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Association between fatty acid signature and growth rate of larval Pacific bluefin tuna in two major spawning grounds

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ABSTRACT: Fatty acid composition and total fatty acids of field-caught Pacific bluefin tuna (PBF) *Thunnus orientalis* larvae were investigated to identify relationships between spawning ground, physiological condition, and growth rate. Multivariate statistical analyses revealed that both growth rate and environmental conditions at spawning grounds were substantially associated with variations in fatty acid compositions. Fast-growing larvae typically contained more α-linolenic acid and 22:5n-3, which are important as metabolic precursors of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Larval PBF fatty acid compositions differed by spawning ground; larvae caught around the Nansei Islands contained more 15:0, 17:0, 19:0, and 22:5n-6, and less EPA and arachidonic acid (ARA) than those from the Sea of Japan. Differences in larval odd-numbered fatty acid compositions might indicate different degrees of dependence on microbial loop energy supply between spawning grounds. Environments subject to sudden changes, such as those in water temperature and prey density in the Sea of Japan, might cause variability in fatty acid profiles, including extremely low %DHA. We suggest that continuous food intake and subsequent fatty acid catabolism for energy generation would be needed to facilitate fast growth.

KEY WORDS: Daily growth increments · Essential fatty acid · Factor analysis · Fatty acid signatures · Larval nutritional condition · *Thunnus orientalis*

1. INTRODUCTION

Marine fish species generally experience high mortality during their early life stages, with larval and juvenile growth rates considered to be the most important factors determining survival (Takasuka et al. 2003, Tanaka et al. 2006). Annual variation in mortality is a main cause of fluctuations in fish stocks (Bailey & Houde 1989). Various hypotheses have been advanced to explain mechanisms underlying early life stage survival.

Environmental factors, especially water temperature and prey abundance, have been proposed to explain variations in larval growth (Heath 1992, Meekan et al. 2003). However, while otolith microstructure can reveal the growth trajectories of individual fish, this method does not identify the cause of any variation. To better understand the causes of growth variation, it is important to consider the physiological condition of larval stages.

The quality and quantity of long-chain polyunsaturated fatty acids (LC-PUFAs), especially docosa-

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hexaenoic acid (DHA: 22:6n-3), are vital to development and survival of newly hatched wild and farmed larvae (Sargent et al. 1999, Izquierdo & Koven 2011). The importance of LC-PUFAs to larval development is based on their importance in cell membrane structure and function (Izquierdo & Koven 2011). Tuna have relatively high levels of body DHA (Tocher 2003, Mourente & Tocher 2009) and require diets high in it for farming (Seoka et al. 2007, 2008, Mourente & Tocher 2009). In this context, an evaluation of the fatty acid composition of field-caught larvae might provide an indication of their physiological condition, affecting their survival before recruitment (Bell & Sargent 1996).

Pacific bluefin tuna (PBF) *Thunnus orientalis* occur widely throughout the Pacific Ocean, are exploited by many countries, and represent one of the most valuable and iconic of global fisheries (ISC 2020). Although 2 major spawning grounds and seasons for this species are recognised (Ashida et al. 2015, Okochi et al. 2016, Ohshimo et al. 2017), other spawning areas exist (Ohshimo et al. 2018b, Tanaka et al. 2020). The main spawning ground is located northeast of the Philippines and extends to the Nansei Islands in the northwestern Pacific Ocean, where spawning occurs from April to June (Yabe et al. 1966, Ashida et al. 2015, Shimose et al. 2016, Tawa et al. 2020). The second major spawning area is in the Sea of Japan from June to August (Okochi et al. 2016, Ohshimo et al. 2018a).

The growth rate of PBF during the first 2 wk post hatching is critical to their survival to recruitment (Tanaka et al. 2006, Watai et al. 2017, 2018). Growth trajectory analysis using otolith microstructure implies that different survival processes occur in different spawning grounds (Watai et al. 2018, Ishihara et al. 2019). Larval growth rate in the Sea of Japan is more variable than around the Nansei Islands (Watai et al. 2018). Larval growth to the flexion stage is adversely affected by lower Sea of Japan temperatures, which are believed to more greatly impact their survival than around the Nansei Islands. Survival past the flexion stage is a major ontogenetic milestone in the Sea of Japan (Ishihara et al. 2019). PBF larval and egg fatty acid contents in the 2 spawning grounds also differ, with those in the Sea of Japan having proportionally more eicosapentaenoic (EPA: 20:5n-3) and arachidonic (ARA: 20:4n-6) acids than those around the Nansei Islands (Matsumoto et al. 2018, Hiraoka et al. 2019).

Nutritional condition evaluated by fatty acid content significantly influences wild larval growth and survival (Hiraoka et al. 2014, Paulsen et al. 2014a,b). The highest growth rates, and %DHA and %EPA, of wild larval herring *Clupea harengus* coincide with high food quality (Paulsen et al. 2014b). Larval %DHA also correlates with instantaneous growth rate as estimated by the RNA:DNA ratio (Paulsen et al. 2014a,b).

Eggs and larval fatty acid compositions generally vary as a result of maternal provisioning and larval feeding (Fuiman & Perez 2015), with both differing in PBF spawning grounds (Hiraoka et al. 2019, Kodama et al. 2020). Here, we identified differences in fatty acid composition and growth rates of larvae from around the Nansei Islands and Sea of Japan spawning grounds. We then examined relationships between %DHA and total fatty acids to ascertain if fatty acid profiles can be associated with growth rates.

2. MATERIALS AND METHODS

2.1. Field observations

PBF larvae were collected from around the Nansei Islands (25° 30′–27° 40′ N, 125° 25′–128° 00′ E) and Sea of Japan (36° 04′–37° 40′ N, 133° 27′–135° 25′ E) aboard RV 'Shunyo-maru' (Japan Fisheries Research and Education Agency): collection dates were 19–28 June 2016 and 22–25 June 2018 (Nansei Islands), and 28 July–4 August 2016 and 26 July 2018 (Sea of Japan) (Table 1). Fish larvae were collected by ring net (2 m diameter, 0.34 mm mesh) horizontally towed on the surface, or a Tucker trawl net (1 m square, 0.5 mm mesh) towed horizontally within 30, 20, and 10 m vertical depth bins. Larvae were quickly sorted from the plankton catch, then stored individually in plastic containers at −25°C.

Temperature and chlorophyll *a* (chl *a*) fluorescence at 10 m depth were measured at all sites using a conductivity, temperature, and depth sensor (SBE9plus, Seabird Electronics) with a fluorometer (Seapoint Sensors). Sea surface temperature (SST) was measured using a calibrated mercury thermometer.

2.2. Larval treatment

Larvae were individually photographed using a digital camera mounted on a dissecting microscope in the laboratory. Body length (notochord length in pre-flexion and flexion larvae, and standard length for post-flexion larvae) was measured from images using ImageJ software (https://imagej.nih.gov/ij/). With a dissecting needle, each larva was divided into head and body (including trunk and tail) sections,

Table 1. Details of field sampling and environmental indices. SST: sea surface temperature. Dates are given as yr-mo-d

Sampling date	Area	Latitude (N)	Longitude (E)	Larvae (n)	SST (°C)	Chl *a* (µg l^{-1})
2016-06-19	Nansei	25° 33′	126° 05′	20	28.9	0.027
2016-06-26	Nansei	27° 56′	127° 57′	5	29.2	0.069
2016-06-27	Nansei	27° 32′	127° 18′	4	29.1	0.110
2016-06-28	Nansei	27° 14′	127° 57′	8	28.2	0.074
2018-06-22	Nansei	25° 47′	127° 11′	12	28.0	0.068
2018-06-25	Nansei	25° 25′	126° 20′	1	28.5	0.067
2016-07-28	Sea of Japan	37° 18′	135° 18′	12	25.5	0.107
2016-07-31	Sea of Japan	37° 00′	134° 32′	12	27.8	0.070
2016-08-02	Sea of Japan	35° 55′	133° 57′	1	27.9	0.109
2016-08-04	Sea of Japan	37° 00′	133° 57′	1	27.0	0.062
2018-07-26	Sea of Japan	37° 16′	135° 00′	3	26.7	0.203
2018-07-26	Sea of Japan	36° 00′	135° 00′	5	28.2	0.160

and its digestive tract was removed and discarded (Hiraoka et al. 2014, Matsumoto et al. 2018).

Sagittal otoliths were extracted from the head and digitally photographed (YCU-300F, Yashima Optical). Otolith increments were counted, and increment width and otolith radius were measured from the digital image. Daily age is the number of increments plus 3 (Itoh et al. 2000); back-calculated body length was estimated using the biological intercept method (Campana 1990), all following procedures used by Ishihara et al. (2019). To exclude the effects of increment widths varying with age (Pepin et al. 2001), standardised growth rates were estimated as follows: $z_{ij} = 1/(x_{ij} - x_j)S_j$, where x_{ij} is the growth rate (mm d^{-1}) of individual i at age j (d), and x_j and S_j are the mean and SD, respectively, of growth rates at age j (Baumann et al. 2008). For the growth index, 3 d averaged standardised growth rates before the catch day were calculated for individuals, which were classified as 'high' or 'low' using median values as a threshold. Because the first otolith increment forms 4 d post-hatching (dph) in culture (Itoh et al. 2000), larvae younger than 7 dph (n = 10) were excluded from analyses in estimates of 3 d average growth rates.

2.3. Fatty acid analysis

A simplified direct saponification/methylation method (Matsumoto et al. 2018) was used for fatty acid analysis. Bodies were individually transferred to pre-weighed screw-capped glass vials (1.5 ml), dehydrated in 99.5% ethanol (50 µl), and dried by blowing a stream of nitrogen through the vial. The body was further dried *in vacuo* in the same vial until constant weight was reached.

The dried body was directly saponified in a mixture of methyl tricosanoate in toluene (2 mg ml^{-1}, 1 µl) and 1 M KOH in 95% ethanol (50 µl) overnight in the dark at 23°C. The reaction was stopped by adding 2 M HCl (50 µl); solvents and excess HCl were evaporated off by passing a stream of nitrogen through the vial. Fatty acids released into the vial were methylated in a mixture of toluene (80 µl), methanol (20 µl), and a 10% solution of trimethylsilyldiazomethane in hexane (50 µl) at 23°C for 15 min. The reaction was stopped by adding acetic acid (5 µl), and all solvents were again removed by passing a stream of nitrogen through the vial. Fatty acid methyl esters were purified by column chromatography using silica gel 60 (Merck), and hexane/diethyl ether (90:10, v/v) for elution.

Gas chromatography was performed using a GC-4000 gas chromatograph (GL Sciences) equipped with a flame-ionization detector and capillary column of FAMEWAX (30 m × 0.32 mm i.d., 0.25 µm film thickness; Restek). Column temperature was programmed from 90°C (0 min) to 170°C at 20°C min^{-1}, then to 240°C at 4°C min^{-1}, and finally maintained at 240°C for 25 min. Injector and detector temperatures were maintained at 240°C. The carrier gas was helium at a linear velocity of 33.5 cm s^{-1} at 170°C (85 kPa); methyl esters dissolved in hexane were injected using the splitless injection mode, and held for 1 min. Peak areas were measured in a Shimadzu C-R3A integrator. Blank analyses with no larvae were performed for all analytical processes.

2.4. Statistical analysis

Generalized linear mixed models were applied to evaluate which factors (year, area, dph) influenced

variation in %DHA and total fatty acid content (µg mg^{-1}). Gaussian error distribution with identity link was assumed. Because larvae were caught in relatively few tows (Table 1), 'tow' was treated as a random effect in the models, using the 'lme4' package (Bates et al. 2015) in R (R Core Team 2019). Spearman's rank correlation was used to consider relationships between 2 factors. Differences in fatty acid fractions (e.g. %DHA) were compared between growth indices (high or low) and sampling area (Nansei Islands or Sea of Japan) using Wilcoxon rank sum tests.

Multivariate statistical analysis included profiles for 21 fatty acids which contributed at least 0.1% to the total fatty acid composition on arcsine square-root transformed values. A principal component factor analysis was undertaken to identify which fatty acid(s) contributed to area or growth differences. To test factorial adequacy of a correlation matrix, the Kaiser-Meyer-Olkin (KMO) index was calculated. The number of factors to be extracted was based on their eigenvalue (>1). The maximum likelihood method was used to estimate parameters. Factor readability was improved through a varimax rotation; factor loading scores were generated for each larva. Analysis was performed using the 'psych' package (Revelle 2021) in R (R Core Team 2019).

Other multivariate statistical analyses were performed using PRIMER-7 software (Clarke & Gorley 2015). A Bray-Curtis similarity matrix and permutational multivariate ANOVA (PERMANOVA) were used to investigate how fatty acid profiles varied between growth indices.

3. RESULTS

3.1. Daily variations in %DHA, total fatty acids, and growth rate

In total, 84 PBF larvae were analysed for fatty acid composition and daily growth rate. Of these, total fatty acids (µg mg^{-1}) were not evaluated for 2 larvae because of difficulties in measuring body dry weights. The size range of Sea of Japan larvae (body length 3.4–6.6 mm, n = 34) was smaller than that of larvae from around the Nansei Islands (body length 4.2–7.0 mm, n = 50). Age estimates were 8–11 dph for larvae from the Nansei Islands, and 5–11 dph for those from the Sea of Japan.

Larval %DHA did not differ in any factor (area, dph, year: $p > 0.050$). Variation in %DHA among daily ages for Sea of Japan larvae was relatively greater than for those from around the Nansei Islands (coefficient of variation; Sea of Japan: 2.4–27.7%, Nansei Islands: 3.4–7.8%; Fig. 1A), although median value ranges did not vary greatly between the Sea of Japan (23.3–27.0%) and the Nansei Islands (25.7–28.8%).

Total fatty acid contents were 0.72–13.8 µg mg^{-1} for the Nansei Islands, and 1.1–10.7 µg mg^{-1} for the Sea of Japan (Fig. 1B). No significant difference was observed in any factor (area, dph, year: $p > 0.050$).

Estimated daily growth rate varied highly, but standardised growth remained around zero because mean values were adjusted to zero (Fig. 2). Median values of standardised growth applied as thresholds to the growth index were 0.13 from the Nansei Islands and 0.19 in the Sea of Japan.

3.2. Relationships between fatty acid compositions, area, and growth index

The KMO measure of sampling adequacy suggests that the sample was factorable (KMO = 0.68). Six factors were extracted based on eigenvalues. Individual factor loading scores were plotted with factors 1 and 2 as axes in Fig. 3A. Factors 1 and 2 explained 21.6

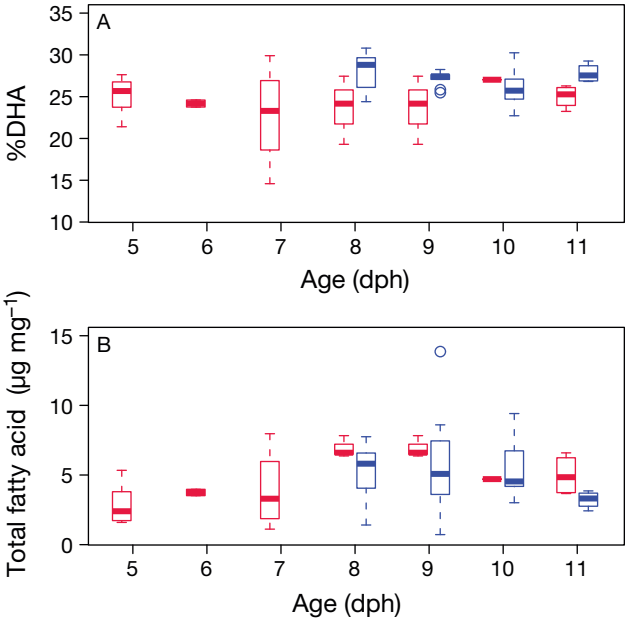

Fig. 1. (A) Percent docosahexaenoic acid (DHA) and (B) total fatty acids by larval age (dph: days post hatch) of *Thunnus orientalis* around the Nansei Islands (blue) and Sea of Japan (red). Boxes delimit the first and third quantiles, the thick horizontal line is the median, and whiskers represent 1.5× the interquartile range above and below the first and third quartiles. Outliers are plotted individually

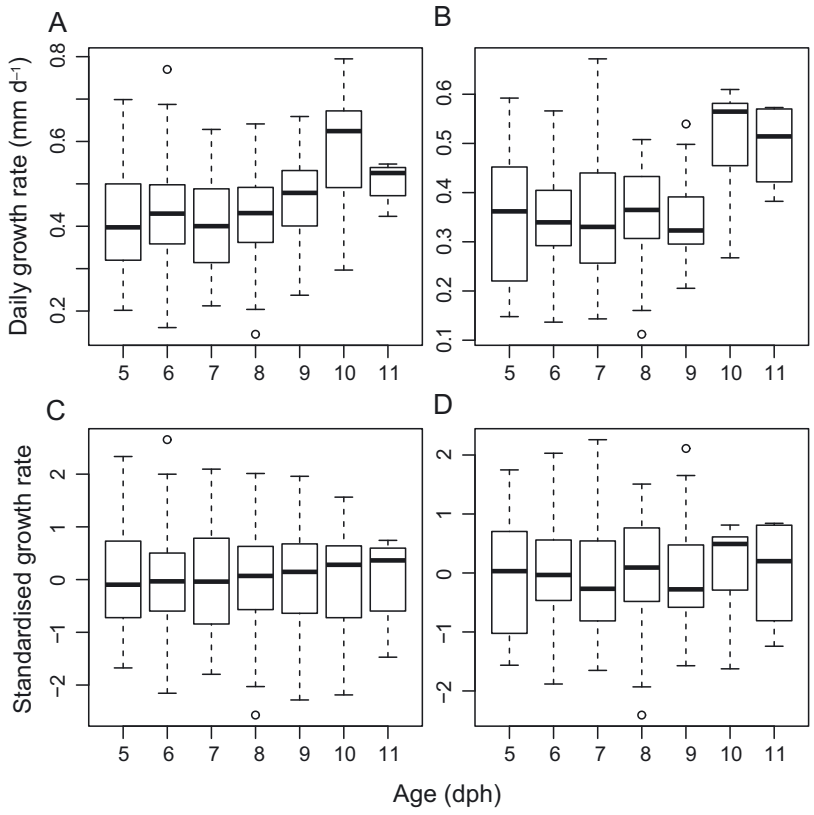

Fig. 2. (A,B) Daily growth rate and (C,D) standardised growth rate of *Thunnus orientalis* larvae by age (days post hatch, dph) around the Nansei Islands (A,C) and the Sea of Japan (B,D). Box plot parameters as in Fig. 1

and 20.2% of the total variance, respectively. Loading scores for factor 1 (comprising mainly 14:0, 7–Me–16:1 and 18:1n-13, and inverse loadings of 18:1n-7, 18:3n-3 [α-linolenic acid] and 22:5n-3) differed significantly between growth indices ($p < 0.01$). Additionally, a significant correlation was found between factor 1 loading scores and the standardised growth for larvae caught ($p < 0.01$: Fig. 3B). Within n-3 PUFAs, loading scores of 22:5n-3 were relatively high (–0.61).

Loading scores for factor 2 (comprising mainly positive loadings from the proportions of 15:0, 17:0, 19:0, and 22:5n-6, and inverse loadings of EPA and iso-18:0; Table 2) clearly separated by sampling area. There were no clear relationships between area or growth index and the third or subsequent factors.

Fatty acid compositions differed significantly between high and low growth indices in both areas (PERMANOVA: Nansei Islands, $p < 0.001$; Sea of Japan, $p = 0.045$; Table 3).

3.3. Area-specific differences in fatty acid signatures and environmental factors

A significant correlation between %DHA and growth rate occurred only around the Nansei Islands ($p = 0.001$ vs. Sea of Japan, $p = 0.30$; Fig. 4A). Extremely low %DHA (<20%) of larvae was found in the Sea of Japan (Fig. 4A). For total fatty acids, no significant relationship was observed in either area (Fig. 4B). Larvae with low growth rates showed larger variations of total fatty acid than those with high growth

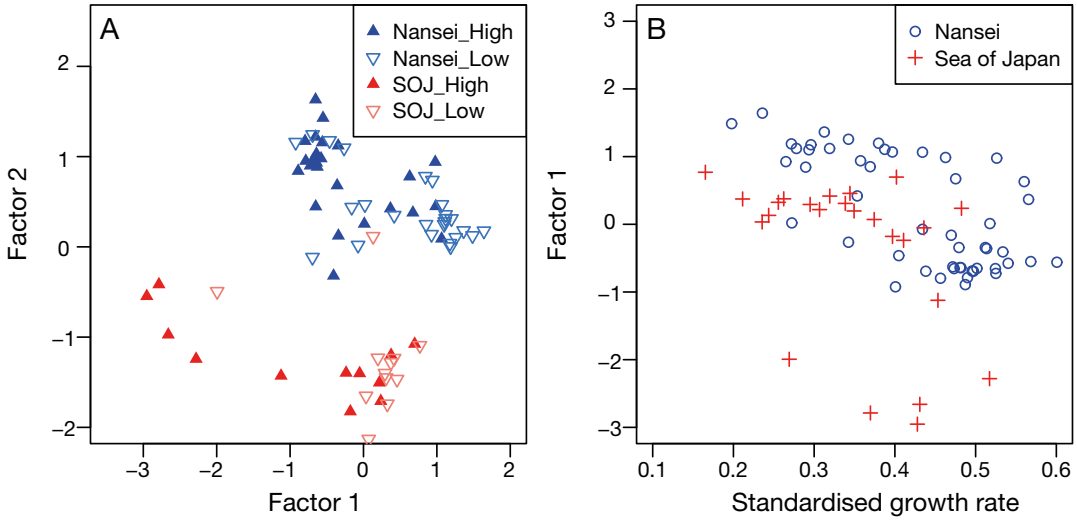

Fig. 3. Relationships (A) between loading scores for factors 1 and 2, and (B) loading scores for factor 1 and standardised growth rate. SOJ: Sea of Japan

Table 2. Fatty acids and factor loadings for varimax orthogonal 6-factor solution

Fatty acid	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6
14:0	0.673	−0.004	0.236	0.347	−0.466	−0.362
15:0	0.195	0.584	0.125	0.159	−0.626	−0.401
16:0	0.488	−0.147	0.751	−0.223	−0.185	−0.069
7-Me-16:1	0.843	0.348	0.139	0.085	−0.051	−0.013
17:0	−0.140	0.931	−0.001	−0.116	−0.012	0.059
iso-18:0	0.179	−0.610	0.165	−0.064	0.086	−0.072
18:0	−0.225	0.101	0.561	−0.408	0.364	0.481
18:1n-13	0.656	0.019	−0.016	0.116	−0.025	0.116
18:1n-9	0.163	−0.626	0.392	−0.227	0.326	0.388
18:1n-7	−0.811	0.282	0.131	0.337	−0.270	0.212
18:2n-6	−0.448	−0.452	0.288	0.180	0.608	−0.089
19:0	0.463	0.634	−0.193	−0.195	0.259	0.224
18:3n-3	−0.822	0.012	0.160	0.514	0.027	−0.145
18:4n-3	0.052	−0.181	0.015	0.851	−0.118	−0.185
20:0	0.170	0.051	0.057	−0.142	0.173	0.805
20:4n-6	−0.402	−0.479	−0.297	−0.047	−0.007	−0.004
20:5n-3	−0.370	−0.777	−0.354	0.187	−0.001	−0.060
22:5n-6	0.252	0.732	−0.274	−0.342	0.226	−0.101
22:5n-3	−0.611	−0.038	−0.381	−0.090	0.043	−0.265
22:6n-3	0.082	0.097	−0.910	−0.238	0.094	−0.047
24:1n-9	0.104	0.142	−0.124	−0.107	0.433	0.152
Sums of squared loadings	4.530	4.246	2.595	1.874	1.697	1.616
% of variance	21.6	20.2	12.4	8.9	8.1	7.7
Cumulative %	21.6	41.8	54.1	63.1	71.2	78.8

Table 3. One-way PERMANOVA results for *Thunnus orientalis* growth index and spawning ground based on a Bray-Curtis transformed matrix of fatty acid composition. Pseudo-*F*: *F*-value by permutation, Perms: number of permutations; p-values are based on >9000 permutations

Factor	df	SS	MS	Pseudo-*F*	p	Perms
Nansei						
Growth index	1	30.92	30.92	6.90	**<0.001**	9948
Residuals	48	215.02	4.48			
Total	49	245.94				
Sea of Japan						
Growth index	1	44.36	44.36	2.41	**0.045**	9927
Residuals	22	405.47	18.43			
Total	23	449.83				

rates in both areas (Fig. 4B). Opposite trends were observed between the 2 areas for relationships between SST and %DHA: a positive correlation was apparent in the Sea of Japan ($p = 0.005$) and a negative correlation around the Nansei Islands ($p < 0.001$; Fig. 4C). SST was lower and its range higher in the Sea of Japan (25.5–28.2°C) than around the Nansei Islands (28.0–29.2°C) (Table 1, Fig. 4C). Furthermore, %DHA was positively correlated with chl *a* only around the Nansei Islands ($p = 0.007$; Fig. 4D). The chl *a* range in the Sea of Japan was also higher and more variable in the Sea of Japan (0.062–0.203 µg l^{-1}) than around the Nansei Islands (0.027–0.110 µg l^{-1}).

4. DISCUSSION

Multivariate analyses revealed evidence indicating an association between larval fatty acid composition and growth rate (Fig. 3, Tables 2 & 3). A significant correlation between standardised growth rate and loading scores for factor 1 suggests that growth speed is related to larval fatty acid composition. The main roles of fatty acids in fish are (1) as sources of metabolic energy, (2) as structural phospholipid components of cell membranes, and (3) as precursors of bioactive molecules (Sargent et al. 1999, Tocher 2003, Izquierdo & Koven 2011). Although the proportions of fatty acids strongly influencing factor 1 (14:0, 7–Me–16:1, 18:1n-13, 18:1n-7, α-linolenic acid, and 22:5n-3) were not high in larvae (mean value <3%; Table A1 in the Appendix), both α-linolenic acid and 22:5n-3 are important as metabolic precursors of EPA and DHA (Dalsgaard et al. 2003, Tocher 2003). Considering that Atlantic bluefin tuna *Thunnus thynnus* larvae are capable of biosynthesis of LC-PUFAs

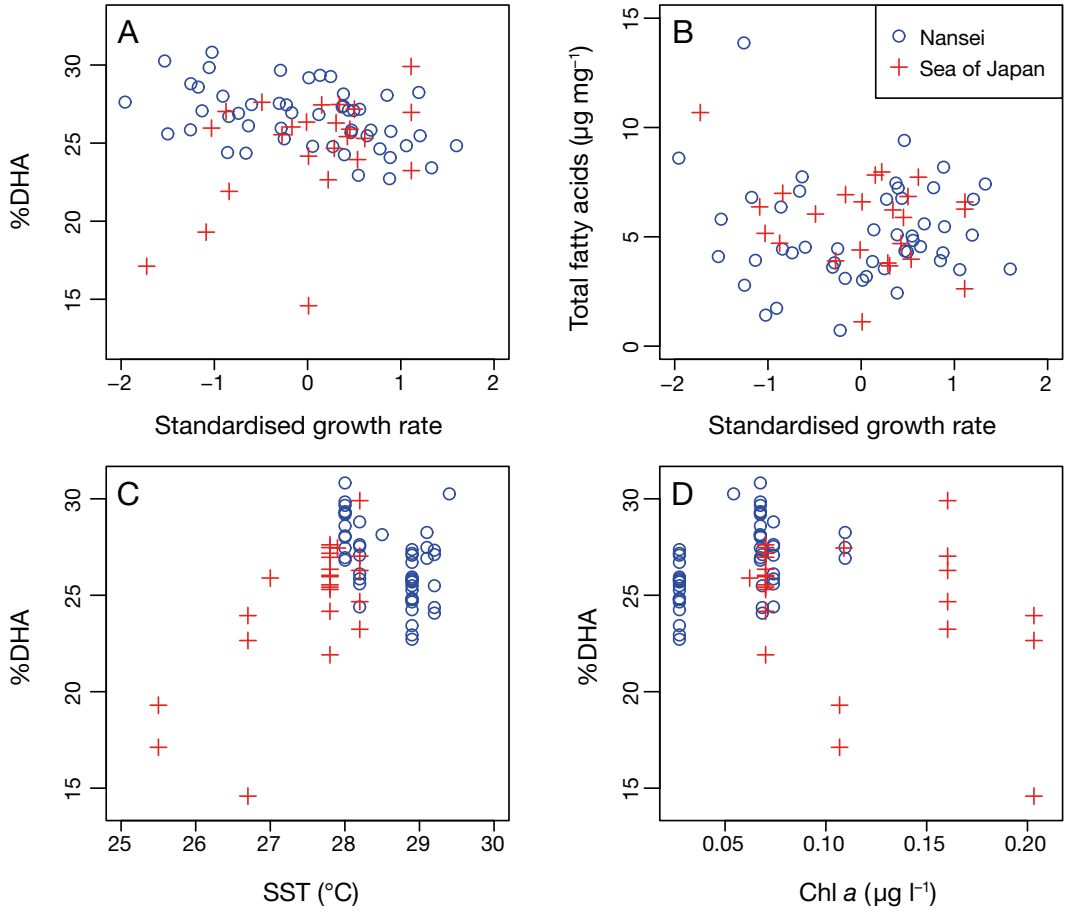

Fig. 4. Relationships between (A) % docosahexaenoic acid (DHA) and standardised growth rate of larval *Thunnus orientalis*, (B) total fatty acids and standardised growth rate, (C) %DHA and sea surface temperature (SST), and (D) %DHA and chlorophyll *a* fluorescence (chl *a*) around the Nansei Islands (blue circles) and Sea of Japan (red crosses)

(Morais et al. 2011), PBF larvae have potential for biosynthesis of EPA and DHA. Fast-growing larvae typically contain more α-linolenic acid and 22:5n-3, but it is difficult to demonstrate an explicit relationship between these fatty acids and growth rate. Growth is a complex process influenced by various environmental factors which may change over time scales of days to weeks. Further studies, including rearing experiments, are needed to better understand relationships between larval growth and larval fatty acid contents.

The environmental conditions at spawning grounds have a substantial impact on variations in fatty acid compositions (Table 2, Fig. 3). Distinct differences in fatty acid compositions of larvae between areas were reported by Matsumoto et al. (2018). Factor analysis revealed that larvae from around the Nansei Islands contained more 15:0, 17:0, 19:0, and 22:5n-6, and less EPA and ARA than those from the Sea of Japan (Tables 2 & A1).

The percentage of odd-numbered fatty acids (15:0, 17:0, 19:0) may be influenced by larval feeding habits. Odd-numbered and branched-chain fatty acids generally represent biomarkers for bacterial input (Sargent et al. 1983, Dalsgaard et al. 2003). Cultured PBF larvae may use energy and nutrients from the microbial loop, where bacteria represent the starting point of energy flow (Azam et al. 1983), because they selectively consume dinoflagellates (Nakagawa et al. 2007). Kodama et al. (2020) reported a difference in feeding habits of PBF larvae between spawning grounds using integrated morphological and metagenetic analysis, although phytoplankton including dinoflagellates were not identified (Kodama et al. 2017, 2020). Our data and previous studies revealed that wild PBF larvae contain more odd-numbered fatty acids (15:0. 17:0, 19:0) than eggs, with mean values, respectively, for larvae of >0.9, >1.9, and >0.3%, and for eggs of <0.4, <0.6, and <0.05%) (Table A1; Matsumoto et

al. 2018, Hiraoka et al. 2019), indicating that PBF larvae accumulate these fatty acids by feeding on prey after hatching. Fatty acid composition provides evidence that wild PBF larvae also use a microbial loop. Consequently, larval differences in odd-numbered fatty acid compositions might indicate differences between spawning grounds in their dependence on energy from microbial loops.

PBF larval growth was unexpectedly negatively correlated with %DHA around the Nansei Islands (Fig. 4A). In laboratory experiments with PBF larvae, larvae achieved high growth and survival rates with high DHA contents (Biswas et al. 2006, Seoka et al. 2007, 2008). For example, Seoka et al. (2008) demonstrated that PBF larvae fed with the yolk-sac larvae of Japanese parrotfish *Oplegnathus fasciatus* accumulated levels of DHA (25.6%) more than PBF larvae fed with enriched *Artemia* (8.7–19.3%), and that high-DHA larvae grew faster after 9 d of rearing. However, the low levels of DHA (8.7–19.3%) in the experiment by Seoka et al. (2008) were lower than the lowest %DHA around the Nansei Islands (22.7%). Therefore, PBF larvae with >22.7% DHA were not DHA-deficient.

Compared to larvae around the Nansei Islands, slow-growing larvae with low levels of DHA occurred in the Sea of Japan (Fig. 4A). While the direct cause of extremely low %DHA in Sea of Japan larvae was not determined, wide variations of environmental factors, including in water temperature and prey density, and their sudden changes, in the Sea of Japan (Table 1, Fig. 4C,D; Watai et al. 2018, Kodama et al. 2020) may have contributed. Because of the importance of DHA to larvae, DHA-deficient larvae in the Sea of Japan do not survive to recruitment. DHA-deficient larvae may be quickly removed by predators around the Nansei Islands because this deficiency reduces their visual capacity (Bell et al. 1995) and schooling and escape behaviours (Masuda et al. 1999, Ishizaki et al. 2001, Fuiman & Perez 2015).

Total fatty acid levels may vary depending on food intake and catabolism (Tocher 2003). Larval total fatty acid concentrations did not differ significantly between year, area, and dph (Fig. 1B). Low variability in total fatty acid concentrations between spawning grounds suggests that any surviving larvae were of similar quality. However, relatively smaller variations were observed in larvae with high growth rates than in those with low growth rates in both areas (Fig. 4B). This fact suggests that continuous food input and subsequent fatty acid catabolism for energy generation would be directed toward growth and development before lipid accumulations.

Because larval survival strongly depends on growth, regardless of spawning ground (Tanaka et al. 2006, Watai et al. 2017, 2018, Ishihara et al. 2019), physiological differences determined by fatty acid signatures imply different survival processes in each spawning ground. Our sample size was small (n = 84) relative to other studies using field-caught larvae, with larvae collected from several tows only because of field-sampling and fatty acid analysis problems. Additionally, the headless bodies of larvae used in fatty acid analysis might have affected results because the larval tuna head represents a large proportion of the total body mass, and brain and eye tissues have higher %DHA than other body parts (Mourente 2003). If these limitations are taken into consideration in future studies, our findings will contribute to further identification of mechanisms responsible for fluctuations in PBF recruitment.

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Appendix.

Table A1. Mean, SD, and fatty acid p-values (as % of total fatty acids) for *Thunnus orientalis* larvae from around the Nansei Islands and Sea of Japan. Totals include some minor components (not listed). Values in **bold** are significant at $p < 0.05$

	Nansei		Sea of Japan		
Fatty acid	Mean	SD	Mean	SD	p
14:0	2.7	0.38	2.5	0.92	0.264
15:0	1.2	0.11	0.9	0.20	**<0.001**
16:0	23.8	1.22	23.8	2.92	0.882
17:0	2.4	0.15	1.9	0.22	**<0.001**
iso-18:0	0.3	0.17	0.5	0.06	**<0.001**
18:0	8.7	0.34	8.8	1.55	0.655
19:0	0.5	0.06	0.4	0.08	**<0.001**
20:0	0.4	0.04	0.4	0.10	**0.002**
Total saturated	41.2	1.49	40.2	4.35	0.202
7-Me-16:1	0.9	0.14	0.8	0.20	**<0.001**
18:1n-13	0.1	0.03	0.1	0.06	0.583
18:1n-7	2.2	0.49	2.3	0.40	0.220
18:1n-9	4.6	0.55	6.0	1.00	**<0.001**
24:1n-9	0.5	0.29	0.5	0.34	0.287
Total monoenes	13.9	1.24	14.2	2.03	0.505
18:2n-6	1.5	0.16	1.8	0.34	**<0.001**
18:3n-3	0.5	0.16	0.6	0.17	**0.002**
18:4n-3	1.1	0.20	1.4	0.47	**<0.001**
20:4n-6 (ARA)	1.6	0.20	1.9	0.28	**<0.001**
20:5n-3 (EPA)	7.9	0.48	10.2	1.43	**<0.001**
22:5n-3	0.6	0.12	0.7	0.28	0.163
22:5n-6	2.1	0.22	1.4	0.25	**<0.001**
22:6n-3 (DHA)	26.6	1.93	24.8	3.12	**0.003**
Total polyenes	44.9	1.86	45.7	5.06	0.420

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