Growth characteristics and anti-predator performance of hatchery-reared larval Japanese anchovy, *Engraulis japonicus*: effects of temperature and food density

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1. Introduction

1.1. General background

Marine fish generally experience heavy mortality during early life stage, resulting in the fluctuation of fisheries resource. Survival during early life and its underlying mechanisms have been studied for a long time. The present research is based on the following hypotheses:

i. "Critical period" hypothesis (Hjort, 1914): Larval survival and recruitment are greatly affected by feeding success at the first-feeding stage, the so-called, "critical period".
ii. "Match-mismatch" hypothesis (Cushing, 1975): The spatiotemporal match or mismatch between larvae and their prey is the main factor of recruitment fluctuations.
iii. "Ocean stability" hypothesis (Lasker, 1975): Environmental stability of ocean plays a key role in the feeding success and survival of larvae.

Above hypotheses focused on the effect of starvation during the larval stage. However, recent studies have revealed that not only starvation but also predation is an important factor of recruitment variability, leading to the following hypotheses:

iv. "Bigger is better" hypothesis (Miller et al. 1988): Large individuals survive better than smaller ones due to higher escape potential from predators.
v. "Stage duration" hypothesis (Chambers & Leggett 1987): Fast-growing larvae go through the high predation mortality stages more quickly and thus optimize survival rate.
vi. "Growth-mortality" hypothesis (Anderson, 1988): Combining iv. (in the sense of scale of

somatic size) and v. (in the sense of temporal scale). Any factor increasing growth rate should benefit survival.

vii. "Growth-selective predation" hypothesis (Takasuka et al., 2003): Growth performance itself affects larval survival, independent of size. The hypothesis is complementary, but independent to mechanisms iv and v.

The key importance of growth rate in regulating survival has been emphasized by the three potential mechanisms described above.

1.2. Japanese anchovy

Japanese anchovy, *Engraulis japonicus*, belongs to order Clupeiforms and family Engraulidae, and is one of the most dominant pelagic fishes in the Northwest Pacific Ocean. Average catch in Japan reached 393,900 tons per year during 2001 to 2010 period, representing 6.9% of total landings (Statistics of the Ministry of Agriculture, Forestry and Fisheries of Japan). Japanese anchovy at various developmental stages are consumed in Japan and play important roles in its food culture. Late larvae are called *Shirasu* and are consumed in boiled form. Boiled and dried adult anchovy are called *Niboshi* and are used to make soup stock. Adults are also consumed as a protein source in daily diet. Beyond these, adults constitute an important bait source in the skipjack tuna fishery.

Japanese anchovy occupies a relatively low trophic level in the marine pelagic ecosystem, consuming mesozooplankton and being preyed upon by predators such as red barracuda *Sphyraena pinguis*, Japanese sea bass *Lateolabrax japonicus*, white croaker *Pennahia argentatus*, Japanese jack mackerel *Trachurus japonicus*, Pacific round herring *Etrumeus teres*, juvenile anchovy (Takasuka et al., 2003), Japanese flounder *Paralichthys olivaceus* (Tomiyama et al., 2011), chub mackerel

Scomber japonicus, spotted *mackerel S. australasicus* (Robert et al., 2010), Japanese amberjack *Seriola quinqueradiata* and other commercially important species (Hashimoto et al., 1989). Anchovy thus plays a key role transferring energy from lower to upper trophic levels through the food web.

The genus *Engraulis* is distributed in all temperate oceans: Peruvian anchoveta *E. ringens* in the southeast Pacific Ocean, northern anchovy *E. mordax* in northeast Pacific Ocean, European anchovy *E. encrasicolus* in Atlantic Ocean and other anchovies contribute a large proportion of the global fishery. Biological information about the genus is required to grasp trends in worldwide fisheries resource fluctuations.

1.3. Resource fluctuations and "optimum growth temperature" hypothesis

Landings of some abundant pelagic species such as anchovy and sardine *Sardinops melanostictus* are known to fluctuate out of phase in Japanese fisheries history (Fig. 1.1). Chaves et al. (2003) revealed that sardine, anchovy and productivity of ocean ecosystems run on cycles of approximately fifty years in the Pacific Ocean. Takasuka et al. (2007[b]) proposed the "optimum growth temperature" hypothesis, based on the existence of an optimum temperature for larval growth, to explain how worldwide climate regime shifts may affect the standing stock of each of these clupeiform species.

Optimum growth temperature has also been revealed in non-clupeiform fishes. For example, Buckley et al. (2004) examined the growth of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) on Georges Bank offshore northeastern USA, and revealed optimum growth at 7°C, although they did not refer to temperature-dependant food fluctuations. Few past laboratory studies yielded results consistent with the "optimum growth temperature" hypothesis and its direct effect on larval survival.

1.4. Aim of the present research

The general objective of the present study was to improve our understanding of the mechanisms driving Japanese anchovy fluctuations in stock size. Specific objectives consisted in:

(1) Improving methodology of otolith microstructure analysis and RNA/DNA ratio analysis in Japanese anchovy;

(2) Assessing growth-dependent survival mechanisms in larval anchovy;

(3) Testing the "optimum growth temperature" hypothesis in laboratory-reared Japanese anchovy;

(4) Assessing links between growth rate and vulnerability to predation in larval anchovy.

1.5. Outline of the present research

This research includes three sections: (1) broodstock rearing, (2) growth examination of hatchery-reared larval anchovy, and (3) predation experiment. First, broodstocks for egg and larval rearing were assembled with wild adult anchovy. Second, larval anchovy obtained from these broodstocks were reared in various experimental conditions and their growth was monitored. Three methods were used to derive growth rate: measurement of somatic size at age, analysis of the otolith microstructure, and analysis of the RNA/DNA ratio. Third, anti-predator performance of larval anchovy reared in different conditions of temperature and food was tested. This allowed the assessment of the effect of variability in larval growth and condition on vulnerability to predation.

1.5.1. Otolith microstructure analysis

Otolith microstructure analysis is one of the most common methods to examine growth of larval fish. Because otolith and somatic growth have proportional relationships, growth trajectory can be back-calculated from daily increments of otolith. This biological intercept method was developed by Campana & Nelson (1985) and Campana (1990). As well as for many fish species, otoliths of larval anchovy show a distinct ring deposition pattern and Tsuji & Aoyama (1984) confirmed that increments are formed on a daily basis. Since then, otolith-based aging and otolith growth estimation was widely performed in larval anchovy (e.g. Takasuka et al., 2003, 2004[a], 2004[b] and 2007[b], Takasuka & Aoki, 2006, Takahashi et al., 2001, Takahashi & Watanabe, 2004 and Wang & Tzeng 1999).

1.5.2. RNA/DNA ratio analysis

RNA/DNA ratio analysis is a powerful method to quantify recent growth rate of organisms. Buckley (1984) correlated growth performance to RNA/DNA ratio in eight temperate fish species and determined a general function which explained well variability in growth rate. The principle of RNA/DNA ratio analysis is as follows: somatic growth can be interpreted as the amount of protein synthesis *in vivo*. The amount of RNA synthesis is directly linked to protein synthesis. Because the amount of DNA per cell is constant, RNA/DNA ratio is a good indicator of growth. Many researchers have used this method to examine larval growth (e.g. Sato et al., 1995, Malloy et al., 1996 and Kono et al., 2003).

1.5.3. Predation experiment

Few studies provided direct evidence for a linkage between growth rate and vulnerability to predation. In this section, larval anchovy reared in various conditions of temperature and food were exposed to moon jellyfish, *Aurelia aurita*, one of the most common nearshore predators for fish larvae in Japan (Purcell & Arai 2001). This experiment aimed to reveal whether the "growth-selective predation" mechanism is can be applied for moon jellyfish predation. If "growth-selective predation" hypothesis prevails, moon jellyfish would selectively prey on anchovy larvae characterized by inferior growth.

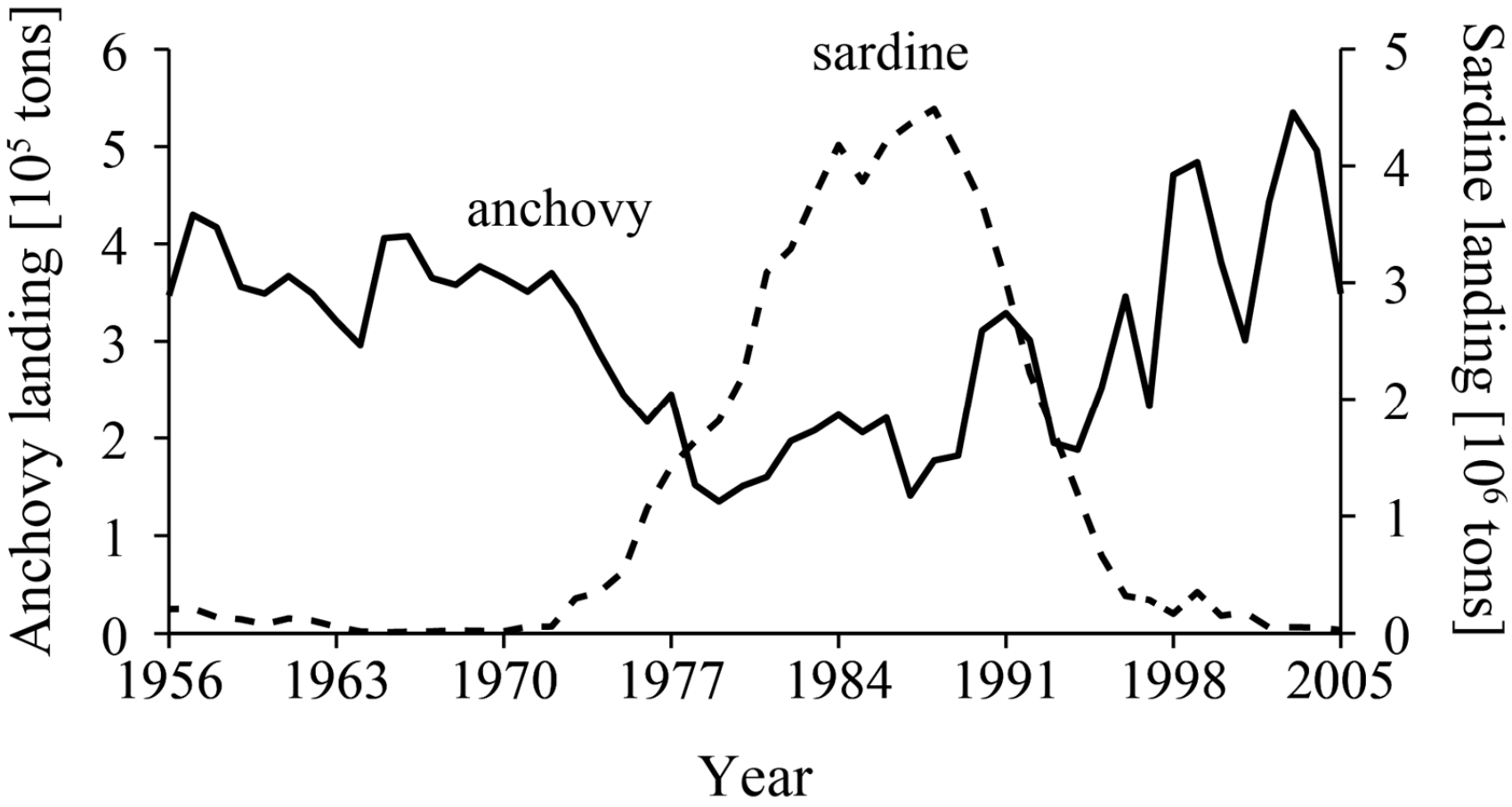

Fig. 1.1. Fluctuations in total landings of anchovy and sardine in Japan from 1956 to 2005. Data were obtained from the statistics of agriculture, forestry and fisheries of Japan (Ministry of Agriculture, Forestry and Fisheries of Japan).

2. Materials and methods

2.1. Broodstock rearing

A total of ~400 adult Japanese anchovy were collected in a set-net near the Maizuru Fisheries Research Station of the Field Science Education and Research Center, Kyoto University in Maizuru-city, Kyoto-prefecture, Japan (in collaboration with Tai Fisheries Cooperative Association) (Fig. 2.1) on 19 March and 9 April 2009. Spawners were quickly transported to the MFRS using four aerated 70L tanks. After transportation, they were divided into two circular broodstock tanks of 4.0 m diameter and 1.6 m depth (Tank 1, 2). Both tanks were oxygenated and supplied with filtered sea water. Water temperature was monitored and thawed frozen krill (a commercial product of the Kyoto-Prefectural Fisheries Cooperative Association Union) was fed twice a day until saturation. Food leftovers and dead fishes were removed as frequently as possible.

Some adult anchovy died during the transportation process from the set net to broodstock tanks. For those individuals, standard length (SL), wet weight (WW) and gonad weight (GW) were measured and Gonadosomatic Index (GSI) was calculated from WW and GW using formula (1).

$$GSI = \frac{GW}{WW - GW} \times 100 \quad (1)$$

2.2. Egg sampling

Japanese anchovy spawns pelagic eggs and Kawaguchi et al. (1990) reported that Japanese anchovy reared in the laboratory initiated spawning 1-1.5 hours after switching off the light for a total duration of 3.5-4 hours. The buoyant eggs of anchovy were collected using 0.3 m diameter ring nets of 0.3 μm mesh set after sunset in a small basin at the end of the surface exhaust pipe of each broodstock tank.

Some of anchovy eggs were sampled daily for the monitoring of spawning activity and egg condition. Because anchovy eggs are prolate spheroid, length (*L*) and width (*W*) were measured to calculate egg volume (*V*) with the following formula (2).

$$V\,[\mathrm{mm}^3] = \frac{4}{3} \times \pi \times L\,[\mathrm{mm}] \times W^2[\mathrm{mm}^2] \qquad (2)$$

2.3. Larval rearing

Larvae were reared under different combinations of temperature and food density conditions. Cross experimental design with 3 temperature treatments and 3 food treatments was employed (Factor = Temperature [16, 21, 25°C] × Food [0, 4, 8 rotifers ml^{-1}]). For predation experiment, an extra treatment of 12 rotifers ml^{-1} at each temperature was added. Larval rearing conditions were duplicated in some treatments. In each larval rearing experiment, 5000 eggs were stocked in one 200L experimental tank.

2.3.1. Temperature conditioning

To maintain water temperature in each experimental tank, heating/chilling devices controlled with a thermostat were used. Air temperature in the experimental room was maintained at 21°C to moderate the effect of atmospheric temperature variations.

2.3.2. Food conditioning

Rotifers *Brachionus plicatilis* were cultured in a chlorella solution and used as food for anchovy larvae. Before being fed to fish larvae, rotifers were enriched with DHA and *Nannochloropsis*. Density of rotifers in the experimental tanks and culture tanks was monitored by counting individuals of a given fraction under a stereomicroscope. Following these counts, density of rotifers in experimental tanks was adjusted twice a day. *Nannochloropsis* was also added to experimental tanks to insure that rotifers remained well fed. In experimental tanks where larvae reached a standard length of >20 mm, *Artemia* (brine shrimp) naupli were fed at a concentration of 2 individuals ml^{-1}.

2.3.3. Conditioning of other factors

Marine fish larvae are visual feeders and the importance of light condition for their feeding behavior has been demonstrated in several studies (Puvanendran & Brown, 2002 and Buckley et al., 2006). Photoperiod was set at 12 : 12 hour for dark : light condition. Sea water exchange in experimental tanks was set at a minimum level (200% per day) to avoid stressing larvae. To prevent contamination of other small organisms and particles, supplied water was filtered through 10 nm

filters. Aeration of experimental tanks was also maintained at a minimum level to avoid stressing larvae.

2.4. Sampling

A minimum of 5 larvae were sampled once every 3 days for analyses. Larvae were carefully sampled from experimental tanks using a pipette. Individuals sampled for otolith microstructure analysis were preserved in 95% ethanol. Samples for RNA/DNA ratio analysis were preserved at -80°C to prevent RNA degradation. Sampling was continued until all larvae were removed in each experimental tank.

2.5. Otolith microstructure analysis

Standard length (SL) of larvae preserved in 95% ethanol was measured with Image analyzer. Because larvae tend to shrink when preserved in ethanol, shrinking rate was examined. SL of 120 larvae were measured pre- and post-fixation with developmental stages ranging from post-hatching to juvenile (1-26 dph (day past hatching), 3.12-25.54 mm). SL of preserved individuals was divided by the estimated shrinking rate of 96.7 ± 0.06% (arithmetic mean ± standard error (Fig. 2.2)). Larvae were then dissected on a glass slide under the binocular and otoliths were extracted. Otoliths were fixed into acrylic resin. Sagittae were used for the analysis. Otolith radius (OR), core radius, the number of daily increments (Increment count, IC) and increment width were measured under microscope with the Otolith Daily Ring Measurement Software (RATOC System Engineering Co).

The biological intercept method (Campana 1990) was employed for estimation of averaged growth

during the three days prior to sampling (recent three-day growth). The otolith edge was not considered as a full increment and was not included in the analysis.

2.6. RNA/DNA ratio analysis

RNA/DNA ratio analysis was built up with two processes of extraction and measurement of nucleic acids, following the protocol described in Belchier et al. (2004). Frozen larvae were thawed and their SL was measured. Larval body was homogenized and incubated for 15 minutes at 37°C with Proteinase *K*. The solution was deproteinized, degreased and other components were removed to purify nucleic acids using the phenol-chloroform method. The solution was divided into two vials, one of which was treated with RNase. Mixture solution of DNA and RNA emits 590 nm fluoric wavelength against 365 nm excitation fluoric wavelength, the intensity of which is known to be proportional to combined DNA and RNA concentration. To measure the fluoric intensity of nucleic solutions, ethidium bromide was added into both vials. Concentration of ethidium bromide was determined following Le Pecq & Paoletti (1966) and Clemmesen (1993). Fluoric intensity of total nucleic acids (I_{DNA} + I_{RNA}) and DNA (I_{DNA}) were measured with fluoric intensity meter and the fluoric intensity of RNA (I_{RNA}) was determined by subtraction using formula (3). Concentrations of DNA and RNA (C_{DNA}, C_{RNA}) were determined by multiplying coefficients of standard curves (a, b) obtained with standard material of DNA and RNA from formula (4) and (5). RNA/DNA ratio was determined with formula (6).

$$I_{DNA+RNA} = I_{DNA} + I_{RNA} \quad (3)$$

$$C_{DNA} = a \cdot I_{DNA} \quad (4)$$

$$C_{RNA} = b \cdot I_{RNA} \quad (5)$$

$$RNA/DNA\ ratio = \frac{C_{RNA}}{C_{DNA}} \quad (6)$$

2.7. Predation experiment

Predation trials were carried out for 3 larval developmental stages for several rearing conditions of temperature and prey density. First-feeding individuals, as well as larvae of 7 mm and 12 mm SL were used for the experiment. In first-feeding larvae, experimental design was Factors = Temperature [16, 21, 25°C] × Food [0, 12 rotifers ml^{-1}]. For larvae at 7 mm, experimental design was Factors = Temperature [16, 21, 25°C] × Food [4, 8, 12 rotifers ml^{-1}]. Larvae at 12 mm were available in sufficient numbers only in the treatments of 21°C / 12 rotifers ml^{-1} and 16°C / 8 rotifers ml^{-1} As predators, a total of 45 moon jellyfish (ca. 10.45 ± 0.66 mm (average ± SD) in bell diameter) were captured in front of MFRS and reared in a predator stock tank. Before the predation experiment, 3 predators were conditioned in a 10L transparent experimental tank at the same temperature as the rearing temperature of larvae being subjected to experimentation.

Prior to predation trials, pulsation rate of predatory jellyfish was monitored as an indicator of activity. At the start of the experiment, a larva was put in the experimental tank carefully. Time until capture by one of the predators, defined as survival duration, was measured for a maximum duration of 300 seconds. In most treatments, 20 individual larvae (defined as N_0 = 20) were tested for each

treatment, with the exceptions of 21°C / 12 rotifers ml^{-1} at 7 mm (N_0 = 17) and 21°C / 12 rotifers ml^{-1} at 12 mm (N_0 = 6). Survival duration was represented in the form of Kaplan-Meier's survival curves and fitted with an exponential mortality model. Mortality coefficient z in the fit was calculated from formula (7), in which N_t was number of surviving larvae at time of t seconds.

$$N_t = N_0 e^{-zt} \quad (7)$$

2.8. Statistics

Sexual differences in adult anchovy physiological condition were assessed with Student's t-test. The relationship between water temperature and the number of spawned eggs was examined by quadratic regression analysis. The link between egg size and date was examined by linear regression analysis and analysis of variance (ANOVA).In otolith growth analysis, SL, IC, OR and cumulative OR, were log-transformed, if needed, before differences among larval rearing treatments were assessed by analysis of covariance (ANCOVA). Between-treatment comparisons effected in this study are summarized in Fig. 2.3. Differences in three-day recent growth among treatments were assessed by multivariate analysis of variance (MANOVA). In predation experiment section, differences in survival rate were tested by log-rank test. A total of 16 comparisons were done (Fig. 2.4). All statistical analyses were conducted using JMP software (SAS Institute Inc.).

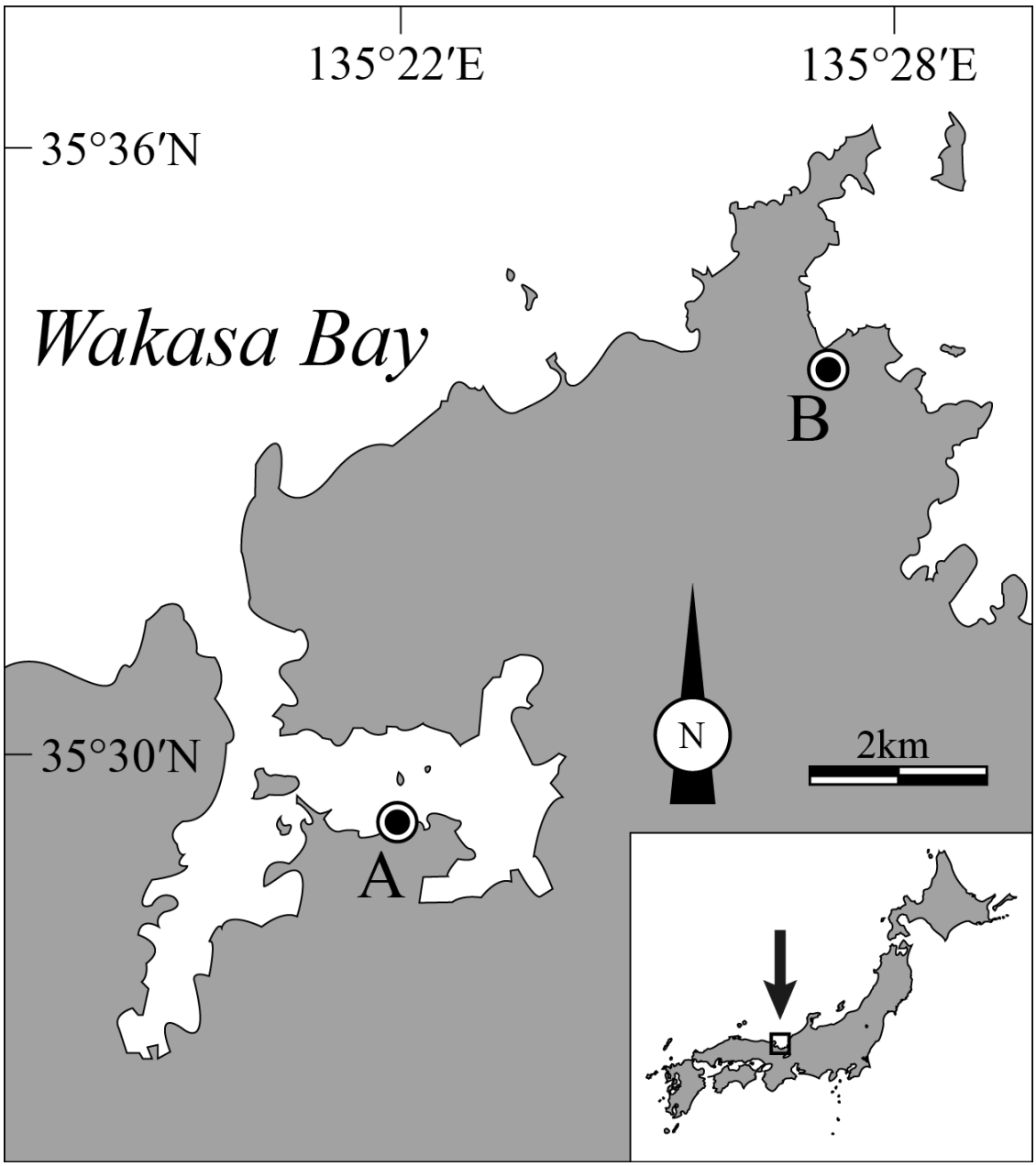

Fig. 2.1. Location of the Maizuru Fisheries Research Station (MFRS, marked as A) and Tai Fisheries Cooperative Association (marked as B) in Wakasa Bay, northern Kyoto Prefecture, Japan.

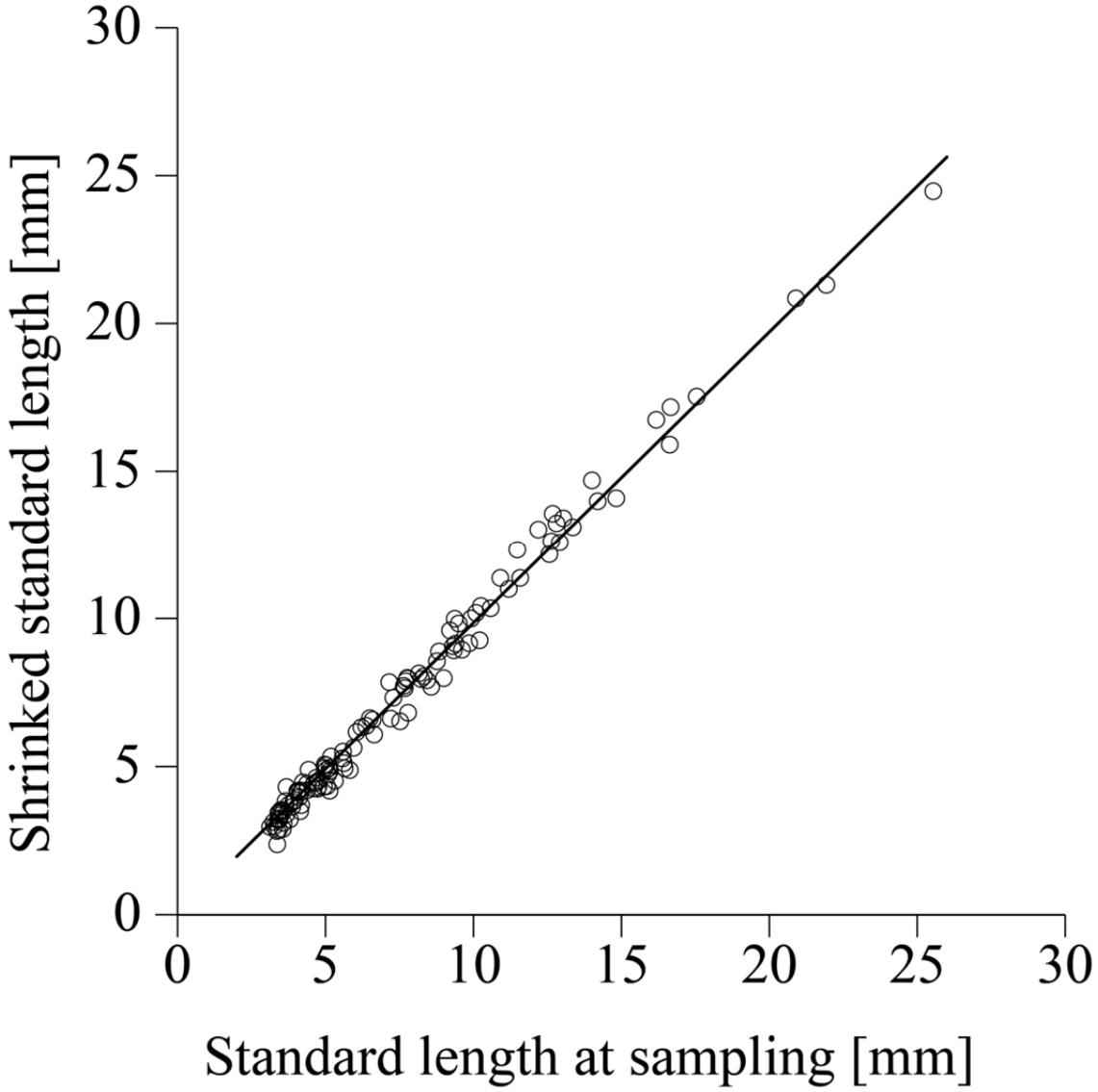

Fig. 2.2. Relationship between fresh length and preserved length in anchovy larvae preserved in 95% ethanol. SL of 200 individuals were measured pre- and post-fixation. Solid line represents the regression line: y = 0.981 x (R^2 = 0.990, p < 0.001).

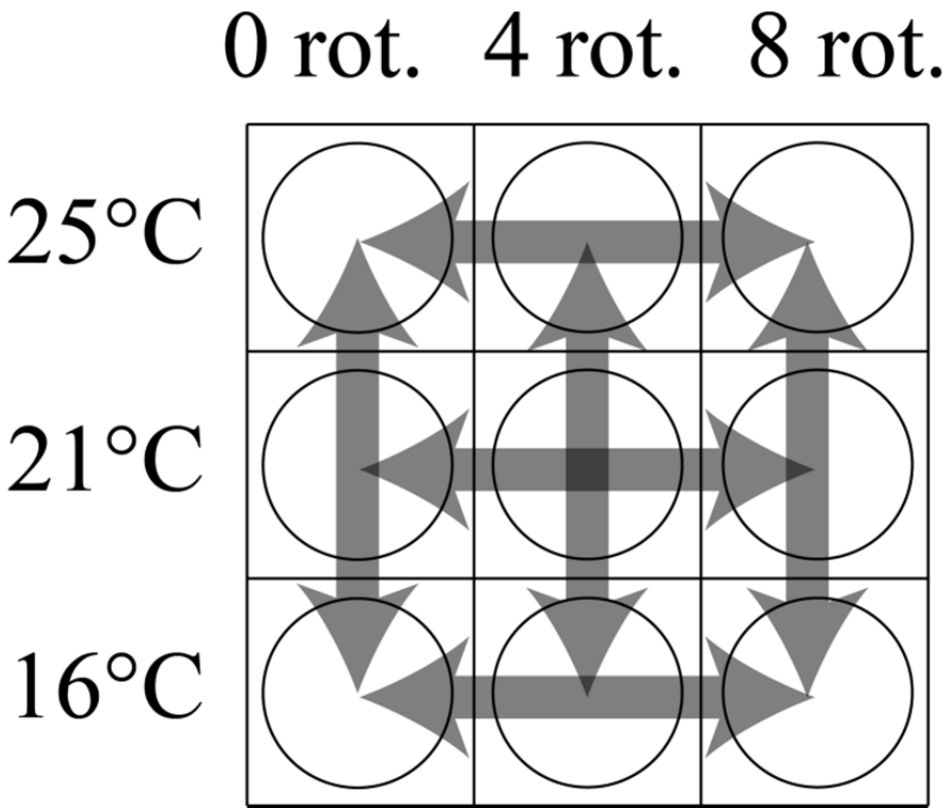

Fig. 2.3. Pairs of environmental conditions compared by ANCOVA in otolith growth analysis experiments. Each circle represents one experimental treatment. Treatments linked by arrows were statistically compared in the present research. Temperature comparisons were effected for the 3 levels of food density, and food density was compared for the 3 levels of temperature.

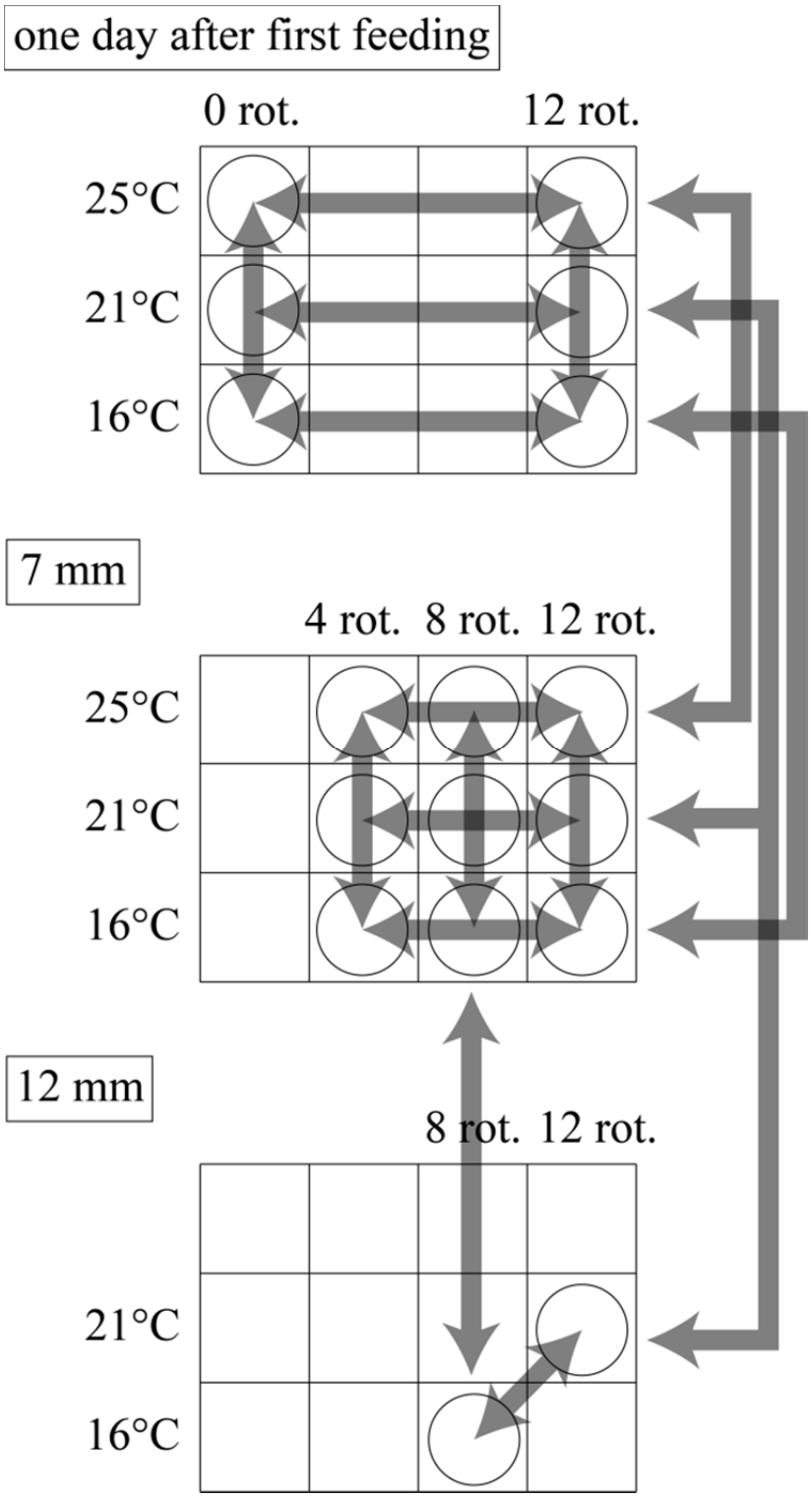

Fig. 2.4. Summary of treatment comparisons by log-rank test in the predation experiment. A total of 16 comparisons were conducted among conditions of temperature and food density, as well as developmental stages.

3. Results

3.1. Broodstock rearing

A total of 54 dead adult anchovy were sampled in the first three weeks post-capture. SL was 12.48 ±0.92 cm and 11.96 ± 0.58 cm, WW was 19.47 ± 5.17 g and 16.29 ± 2.24 g, GSI was 3.36 ± 1.39% and 4.30 ± 1.17%, in female and male, respectively (mean ± SD, Table 3.1). Females were larger than males, but males showed higher GSI than females (Student's t-test, SL: $p < 0.05$, WW: $p < 0.01$, GSI: $p < 0.01$).

3.2. Spawning

Temperature of broodstock tanks ranged from 10.3°C to 25.8°C during the experiment (Fig. 3.1). First spawning was observed on 22 April in Tank 2, at a temperature of 14.8°C. Spawning in Tank 1 started on 8 May, when temperature reached 15.7°C. Spawning continued until 23 August, during which maximum temperature was 25.8°C in Tank 2.

The number of eggs spawned followed a dome-shaped relationship with time and temperature (Fig. 3.2). Temperature explained 42% and 32% of the variation spawning in Tank 1 and Tank 2, respectively (quadratic regression analysis, Tank 1 and Tank 2: $p < 0.01$). Peak spawning occurred near 21°C in both tanks.

3.3. Egg condition

Seven subsamples totalizing 207 anchovy eggs were subsampled in Tank 1 to examine condition on 27 May, 31 May, 3 June, 8 June, 12 June, 17 June, 23 June, 5 July and 16 July. The width, length and volume of anchovy eggs averaged 0.67 ± 0.03 mm, 1.34 ± 0.08 mm and 2.54 ± 0.32 mm^3 (average ± SD). The three variables showed significant negative relationships with the continuous factor of date, which was represented by the number of days past the day of first examination (27 May) (regression analysis, Table 3.3). The categorical factor of date had significant effect on the three variables (ANOVA, $p < 0.01$, Table 3.2). Egg volume first increased, and then decreased during this period (Fig. 3.3, Student's t-test with Bonferroni multiple comparison, $p < 0.05$). Egg length, width and volume significantly decreased past the peak throughout the season (Table 3.3, linear regression analysis, $p < 0.01$).

3.4. Basic growth analysis

Except for the 25°C / 0 rotifers ml^{-1} treatment, significant relationships were always found between $\log_{10}$ SL and age (Fig. 3.4 and Table 3.4, linear regression analysis, $p < 0.05$). Age, temperature and the interaction factor between age and temperature had significant effect on SL in the temperature comparisons at prey densities of 4 and 8 rotifers ml^{-1} (Fig. 3.5 a, b and c, Table 3.5, ANCOVA, $p < 0.01$). Age and food had a significant effect on SL in food density comparisons at 16°C, 21°C and 25°C (Fig. 3.5 d, e and f, Table 3.5, ANCOVA, $p < 0.01$). Interaction factor between age and food had no significant effect on SL (Fig. 3.5 and Table 3.5, ANCOVA, $p > 0.05$). Survival duration tended to be longer for lower temperature and higher food density treatments (Fig. 3.6).

3.5. Otolith information

3.5.1. Daily increment

A total of 303 larvae were subsampled for otolith microstructure analysis. Age and the number of increments were strongly correlated in all treatments except for 25°C / 0 rotifers ml^{-1} (Fig. 3.7 and Table 3.6, linear regression analysis, $p < 0.01$).

Age, temperature and the interaction factor between age and temperature showed significant effect on increment count in the temperature comparison at 8, 4 and 0 rotifers ml^{-1} (Table 3.7, ANCOVA, $p < 0.05$). Food density had a significant effect on increment count at 21°C and 25°C (Table 3.7, ANCOVA, $p < 0.05$). Interaction between age and food had significant effect at 16°C and 21°C (Table 3.7, ANCOVA, $p < 0.01$). Age and increment count had a 1:1 relationship in most treatments, with the exception of 16°C treatments.

3.5.2. Otolith radius

Except for 16°C / 0 rotifers ml^{-1}, 21°C / 0 rotifers ml^{-1}, 25°C / 0 rotifers ml^{-1} and 16°C / 4 rotifers ml^{-1} treatments, a significant relationship was observed between $\log_{10}$ OR and SL (Fig. 3.8 and Table 3.8, linear regression analysis, $p < 0.01$). When comparing the different temperature treatments, both SL and temperature had significant effect on OR at 4 and 8 rotifers ml^{-1} (Fig. 3.9 and Table 3.9, ANCOVA, $p < 0.05$). Interaction factor between SL and temperature had significant effect at 8 rotifers ml^{-1} (Fig. 3.9 and Table 3.9, ANCOVA, $p < 0.01$). When comparing the different food density treatments, SL had significant effect on OR at 16°C, 21°C and 25°C (Fig. 3.9 and Table 3.9, ANCOVA, $p < 0.01$), while food density had significant effect at 21°C (Fig. 3.9 and Table 3.9, ANCOVA, $p < 0.01$).

3.5.3. Three-day recent growth

Temperature had significant effect on the growth achieved during the last three days of life (Fig. 3.10, Fig. 3.11 and Table 3.10, MANOVA, $p < 0.01$). Food density had no significant effect on the three-day recent growth (Fig. 3.10, Fig. 3.11 and Table 3.10, MANOVA, $p = 0.400$)

3.6. RNA/DNA ratio Analysis

Value of RNA/DNA differed with SL and it diverged at smaller SL (Fig. 3.12). No clear tendency was found among treatments (Fig. 3.13).

3.7. Predation experiment

Kaplan-Meier's survival curves and reciprocals of mortality rate (*1/z*) were described (Fig. 3.14, Fig. 3.15, Fig. 3.16 and Table 3.11). In temperature and food density comparisons at SL of 7 mm, larvae from 25°C / 4 rotifers ml^{-1} treatment showed significantly higher mortality rate ($p < 0.01$, Fig. 3.18 and Table 3.12). No significant difference was found among other treatments (Fig. 3.17, Fig. 3.19 and Table 3.12). At 12 mm, 21°C / 12 rotifers ml^{-1} showed higher survival rate than 16°C / 8 rotifers ml^{-1} ($p < 0.01$, Fig. 3.19 and Table 3.12). In comparison between developmental stages, 12 mm larvae showed a higher survival rate than 7 mm at 16°C / 8 rotifers ml^{-1} treatment ($p < 0.01$, Fig. 3.20 and Table 3.13).

Table 3.1. Standard length (SL), wet weight (WW) and gonadosomatic index (GSI) of adult Japanese anchovy at the beginning of rearing. SD represents standard deviation. One female died on 19 March and 24 females and 26 males died on 9 April, on both capture days. One female and two males died within 3 weeks after capture.

Sex / difference	SL ± SD [cm]	WW ± SD [g]	GSI ± SD [%]
Female	12.48 ± 0.92 (n = 26)	19.47 ± 5.17 (n = 26)	3.36 ± 1.39 (n = 26)
Male	11.96 ± 0.58 (n = 28)	16.29 ± 2.24 (n = 27)	4.30 ± 1.17 (n = 27)
Sexual difference	$*p = 0.0139$	$**p = 0.0052$	$**p = 0.0010$

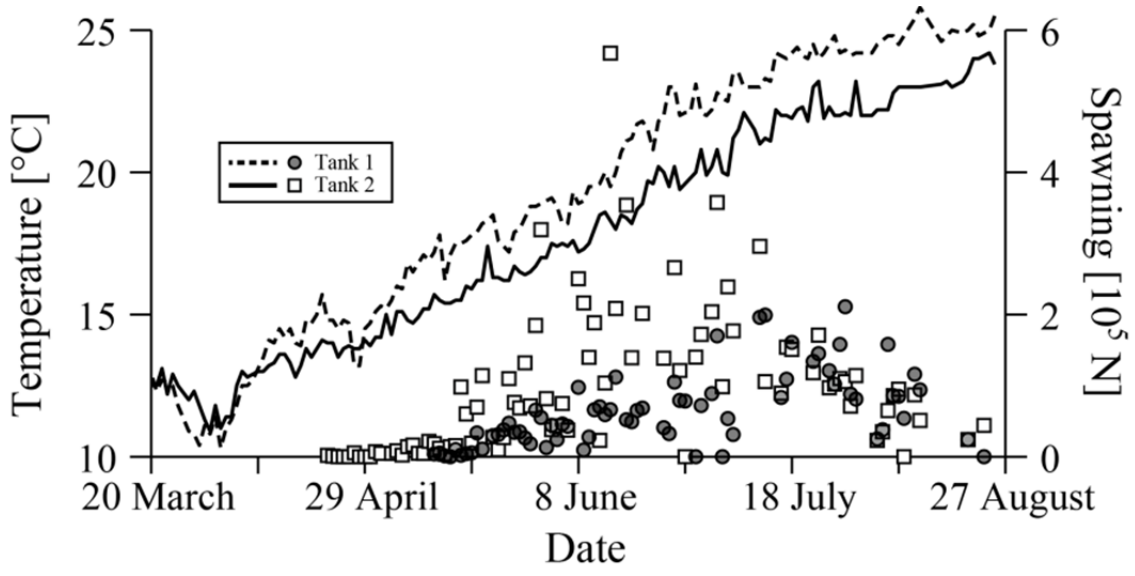

Fig. 3.1. Temporal change in temperature (Tank 1: solid line, Tank 2: broken line) and number of eggs spawned (Tank 1: ●, Tank 2: □) throughout the spawning season.

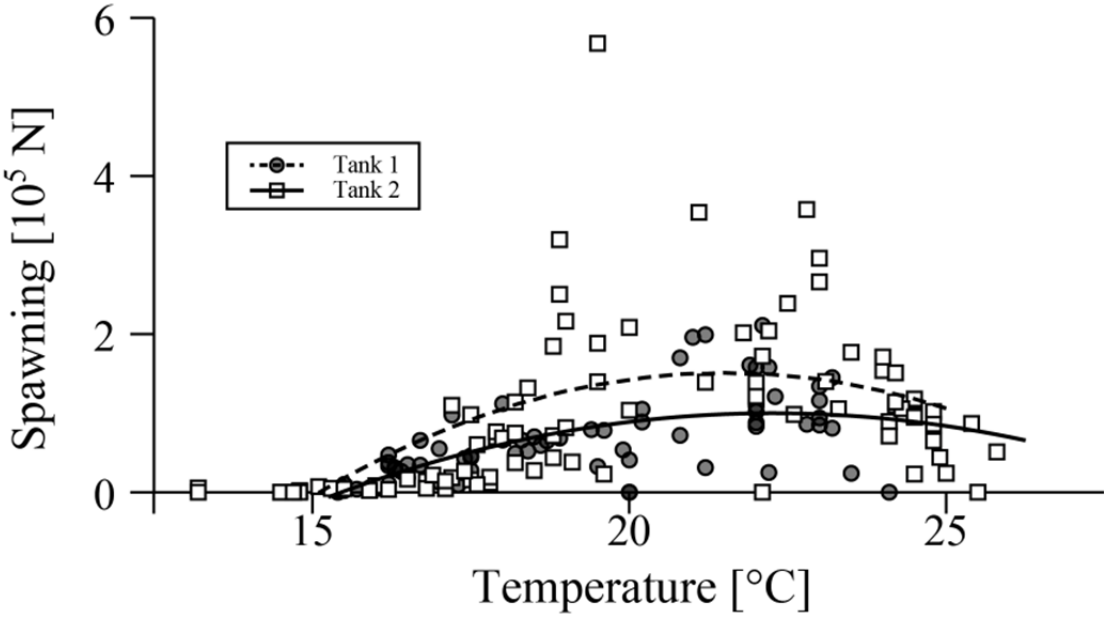

Fig. 3.2. Relationships between water temperature and the numbers of egg spawned. The number of egg spawned in Tank 1 and Tank 2 showed significant relationships with water temperature. Tank 1 (●, Solid line): $y = -2160\,x^2 + 96176\,x - 970366$ ($R^2 = 0.420$, $p < 0.001$); Tank 2 (□, broken line:) $y = -3686\,x^2 + 158555\,x - 2000000$ ($R^2 = 0.320$, $p < 0.001$)

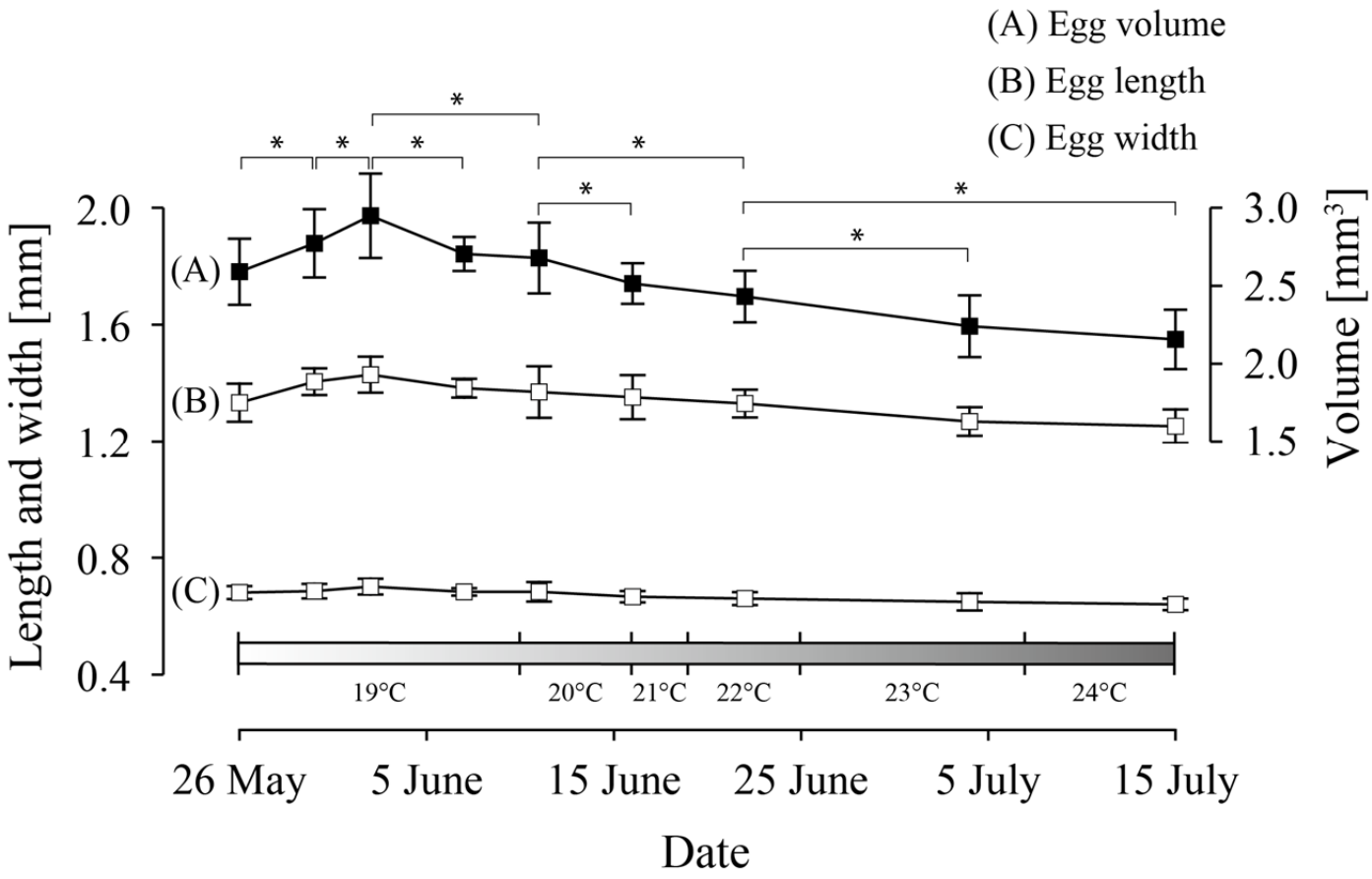

Fig. 3.3. Seasonal change in egg volume (A), length (B) and width (C). Errors bars represent SD. The Least number of pairs of date groups to express their significant differences were picked up and exhibited with an asterisk ($p < 0.05$).

Table 3.2. Summary of ANOVA statistics for egg volume. The categorical factor of Date includes [27 May, 31 May, 3 June, 8 June, 12 June, 17 June, 23 June, 5 July, 16 July]. Factor of date had significant effect on egg volume. *Df*, *SS*, *F* and *p* mean degree of freedom, sum squared, *F* value and probability of significance, respectively.

factor	*df*	*SS*	*F*	*p*
Date	8	13.155	40.256	**< 0.0001
error	198	8.088		
total	206	21.244		

Table 3.3. Summary of linear regression analysis of egg width, length and volume as a function of date, which was represented by the number of days past the day of first examination (27 May). R^2 is contribution rate of regression.

Response variable	Intercept	Parameter for Date [day]	*N*	R^2	*p*
Width [mm]	0.695	-0.001	207	0.332	**< 0.001
Length [mm]	1.413	-0.003	207	0.384	**< 0.001
Volume [mm^3]	2.849	-0.014	207	0.517	**< 0.001

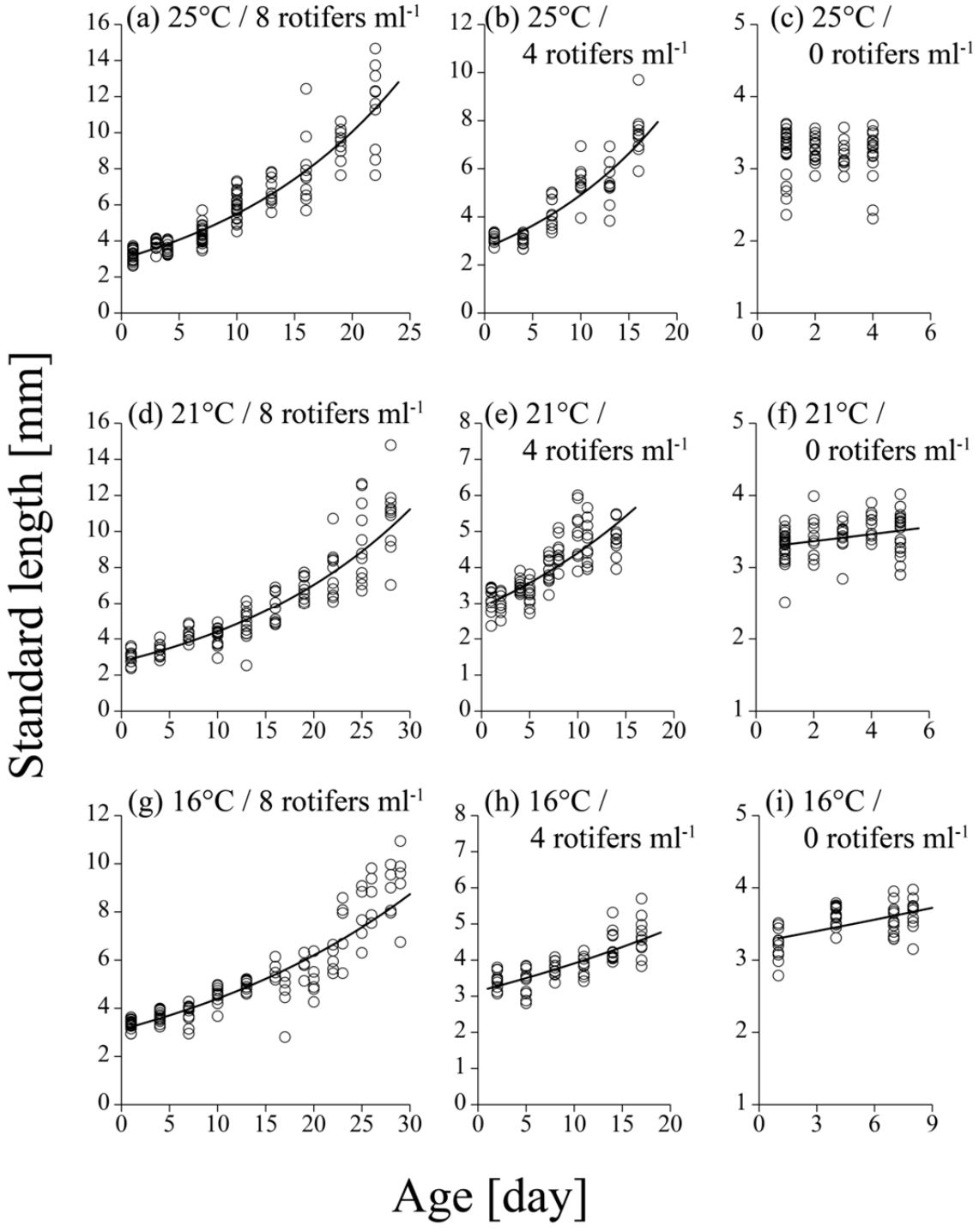

Fig. 3.4. Length at age relationship in all experimental treatments. Solid lines represent exponential functions.

Table 3.4. Summary of linear regressions between Log_{10} SL and Age.

Treatment		Intercept	Parameter for Age	*N*	R^2	*p*
Temperature (°C)	Food (rotifers ml^{-1})					
16	8	0.494	0.015	112	0.845	**< 0.001
	4	0.496	0.010	62	0.589	**< 0.001
	0	0.512	0.007	46	0.282	**0.001
21	8	0.441	0.020	108	0.860	**< 0.001
	4	0.463	0.018	94	0.673	**< 0.001
	0	0.512	0.007	74	0.077	*0.016
25	8	0.478	0.026	127	0.893	**< 0.001
	4	0.431	0.026	60	0.811	**< 0.001
	0	0.511	0.012	61	0.004	0.615

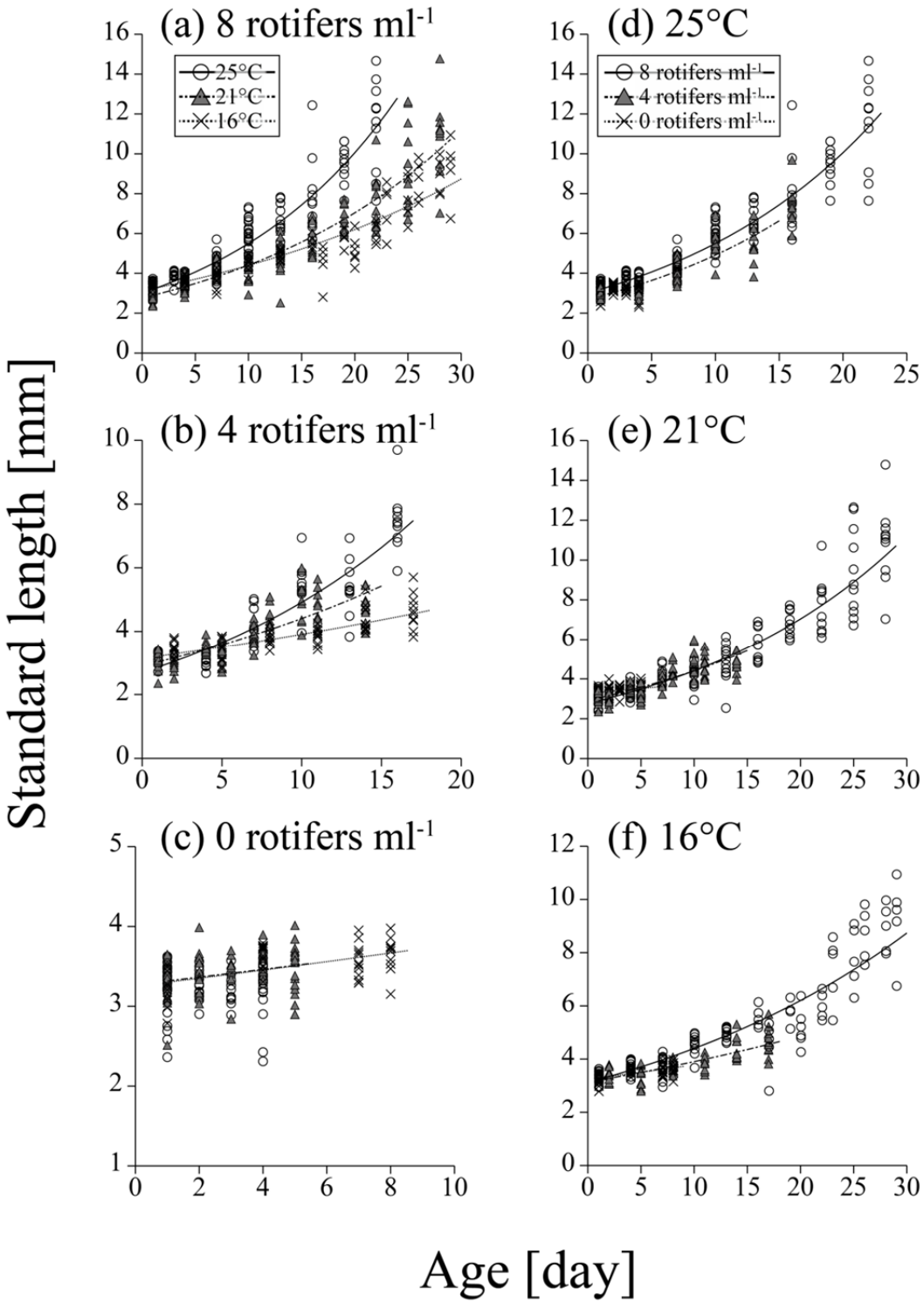

Fig. 3.5. Growth curves comparisons between treatments. (a), (b) and (c) compare temperature effect at 0, 4 and 8 rotifers ml^{-1}, respectively. (d), (e) and (f) compare food effect at 16°C, 21°C and 25°C, respectively.

Table 3.5. Summary of ANCOVA of $\log_{10}$ SL with the covariate of Age. The regressors "temp." and "rot." represent temperature and the number of rotifers ml^{-1}, respectively.

		factor	*df*	*SS*	*F*	*p*
Temperature comparison (16°C vs. 21°C vs. 25°C)	at 8 rot.	Age	1	6.814	1868.723	**<0.0001
		Temp.	2	0.670	91.896	**<0.0001
		Age × Temp.	2	0.288	39.494	**<0.0001
	at 4 rot.	Age	1	0.887	354.303	**<0.0001
		Temp.	2	0.039	7.860	**0.0005
		Age × Temp.	2	0.083	16.583	**<0.0001
	at 0 rot.	Age	1	0.000	0.284	0.595
		Temp.	2	0.008	2.995	0.0533
		Age × Temp.	2	0.003	1.061	0.349
Food density comparison (4 rot. vs. 8 rot.)	at 16°C	Age	1	0.419	203.021	**<0.0001
		Food	1	0.037	17.903	**<0.0001
		Age × Food	1	0.000	0.114	0.7366
	at 21°C	Age	1	0.909	277.775	**<0.0001
		Food	1	0.005	1.533	0.2175
		Age × Food	1	0.006	1.848	0.176
	at 25°C	Age	1	2.729	770.576	**<0.0001
		Food	1	0.065	18.390	**<0.0001
		Age × Food	1	0.006	1.786	0.1833

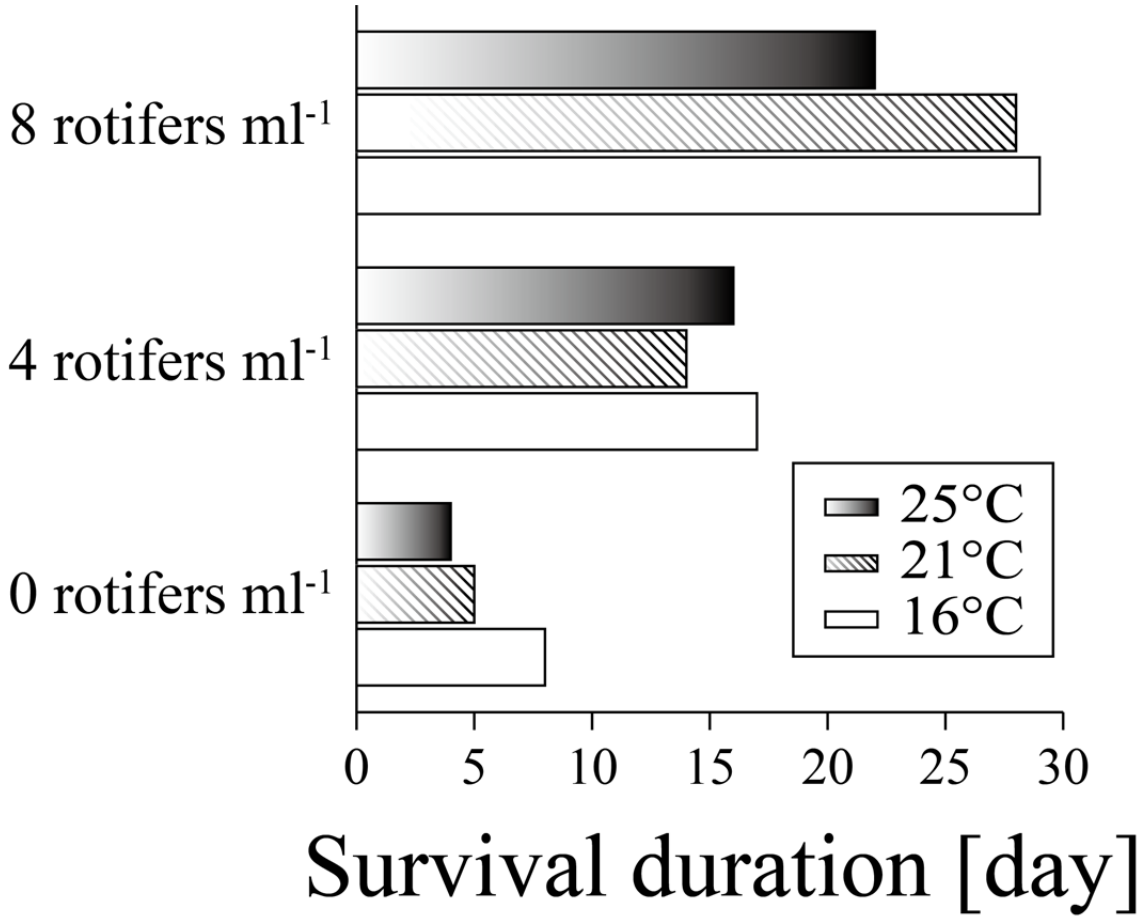

Fig. 3.6. Comparisons of survival duration defined as maximum larval age for each treatment.

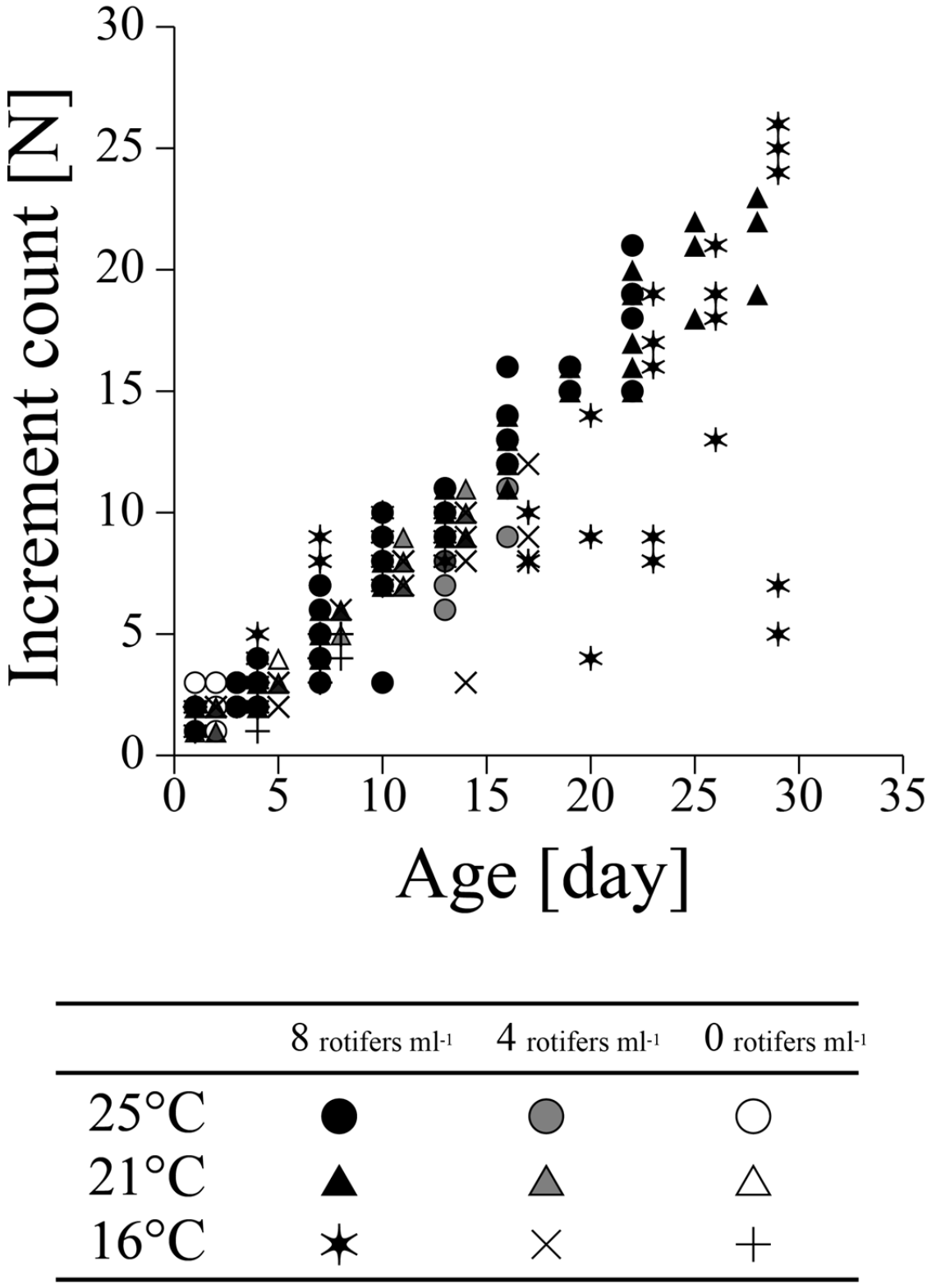

Fig. 3.7. Age-increment count relationships.

Table 3.6. Summary of the linear regression between increment count and age.

Treatment		Intercept	Parameter for age	*N*	R^2	*p*
Temperature (°C)	Food (rotifers ml^{-1})					
16	8	1.473	0.542	53	0.617	**< 0.0001
	4	0.582	0.551	22	0.761	**< 0.0001
	0	0.029	0.569	20	0.773	**< 0.0001
21	8	-0.925	0.836	54	0.975	**< 0.0001
	4	0.119	0.685	49	0.942	**< 0.0001
	0	1.425	0.350	16	0.555	**0.0009
25	8	-0.193	0.831	58	0.948	**< 0.0001
	4	0.028	0.664	21	0.853	**< 0.0001
	0	2.016	0.0787	19	0.032	0.4627

Table 3.7. Summary of ANCOVA of Increment count and the covariate Age. The regressors "temp." and "rot." represent temperature and the number of rotifers ml^{-1}, respectively.

		factor	*df*	*SS*	*F*	*p*
Temperature comparison (16°C vs. 21°C vs. 25°C)	at 8 rot.	Age	1	3756.009	739.023	**< 0.0001
		Temp.	2	132.074	12.993	**< 0.0001
		Age × Temp.	2	296.829	29.202	**< 0.0001
	at 4 rot.	Age	1	443.023	394.039	**< 0.0001
		Temp.	2	9.241	4.110	*0.0203
		Age × Temp.	2	7.709	3.428	*0.0377
	at 0 rot.	Age	1	14.770	39.721	**< 0.0001
		Temp.	2	2.697	3.627	*0.0357
		Age × Temp.	2	6.133	8.247	**0.001
Food density comparison (4 rot. vs. 8 rot.)	at 16°C	Age	1	286.166	58.795	**< 0.0001
		Food	1	0.810	0.166	0.6848
		Age × Food	1	45.317	9.311	**0.0034
	at 21°C	Age	1	1316.328	1456.654	**< 0.0001
		Food	1	4.180	4.626	*0.035
		Age × Food	1	18.483	20.453	**< 0.0001
	at 25°C	Age	1	538.017	378.835	**< 0.0001
		Food	1	9.837	6.926	*0.0107
		Age × Food	1	2.676	1.884	0.1747

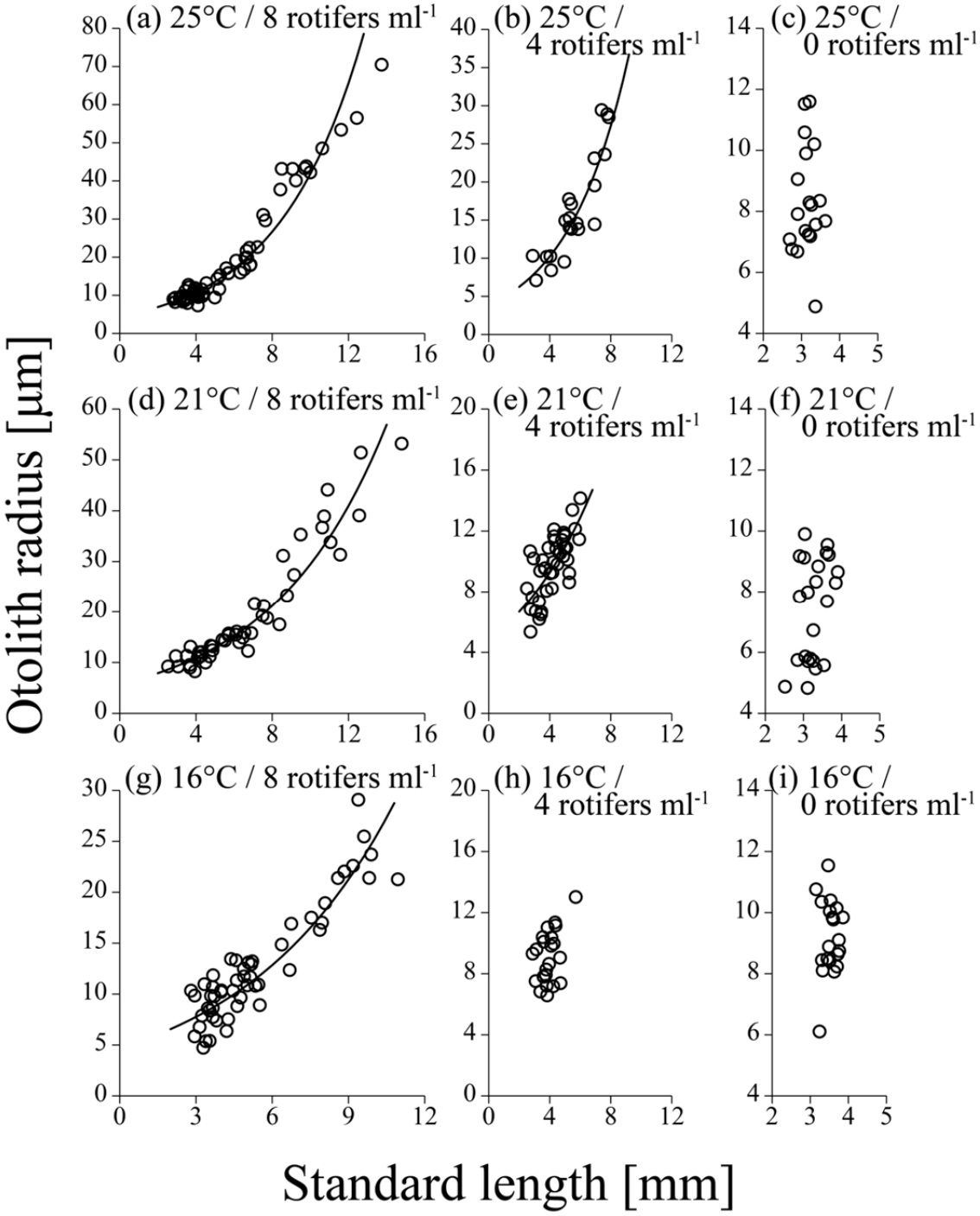

Fig. 3.8. SL-OR relationships for the 9 different experimental treatments. Solid lines represent exponential fit curves.

Table 3.8. Summary statistics of linear regression between SL by $\log_{10}$ OR for each experimental treatment.

Treatment		Intercept	Parameter for SL	*N*	R^2	*p*
Temperature (°C)	Food (rotifers ml^{-1})					
16	8	0.671	0.073	53	0.766	**< 0.0001
	4	0.751	0.051	22	0.152	0.0730
	0	0.832	0.036	20	0.013	0.6317
21	8	0.754	0.071	46	0.922	**< 0.0001
	4	0.681	0.071	41	0.488	**< 0.0001
	0	0.453	0.124	23	0.167	0.0527
25	8	0.644	0.098	58	0.937	**< 0.0001
	4	0.582	0.107	21	0.816	**< 0.0001
	0	0.867	0.014	19	0.001	0.8807

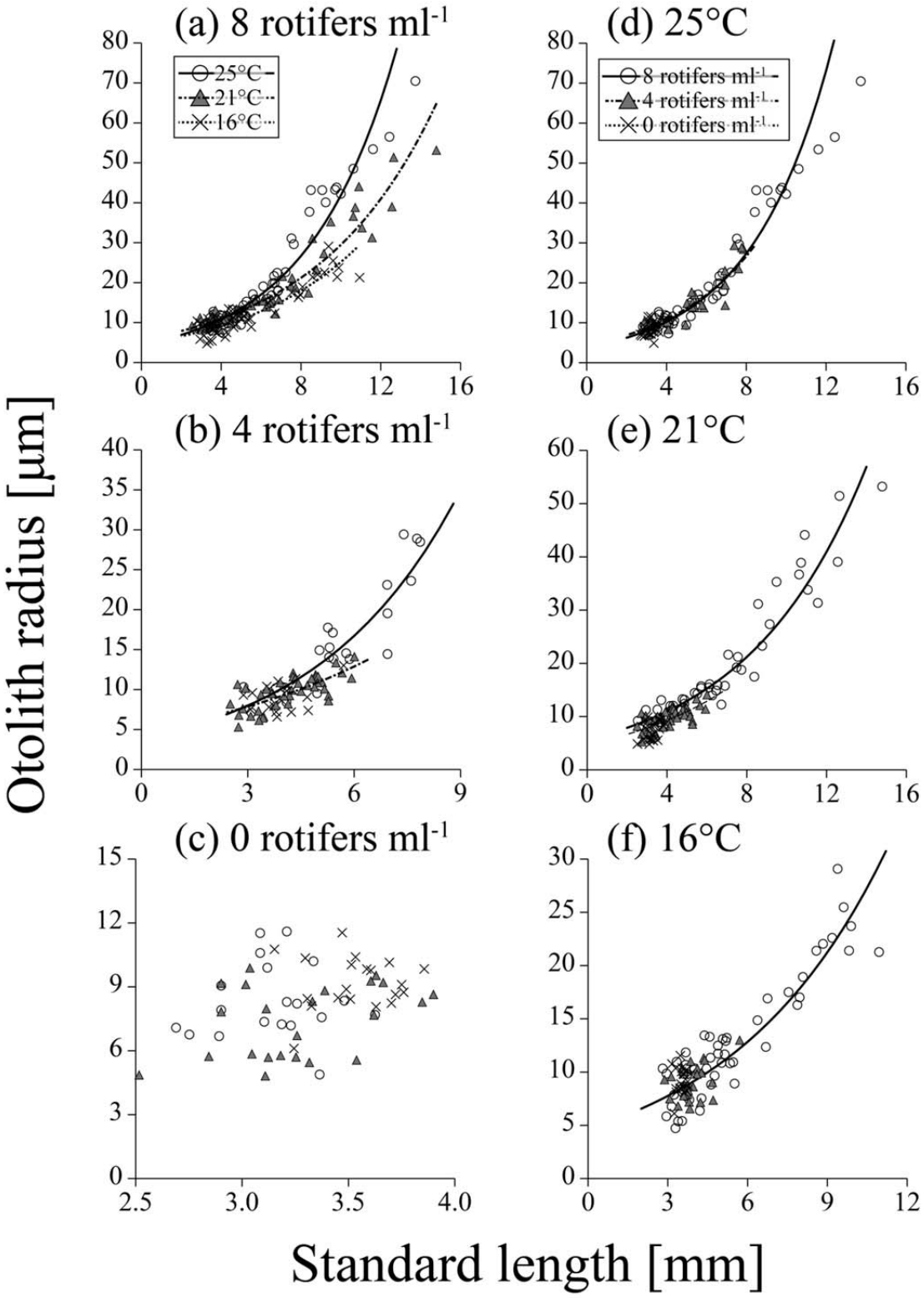

Fig. 3.9. Otolith growth curve comparisons. (a), (b) and (c) compare the temperature effect at each food treatment, and (d), (e) and (f) compare food effect at each temperature treatment.

Table 3.9. Summary of ANCOVA for SL-OR relationships. The regressors "temp." and "rot." represent temperature and the number of rotifers ml^{-1}, respectively.

		factor	*df*	*SS*	*F*	*p*
Temperature comparison (16°C vs. 21°C vs. 25°C)	at 8 rot.	SL	1	5.1309165	972.0167	**< 0.0001
		Temp.	2	0.3809960	36.0885	**< 0.0001
		SL × Temp.	2	0.1616971	15.3162	**< 0.0001
	at 4 rot. ex. 16°C	SL	1	0.23617825	43.2285	**< 0.0001
		Temp.	1	0.02624977	4.8046	*0.0334
		SL × Temp.	1	0.01061383	1.9427	0.1699
	at 0 rot.	SL	1	0.00992920	1.1252	0.2945
		Temp.	2	0.05474107	3.1016	0.0547
		SL × Temp.	2	0.00461101	0.2613	0.7712
Food density comparison (4 rot. vs. 8 rot.)	at 16°C	SL	1	0.08834543	10.7927	**0.0018
		Food	1	0.00038141	0.0466	0.8299
		SL × Food	1	0.00164235	0.2006	0.6560
	at 21°C	SL	1	0.15039746	35.0225	**< 0.0001
		Food	1	0.05267450	12.2661	**0.0009
		SL × Food	1	0.01411851	3.2877	0.0751
	at 25°C	SL	1	1.1375872	273.9173	**< 0.0001
		Food	1	0.0005133	0.1236	0.7263
		Age × Food	1	0.0052776	1.2708	0.2640

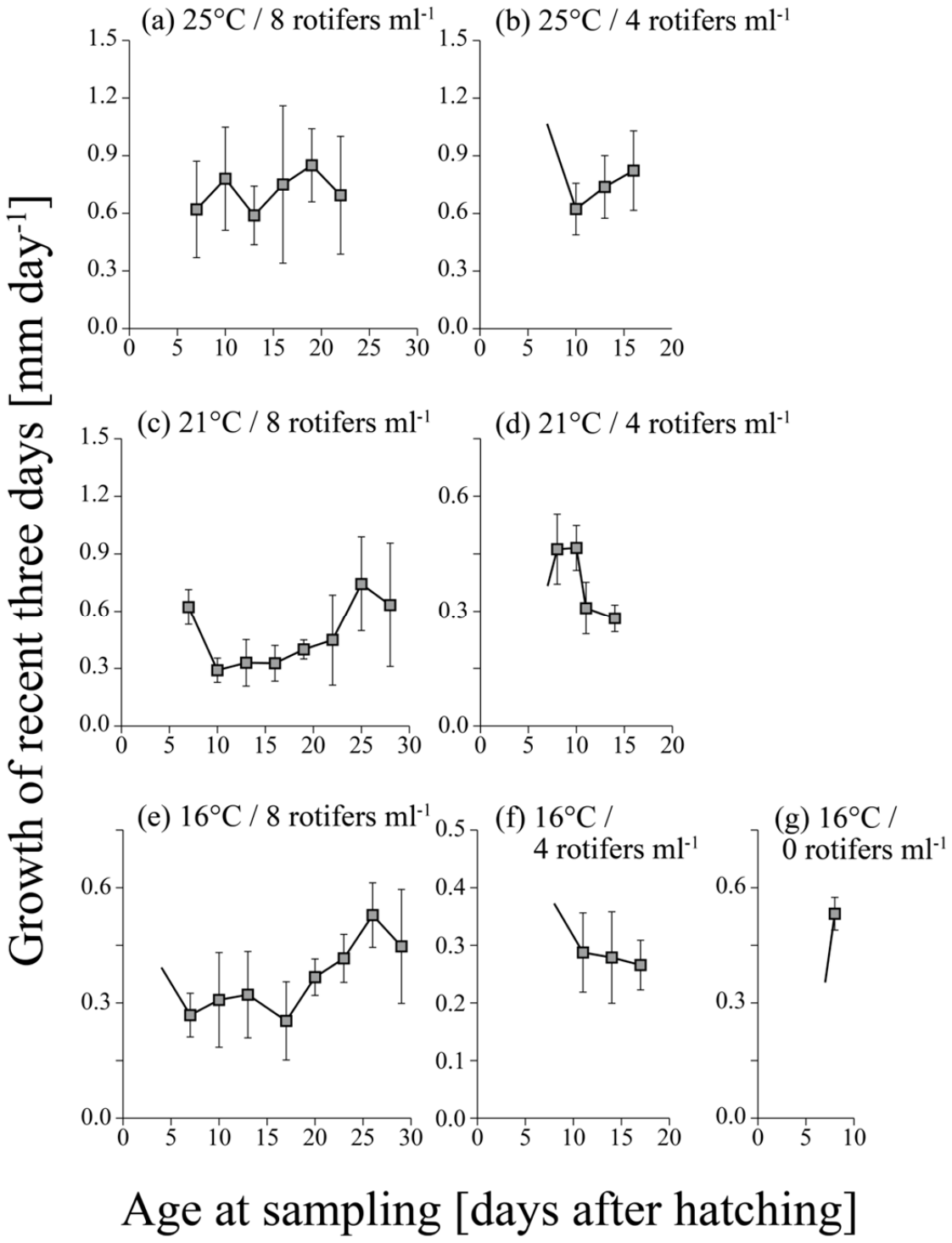

Fig. 3.10. The three-day recent growth with SD for the different experimental treatments.

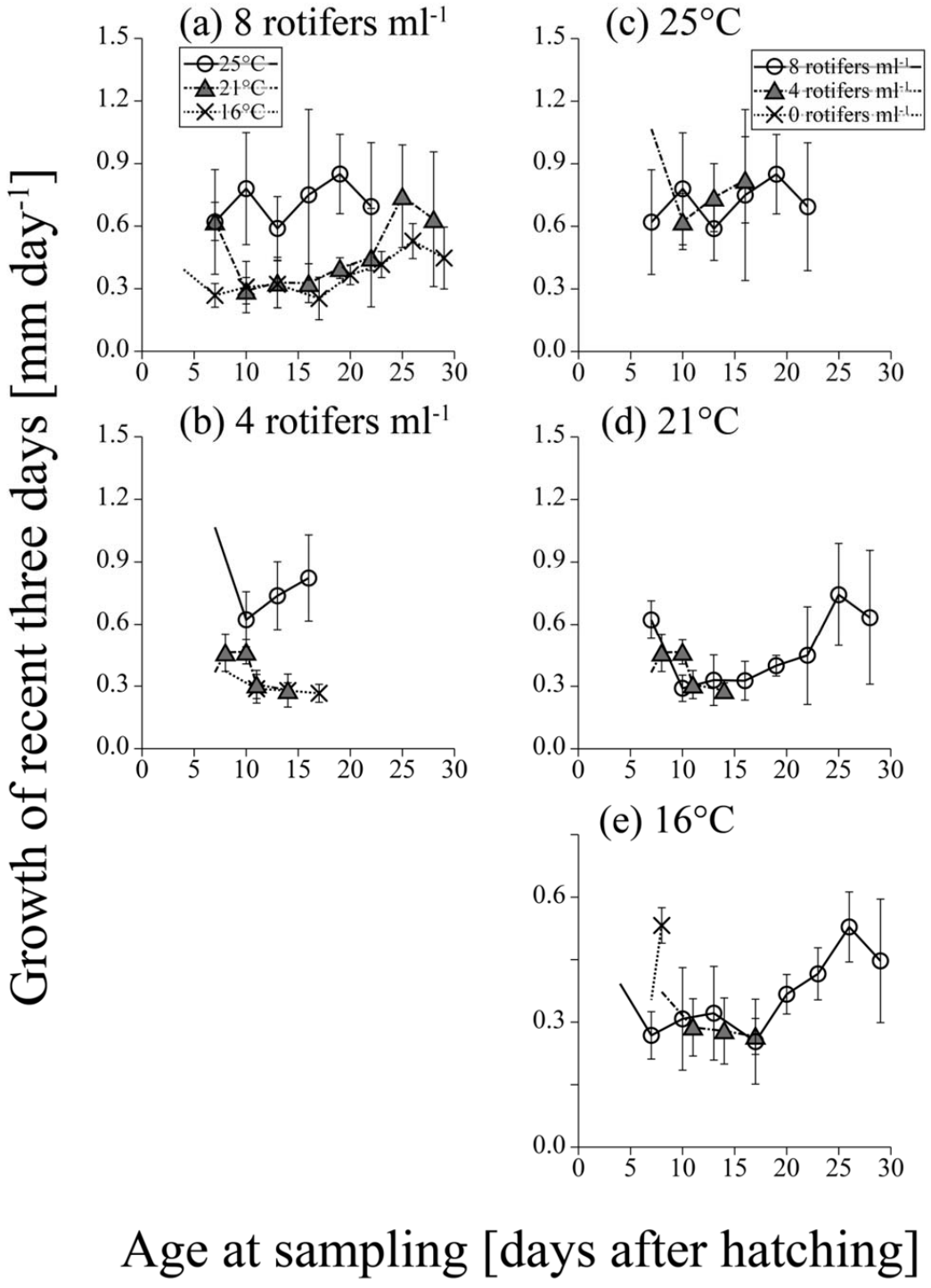

Fig. 3.11. Temperature and food density comparisons of the growth of recent three days with SD. (a) and (b) compare temperature effect at each food density treatments, and (c), (d) and (e) compare food density at each temperature.

Table 3.10. Summary of MANOVA statistics for three-day recent growth.

factor	*df*	*SS*	*F*	*p*
Temperature	2	4.295812	54.9843	**< 0.0001
Food	2	0.072053	0.9222	0.400
Age	1	0.02657	0.6802	0.411
Temperature × Age	2	0.07366	0.9428	0.392
food × Age	2	0.187996	2.4063	0.093

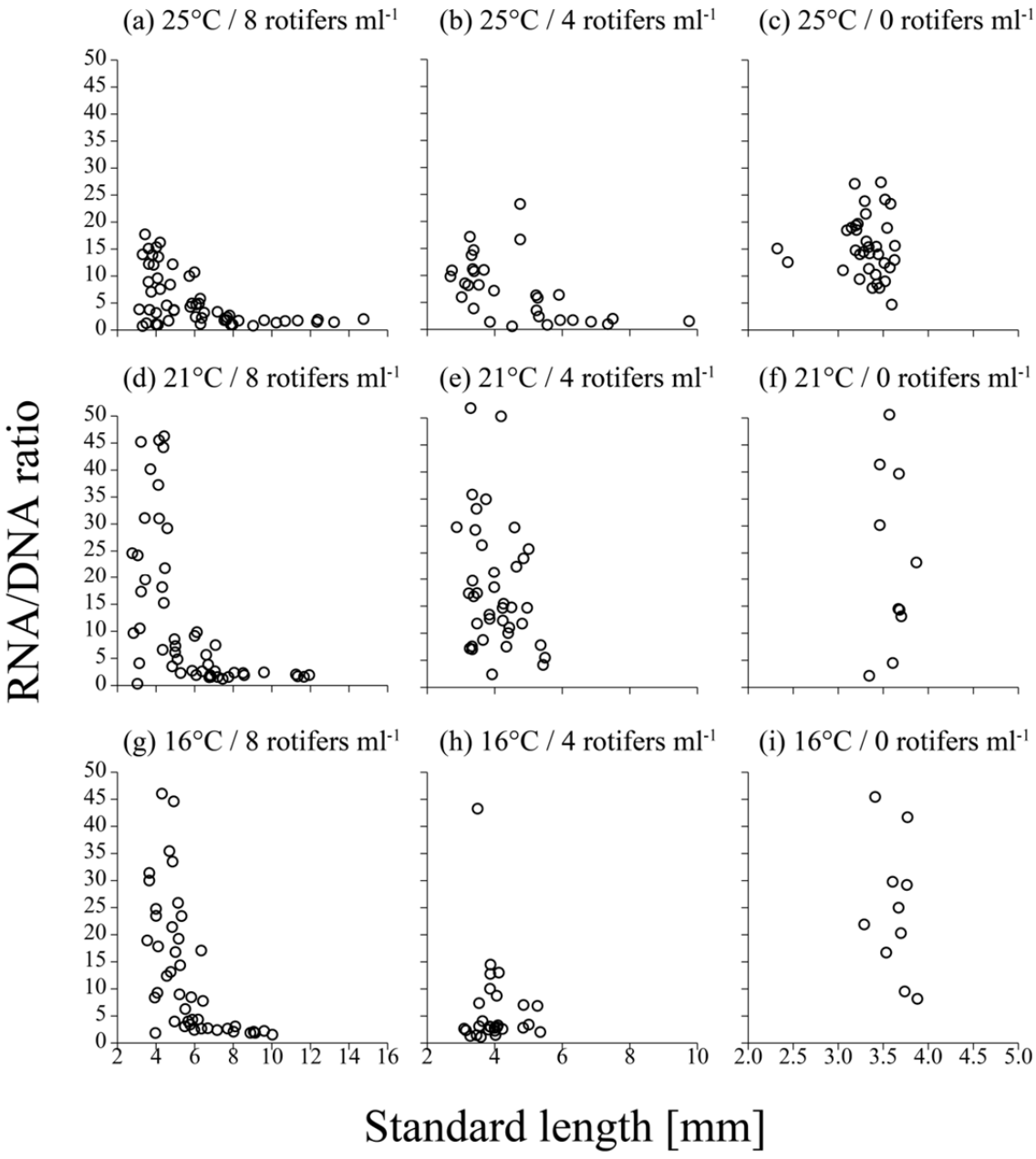

Fig. 3.12. Relationship between SL and RNA/DNA ratio for the different experimental treatments.

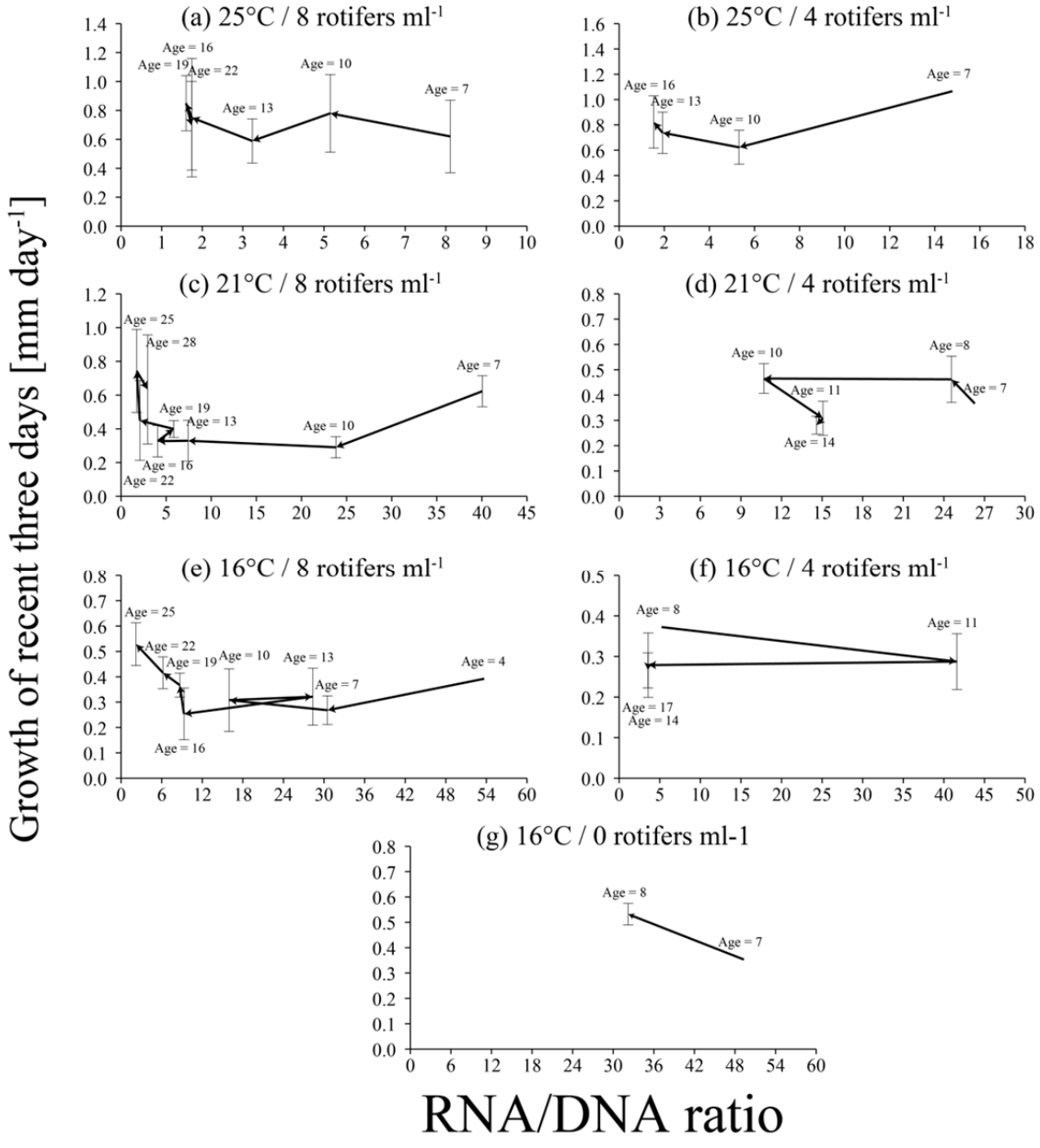

Fig. 3.13. Temporal transition of relationship between RNA/DNA ratio and growth with SD.

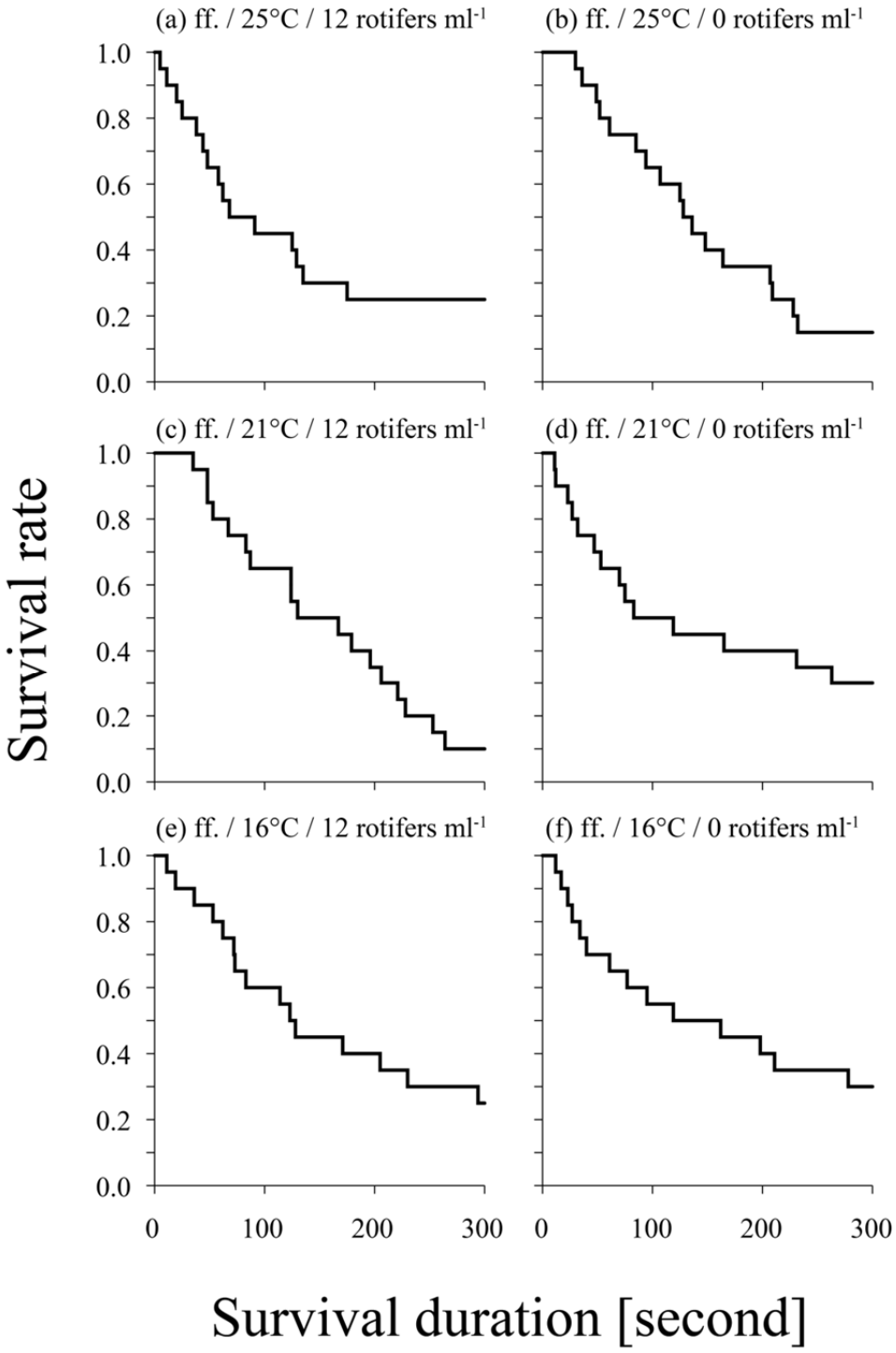

Fig. 3.14. Survival ratio in the predation experiment expressed as Kaplan-Meier's survival curve in larvae reared in different experimental conditions at one day after first-feeding. Each step in graphs represents an event of predation at a given time. The abbreviation ff. stands for one day after first-feeding.

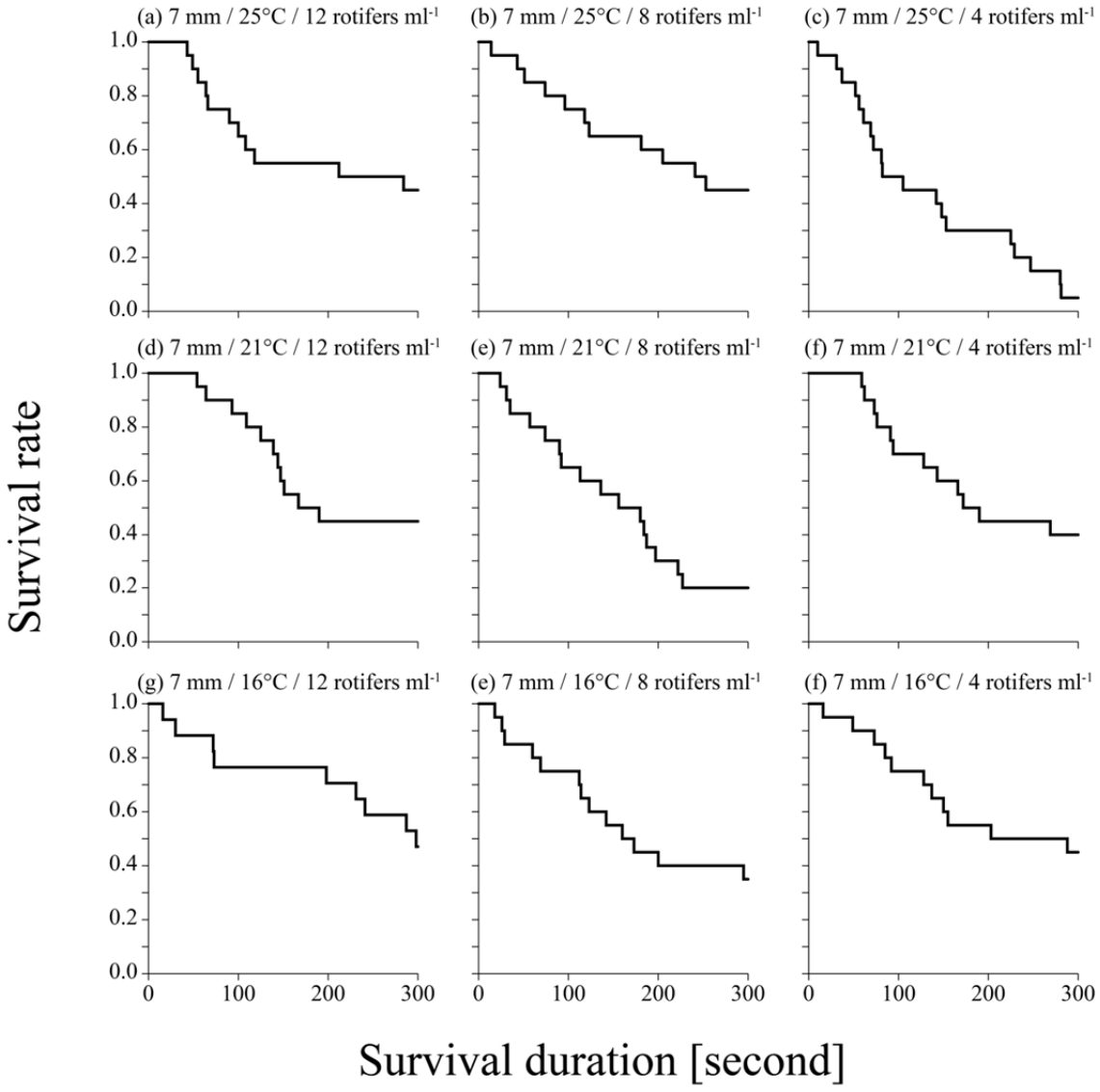

Fig. 3.15. Survival rate as a function of time in the predation experiment in 7 mm larvae reared in different conditions of temperature and food density.

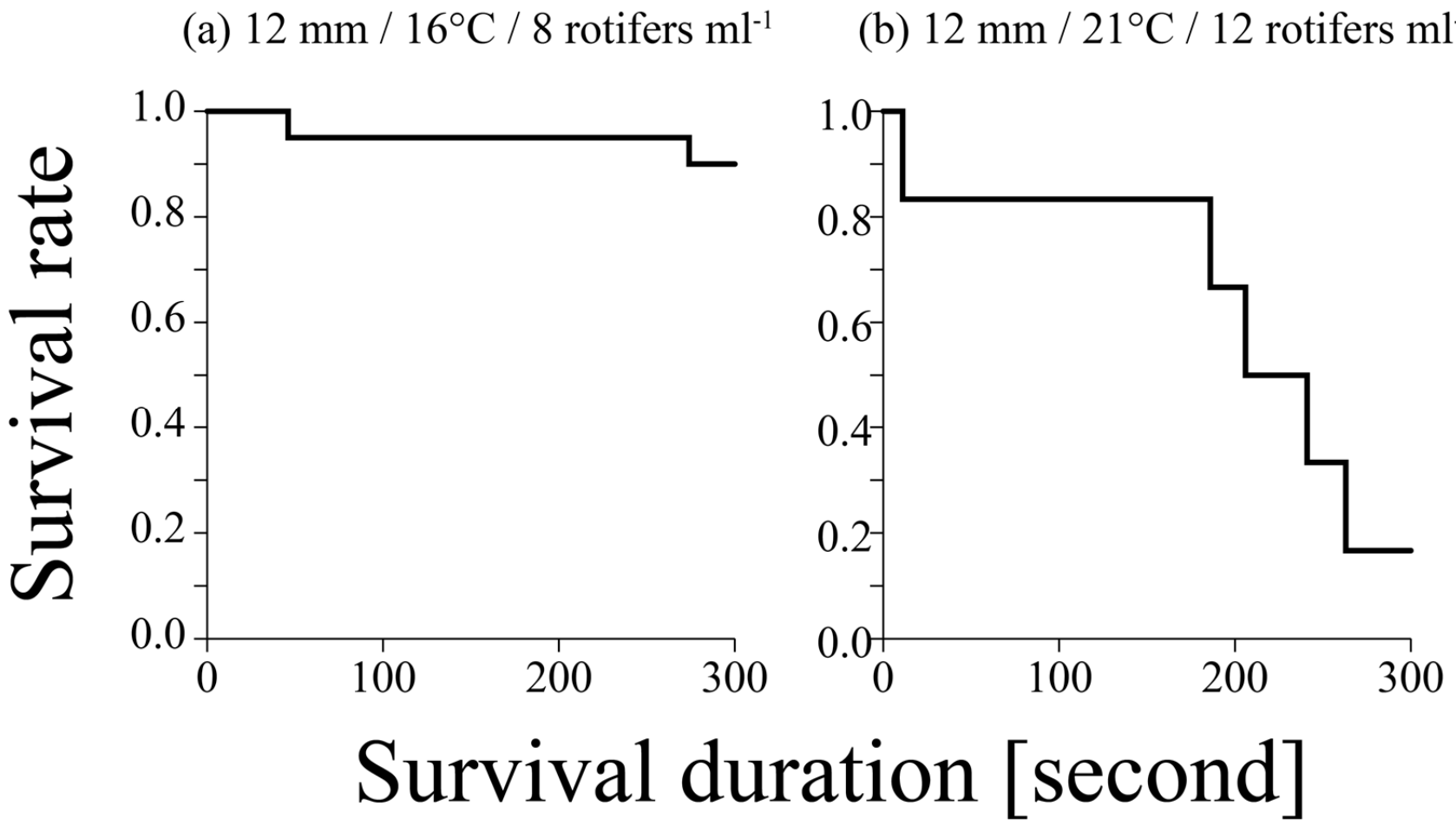

Fig. 3.16. Survival rate as a function of time in the predation experiment in 12 mm larvae reared in different conditions of temperature and food density.

Table 3.11. Summary of the parameter for survival rate. The parameter was expressed as recipricals. CI is confident interval. N_0 and N_{300} are numbers of individuals at start and end of the experiment, respectively.

Developmental stage	temperature (°C)	food (rotifers ml^{-1})	1/z	95% CI	predated / survived (N_o - N_{300} / N_{300})
one day after first-feeding	16	0	225	[139, 400]	14 / 6
		12	212	[133, 368]	15 / 5
	21	0	215	[133, 382]	14 / 6
		12	173	[113, 285]	18 / 2
	25	0	176	[113, 295]	17 / 3
		12	169	[106, 294]	15 / 4
7 mm	16	4	371	[216, 714]	11 / 9
		8	279	[169, 506]	13 / 7
		12	427	[237, 889]	9 / 11
	21	4	327	[195, 610]	12 / 8
		8	200	[127, 342]	16 / 4
		12	371	[217, 715]	11 / 6
	25	4	140	[92, 228]	19 / 1
		8	373	[218, 718]	11 / 9
		12	354	[206, 681]	11 / 9
12 mm	16	8	2860	[-1104, 17200]	2 / 18
	21	12	241	[112, 673]	5 / 1

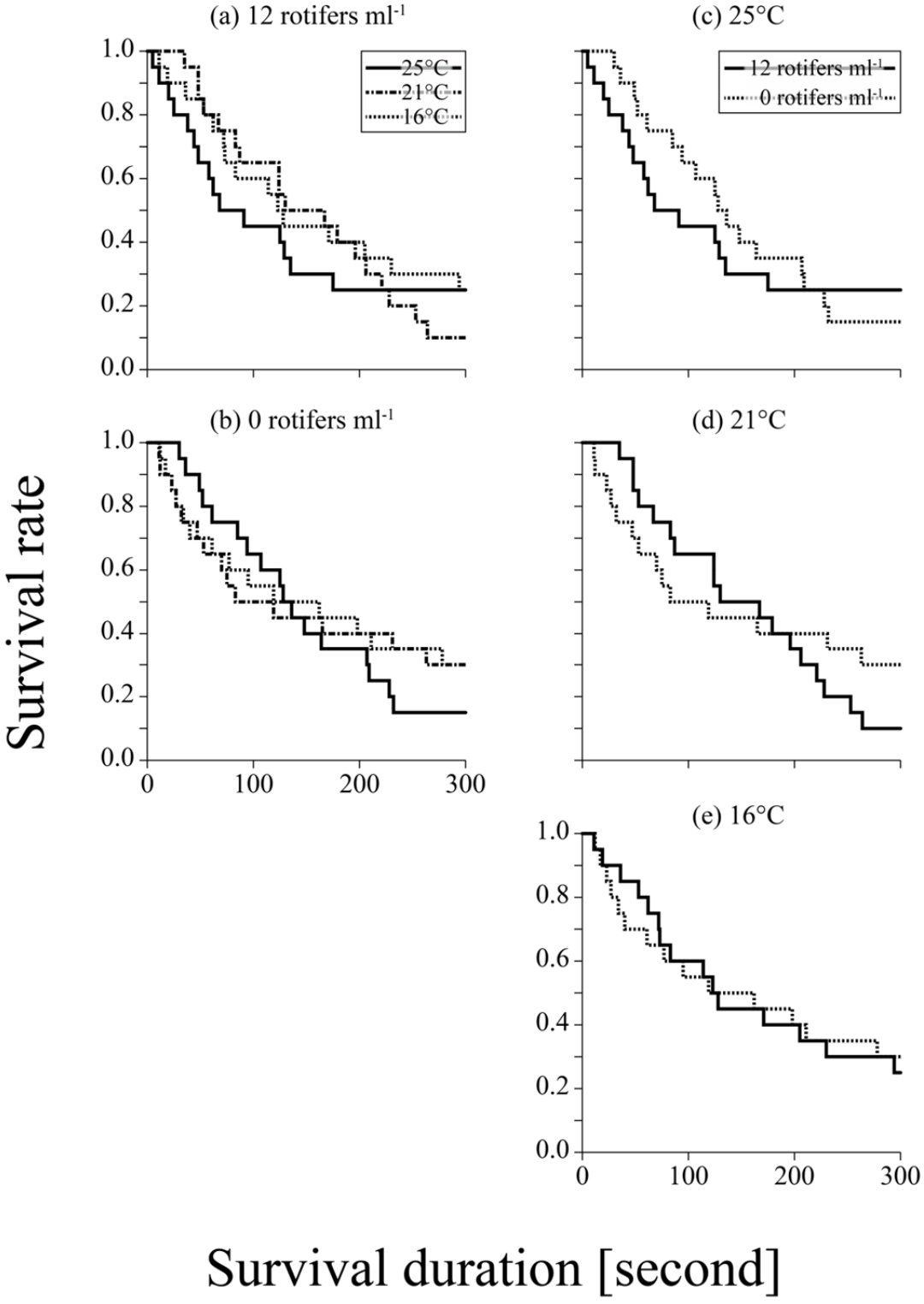

Fig. 3.17. Temperature and Food density comparisons of Kaplan-Meier's survival curves in larvae at one day after first-feeding. (a) and (b) compare temperature effect at each food density treatments, and (c), (d) and (e) compare food density at each temperature treatment.

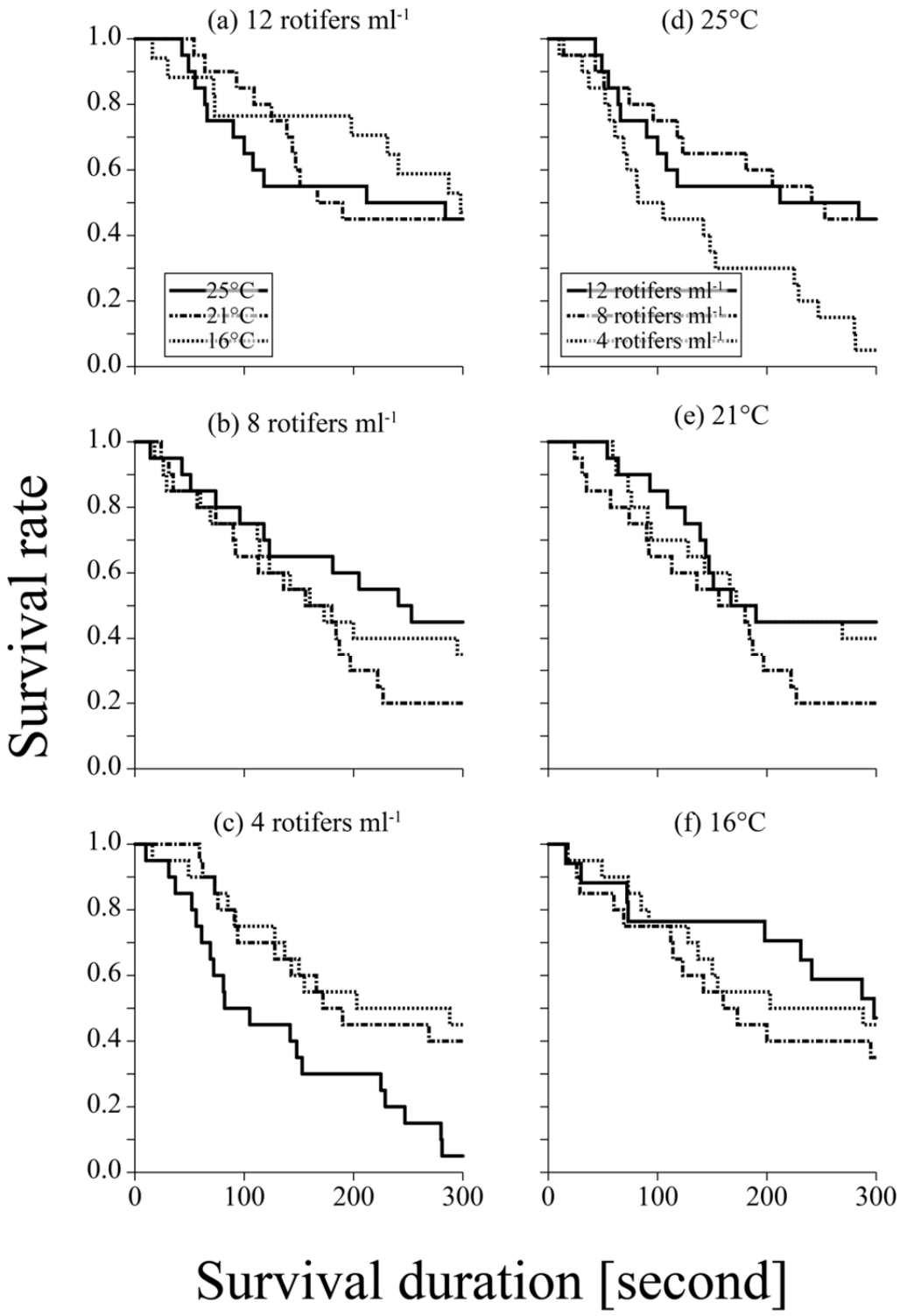

Fig. 3.18. Comparisons of Kaplan-Meier's survival curves in 7 mm larvae. (a), (b) and (c) compare temperature effect at each food density treatments, and (d), (e) and (f) compare food density at each temperature treatment.

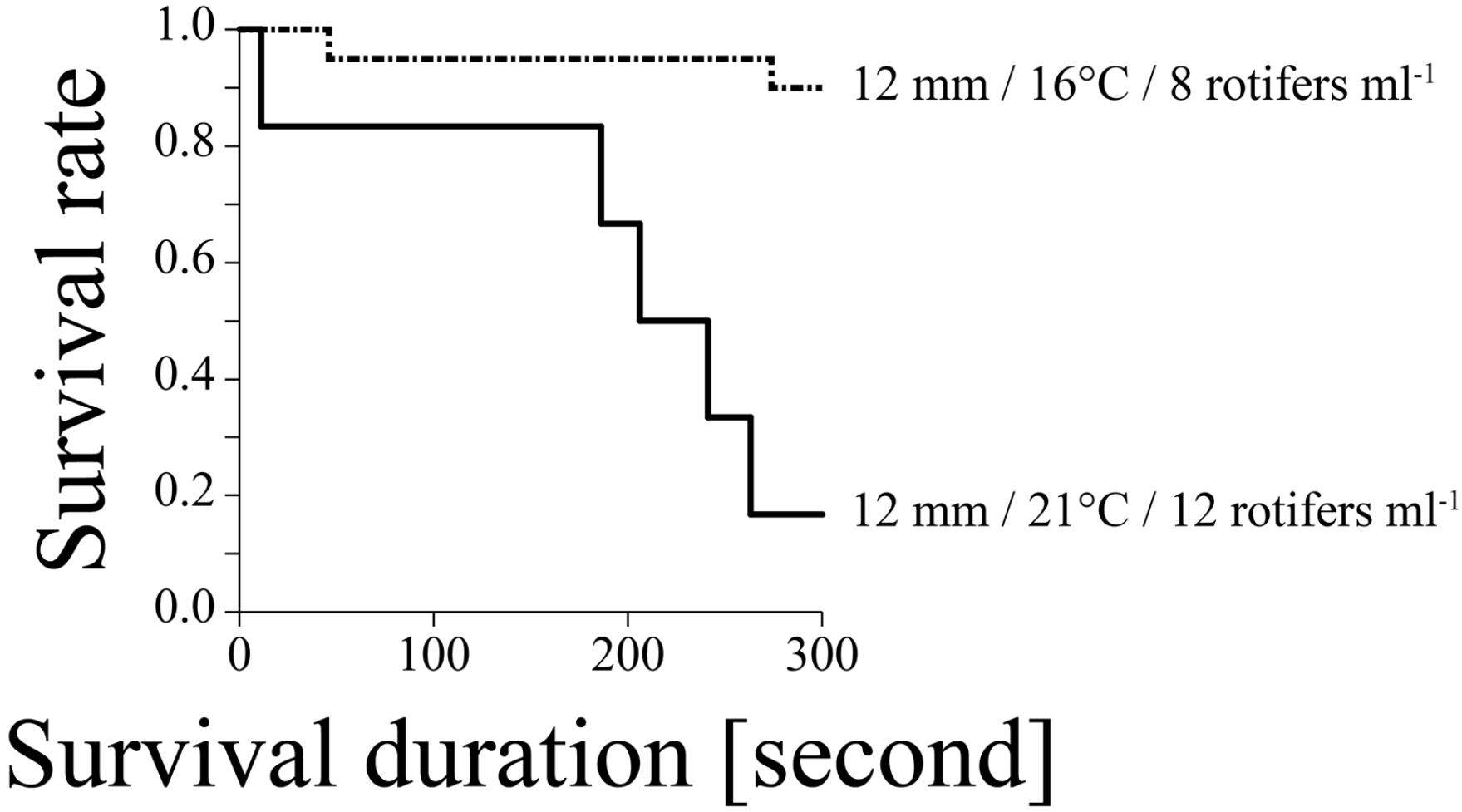

Fig. 3.19. A comparison of Kaplan-Meier's survival curves in 12 mm larvae.

Table 3.12. Summary of log-rank test for temperature and food density comparisons at each experimental stage.

Developmental stage	comparison	Food or Temp. condition	χ^2	*df*	*p*
one day after first-feeding	25°C vs. 21°C vs. 16°C	12 rot.	0.423	2	0.810
		0 rot.	0.329	2	0.848
	12 rotifers ml^{-1} vs. 0 rotifers ml^{-1}	16°C	0.009	1	0.925
		21°C	0.327	1	0.567
		25°C	0.098	1	0.754
7 mm	25°C vs. 21°C vs. 16°C	12 rot.	0.209	2	0.901
		8 rot.	2.623	2	0.269
		4 rot.	10.519	2	**0.005
	12 rotifers ml^{-1} vs.	25°C	9.941	2	**0.007
	8 rotifers ml^{-1} vs.	21°C	2.656	2	0.265
	4 rotifers ml^{-1}	16°C	1.056	2	0.590
12 mm	21°C / 12 rotifers ml^{-1} vs. 16°C / 8 rotifers ml^{-1}		16.993	1	**<.0001

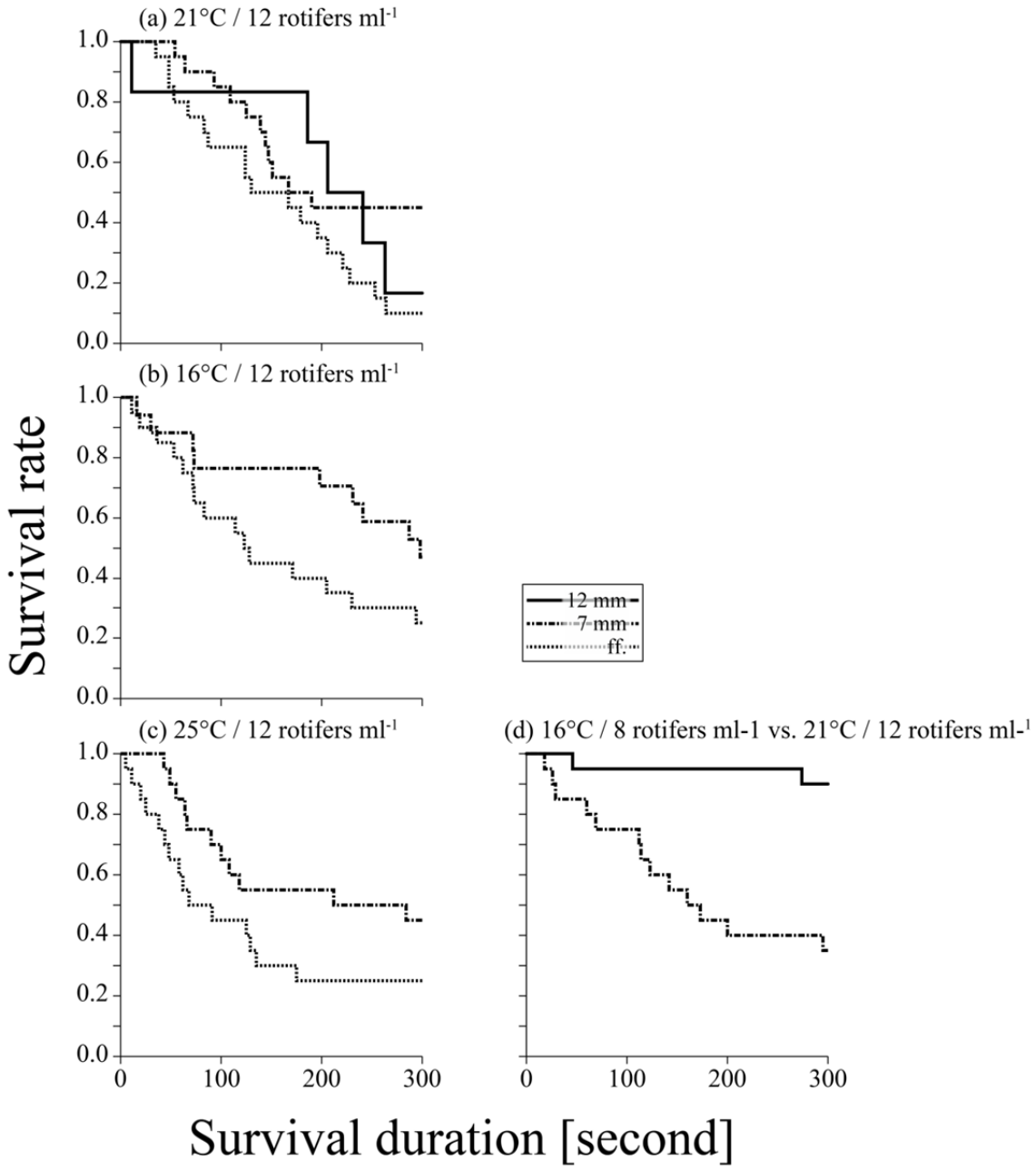

Fig. 3.20. Comparison of Kaplan-Meier's survival curves between stages.

Table 3.13. Summary of log-rank test statistics for stage comparison of survival rate. Stage of one day after first-feeding was abbreviated as ff.

Food and Temperature condition	comparison	χ^2	*df*	*p*
16°C / 12 rotifers ml^{-1}	ff. vs. 7 mm	2.780	1	0.096
21°C / 12 rotifers ml^{-1}	ff. vs. 7 mm vs. 12 mm	4.632	2	0.099
25°C / 12 rotifers ml^{-1}	ff. vs. 7 mm	2.731	1	0.098
16°C / 8 rotifers ml^{-1}	7 mm vs. 12 mm	13.191	1	**0.0003

4. Discussion

4.1. Broodstock rearing and spawning

Funamoto et al. (2004) reported that average maturity size of anchovy in Wakasa Bay was 8.53 cm and that GSI of female and male ranged from approximately 2 to 6 from April to March in the years 1999 and 2000. In the present research, fish were larger than minimum maturity size and the GSI was in the same range (Table 3.1 and Fig. 3.1). Values of GSI of males were higher than that of females. Lapolla (2001) reported higher GSI and earlier maturation of male in the case of bay anchovy *Anchoa mitchilli*. This is also the case in pacific herring *Clupea pallasii*, Japanese sardine (Takayanagi & Ishida, 2002) and several other pelagic fish species (Kawasaki, 1982), showing that males usually depend upon females for reproduction.

Japanese anchovy is known to initiate spawning at 14°C (Kawaguchi et al., 1990) and the spawning season ranges from April to August in Wakasa Bay (Funamoto et al., 2004). In the present study, adult anchovy also started spawning at a temperature of 14.8°C on 22 April and continued until 23 August (Fig. 3.1). The peak spawning occurred at about 21°C (Fig. 3.2), which is reported as the optimal temperature for the larval growth of Japanese anchovy (Takasuka, 2003). If the "optimum growth temperature" hypothesis prevails, this spawning ecology would reflect an adaptation to optimize growth rate in a maximum offspring number. This can be interpreted as inductive evidence for "optimum growth temperature" hypothesis. However, there is no direct evidence that the timing of maximum spawning in our broodstock tanks was driven from adaptive behavior. Relative batch fecundity and spawning frequency of Japanese anchovy are known to increase with water temperature in natural condition (Takasuka et al., 2005). In this study, spawners frequently died through the summer, which may explain the observed decrease in total egg production from

mid-summer. While the apparent “optimum growth temperature” may partly reflect such rearing artifact, we cannot discard the hypothesis of adaptive behavior described above.

4.2. Egg condition

Size and volume of eggs significantly decreased during the second half of the spawning season (Table 3.3), when the water temperature simultaneously increased (Fig. 3.1). There are two possible ecological hypotheses which might have worked independently or in synergy. First, larger fish may spawn larger eggs at lower temperature, so that average egg size decreases as temperature rises when smaller fish start to spawn smaller eggs. Second, several adaptive aspects of reproduction strategy can be assumed. Imai & Tanaka (1987) also demonstrated the negative correlation between surface water temperature and egg size of Japanese anchovy in both field and hatchery, and suggested their correspondence with the shift of the size of copepod from larger cold water to smaller tropical or temperate species, which is most important food for fish larvae. Iguchi et al. (1999) investigated seasonal changes in the copepod assemblage as food for larval anchovy in western Wakasa Bay and reported changes from temperate species in spring to sub-tropical species in autumn. Larger larvae hatched from larger eggs with larger mouth should thus select for larger food organisms and show higher resilience to starvation. In addition, Imai (2001) discussed that the emergence of a large number of smaller larvae may also provide an advantage at the population level against predation mortality, which usually increases with temperature, from *r* / *K* selection theory (MacArthur & Wilson, 1967). Variability in egg size may thus reflect trade-offs between starvation tolerance and predation avoidance.

In the present study, both size and volume of anchovy eggs increased during the first week of spawning, and then started to decline. This could be explained by the combination of the two

following factors (Fig. 4.1):

(a) Egg size frequency distribution from certain individuals would follow a dome shape function.

(b) A decreasing trend would occur by adaptive mechanisms described above.

(c) Actual egg size frequency distribution was a product (a) and (b).

The hypothesized mechanism of (a) cannot be tested directly, although various effects on egg size are likely to be complex enough to lead to such a trend. Furthermore, if adult anchovy maximize the number of eggs spawned at the "optimum growth temperature", egg size frequency would distribute as in (a). Further research is needed to test these proposed mechanisms.

4.3. Basic growth analysis

Temperature obviously had a larger effect than food density on larval growth (Fig. 3.5 and Table 3.5). "Optimum growth temperature" hypothesis was not confirmed in the present rearing experiment. This could be partly explained if the prey density of 4 rotifers ml^{-1} was beyond the satiation level for anchovy larvae. However, larvae survived better in the 8 rotifers ml^{-1} despite the absence of difference in observed growth (Fig. 3.6). Differences in survival rate between the 2 food treatments may be due to a sudden ontogenetic change in energy demand, which would have resulted in prey shortage at relatively high temperature (25°C) and low prey availability (4 rotifers ml^{-1}) In larval Japanese anchovy, growth may not always be an ideal indicator of larval condition and survival.

In this section, I examined physiological aspect of "optimum growth temperature" hypothesis in

laboratory-reared larvae. However, the “optimum growth temperature” likely reflects the combination of several complex processes. In a physiological sense, the “optimum growth temperature” is a trade-off between metabolism and energy intake. In an ecological sense, environmental factors such as the match or mismatch between larvae and their prey, may also be influenced by temperature and modulate the “optimum growth temperature”.

4.4. Otolith information

Age and increment count of otolith showed linear relationships in most treatments except for the lowest temperature of 16°C (Fig. 3.7 and Table 3.6). Folkvord et al. (2004) also reported significantly lower marking success rate in increment deposition of otolith in Atlantic herring *Clupea harengus* reared at 4°C relative to 12°C. Marking failure in lower temperature may be a characteristic of Clupeiforms and is likely to be induced by low metabolism or low feeding activity.

The effect of food density was minor, whereas temperature greatly affected the size-specific growth of otolith (Fig. 3.9 and Table 3.9). Temperature dependency of otolith growth was thus revealed in Japanese anchovy for the first time to our knowledge. This phenomenon can lead to a large error in the biological intercept method.

Folkvord et al. (2004) examined temperature dependency of otolith growth in Atlantic herring and reported that the growth rate of otolith was more than four times higher in the group reared at 12°C relative to that reared at 4°C. Mosegaard et al. (1988) studied the effects of temperature on both somatic and otolith growth rate in Arctic char *Salvelinus alpinus* and found discrepancies between these two growth rates. On the other hand, Otterlei et al. (2002) reported no clear difference in otolith growth at different temperature in Atlantic cod *Gadus morhua*. Temperature dependency of otolith growth is probably species-specific but is obvious in Clupeiform fishes. Further studies on

this subject are required to exclude any potential bias in the biological intercept method for this and other commercially important species.

4.5. RNA/DNA ratio Analysis

Values of RNA/DNA ratio diverged in smaller SL and no clear trend was found among treatments (Fig. 3.12). Moreover, RNA/DNA did not correlate with three-day recent growth index derived from otolith analysis (Fig. 3.13). Failure of this analysis was probably due to the insufficient body mass of sampled larvae. Rearing needs to be conducted at larger scale to obtain a sufficient number of larvae for RNA/DNA ratio analysis.

To date, no standard technique for RNA/DNA analysis has been established and methodological detail can strongly affect the estimated RNA/DNA ratio. Differences in analytical protocol can explain 57.1% of the variations (Caldarone et al., 2006). RNA/DNA ratio analysis is therefore a delicate method so that sophistication of experimental technique and well-equipped laboratory are needed.

4.6. Predation by moon jellyfish

In the comparison of larval survival rate in the presence of jellyfish predators within developmental stages, no clear difference was found among experimental treatments, except for 4 rotifers ml^{-1} / 25°C at 7 mm, where the lowest survival rate was observed. The food limitation in 4 rotifers ml^{-1} discussed in chapter 4.4 may also have affected larval anti-predator performance.

Feeding strategy of moon jellyfish may have driven the present result. Because the moon jellyfish

is a tactile predator which ambushes and captures prey following an encounter, mortality rate may largely depend on larval activity, which is in turn affected by temperature. At higher temperature, larvae may encounter the ambush predator more frequently. In this sense, moon jellyfish could be considered as a "non-growth-selective predator" (Takasuka et al., 2007[a]) at least until larvae attain certain size, and perhaps as a "negative-growth-selective predator" because encounter rate seems to increase with growth. In contrast, survival comparisons among life stages (Fig. 3.20) revealed that 12 mm larvae survived better than 7 mm larvae at 16°C / 8 rotifers ml^{-1}. At a SL of 12 mm, anchovy might have already attained a size refuge and were able to escape effectively from moon jellyfish. The "negative-growth-selective predation" pressure would occur in larvae smaller than 12 mm.

4.7. Effect of climate change on anchovy

In this study, temperature had a positive effect on both growth and mortality of larval Japanese anchovy. Populations of Japanese anchovy will thus likely respond to global climate warming. At higher temperature, while growth of larval anchovy is expected to increase, vulnerability to starvation would be magnified due to the increase of basal metabolism at high temperature. Moreover, species composition of the copepod assemblage, which represents the most important food source for Japanese anchovy, would shift to smaller warm-water species (Beaugrand & Reid, 2003). This could have consequences on feeding success and survival rate. Because anchovy plays a major role transferring energy from secondary producers to upper trophic levels, any change in population dynamics will strongly impact the coastal marine ecosystem.

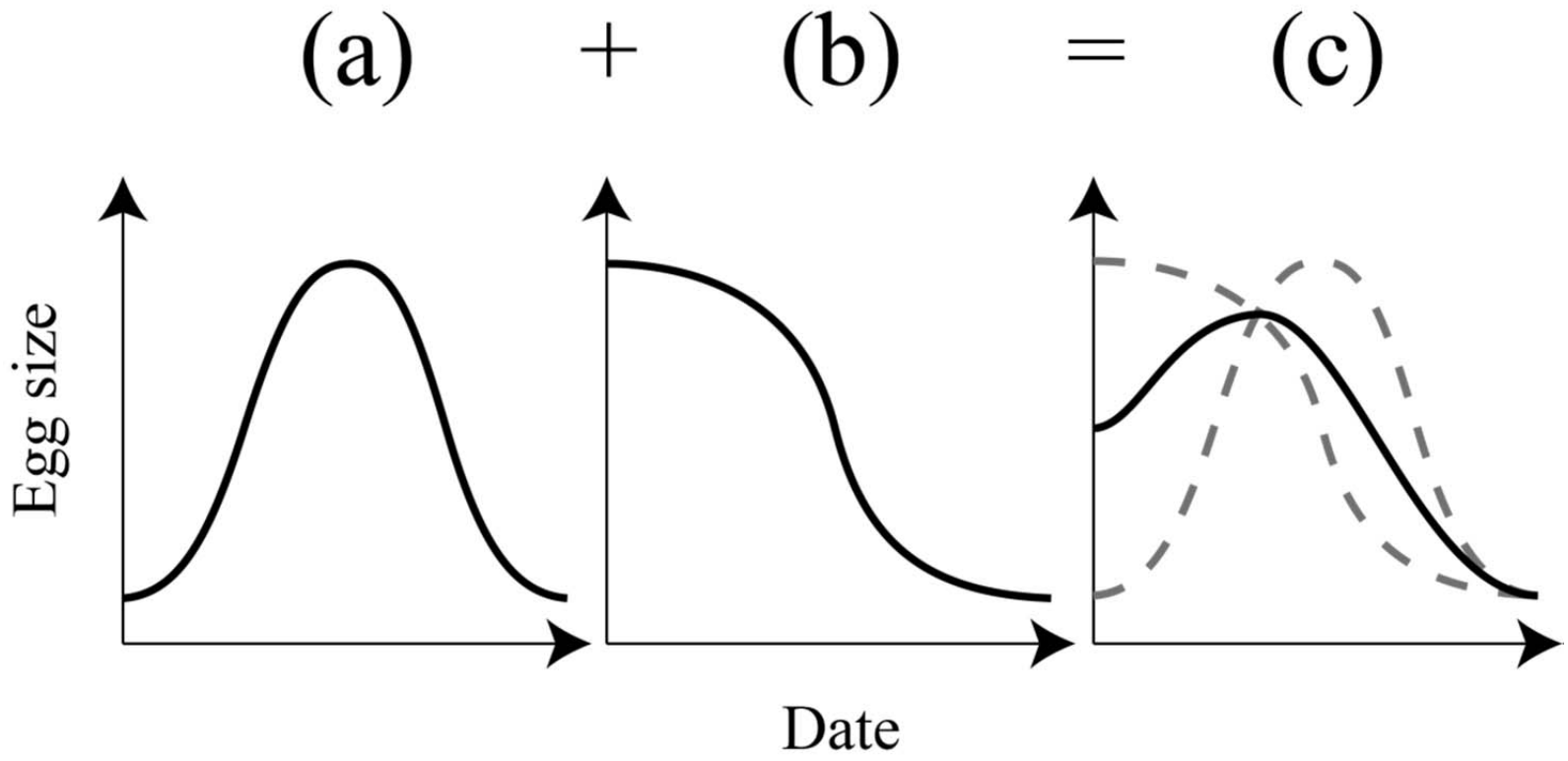

Fig. 4.1. Hypothetical mechanisms of decreasing egg size during the spawning season. In (a), Egg size frequency distribution from certain individuals would follow a dome-shaped function. In (b), a decreasing trend would occur through adaptive mechanisms described above. In (c), actual egg size frequency distribution is a product (a) and (b).

5. Conclusion

(1) The number of eggs spawned followed a dome-shaped relationship and peak spawning occurred near 21°C, which is reported as optimum temperature in the literature. This is likely an outcome of reproductive adaptation.

(2) Temperature had a positive effect on larval growth of Japanese anchovy, and a negative effect on their survival. Food density did not have a major effect on growth but had positive effect on survival.

(3) Temperature dependency of otolith growth rate and the lack of daily increment deposition at low temperature was revealed in Japanese anchovy for the first time. This phenomenon can result in considerable bias when using the biological intercept method to back-calculate growth trajectory.

(4) Higher temperature enhanced growth of anchovy larvae as well as their swimming activity. This raised encounter rate with jellyfish ambush predators, suggesting that moon jellyfish is a "non-growth-selective predator" (Takasuka et al., 2007[b]) or even a "negative-growth-selective predator".

(5) Climate change can affect growth and anti-predator performance of Japanese anchovy. Fluctuations in the dynamics of populations will impact coastal marine ecosystems.

6. Abstract

Temperature and food availability are two major factors regulating larval growth, survival, and recruitment of marine fish populations. The survival rate of Japanese anchovy *Engraulis japonicus* larvae is predicted by the “optimum growth temperature” hypothesis, stipulating that survival follows a dome-shaped relationship as a function of temperature. The hypothesis was however solely derived from field-based observations and laboratory experimentation is required to refine our understanding of the mechanisms driving this function. The present study aimed to assess the effect of variations in temperature and food density on the growth and survival rates of hatchery-reared anchovy, providing a direct test for the “optimum growth temperature” hypothesis.

A broodstock of mature Japanese anchovy was reared in the laboratory, and the size and number of spawned eggs were monitored. Upon spawning, eggs were rapidly transferred to larval rearing tanks in a temperature- and photoperiod-controlled room. Larvae were reared in different conditions of temperature and food density, and at determined time intervals, each tank was sampled to estimate somatic growth, otolith growth and vulnerability to a jellyfish predator. Larval growth was estimated through the measurement of standard length, RNA/DNA ratio, and otolith microstructure analysis. The moon jellyfish *Aurelia aurita* was used for the predation experiment. Experimental treatments were defined as Factor = Temperature [16, 21, 25°C] × Food [0, 4, 8, 12 individuals of rotifers ml-1)].

The number of eggs spawned daily followed a dome-shaped relationship as a function of temperature, with peak spawning occurring near 21°C, which is reported as the optimum temperature for larval growth. This may reflect adaptation to optimize growth rate for a maximum number of offspring. Egg size significantly decreased as temperature increased through the season. Because egg size determines larval hatching size, we argue that the variation in egg size reflects a

trade-off strategy in r / K selection theory, in which a relatively small number of large larvae may tolerate starvation early in the season, while the bulk of larvae hatching later at smaller size benefit from optimum growth conditions and experience lower cumulative predation pressure. Larval growth was positively linked to temperature, but no significant relationship emerged between growth and food density. Despite the absence of a relationship linking growth to prey density, larval survival rate increased with prey density. This suggests that growth is not always an indicator of larval condition and survival potential. The present research conducted in controlled environmental conditions did not fully support the "optimum growth temperature" hypothesis. Other ecological processes, such as "match-mismatch" between larvae and their prey, may combine with the "optimum growth temperature" mechanism in natural conditions. The temperature dependency of otolith growth and the lack of daily increment deposition at low temperature were revealed in Japanese anchovy for the first time. These aspects can result into large sources of error in the back-calculation of growth rate, and some previous studies on Japanese anchovy may therefore need reconsideration on this potential bias. In the predation experiment, one of the trials at 25°C was characterized by the significantly lower survival rate. Higher temperature likely enhanced larval growth, but also metabolism and activity level of both anchovy larvae and jellyfish, resulting in increased encounter rate between the larvae and their ambush predators. The moon jellyfish could thus be considered as a "non-growth-selective predator", or even a "negative-growth-selective predator".

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