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Title	Maternal effects and larval survival of marbled sole Pseudopleuronectes yokohamae
Author(s)	Higashitani, Tomomi; Takatsu, Tetsuya; Nakaya, Mitsuhiro et al.
Citation	Journal of Sea Research, 58(1), 78-89 https://doi.org/10.1016/j.seares.2007.01.005
Issue Date	2007-07
Doc URL	https://hdl.handle.net/2115/25160
Type	journal article
File Information	JSR.pdf

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Maternal effects and larval survival of marbled sole *Pseudopleuronectes yokohamae*

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Received 5 October 2005; accepted 23 January 2007

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Abstract

Maternal effects of animals are the phenotypic influences of age, size, and condition of spawners on the survival and phenotypic traits of offspring. To clarify the maternal effects for marbled sole *Pseudopleuronectes yokohamae*, we investigated the effects of body size, nutrient condition, and growth history of adult females on egg size, larval size, and starvation tolerance, growth, and feeding ability of offspring. The fecundity of adult females was strongly dependent on body size. Path analysis revealed that the mother's total length positively affected mean egg diameter, meaning that large females spawned large eggs. In contrast, the relative growth rate of adult females negatively affected egg diameter. Egg diameters positively affected both notochord length and yolk sac volume of the larvae at hatching. Under starvation conditions, notochord length at hatching strongly and positively affected days of survival at 14°C but not at 9°C. Under adequate food conditions (1000 rotifers L^{-1}), the notochord length of larvae 5 days after hatching positively affected feeding rate, implying that large larvae have high feeding ability. In addition, the mean growth rate of larvae between 0 and 15 days increased with increasing egg diameter under homogenous food conditions, suggesting that larvae

hatched from large eggs might have a growth advantage for at least to 15 days after hatching. In marbled sole, these relationships (i.e., mother's body size–egg size–larval size–larval resistance to starvation–larval feeding ability) may help explain recruitment variability.

Keywords: Marbled sole; Egg and larval size; Starvation tolerance; Feeding ability; Maternal effect

1. Introduction

The earliest hypotheses to explain recruitment variability in marine fishes were based on concepts of a critical period and transport (Hjort, 1914) and noted the importance of starvation and advection in the early life stages. Many recruitment hypotheses have been presented, such as the match-mismatch (Cushing and Dickson, 1976), predation (Hunter, 1981; Van der Veer and Bergman, 1987), and growth (Houde, 1987; Anderson, 1988; Watanabe et al., 1995; Campana, 1996) hypotheses. The 'bigger is better' hypothesis focuses on size-dependent mortality and the premise that large larvae may have high starvation resistance and less vulnerability to predation (Miller et al., 1988). The 'maternal effects' hypothesis for marine fishes (Chambers and Leggett, 1996; Kjesbu et al., 1996; Solemdal, 1997) highlights the influence of age, size, and condition of spawning females on the size, condition, and viability of offspring. Thus, clear-cut phenotypic responses rather than genetic traits are primarily targeted by the maternal effects hypothesis (Bernardo, 1996; Johnston and Leggett, 2002).

Many relevant papers exist on the relationships between egg or larval size and the attributes of the mother in marine fishes such as cod *Gadus morhua* (Chambers and Waiwood, 1996; Kjesbu et al., 1996; Solemdal, 1997; Marteinsdottir and Steinarsson, 1998), sardine *Sardina pilchardus* (Riveiro et al., 2000), haddock *Melanogrammus aeglefinus* (Trippel and Neil, 2004), black rockfish *Sebastes melanops* (Berkeley et al., 2004), and clownfish *Amphiprion melanopus* (Green and McCormick, 2005). In flatfishes, maternal influences on variations in egg size have been clarified in detail for winter flounder *Pseudopleuronectes americanus* as a repeat spawner (Buckley et al., 1991a, b) and plaice *Pleuronectes platessa* (Fox et al., 2003). However, the maternal effects in species that spawn only

once in a spawning period, such as the marbled sole *Pseudopleuronectes yokohamae* (Oshiro and Hibiya, 1981), are unknown. In addition, the relationships between the growth rate of the mother and the attributes of her offspring remain unclear for flatfishes.

Marbled sole are widely distributed in the coastal waters around Japan. Juvenile marble sole reared from artificially fertilised eggs have been released in Japan, and the number released is second among flatfishes to that of the Japanese flounder *Paralichthys olivaceus* (Fukuhara, 1988). Marbled sole have the highest biomass in the demersal fish assemblage in Kikonai Bay close to Tsugaru Strait (Fig. 1; Takagi et al., 1997) and are commercially caught by bottom gill nets and set nets. Kikonai Bay and Hakodate Bay are the northern distributional limits for marbled sole, where they form a single population within these two bays (Takahashi, 1998). Many marbled sole females mature at the end of their third year (i.e., reach age-3 and approx. 26 cm in total length in March of their third year; Shafieipour et al., 1999, 2004) and can live as long as 10 years. Female marbled sole exhibit the characteristic synchronous ovary type of gonadal maturation: Females release eggs only once in the spawning period (single batch spawner; Oshiro and Hibiya, 1981), and eggs are demersal and adhesive (Yusa, 1960). Adult females spawn on the bottom along a 20-m isobath off Hakodate Bay and Kikonai Bay in February–April, but rarely in February (Fig. 1; Nakagami et al., 2001; Shafieipour et al., 2004). Pelagic larvae are distributed chiefly in Hakodate Bay (Nakagami et al., 2001, Fig. 1); the first feeding stage larvae prey chiefly on tintinids, rotifers, and copepod nauplii; and the principal prey shifts to copepod nauplii with larval growth (Nakagami, 2001). To clarify the causes of fluctuations in the population of marbled sole in Kikonai Bay and Hakodate Bay, we previously studied the early life stages of the fish. However, the maternal influence on the size and viability of marbled sole eggs and larvae is still unknown. Maternal effects are important because they are the only recruitment mechanism that can be directly managed via fishing regulations (Solemdal, 1997; Trippel et al., 1997).

In the present study, we investigated: (1) the effects of body size/age and growth history of female marbled sole on the size of spawned eggs; and (2) the effects of egg size on larval size, growth, starvation tolerance, and feeding ability. We then considered the role of maternal effects on the marbled sole population in Hakodate and Kikonai Bays.

2. Materials and methods

In this study, the scientific name of the marbled sole, *Pseudopleuronectes yokohamae*, follows Cooper and Chapleau (1998), which corresponds to *Limanda yokohamae* (GÜNTHER, 1877) and *Pleuronectes yokohamae* GÜNTHER, 1877 (Sakamoto, 1984).

2.1. Sampling of adult females and egg size measurement

Adult and immature marbled sole were collected with commercial bottom set nets and gill nets off Toubetsu and Kikonai, Hokkaido, Japan from March to April in 2001–2003 (Fig. 1; Table 1). Ripe females were distinguishable from others by the external appearance of well-swollen gonads. Immature females were used only for estimation of the von Bertalanffy formula for age and ring radius of otoliths (described later). One-hundred-thirty-two mature females and >56 mature males were transported live to the Field Science Center for Northern Biosphere Usujiri Fisheries Station, Hokkaido University. Sixty-seven mothers were randomly selected from 132 mothers due to limitation of laboratory space, and eggs from 67 mothers were stripped and artificially fertilised by mixed sperm from three random males using the dry method (Yusa, 1960). Fertilised eggs from each mother were attached to hemp-palm trays, and incubated in a 5-L basket in flowing, aerated, sterilized, and micro-filtered seawater at 14°C under a 12 h light:12 h dark regime. Eggs from 132 fully mature females with freely running eggs were stripped, and 50 eggs from each female were measured for egg diameter (*ED*) under a binocular microscope with a micrometer (accuracy 0.05 mm).

All 132 females were sacrificed after egg stripping and measured for total length (*TL*, mm) and weighed for body weight (*BW*, accurate to 0.1 g); sagittal otoliths were then extracted. Missing data of *TL* (two ripe 3-y-old individuals (age-3) with broken tails collected in 2003 (on 14 April), $n = 2$) were excluded from later analyses. For estimation of fecundity, non-stripped gonads from 20 females collected in 2003 (exclusive of the above 132 females) were fixed in 10% buffered seawater–formalin solution and weighed later. The total number of eggs in a gonad was estimated by extrapolation from the number of eggs per gram wet weight (Hattori et al., 1995); this is a valid method for species in which ovary development is synchronous and females release all of their eggs only once a year (Oshiro

and Hibiya, 1981).

Sagittal otoliths were measured to determine age and relative growth rate (*RG%*) of individual females. The ocular side otolith grows faster on the axis toward the anterior part of the body in marbled sole (Shafieipour et al., 1999). The otolith radius (*R*) and ring radii (from r_1 to r_t; Fig. 2) from 306 females (132 ripe and 174 immature females; Table 1) were measured to the nearest 0.05 mm along the longest axis of the otolith, which was ground on the concave side with fine sandpaper (#600 grit). Because the period for each translucent margin formed coincided with the sampling season of females in this study (March–April), we hypothesised that the outermost margin was the otolith radius *($r_t = R$)* and the hatch date of the population was 1 March. Parameters of the Von Bertalanffy formula for age and ring radius (r_1 to r_t) were estimated by the least-squares method using Microsoft Excel. The relative growth rate (*RG%*) was calculated for females as follows:

$$RG\% = (R - D_t) \times 10^2 / D_t$$

where R (mm) is the otolith radius at capture and D_t (mm) is the radius estimated from the von Bertalanffy formula at captured age t. *RG%* values can range from positive, which means a high relative growth rate, to negative. We chose to use *RG%* rather than the raw otolith radius data because raw otolith radius data might include information about the maternal growth trajectory and maternal age. We wanted to compare only the past growth trajectory, without female age (relative otolith radius in the same age group). Individual *RG%* can compare relative otolith radius in the population independent of its age. Missing datum of *RG%* (a ripe age-3 individual with defective otolith collected in 2003 (on April 22), $n = 1$) was excluded from later analyses.

To determine plumpness of mothers, a condition factor *K'* was estimated for each female as $K' = BW \times 10^6 / TL^a$, where $a = 3.318$ is the exponent of the *TL*–*BW* regression ($\mathrm{Log}_{10}BW = -5.669 + 3.318 \times \mathrm{Log}_{10}TL$, $n = 130$, $r = 0.96$, $P < 0.001$). *K'* compares the female plumpness in the population independent of other variables such as *TL* and *RG%*. We did not use Fulton's condition factor *K* ($a = 3$) because *K* has multicollinearity to other variables in marbled sole, and non-multicollinearity between variables is a requirement for path analysis (described later).

2.2. Measurements of hatched larvae and larval survival without food

The larvae hatched from the eggs stripped from 67 mothers were reared independently (i.e., 67

groups of larvae) and incubated in a 5-l basket in flowing seawater at 14°C under a 12 h light:12 h dark regime. Seawater was aerated, sterilised, and micro-filtered outside the baskets. For each mother, 20 individual larvae were sacrificed and stored in 90% ethanol solution after being anesthetised with MS-222 on hatching (0 days) to alleviate pain. These larvae (n = 1340 individuals) were then measured for notochord length (*NL*) to the nearest 0.05 mm under a dissecting microscope with a micrometer. Yolk sac length (*YL*), depth (*YD*), and width (*YW*) were also measured for larvae from 45 mothers randomly selected from 67 mothers (n = 900 larvae). Body length shrinkage was not considered a problem in this fixative for marbled sole larvae (Joh et al., 2003). The volume (*YV*) of the yolk sac, which we assumed to be an ellipsoid shape, was estimated using the following formula: $YV = (1/6)\pi YL \times YD \times YW$.

Two beakers (200 ml volume) with 20 larvae each from 26 mothers (i.e., 52 beakers) were prepared for starvation tolerance and kept at 9°C, and two beakers with 20 larvae each from 33 mothers (i.e., 66 beakers) were kept at 14°C. The numbers of dead larvae were counted and removed daily. Mean survival days for 50% of the individuals (starved at 9°C and at 14°C) were calculated as starvation tolerance.

2.3. Rearing and measurements of larvae with food

In parallel, four beakers (1 L volume) with 20 larvae each from 41 mothers (164 beakers and 3280 larvae in total) were prepared to study feeding condition and growth rate. Beakers were filled with sterilised, micro-filtered seawater at 14°C and aeration was carefully adjusted so as not to interfere with larval behaviour. Larvae were fed cultured rotifers *Brachionus plicatilis* enriched with shark egg powder (Aquarun; BASF Japan, Tokyo) from 3 d after hatching (DAH) and the density was adjusted to 1000 rotifers L^{-1} at 08:00 and 16:00 h every day. We carefully performed a 50% water change and removed dead larvae every day. Every fifth day (at 5, 10, 15, and 20 DAH), all larvae alive in one beaker were sacrificed and stored in 90% ethanol solution between 10:00 and 12:00 h after exposure to MS-222 anaesthetic. Later, these larvae were measured for *NL*. A subsample of the larvae at 5 DAH from 18 mothers collected in 2003 (360 larvae) from 41 mothers were measured for mouth width (*MW*), and the numbers of larvae with rotifers in their gut were counted to estimate feeding rate (*FR%*). *FR%* was defined as the percentage of larvae with rotifers out of the total number

of larvae examined. We did not use the *NL* data from 20 DAH because more than 10 out of 20 larvae died between 16 and 20 DAH in 31 of 41 beakers; thus, the *NLs* at 20 DAH might not truly represent the 20 DAH larval population. We could not rear the larvae at 9°C because they did not feed on the rotifers that were inactive due to the low water temperature.

2.4. Data analysis

The mean values of offspring's attributes (*ED, NL* at hatching and 5 days, *YV* at hatching, starvation days at 9°C and 14°C, and *MW* at 5 days) for each individual female were used as representative values. One-way ANOVAs were used to test for differences in *TL* between sampling areas and between starvation days at 9°C and 14°C. ANCOVA was performed to compare *TL* and sampling date of females among years. Regression analyses were performed between *TL* of females and log-transformed fecundity [Log_{10}(number of eggs + 1)] and between *BW* and fecundity. A two-factor ANOVA was used to test for differences in *NL* in the feeding condition with DAH and *ED* class. Pearson's correlation coefficients (*r*) were estimated between two parameters (sampling date of females, *TL, K', RG%, ED, NL* at hatching and 5 days, log-transformed *YV* at hatching, starvation days at 9°C and 14°C, *MW* at 5 days, and *FR%* at 5 days). Spearman's rank correlation coefficient (r_s) was used to look for relationships between female age and other parameters because female age data were not normally distributed, as indicated by the Kolmogorov–Smirnov test for goodness of fit. The level of significance in all tests was 5%. In addition, we set a level of 0.42% for 12 items for Bonferroni-corrected multiple comparisons. Missing data of mother's attributes (*TL* and following *K': n* = 2; and *RG%: n* = 1, mentioned before) were excluded from the Pearson's correlation analysis. Data of offspring's attributes (*ED, NL* at hatching and 5 days, *YV* at hatching, and starvation days at 14°C) from a ripe age-2 individual collected in 2002 (on April 10) were treated as outliers to avoid underestimation, and those were excluded from calculations of the correlation coefficient and from path analysis (described later).

A maximum likelihood path analysis was executed using AMOS Ver. 5.0 (SmallWaters Corporation, Chicago, USA) to examine the interaction of mother's (*TL, K',* and *RG%* as exogenous variables) and offspring's attributes (*ED, NL* at hatching and 5 days, *YV* at hatching, starvation days at 9°C and 14°C, *MW* at 5 days, and *FR%* at 5 days as endogenous variables). Path analysis allows

researchers to test the fit of the correlation matrix against two or more causal models with statistical robustness. Mother's age was excluded from the path analysis to avoid multicollinearity between the model's exogenous variables. AMOS provides full information maximum likelihood (FIML), and the 'estimate means and intercepts' option was used to handle missing data. Path model selection, whether indirect paths between non-neighbouring factors are inclusive or not, was based on goodness-of-fit statistics (i.e., whole model's *P*-value of chi-square, r^2 of endogenous variables, the comparative fit index (*CFI*), the parsimony-adjusted comparative fit index (*PCFI*), the root mean square of approximation (*RMSEA*)).

3. Results

3.1. Seasonal change in female body size

No significant difference was found during 2001–2003 for the relationship between sampling date and maternal *TL* (ANCOVA; slope, $F_{2,124} = 1.04$, $P = 0.35$; intercept, $F_{2,126} = 2.24$, $P = 0.11$). Total length combined for all years decreased during the spawning season ($r = –0.29$, $n = 130$, $P < 0.001$; Fig. 3). Mean *TL* collected off Toubetsu (348 mm) was significantly larger than that off Kikonai (319 mm; one-way ANOVA; $F_{1,128} = 14.16$, $P < 0.001$).

3.2. Relationships between female attributes and egg parameters

The regression equations for the relationship between *TL* of females collected in 2003 and log-transformed fecundity and between *BW* of females and fecundity are shown in Table 2. Females spawned a greater number of eggs as they grew larger (Fig. 4a, b).

Relationships between female attributes and *ED* are shown in Fig. 5. Only one ripe 2-y-old individual (age-2) was collected in 2002 (on 10 April 10). Data from this individual were treated as outliers to avoid underestimation. Significantly positive correlations existed between *TL* and *ED* ($r = 0.20$, $n = 129$, $P = 0.023$; Fig. 5a, Table 3) and between female age and *ED* ($r_s = 0.26$, $n = 131$, $P = 0.003$; Fig. 5b, Table 3), but the correlation between female *K'* and *ED* was not significant ($r = 0.04$, $n = 129$, $P = 0.65$; Fig. 5c).

Results of the Von Bertalanffy analysis relating otolith radius D_t (mm) and age t (i.e., number of transparent rings) are shown in Table 2. To compare the relative otolith sizes of females in the same age group, individual *RG%* was estimated as the ratio of otolith radius for each female at age t (R) to that for the population (D_t). There was a significant negative correlation between *RG%* and *ED* (r = –0.19, n = 130, P = 0.031; Fig. 5d, Table 3).

3.3. Relationships between egg size and larval attributes

Significant positive correlations existed between *ED* and *NL* at hatching (r = 0.36, n = 66, P = 0.003; Fig. 6a) and between *ED* and *YV* (r = 0.31, n = 44, P = 0.041; Fig. 6b). These findings indicate that relatively large eggs hatched large larvae and had large yolk sacs.

3.4. Rearing experiments of larvae without food

Under starvation conditions, mean survival for larvae was 8.1 d at 14°C (range 6.6–9.7 d; n = 32) and 11.0 d at 9°C (range 5.6–14.0 d; n = 26); survival at 9°C was significantly longer than at 14°C (one-way ANOVA; $F_{1,56}$ = 45.8, P < 0.001). *NL* at hatching was strongly correlated with survival days at 14°C (r = 0.57, n = 32, P < 0.001) but not at 9°C (r = 0.07, n = 26, P = 0.74; Fig. 7).

3.5. Rearing experiments of larvae with food

A significant and positive correlation was observed between *NL* at hatching (0 days) and *NL* at 5 days (r = 0.41, n = 40, P = 0.008; Fig. 8). Mean *NL* and *MW* at 5 days were 4.2 and 0.21 mm, with ranges of 3.6–4.7 and 0.18–0.24 mm, respectively. A significant and positive correlation was observed between *NL* and *MW* at 5 days (r = 0.87, n = 18, P < 0.001; Fig. 9a). Mean *FR%* and range at 5 days during 10:00–12:00 h were 61% and 20–90%, respectively. A significant and positive correlation was found between *NL* and *FR%* at 5 days (r = 0.63, n = 18, P = 0.005; Fig. 9b).

Forty-one sets of four beakers (representing 41 mothers), each containing 20 larvae at hatching, were used for growth experiments, and the larvae from one beaker of each set were sacrificed every 5 days. At 5 DAH, more than 10 of the 20 larvae survived for all 41 mothers. At 10, 15, and 20 DAH, more

than 50% of the larvae survived for 33, 19, and 10 mothers, respectively. However, the data for *NL* at 20 DAH were not used, because >10 dead larvae were observed in 31 of 41 beakers. For the change in *NL* from hatching to 15 days, the interaction between DAH and *ED* class was significant ($F_{1,5} = 16.9$, $P = 0.009$; Fig. 10): The mean (± standard deviation) growth rate between 0 and 15 days increased with *ED* (0.137 ± 0.039, 0.143 ± 0.036, and 0.159 ± 0.043 mm d^{-1} for *ED* <0.83, 0.83–0.85, and >0.85 mm, respectively).

3.6. Relationships between maternal and larval attributes

Of the 28 correlations between maternal and larval attributes, two were significant: maternal age and larval *NL* at hatching ($r_s = 0.26$, $n = 66$, $P = 0.035$) and maternal age and *YV* at hatching ($r_s = 0.31$, $n = 44$, $P = 0.039$; Table 3). No significance for a Bonferroni corrected α value of 0.0042 was found for all 28 correlations.

For correlations between two larval attributes, almost all between non-neighbouring factors were insignificant. Seven of 21 correlations for an uncorrected α value of 0.05 and two of 21 correlations for a corrected α value of 0.0042 were significant (Table 3).

Path analysis between variables was conducted based on the life history of marbled sole. Maternal age was excluded from the path analysis to avoid multicollinearity between the exogenous variables. The best model showed similar tendencies to simple correlation analyses (Fig. 11). Maternal *TL* positively affected *ED* (standardised path coefficient $\beta = 0.21$, $P = 0.013$), but *RG%* negatively affected *ED* ($\beta = -0.21$, $P = 0.011$). The squared multiple correlation (r^2) of *ED* was 0.093, meaning that 9.3% of the variation was explained by three exogenous variables. *ED* positively affected both *NL* and *YV* of the larvae at hatching ($\beta = 0.36$, $P = 0.002$ and $\beta = 0.40$, $P = 0.003$, respectively). Under starvation conditions, *NL* at hatching strongly and positively affected survival days at 14°C ($\beta = 0.62$, $P < 0.001$), but did not at 9°C ($\beta = 0.11$, $P = 0.63$). Under conditions in which food was supplied (1000 rotifers L^{-1}), *NL* at hatching positively affected *NL* at 5 DAH ($\beta = 0.49$, $P < 0.001$), and *NL* at 5 DAH positively affected both *FR%* ($\beta = 0.56$, $P = 0.005$) and *MW* ($\beta = 0.82$, $P < 0.001$) at 5 DAH. Models including indirect paths between non-neighbouring factors were not selected because of insignificant chi-squares (all $P > 0.05$) with poor goodness-of-fit statistics.

4. Discussion

The maternal effects hypothesis provides a persuasive explanation of recruitment variability in marbled sole. Maternal effects are defined generally as the direct effects of the mother's phenotype on the phenotype of her offspring, which have a variety of characteristics such as egg size, larval body size, growth rate, development trajectory, and survival rate of the immature stages (Bernardo, 1996). In this study, maternal effects explained the pathways from female attributes to egg size to larval size to larval resistance to starvation or feeding ability in marbled sole, as determined by path analysis. Larger and older marbled sole females produced larger eggs. Similar relationships between maternal attributes and egg size, larval size, or larval quality have been reported for many fishes (e.g., Buckley et al., 1991a, b; Chambers and Leggett, 1992; Chambers and Waiwood, 1996; Kjesbu et al., 1996; Solemdal, 1997; Marteinsdottir and Steinarsson, 1998; Keckeis et al., 2000; Riveiro et al., 2000; Johnston and Leggett, 2002; Fox et al., 2003; Berkeley et al., 2004; Trippel and Neil, 2004; Green and McCormick, 2005). Although we found that larger larvae had higher feeding ability, direct correlations between feeding rate and maternal attributes were not significant. Similar weak correlations between non-neighbouring factors have been found in other fish populations (Buckley et al., 1991b; Marteinsdottir and Steinarsson, 1998; Keckeis et al., 2000; Berkeley et al., 2004). In marbled sole in this study area, however, the only negative correlation observed was between female growth rate and egg diameter. In addition, mean growth rate of marbled sole larvae between 0 and 15 d increased with egg diameter class under the homogenous food condition; thus larvae that originated from large eggs might have had a growth advantage, at least to 15 DAH. Overall, it is possible that large eggs originated from large and slow-growing females have better survival potential to the first feeding larval stage.

Because plumpness of females (*K'*) was not correlated with egg size, recent somatic condition of females may not influence egg size in marbled sole. In contrast, females exhibiting relatively slower growth tended to produce larger eggs; therefore, slow-growing females may have enhanced egg quality. Egg size of marbled sole may be related to maternal growth history for a relatively long period in the past. A similar negative effect of maternal relative growth rate on egg size has been reported in freshwater walleye *Stizostedion vitreum*, and the timing of resource availability of females may influence the size and number of eggs produced (Johnston and Leggett, 2002). The relationship

between maternal growth trajectory and egg size has been examined in anadromous Atlantic salmon *Salmo salar* (Thorpe et al., 1984) and landlocked white-spotted char *Salvelinus leucomaenis* (Morita et al., 1999); these studies showed that rapid juvenile growth produced smaller eggs. The implication of these studies is that realised juvenile growth of individual females may be influencing their future egg size (Johnston and Leggett, 2002). It is unclear, however, which factor influences the female growth trajectory in marbled sole, and further studies are needed to clarify the factor.

Rideout et al. (2005) showed that survival time under the starvation condition of haddock *Melanogrammus aeglefinus* larvae increased linearly with original egg size. In marbled sole, a similar phenomenon was observed, and survival times under starvation conditions for large larvae were longer than those for small larvae at a water temperature of 14°C; however, there was no difference between them at 9°C. Oxygen consumption in the larval period increases with increasing water temperature, and oxygen consumption per unit weight decreases with body size enlargement. It is possible that large larvae with low metabolic consumption can survive longer than small larvae under high water temperature condition. Under low temperature conditions, however, the metabolic difference may be small between large and small larvae, as consumption would be kept low by inactive swimming behaviour, thereby leading to similar survival times. The difference in starvation tolerance between different larval sizes might have been small in the study area because the water temperature remained low from early March (7.7°C) to late April (10.2°C) in 2002 (Higashitani, unpublished data).

Egg sizes of multi-batch spawners have a tendency to decrease in the course of spawning (Bagenal, 1971; Chambers and Leggett, 1996; Chambers, 1997; Rideout et al., 2005). Large and old females of marbled sole are expected to spawn greater numbers of eggs and potentially surviving offspring, and they shoal in the early spawning period (mainly March). It is possible that the reproductive strategy of marbled sole of the population studied here is a response to poor larval growth conditions and large eggs in the early spawning period mitigate recruitment failure. In Hakodate Bay, water temperature and densities of copepod nauplii and rotifers (the initial prey items for marbled sole larvae) are low in March and increase in April (Nakagami, 2001; Nakaya et al. 2004). It has been shown that smaller larvae may be disadvantaged in terms of food availability and growth under conditions of low prey abundance or inadequate temperature in the wild (Hutchings, 1991; Benoît and Pepin, 1999; Rideout et al., 2005).

In this study, we have illustrated that many characteristics of the eggs and larvae of marbled sole

were influenced by maternal effects. Future research should involve studies of the biochemical components (Buckley et al., 1991a; Keckeis et al., 2000; Riveiro et al., 2000) of marbled sole eggs and the buoyancy of larvae (Saborido-Rey et al., 2003) to estimate their quality.

Acknowledgement

The authors are grateful to Prof. Y. Sakurai and Assoc. Prof. T. Nakatani, Graduate School of Fisheries Sciences, Hokkaido University for their valuable suggestions. Thanks are due to Mr. K. Imura (Laboratory of Marine Bioresource Science, Hokkaido University) for his cooperation in rearing the specimens. We thank Assoc. Prof. H. Munehara, (Usujiri Fisheries Station, Hokkaido University) and T. Sugimoto, T. Matsuda, N. Sato, and other staff members of the Hokkaido Institute of Mariculture for their advice and support in our rearing experiments. We would like to thank three anonymous referees for improving the paper. This work was supported by Primary Study of Fisheries Encouragement to T. Takatsu from the Hakodate City Government and the 21st Century COE Program 'Marine Bio-Manipulation Frontier for Food Production' of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Figure captions

Fig. 1 Sites for sampling of adult and immature marbled sole *Pseudopleuronectes yokohamae* by bottom set nets and gill nets off Toubetsu and Kikonai (open circles) and schematic model of horizontal distribution of each developmental stage.

Fig. 2 Ocular side otolith of marbled sole with four annuli. Opaque and transparent zones are shown in white and black, respectively. F, focus; *R*; otolith radius; r_1–r_4: annual ring radii.

Fig. 3 Seasonal changes in the total length of adult female marbled sole (r = –0.29, n = 130, P < 0.001).

Fig. 4 Regressions between total length (*TL*) and log-transformed fecundity (a) and between body weight (*BW*) and fecundity (b) of adult female marbled sole. The regression equations are shown in Table 2.

Fig. 5 Relationship between the mean egg diameter (*ED*) and attributes of adult female marbled sole: (a) total length (*TL*); (b) age; (c) a condition factor *K'* (=$BW \times 10^6/TL^{3.318}$); and (d) relative growth rate (*RG%*) from the growth curve for otolith radius. The solid circle shows the outlier (age-2 female) that was excluded from calculations of the correlation coefficient and from path analysis.

Fig. 6 Relationship between mean egg diameter (*ED*) and notochord length (*NL,* a) and yolk sac volume (*YV,* b) of larvae at hatching (0 days). The solid circle shows the outlier (age-2 female) that was excluded from calculations of the correlation coefficient and from path analysis.

Fig. 7 Relationship between the notochord length of larvae at hatching (*NL* 0 days) and survival days under starvation conditions at 9°C (Starve 9°C, a) and 14°C (Starve 14°C, b). The solid circle shows the outlier (age-2 female) that was excluded from calculations of the correlation coefficient and from path analysis.

Fig. 8 Relationship between the notochord length of larvae at hatching (*NL* 0 days) and at 5 DAH (*NL* 5 days). The solid circle shows the outlier (age-2 female) that was excluded from calculations of the correlation coefficient and from path analysis.

Fig. 9 Relationship of mouth width (*MW,* a) and feeding rate (*FR%,* b) to notochord length of larvae at 5 DAH (*NL* 5 days).

Fig. 10 Change in mean notochord length of larvae reared with food (1000 rotifers L^{-1}) by egg diameter class. Vertical bars show standard deviations.

Fig. 11 Path model of the interaction of mother's and offspring's attributes (χ^2 = 61.49, *df* = 44, *P* = 0.042, *CFI:* 0.78, *PCFI:* 0.52, *RMSEA*: 0.055). Numerals with arrows and the numbers at the upper right of each box show the standardised path coefficients (β) and squared multiple correlations (r^2), respectively. There was no significant correlation between two exogenous variables (*TL, K',* and *RG%*).

Table 1. Sampling dates and numbers of fish caught in coastal waters off Toubetsu and Kikonai

Date	Location	Gear	Number of fish caught		
			Ripe female	Ripe male	Immature female
5 March 2001	T	S	15 (3)	ND	34
15 March 2001	T	S	20 (10)	ND	8
27 March 2001	T	S	7 (4)	ND	3
9 April 2001	K	S	7 (0)	ND	3
17 April 2001	K	S	7 (4)	ND	3
1 March 2002	T	S	10 (10)	7	21
18 March 2002	T	S	12 (6)	6	15
10 April 2002	K	S	12 (7)	6	4
27 April 2002	K	S	1 (1)	3	1
3 March 2003	T	S	3 (3)	5	31
13 March 2003	T	S	4 (4)	9	27
24 March 2003	T	S and G	13 (3)	6	11
4 April 2003	K	S	10 (6)	5	5
14 April 2003	K	S	8 (3)	6	7
22 April 2003	K	S	3 (3)	3	1
Total			132 (67)	56	174

Numerals in parentheses show numbers of ripe females used for artificial fertilization of stripped eggs.

T, off Toubetsu; K, off Kikonai, shown in Fig. 1. S, bottom set net; G, bottom gill net; ND, no data.

Table 2. Regression equations of marbled sole females in Kikonai Bay and Hakodate Bay

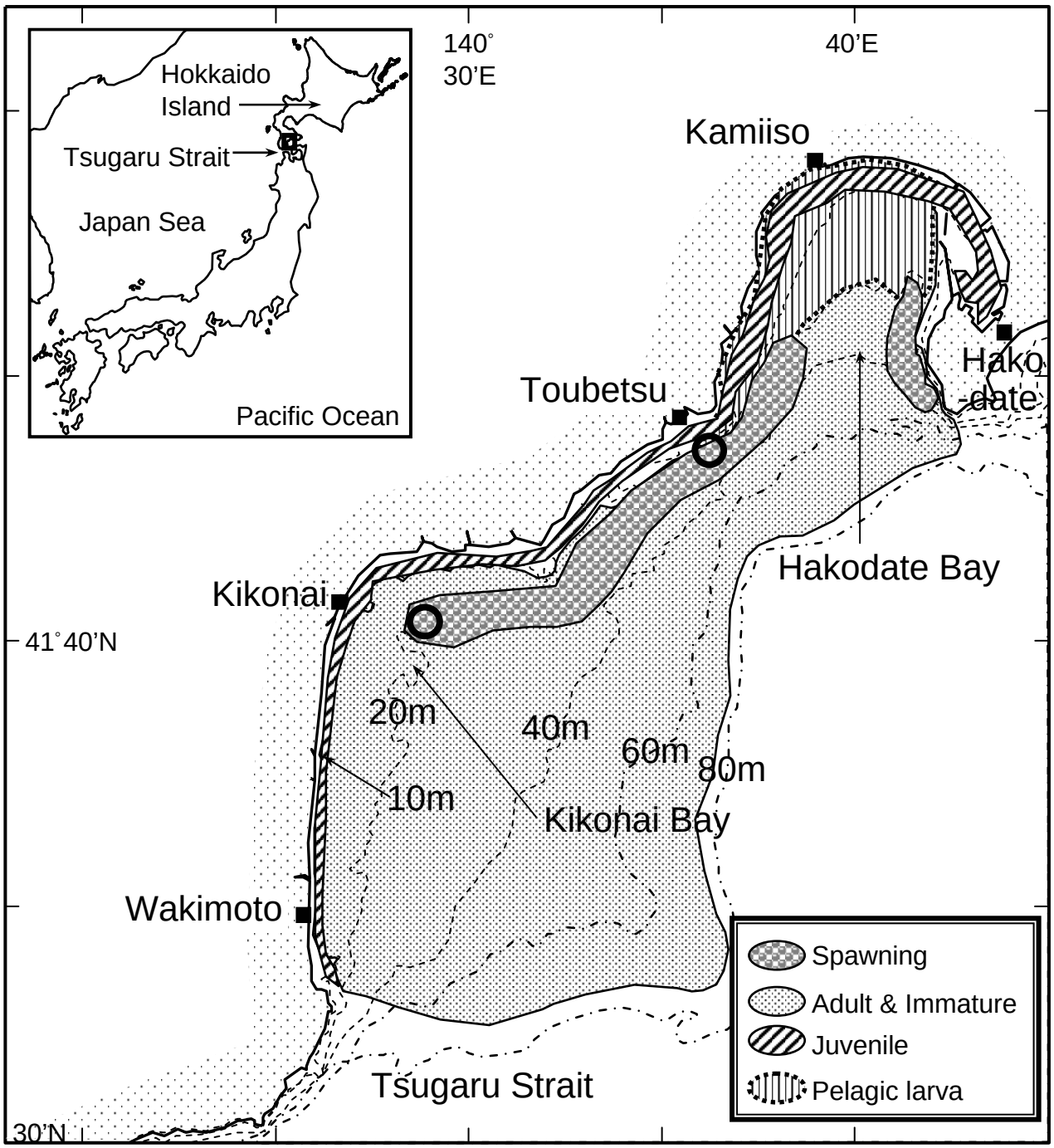
Regression equation	*n*	*r*	*P*
Fecundity			
Log_{10}(*number of eggs*+1)=0.0042·(*Total length*, mm)+4.45	20	0.84	<0.001
Number of eggs=1618·(*Body weight*, g)–134500	20	0.91	<0.001
Otolith growth			
Otolith radius D_t (mm)=4.73(1–$e^{-0.339((age\ t\ \mathrm{in\ year})-0.100)}$)	1295	0.94	<0.001

Table 3. Simple correlations (sample size in parentheses) between maternal, egg and larval attributes

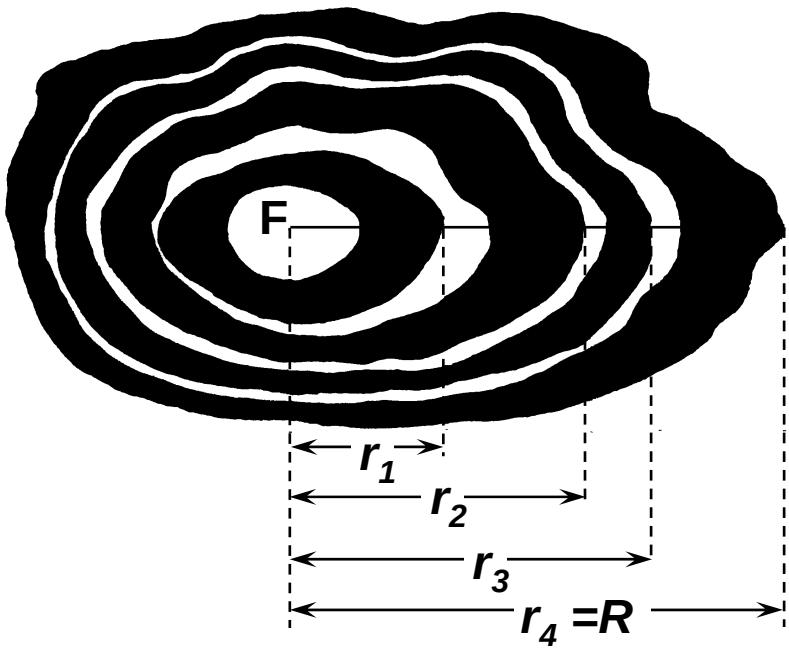
Trait	*ED*	*NL* 0 days	*YV* 0 days	Starve 9 °C	Starve 14 °C	*NL* 5 days	*MW* 5 days	*FR%* 5 days
Female *TL*	0.20* (129)	0.23 (66)	0.25 (44)	–0.29 (26)	0.21 (32)	–0.00 (40)	0.07 (18)	0.05 (18)
Female age	**0.26**** (131)	0.26* (66)	0.31* (44)	–0.19 (26)	0.22 (32)	0.01 (40)	–0.03 (18)	0.28 (18)
Female *K'*	0.04 (129)	–0.11 (66)	0.18 (44)	0.13 (26)	–0.09 (32)	–0.14 (40)	–0.22 (18)	–0.08 (18)
Female *RG%*	–0.19* (130)	–0.21 (66)	–0.22 (44)	–0.13 (26)	–0.16 (32)	0.08 (40)	–0.03 (18)	–0.09 (18)
Egg diameter (*ED*)	–	**0.36**** (66)	0.31* (44)	0.13 (26)	0.25 (32)	0.33* (40)	0.15 (18)	0.06 (18)
Larval *NL* 0 days		–	0.41** (44)	0.07 (26)	**0.57***** (32)	0.41** (40)	0.42 (18)	0.19 (18)
Larval *YV* 0 days			–	–0.16 (26)	0.37* (32)	0.12 (40)	0.10 (18)	–0.10 (18)
Larval starve 9 °C				–	0.22 (33)	–0.18 (40)	–0.13 (18)	–0.27 (18)
Larval starve 14 °C					–	0.24 (40)	0.36 (18)	0.36 (18)
Larval *NL* 5 days						–	**0.87***** (18)	0.63** (18)
Larval *MW* 5 days							–	0.64** (18)
Larval *FR%* 5 days								–

Abbreviations are as in the text. For correlations between female age and other traits, Spearman's r_s was used instead of Pearson's r, because age data were not normally distributed according to the Kolmogorov–Smirnov test for goodness of fit. Missing data of mother's attributes (*TL* and *K'*: two ripe 3-year-old individuals (age 3) with broken tails, $n = 2$; and *RG%*: a ripe age 3 individual with defective otolith, $n = 1$) and data from a female individual of ripe age 2 were excluded from the analysis.

*P<0.05; **P<0.01; ***P<0.001. Bold font indicates significance for a Bonferroni corrected α value of P<0.0042 for 12 items.



Higashitani et al. Fig.1



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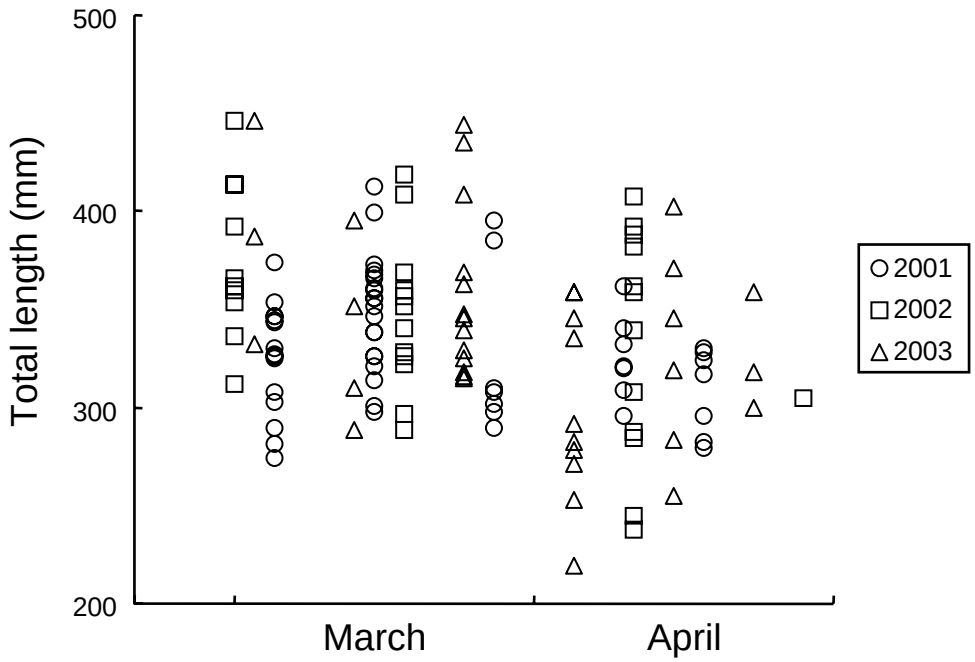

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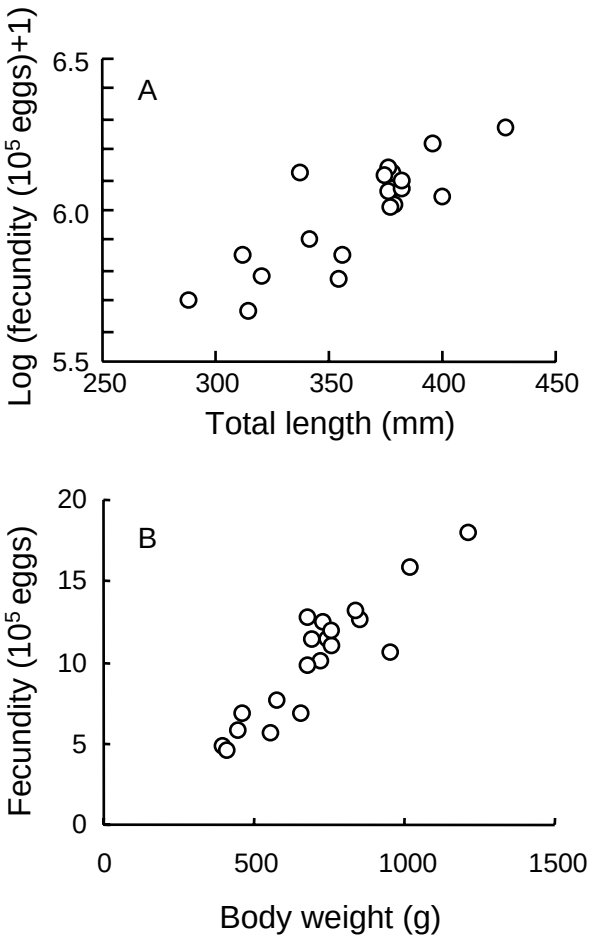

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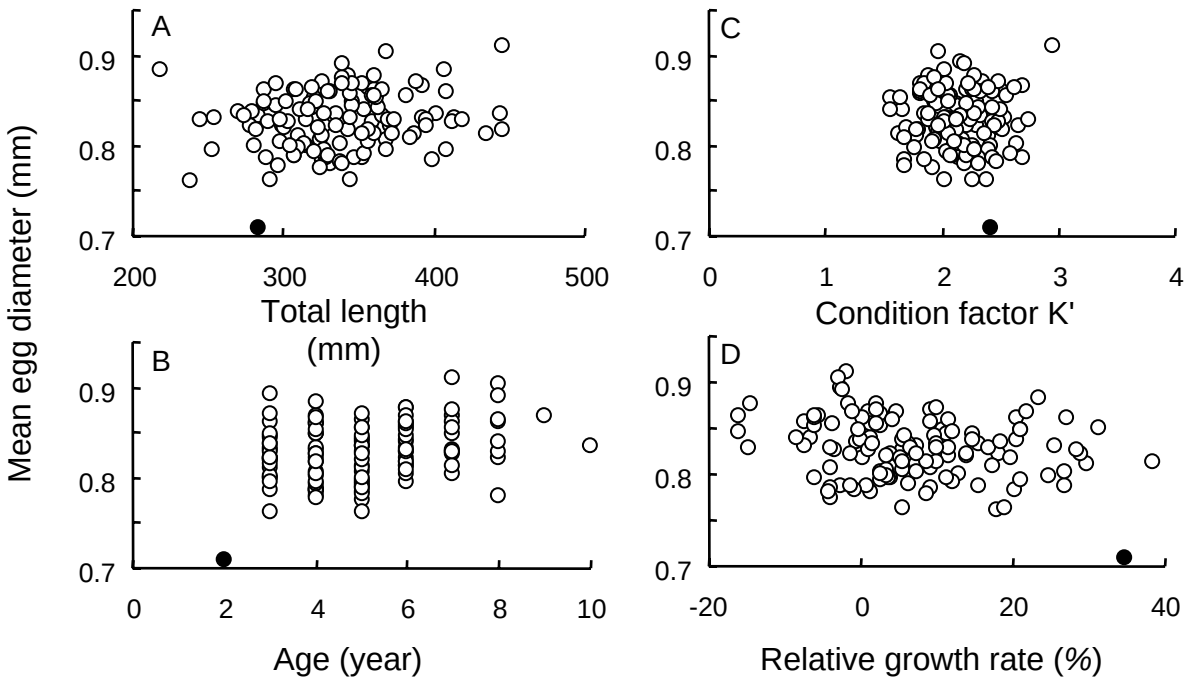

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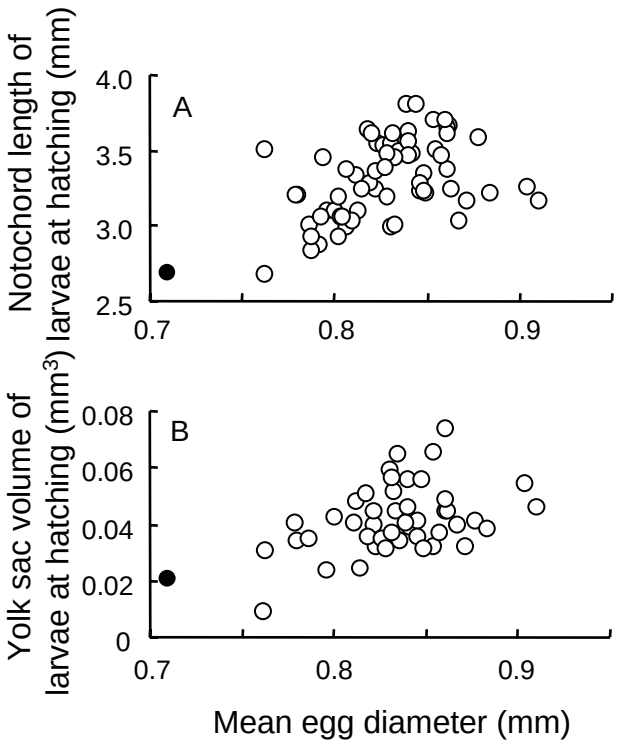

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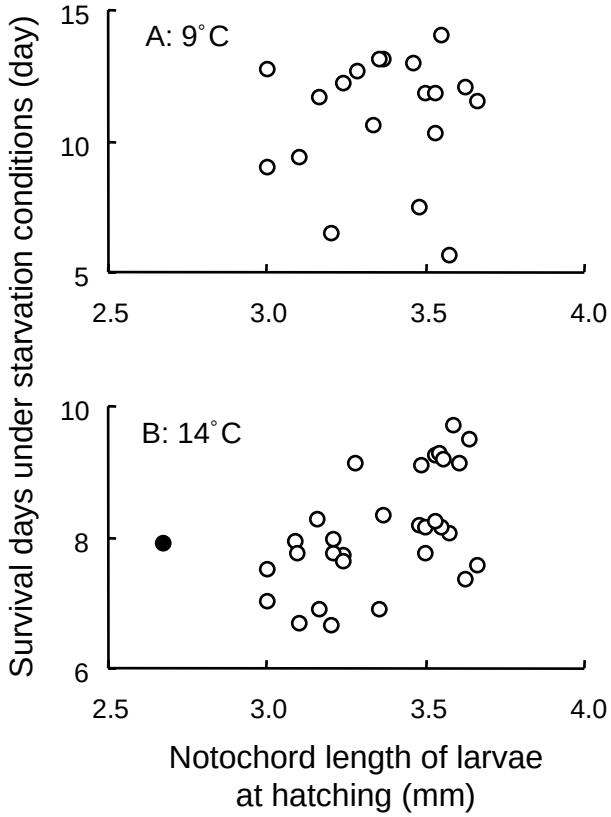

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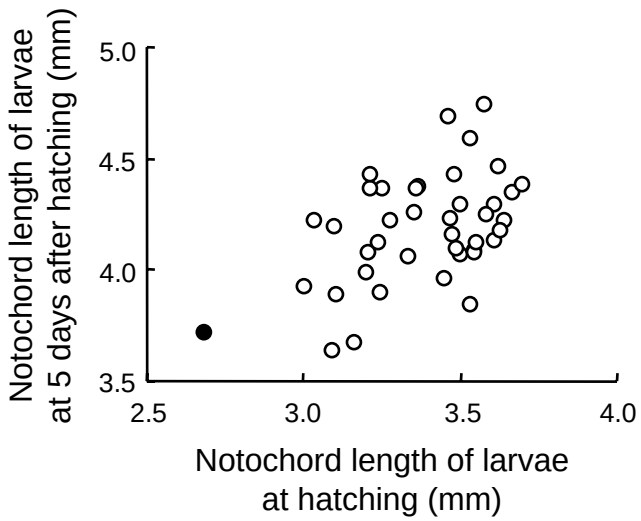

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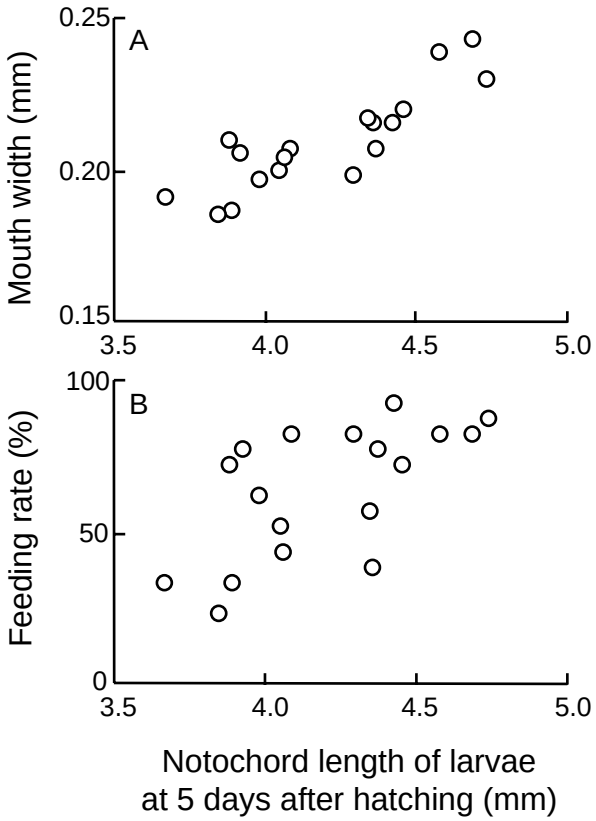
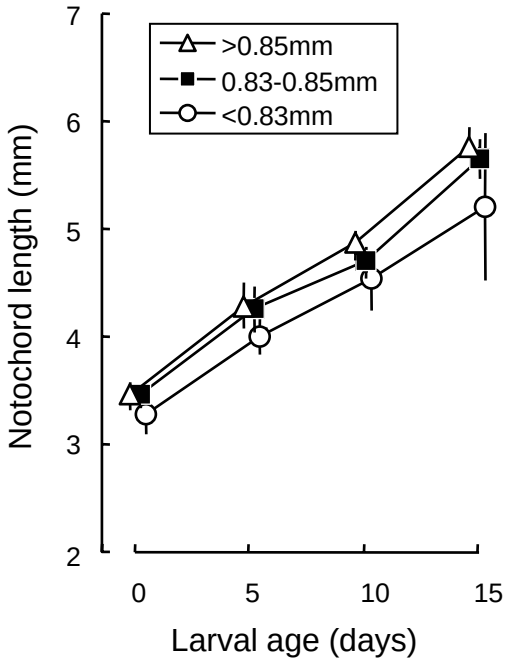
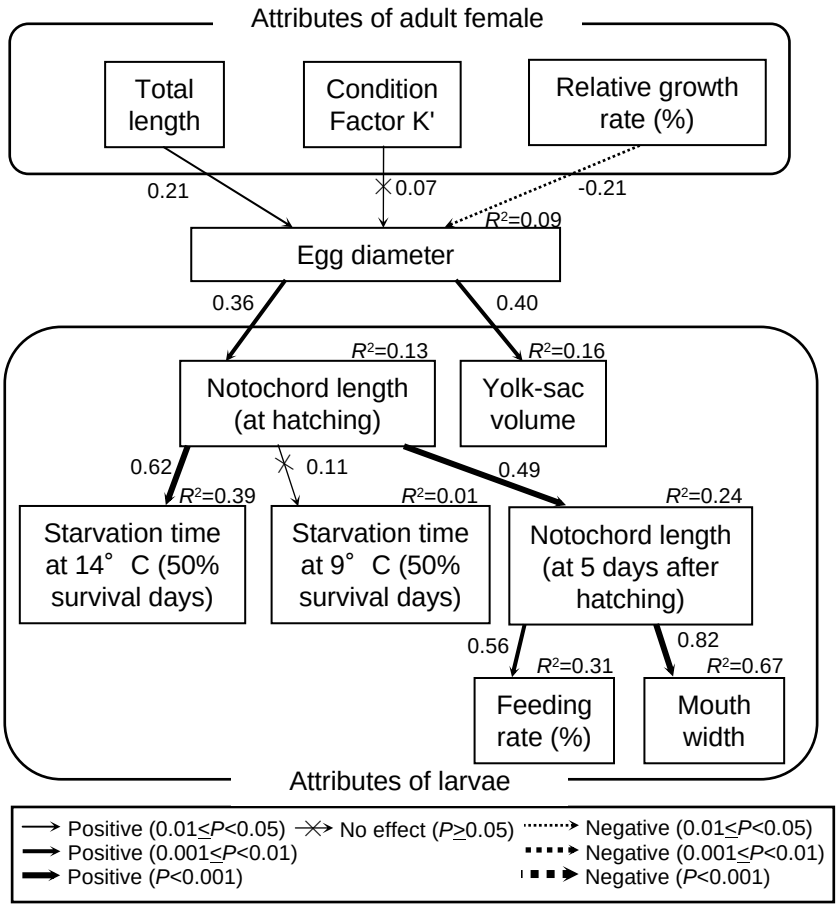


Higashitani et al. Fig.9



Higashitani et al. Fig.10



Higashitani et al. Fig.11