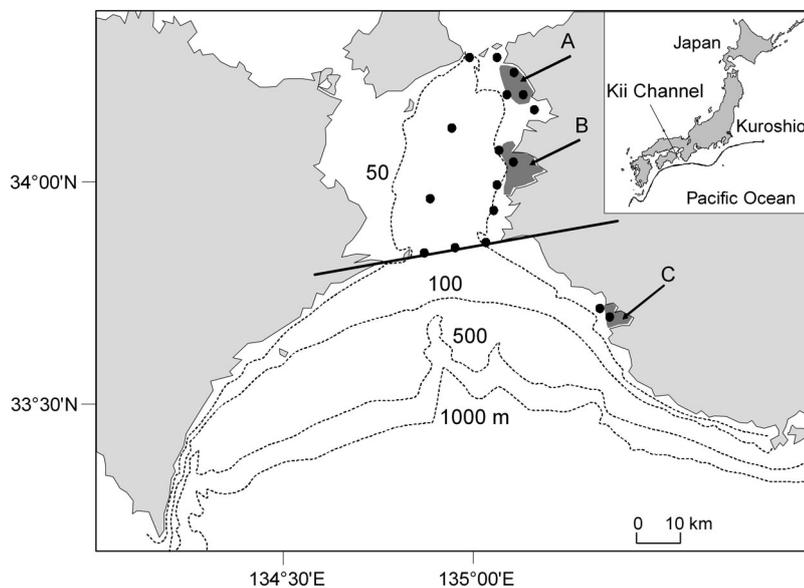

Fig. 1. Study areas in the Kii Channel, Japan. Dark shaded areas, solid circles, the bold line, and dashed lines represent sampling areas for Japanese anchovy *Engraulis japonicus*, and sea surface temperature stations, the boundary between the northern side and the southern side, and the depth of water, respectively
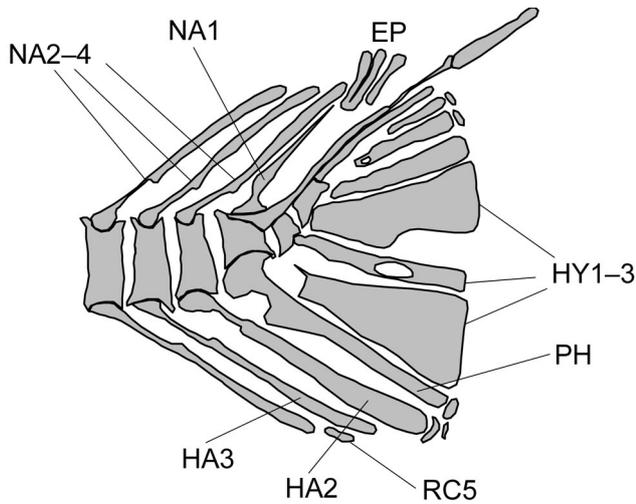
Fig. 2. Schematic diagram of osteological elements of Japanese anchovy *Engraulis japonicus* caudal fin supports. NA1: neural arch and spine 1; NA2–4: neural arch and spine 2–4; EP: epural; HY1–3: hypural 1–3; PH: parhypural; HA2: haemal arch and spine 2; HA3: haemal arch and spine 3; RC5: radial cartilage. Modified from Fig. 2 of Balart (1995)

the Kii Channel and 2 stations on the southern side once a month using a bar thermometer (Fig. 1).

An allometric equation was adopted for the relationship between SL and HL, since it fitted the data better than the linear equation (HL = −0.8378 + 0.2478 SL, n = 1737, r^2 = 0.891, p < 0.001), when all the data with a SL range of <35 mm were included (Fig. 3). The data with a SL range of ≥35 mm were removed from the analysis since the degree of fitness of the equation became low at larger SL. For each daily sample (Table 1), an allometric equation was fitted to the data of SL (<35 mm) and HL (r^2 = 0.856– 0.966, p < 0.001), and then HL at 20, 25 and 30 mm SL was calculated. The Kruskal-Wallis test was used to test differences in HL at 30 mm SL among sampling seasons (March– May: n = 3; June–August: n = 5; September–November: n = 3; December–February: n = 5), although the sample size for each season was limited. The relationships between SST and HL at these SLs were examined using a linear regression analysis. The monthly SST was calculated by averaging the temperature at 15 stations on the northern side of the Kii Channel and 2 stations on the southern side. SST measured on the same

side in the same month for each daily sampling was assumed to represent the ambient temperature condition affecting the development of the anchovy larvae.

The largest 5 individuals among the GD-0 stage fish were assumed to be immediately before the start of guanine deposition; the smallest 5 individuals among the GD-1 stage fish were assumed to be immediately after the start of guanine deposition. One-way ANOVA was used to test differences in SL of the GD-0 stage fish immediately before the start of guanine deposition and SL of the GD-1 stage fish immediately after the start of guanine deposition among sampling dates. Linear regression analysis was used to examine the relationships of the mean SL immediately before the start of guanine deposition and the mean SL immediately after the start of guanine deposition to SST.

For NA1, EP, PH, HA2 and HA3, individuals with both cartilage and bone were assumed to be undergoing the transition from cartilage to bone. ANOVA or Kruskal-Wallis test was used to test differences in SL during this transition from cartilage to bone among sampling months. NA1, EP, PH, HA2, and HA3 were selected because there were individuals with cartilage or bone and individuals with both cartilage and bone. Also, the individuals with 1 to 13 pectoral fin rays (full complement: 14 rays or more) were assumed to be undergoing the process of forming the pectoral fin rays. The Kruskal-Wallis test was used to test differences in SL undergoing the process of forming the pectoral fin rays among sampling months.
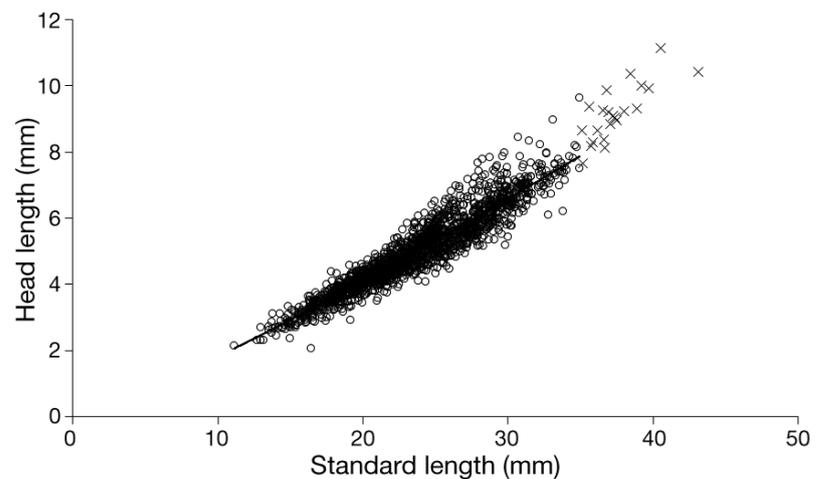

Fig. 3. Relationship between standard length (SL) and head length (HL) for Japanese anchovy *Engraulis japonicus* in the Kii Channel. The allometric equation was fitted to the data with a SL range of <35 mm (open circle); the data with a SL range of ≥35 mm (crosses) were excluded from the analysis. HL = 0.1243 $SL^{1.167}$ (n = 1737, r^2 = 0.906, p < 0.001)
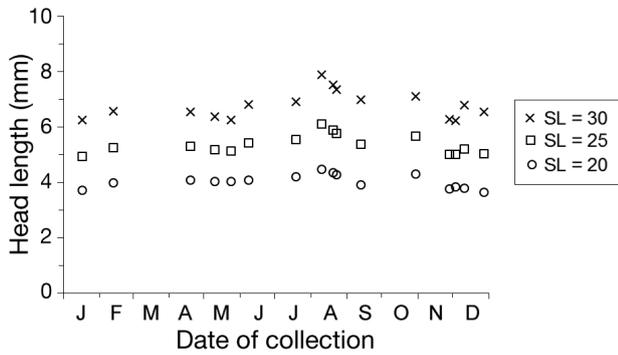
Fig. 4. Seasonal variability in head length at different standard lengths (SLs) for Japanese anchovy *Engraulis japonicus* in the Kii Channel in 2012
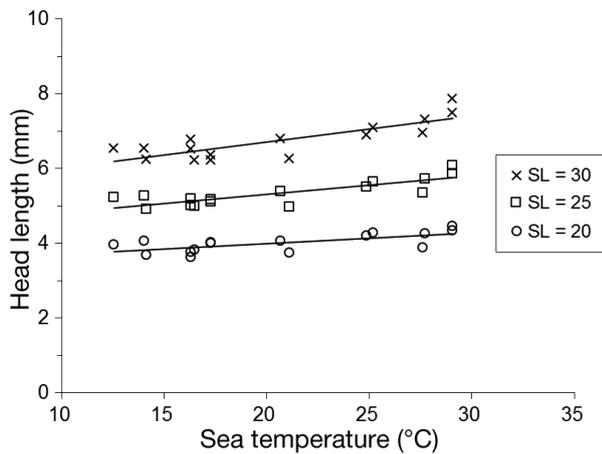

Fig. 5. Relationship between sea surface temperature (SST) and head length (HL) at different standard lengths (SLs) for Japanese anchovy *Engraulis japonicus* in the Kii Channel. HL = 3.41 + 0.0291 SST for 20 mm SL (n = 16, r^2 = 0.473, p < 0.01); HL = 4.35 + 0.0482 SST for 25 mm SL (n = 16, r^2 = 0.665, p < 0.001); HL = 5.30 + 0.0705 SST for 30 mm SL (n = 16, r^2 = 0.689, p < 0.001)
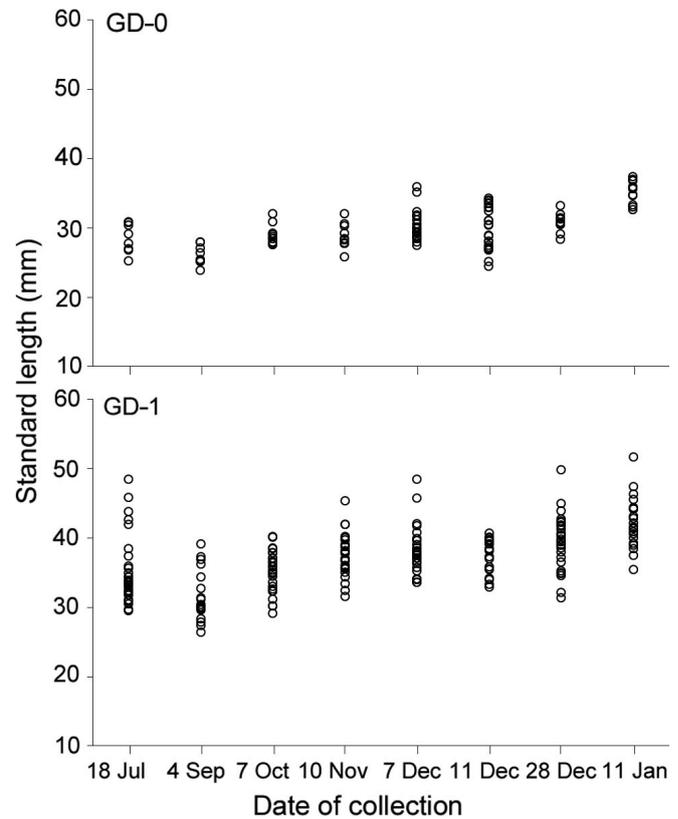

Fig. 6. Seasonal variability in standard length of individuals without guanine deposition on the peritoneal surface (GD-0 stage fish) and those with guanine deposition on the peritoneal surface (GD-1 stage fish) for Japanese anchovy *Engraulis japonicus* in the Kii Channel

RESULTS

Head length

For HL at 30 mm SL, HL reached its maximum in August and its minimum in May (Fig. 4). Similar seasonal trends were observed for 20, 25, and 30 mm SL. HL at 30 mm SL differed among sampling seasons (Kruskal-Wallis test, χ^2 = 9.12, df = 3, n = 16, p < 0.05), indicating seasonal variability of HL at 30 mm SL. HL was larger during June to October. SST ranged from 12.5°C (February) to 29.1°C (August) in the Kii Channel (Table 1). The linear regression analysis showed a significant positive relationship between SST and HL for 20, 25, and 30 mm SL (Fig. 5).

Standard length at the start of guanine deposition

The maximum SL of a GD-0 stage fish was 37.3 mm (Fig. 6). GD−1 stage fish occurred from 26.4 mm SL. The SL immediately before the start of guanine deposition differed among sampling dates (ANOVA, $F_{7,32}$ = 31.46, p < 0.001). The maximum difference of SL between sampling dates was 9.4 mm in the fish collected at 14.1°C and 28.4°C. The SL immediately after the start of guanine deposition also differed among sampling dates (ANOVA, $F_{7,32}$ = 32.23, p < 0.001), indicating seasonal variability of SL at the start of guanine deposition. The maximum difference of SL between sampling dates was 9.0 mm, as in the case of GD-0. The linear regression analysis showed significantly negative relationships between SST and SL immediately before the start of guanine deposition, and between SST and SL immediately after the start of guanine deposition (Fig. 7).
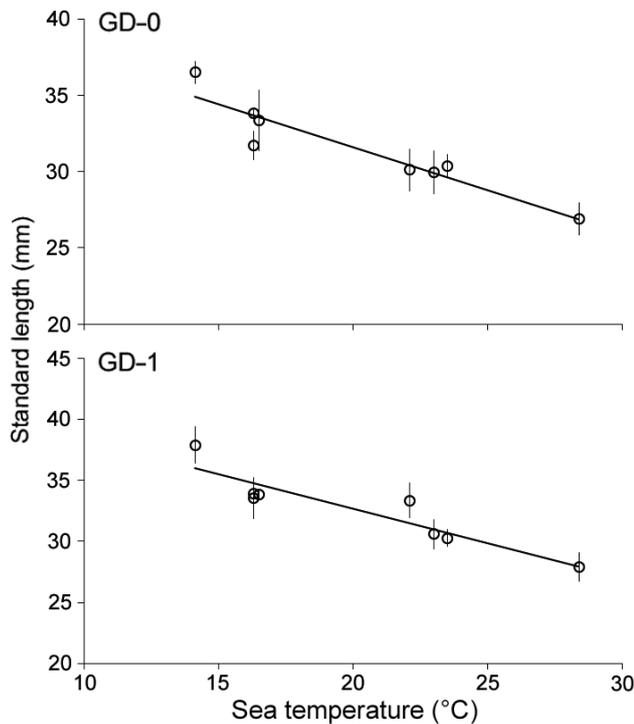
Fig. 7. Relationships between sea surface temperature (SST) and standard length (SL) immediately before the start of guanine deposition (GD-0 stage), and between SST and SL immediately after the start of guanine deposition (GD-1 stage) for Japanese anchovy *Engraulis japonicus* in the Kii Channel. Mean ± standard deviation values are shown at sample level. SL = 42.8 − 0.560 SST for the GD-0 stage (n = 8, r^2 = 0.881, p < 0.001); SL = 43.9 − 0.563 SST for the GD-1 stage (n = 8, r^2 = 0.839, p < 0.01)

Osteological development

The SL range undergoing the transition from cartilage to bone differed among months for NA1, EP, PH, HA2, and HA3 (Table 2). In NA1 and PH, the transition started at smaller SLs in August than in June and January (Fig. 8). The transition from cartilage to bone of NA2–4 and HY1–3 started at smaller SLs in August than in June, although the transition had already started for all individuals in January. In EP, the transition started at smaller SLs in the order of August, June, and January. The transition from cartilage to bone of HA2 and HA3 started and was completed at smaller SLs in August than in June. The SL of the occurrence of RC5 was smaller in August than in June, although RC5 had already occurred for all individuals in January. Although there were varia-

tions among individuals for each osteological element even within the same month, the development of the caudal fin supports was faster in the order of August (warmest month), June (intermediate), and January (coldest).

The smallest individual with pectoral fin rays was 18.9 mm SL in June, 19.6 mm SL in August, and 27.1 mm SL in January (Fig. 9). The smallest individual with a full complement was 32.7 mm SL in June and 26.5 mm SL in August; a full complement of rays was not observed for any individual in January. The SL undergoing the process of forming the pectoral fin rays differed among sampling months (Kruskal-Wallis test, χ^2 = 27.92, df = 2, n = 55, p < 0.001). As in the case of the caudal fin supports, the rays were more developed at the same SL in the order of August, June, and January.

DISCUSSION

The present study revealed substantial seasonal variability in head size, degree of guanine deposition, and the degree of ossification during the transition from larval to juvenile stages of Japanese anchovy in response to seasonal changes in temperature in the Kii Channel. Moreno et al. (2011) reported that changes in the body proportions during the larval to juvenile stages of *Engraulis ringens* do not involve a conspicuous and relatively abrupt change in the body form or structure (e.g. as in pleuronectids), and used the term 'transition period' instead of metamorphosis. The samples of the present study were mainly categorized in the transition period from larvae to juveniles. As in a previous study (Nikolioudakis et al. 2010), the present study adopted an allometric equation for the relationship between SL and HL. At higher temperatures, HL was relatively larger and therefore the trunk length (SL minus HL) was smaller at the same SL. This tendency suggests that

Table 2. Results of ANOVA and Kruskal-Wallis test to test differences in standard length during the transition from cartilage to bone among sampling months (June, August, and January). Schematic diagram of osteological elements of Japanese anchovy *Engraulis japonicus* caudal fin supports is shown in Fig. 2. df: degree of freedom; n: sample size

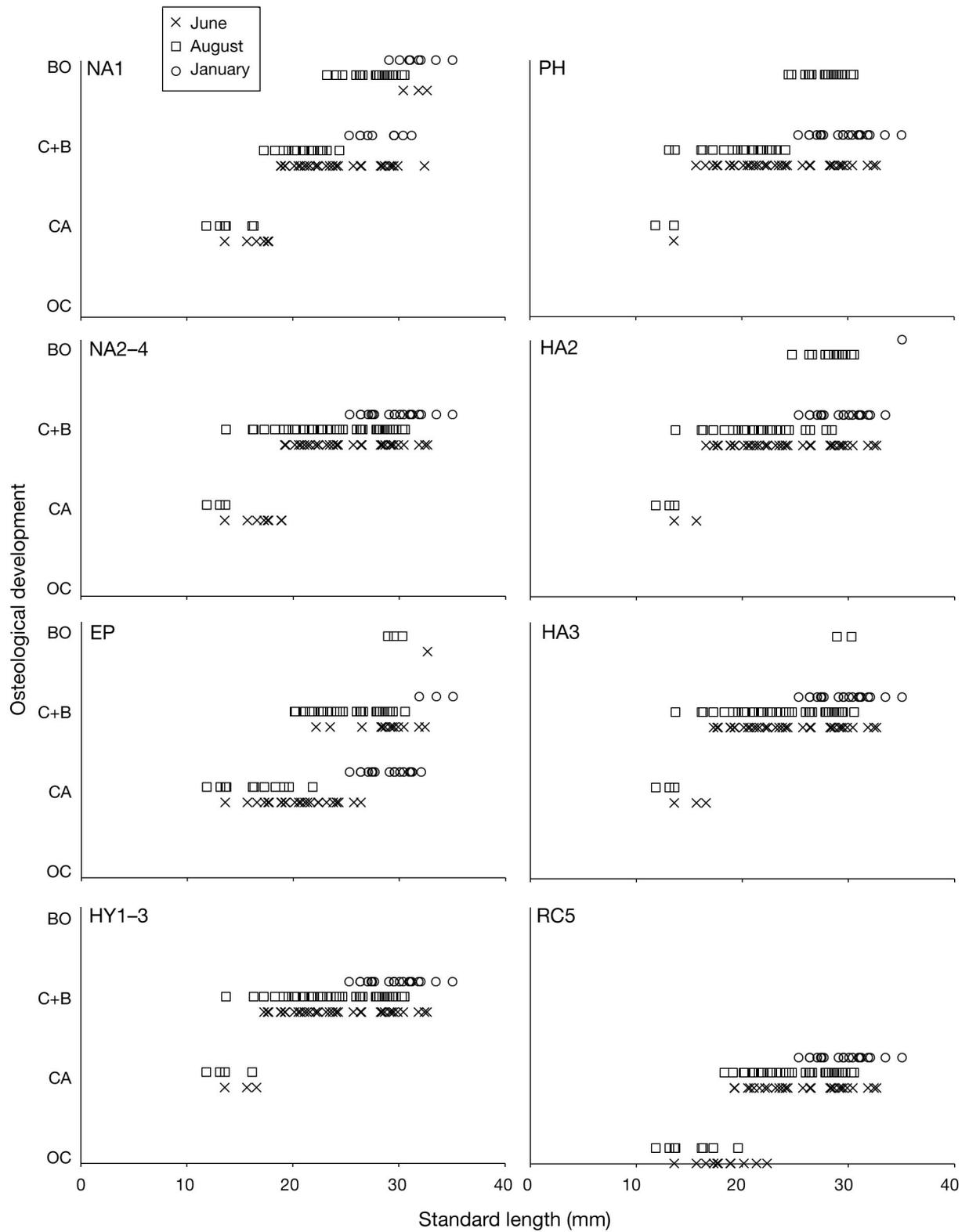| Osteological element	Statistical method	df	Statistic	n	p
Neural arch and spine 1	Kruskal-Wallis	2	χ^2 = 19.34	56	<0.001
Epural	ANOVA	2, 40	F = 13.25	43	<0.001
Parhypural	Kruskal-Wallis	2	χ^2 = 33.26	79	<0.001
Haemal arch and spine 2	Kruskal-Wallis	2	χ^2 = 27.29	81	<0.001
Haemal arch and spine 3	Kruskal-Wallis	2	χ^2 = 22.61	92	<0.001

Fig. 8. Relationships between standard length and osteological development for Japanese anchovy *Engraulis japonicus* in the Kii Channel in January, June and August. The 8 osteological elements (see Fig. 2) were observed based on skeletal specimens. Each osteological element was distinguished as follows: occurrence (OC), cartilage (CA), cartilage and bone (C+B) and bone (BO)
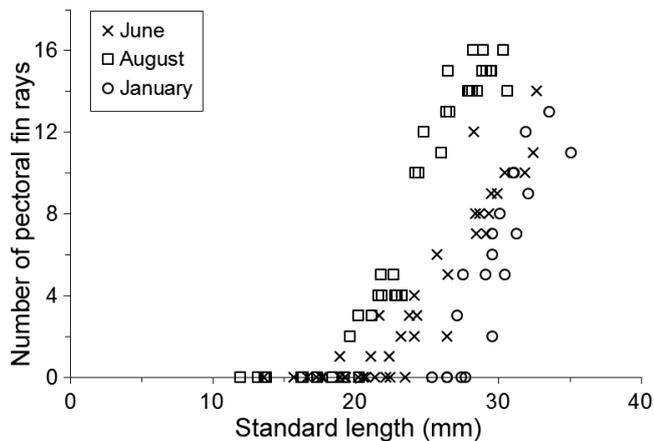
Fig. 9. Relationship between standard length and number of pectoral fin rays (either right or left side) for Japanese anchovy *Engraulis japonicus* in the Kii Channel in different months

the development of the head region is advanced at higher temperatures. Many sense organs for sight, smell, hearing, and taste are located in the head region of Japanese anchovy (Uyan et al. 2006b). For example, larvae (12–31 mm total length) have well-developed eyes with duplex retina, grouped rods and dense retinal tapetum, and canal neuromasts (Uyan et al. 2006a). If larvae developing at warmer temperatures have better-developed sense organs, they would be better equipped to capture prey and avoid predators than similar-sized larvae developing in colder temperatures.

Guanine deposition on the external body surface was not measured in the present study, since guanine on the body surface could be lost during sample collection and preservation. Instead, guanine deposition on the internal peritoneal surface was measured over a SL range of 23.9–51.7 mm. The GD-0 stage is considered to correspond well to 'the larval and early metamorphosing stages', and the GD-1 stage to 'the late metamorphosing stage' or 'the metamorphosed juvenile stage' (Takahashi & Watanabe 2004b). The maximum SL of the GD-0 stage fish was 37.3 mm, and the minimum SL of the GD-1 stage fish was 26.4 mm. In Japanese anchovy, Takahashi & Watanabe (2004b) showed that the maximum SL of fish without guanine deposition on the peritoneal and trunk surfaces was 40.1–45.0 mm and the minimum SL of fish with guanine deposition on the peritoneal surface but not on the trunk surface was 26.4 mm, based on samples collected in the Kuroshio–Oyashio transition region (offshore waters) during May to June in 1998. Sea surface temperature ranged from

13.4°C to 19.7°C in the waters where larval and early juvenile anchovy were distributed in this offshore region during May to June in 1996 and 1997 (Takahashi & Watanabe 2004a). The maximum SL of the GD-0 stage fish and the minimum SL of the GD-1 stage fish in the present study fell within the range of values obtained from the Kuroshio–Oyashio transition region, although the present study dealt with a wider temperature range (14.1–28.4°C). As such, the effects of factors other than temperature on development may be smaller in the Kii Channel than in the Kuroshio–Oyashio transition region. Seasonal variability was observed in SL at the start of guanine deposition. Guanine deposition started at smaller body sizes at higher temperatures. The difference of the developmental degree was 9.0–9.4 mm in SL among seasons, based on the mean SL of the GD-0 and GD-1 stage fish corresponding to immediately before and after the start of guanine deposition. The reflection of light from the external surface of fish can serve as camouflage (Denton & Nicol 1965) potentially reducing risks of detection by predators and offering an advantage to larvae developing at warmer temperatures.

Caudal fin supports play an important role in swimming for fish. Somarakis & Nikolioudakis (2010) provided evidence that the stage of caudal fin formation, initiated by notochord flexion, might be a developmental milestone in the early life history of engraulids, associated with changes in capabilities of the developing fish. In a rearing experiment, the cruise and burst swimming speeds of anchovy increased from 10–21 mm SL to 21–33 mm SL (Masuda 2011). Hence, the present samples correspond to the SL range in which swimming ability increases. The contributions to swimming speed are limited in caudal fin supports with a large cartilaginous area compared to those with a small cartilaginous area. The ossification of the caudal fin supports progressed with increased SL, indicating increased swimming ability. Caudal, dorsal, anal, and pelvic fin rays of anchovy reach the fully developed state at ca. 9, 12, 11, and 19 mm SL, respectively (Balart 1995). Since many individuals were larger than 19 mm SL in the present study, only pectoral fin rays, which are the last to develop, were counted. Pectoral fin rays started to form at 18.9–27.1 mm SL and the full complement was attained at 26.5–32.7 mm SL and more, based on the 3 mo samples. The attainment of full external meristic complements and loss of temporary specializations for pelagic life is a definition of the onset of the juvenile stage (Leis & Carson-Ewart 2000), although there is no agreed definition of the

onset of the juvenile stage (Nikolioudakis et al. 2014). The differences of developmental degree between seasons (temperature difference: 11.3°C) were 8.2 mm in SL based on the start of the formation, and 6.2 mm or more based on the full complement. These differences are less than the 9.0 to 9.4 mm seasonal difference in the developmental degree of guanine deposition examined for the temperature difference of 14.3°C. The beginning and end of the transition period from larvae to juveniles might vary by ~10 mm in SL between seasons, based on the seasonal difference of temperature (12.5–29.1°C) in the Kii Channel. Balart (1995) showed that the smallest individual of anchovy with pectoral fin rays was 17.5 mm SL, and the full complement was attained between 20.9 and 25.1 mm SL based on the laboratory-reared and wild-caught larvae. In a laboratory experiment, the segmentation of pectoral fin rays of anchovy commenced at ca. 21 mm SL and finished at 25 mm SL (Fukuhara 1983). Also, the pectoral fin rays started to develop at ca. 25 mm total length for anchovy in Sagami Bay in summer (Mitani 1990). Overall, there is considerable SL variability in the development of pectoral fin rays. Seasonal variability was observed in the advancement of the development of the caudal fin supports and pectoral fin rays, and the development was considered to be more advanced at higher temperatures at the same SL. Moyano et al. (2016) showed that higher temperatures accelerated development of swimming capacity in Atlantic herring *Clupea harengus* larvae. The larvae under higher temperatures would also have advantages of enhanced predator avoidance and prey capture in terms of caudal fin supports and pectoral fin rays.

The present study showed seasonal variability in development based on the change in head size, degree of guanine deposition, and the ossification (8 osteological elements and pectoral fin rays). These characteristics were selected because they can be readily assessed and because they can influence processes affecting the growth and survival of fish early life stages such as predator avoidance and prey capture, although a variety of characteristics contribute to these processes (Blaxter 1986, Fuiman & Magurran 1994).

A parallel development was observed between the external morphology (head length and pectoral fin rays) and the internal morphology (guanine on the peritoneal surface and caudal fin supports). The energy requirement for development of some characteristics did not result in delayed development of other characteristics. Osse & van den Boogaart (1995) concluded that the patterns of development and growth reflect successive functional priorities (e.g.

feeding, locomotion, and respiration), which, in some cases, can occur at different sizes. The observed parallel development of characteristics would reflect their functional requirements. In the temperature range of 12.5–29.1°C, higher temperatures led to an enhanced morphological development of anchovy. Chambers & Leggett (1987) reported that the effect of temperature on the length at metamorphosis (transition) is not necessarily consistent among studies (species), for example, in winter flounder *Pseudopleuronectes americanus*, cooler temperatures resulted in smaller sizes at metamorphosis. Our results for anchovy are consistent with the results for Japanese flounder *Paralichthys olivaceus* (Seikai et al. 1986) in that metamorphosis (transition) was accelerated at higher temperatures, resulting in a smaller size at metamorphosis.

To relate changes in intrinsic factors of fish and extrinsic (environmental) factors, it is important that field sampling is conducted at the appropriate spatial and temporal scales (Dower et al. 2002). The present study used monthly and station-mean temperature data and compared these to measurements made on fish from commercial catches. The extent of possible bias in temperature due to such a temporal and spatial mismatch can be assumed to be small compared with the seasonal variability in temperature (12.5–29.1°C). Although we focused on the effect of temperature on development of Japanese anchovy, other environmental factors such as prey abundance and type (Kamler et al. 1990) may have co-varied with temperature. However, the strong correspondence observed between seasonal variability in development and temperature suggests that temperature is a major environmental driver of the development of anchovy in the Kii Channel.

Engraulis species have a protracted spawning period in some regions (Gaughan et al. 1990, La Mesa et al. 2009). The seasonal differences in morphology and development of fish may have consequences for changes in potential survival. Growth rate of anchovy larvae was positively related to temperature in the Kii Channel (Yasue & Takasuka 2009). At higher temperatures, therefore, the larvae have a higher growth rate and develop to juveniles at smaller SL, resulting in a shorter larval stage duration. However, other factors should be carefully considered in discussing the actual survival potential. For example, daily mortality rates could increase with increasing temperature for fish larvae (Houde 1989, Pepin 1991). As each species has a thermal tolerance and optimal temperature window (Pörtner & Peck 2010), the growth rate could be depressed under hyper-optimal temperatures

(Takasuka et al. 2007b). The required ingestion to support the growth rate increased at higher temperatures (Houde 1989). Although lower temperatures would result in a longer larval stage duration, lower temperatures may lead to a reduction in swimming activity and therefore in metabolic demand (Hunter 1977). In addition, elongate and transparent bodies of larvae are adaptive to avoid visual predators (Masuda 2011). Another example is a negative relationship between temperature and egg diameter (Imai & Tanaka 1987). That is, size at hatching can be larger at lower temperatures (Llanos-Rivera & Castro 2006). There is a possibility that fish can survive in lower temperatures owing to these advantages. The temperature experienced during the egg and larval stages may have an impact on development during the later stages (Johnston et al. 2001, Koumoundouros et al. 2009). Hence, a future issue is to understand the effects of temperature on development and survival potential throughout the life history.

The 'growth-selective predation' mechanism posits that faster-growing larvae are less vulnerable to predation mortality than slower-growing conspecifics at the same somatic size. This mechanism assumes that faster-growing individuals are in a better physiological condition and thus have a higher potential for anti-predator behavior (Takasuka et al. 2003, 2007a). However, mechanistic processes linking growth rate to anti-predator behavior have yet to be identified. The results of the present study may contribute to the understanding of such processes. Growth rate and morphological development are probably linked tightly, as the seasonal variability of both was mainly driven by seasonal temperature cycles (Yasue & Takasuka 2009, present study). For example, larvae with more progressed ossification would have a higher potential for anti-predator behaviors (predator avoidance) as discussed above. Therefore, variability in development could have direct effects on the potential survival other than stage duration effects. Nonetheless, seasonal variability in environmental factors will affect the abundance, composition, and biological parameters of both predators (e.g. pelagic fish) and prey items (e.g. zooplankton) for the larvae. Therefore, future studies will need to consider seasonal variability in development of the larvae in the context of food web structure.

LITERATURE CITED

Anderson JT (1988) A review of size dependent survival during prerecruit stages of fishes in relation to recruitment. J Northwest Atl Fish Sci 8:55–66

Bacha M, Amara R (2012) Inter-cohort differences in growth, condition and feeding of juvenile anchovy (*Engraulis encrasicolus*) in the Gulf of Béjaia (Algerian coast, SW Mediterranean): implications for recruitment success. Fish Res 129–130:73–81

Balart EF (1995) Development of the vertebral column, fins and fin supports in the Japanese anchovy, *Engraulis japonicus* (Clupeiformes: Engraulididae). Bull Mar Sci 56:495–522

Blaxter JHS (1986) Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. Trans Am Fish Soc 115:98–114

Castello L, Castello JP (2003) Anchovy stocks (*Engraulis anchoita*) and larval growth in the SW Atlantic. Fish Res 59:409–421

Chambers RC, Leggett WC (1987) Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can J Fish Aquat Sci 44:1936–1947

Chiu TS, Chen CS (2001) Growth and temporal variation of two Japanese anchovy cohorts during their recruitment to the East China Sea. Fish Res 53:1–15

Denton EJ, Nicol JAC (1965) Reflection of light by external surfaces of the herring, *Clupea harengus.* J Mar Biol Assoc UK 45:711–738

Dower JF, Pepin P, Leggett WC (2002) Using patch studies to link mesoscale patterns of feeding and growth in larval fish to environmental variability. Fish Oceanogr 11:219–232

Fontes J, Santos RS, Afonso P, Caselle JE (2011) Larval growth, size, stage duration and recruitment success of a temperate reef fish. J Sea Res 65:1–7

Fuiman LA, Magurran AE (1994) Development of predator defences in fishes. Rev Fish Biol Fish 4:145–183

Fukuhara O (1983) Development and growth of laboratory reared *Engraulis japonica* (Houttuyn) larvae. J Fish Biol 23:641–652

Gaughan DJ, Neira FJ, Beckey LE, Potter IC (1990) Composition, seasonality and distribution of the ichthyoplankton in the Lower Swan Estuary, South-western Australia. Aust J Mar Freshwater Res 41:529–543

Grorud-Colvert K, Sponaugle S (2011) Variability in water temperature affects trait-mediated survival of a newly settled coral reef fish. Oecologia 165:675–686

Houde ED (1987) Fish early life dynamics and recruitment variability. Am Fish Soc Symp 2:17–29

Houde ED (1989) Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish Bull 87:471–495

Houde ED (2008) Emerging from Hjort's shadow. J Northwest Atl Fish Sci 41:53–70

Hunter JR (1977) Behavior and survival of northern anchovy *Engraulis mordax* larvae. CCOFI Rep 19:138–146

Imai C, Tanaka S (1987) Effect of sea water temperature on egg size of Japanese anchovy. Bull Jpn Soc Sci Fish 53:2169–2178

Johnston IA, Vieira VLA, Temple GK (2001) Functional consequences and population differences in the developmental plasticity of muscle to temperature in Atlantic

herring *Clupea harengus*. Mar Ecol Prog Ser 213: 285–300

Kamler E, Szlamińska M, Przybył A, Barska B, Jakubas M, Kuczyński M, Raciborski K (1990) Developmental response of carp, *Cyprinus carpio*, larvae fed different foods or starved. Environ Biol Fishes 29:303–313

Kawamura K, Hosoya K (1991) A modified double staining technique for making a transparent fish-skeletal specimen. Bull Natl Res Inst Aquacult 20:11–18 (in Japanese with English Abstract)

Koumoundouros G, Ashton C, Sfakianakis DG, Divanach P, Kentouri M, Anthwal N, Stickland NC (2009) Thermally induced phenotypic plasticity of swimming performance in European sea bass *Dicentrarchus labrax* juveniles. J Fish Biol 74:1309–1322

La Mesa M, Donato F, Giannetti G, Arneri E (2009) Growth and mortality rates of European anchovy (*Engraulis encrasicolus*) in the Adriatic Sea during the transition from larval to juvenile stages. Fish Res 96:275–280

Leggett WC, Frank KT (2008) Paradigms in fisheries oceanography. Oceanogr Mar Biol Annu Rev 46:331–363

Leis JM, Carson-Ewart BM (eds) (2000) The larvae of Indo-Pacific coastal fishes: an identification guide to marine fish larvae. Fauna Malesiana handbooks Vol 2. Brill, Leiden

Llanos-Rivera A, Castro LR (2006) Inter-population differences in temperature effects on *Engraulis ringens* yolk-sac larvae. Mar Ecol Prog Ser 312:245–253

Masuda R (2011) Ontogeny of swimming speed, schooling behaviour and jellyfish avoidance by Japanese anchovy *Engraulis japonicus*. J Fish Biol 78:1323–1335

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

Miller TJ, Crowder LB, Rice JA, Marschall EA (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can J Fish Aquat Sci 45:1657–1670

Mitani I (1990) The biological studies on the larvae of Japanese anchovy, *Engraulis japonica* Houttuyn, in Sagami Bay. Spec Rep Kanagawa Prefectural Fish Exper Stn 5: 1–140 (in Japanese with English Abstract)

Moreno P, Claramunt G, Castro LR (2011) Transition period from larva to juvenile in anchoveta *Engraulis ringens*. Length or age related? J Fish Biol 78:825–837

Moyano M, Illing B, Peschutter P, Huebert KB, Peck MA (2016) Thermal impacts on the growth, development and ontogeny of critical swimming speed in Atlantic herring larvae. Comp Biochem Physiol A Mol Integr Physiol 197: 23–34

Nikolioudakis N, Koumoundouros G, Kiparissis S, Somarakis S (2010) Defining length-at-metamorphosis in fishes: a multi-character approach. Mar Biol 157:991–1001

Nikolioudakis N, Koumoundouros G, Somarakis S (2014) Synchronization in allometric and morphological changes during metamorphosis: comparison among four sparid species. Aquat Biol 21:155–165

Noichi T, Noichi T, Senta T (1997) Comparison of ages, morphology and osteology by birth months of larval Japanese flounder settling at Yanagihama Beach, Nagasaki Prefecture, Japan. Fish Sci 63:169–174

Oozeki Y, Takasuka A, Kubota H, Barange M (2007) Characterizing spawning habitats of Japanese sardine (*Sardinops melanostictus*), Japanese anchovy (*Engraulis japonicus*), and Pacific round herring (*Etrumeus teres*) in the Northwestern Pacific. CCOFI Rep 48:191–203

Osse JWM, van den Boogaart JGM (1995) Fish larvae, development, allometric growth, and the aquatic environment. ICES Mar Sci Symp 201:21–34

Pepin P (1991) Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Can J Fish Aquat Sci 48: 503–518

Pörtner HO, Peck MA (2010) Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. J Fish Biol 77:1745–1779

Potthoff T, Tellock JA (1993) Osteological development of the snook, *Centropomus undecimalis* (Teleostei, Centropomidae). Bull Mar Sci 52:669–716

Radtke RL, Kinzie RA, Shafer DJ (2001) Temporal and spatial variation in length of larval life and size at settlement of the Hawaiian amphidromous goby *Lentipes concolor*. J Fish Biol 59:928–938

Rankin TL, Sponaugle S (2011) Temperature influences selective mortality during the early life stages of a coral reef fish. PLOS ONE 6:e16814

Robert D, Castonguay M, Fortier L (2007) Early growth and recruitment in Atlantic mackerel *Scomber scombrus*: discriminating the effects of fast growth and selection for fast growth. Mar Ecol Prog Ser 337:209–219

Seikai T, Tanangonan JB, Tanaka M (1986) Temperature influence on larval growth and metamorphosis of the Japanese flounder *Palalichthys olivaceus* in the laboratory. Bull Jpn Soc Sci Fish 52:977–982

Somarakis S, Nikolioudakis N (2010) What makes a late anchovy larva? The development of the caudal fin seen as a milestone in fish ontogeny. J Plankton Res 32: 317–326

Takahashi M, Watanabe Y (2004a) Growth rate-dependent recruitment of Japanese anchovy *Engraulis japonicus* in the Kuroshio-Oyashio transitional waters. Mar Ecol Prog Ser 266:227–238

Takahashi M, Watanabe Y (2004b) Staging larval and early juvenile Japanese anchovy based on the degree of guanine deposition. J Fish Biol 64:262–267

Takahashi I, Azuma K, Fujita S, Hiraga H (2000) Differences in larval and juvenile development among monthly cohorts of ayu, *Plecoglossus altivelis*, in the Shimanto River. Ichthyol Res 47:385–391

Takahashi M, Watanabe Y, Kinoshita T, Watanabe C (2001) Growth of larval and early juvenile Japanese anchovy, *Engraulis japonicus*, in the Kuroshio-Oyashio transition region. Fish Oceanogr 10:235–247

Takasuka A, Aoki I, Mitani I (2003) Evidence of growth-selective predation on larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. Mar Ecol Prog Ser 252: 223–238

Takasuka A, Aoki I, Mitani I (2004) Three synergistic growth-related mechanisms in the short-term survival of larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. Mar Ecol Prog Ser 270:217–228

Takasuka A, Aoki I, Oozeki Y (2007a) Predator-specific growth-selective predation on larval Japanese anchovy *Engraulis japonicus.* Mar Ecol Prog Ser 350:99–107

Takasuka A, Oozeki Y, Aoki I (2007b) Optimal growth temperature hypothesis: Why do anchovy flourish and sardine collapse or vice versa under the same ocean regime? Can J Fish Aquat Sci 64:768–776

Takasuka A, Kubota H, Oozeki Y (2008) Spawning overlap of anchovy and sardine in the western North Pacific. Mar Ecol Prog Ser 366:231–244

Takasuka A, Sakai A, Aoki I (2016) Dynamics of growth-based survival mechanisms in Japanese anchovy *Engraulis japonicus* larvae. Can J Fish Aquat Sci, doi: 10.1139/cjfas-2016-0120

Uyan S, Kawamura G, Archdale MV (2006a) Morphology of the sense organs in larval anchovy *Engraulis japonicus* caught by midwater trawl. Aquacult Sci 54:275–282

Uyan S, Kawamura G, Archdale MV (2006b) Morphology of the sense organs of anchovy *Engraulis japonicus*. Fish Sci 72:540–545

Wang YT, Tzeng WN (1999) Differences in growth rates among cohorts of *Encrasicholina punctifer* and *Engraulis japonicus* larvae in the coastal waters off Tanshui River Estuary, Taiwan, as indicated by otolith microstructure analysis. J Fish Biol 54:1002–1016

Yasue N, Takasuka A (2009) Seasonal variability in growth of larval Japanese anchovy *Engraulis japonicus* driven by fluctuations in sea temperature in the Kii Channel, Japan. J Fish Biol 74:2250–2268

Yasue N, Takasuka A, Shirakihara K (2011) Interspecific comparisons of growth and diet among late larvae of three co-occurring clupeoid species in the Kii Channel, Japan. Mar Biol 158:1709–1720

Yasue N, Doiuchi R, Takasuka A (2014) Trophodynamic similarities of three sympatric clupeoid species throughout their life histories in the Kii Channel as revealed by stable isotope approach. ICES J Mar Sci 71:44–55

Zenitani H, Kimura R (1997) Increase in late winter egg production of the Japanese anchovy as related to recovery of the stock size along the Pacific Coast of Japan. Bull Jpn Soc Sci Fish 63:665–671 (in Japanese with English Abstract)