

1 **Ontogeny of swimming speed, schooling behaviour and jellyfish avoidance by**

2 **Japanese anchovy *Engraulis japonicus***

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8 Running headline:

9 Ontogeny of anti-predator behavior in anchovy

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21 The ontogeny of swimming speed, schooling behaviour and jellyfish avoidance was
22 studied in hatchery-reared Japanese anchovy *Engraulis japonicus* to compare its life
23 history strategy with two other common pelagic fishes, jack mackerel *Trachurus*
24 *japonicus* and chub mackerel *Scomber japonicus*. Cruise swimming speed of anchovy
25 increased allometrically from 1.4 to 3.9 folds of standard length per second ($L_S s^{-1}$)
26 during the course from early larval to metamorphosing stage. Burst swimming speed
27 also increased from 6.1 to 28 $L_S s^{-1}$ in these stages. Cruise speed was inferior to that of
28 chub mackerel, as was burst speed to that of jack mackerel. Anchovy larvae were highly
29 vulnerable to predation by moon jellyfish *Aurelia aurita* and were readily eaten until
30 they reached 23 mm L_S , but not at 26 mm L_S . Schooling behavior (indicated by parallel
31 swimming) started at about 17 mm L_S . Average distance to the nearest neighbour was
32 shorter than values reported in other pelagic fishes. The relatively low predator
33 avoidance capability of Japanese anchovy may be compensated for by their transparent
34 and thus less conspicuous body, in addition to their early maturation and high fecundity.

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36 Key words: *Aurelia aurita*; behavioural ontogeny; Engraulidae; population dynamics;
37 predator avoidance; shoaling.

INTRODUCTION

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40 The world's largest fisheries have largely depended on clupeoids such as sardine
41 *Sardinops* spp., herring *Clupea* spp. and anchovy *Engraulis* spp. (Hunter, 1980), among
42 which anchovy are now probably the most dominant in many temperate areas. Periods
43 of increased anchovy populations coincide with the declines of sardine populations off
44 Japan, California, Peru and Chile (Chavez *et al.*, 2003). In the case of Japanese coastal
45 waters, three major pelagic fishes, i.e., chub mackerel *Scomber japonicus* Houttuyn,
46 jack mackerel *Trachurus japonicus* (Temminck & Schlegel), and Japanese anchovy
47 *Engraulis japonicus* (Houttuyn), filled the empty niche resulting from the collapse of
48 sardine *Sardinops melanostictus* (Temminck & Schlegel) populations in the late 1980s.
49 Dominance of one or two of these species occurs at *c.* 10-year intervals (Matsuda *et al.*,
50 1991). Takasuka *et al.* (2008) studied the optimum spawning temperature of these fishes
51 and found that *Sardinops melanostictus* and *Scomber japonicus* are stenothermal species
52 with peak spawning at 16° C and 18° C, respectively, whereas *Trachurus japonicus* and
53 *Engraulis japonicus* are eurythermal with optimum spawning at *c.* 22° C. This is
54 coincident with the theory that fluctuations in pelagic fish populations are governed by
55 global climatic change, the so-called "regime shift theory" (Kawasaki, 1992). Yet at
56 each stage of population replacement, it is still not clear what would be the direct cause
57 of mortality leading to the competitive advantage for any one species. It is necessary to
58 elucidate which species will benefit from certain environmental conditions in order to
59 better understand this cyclic pattern of population dynamics.

60 On the other hand, the population of anchovy *Engraulis encrasicolus* (L.) in the
61 Black Sea has been seriously depleted over the past two decades in part due to predation
62 by a gelatinous predator, an alien ctenophore, *Mnemiopsis leidyi* (Agassiz), that invaded
63 in the 1980s (Shiganova, 1998). Castro et al. (2000) also found an apparent inverse
64 relationship between the anchovy *Engraulis ringens* Jenyns larval density and
65 gelatinous predators (medusae, ctenophores and siphonophores) off Chile. Because
66 recently jellyfish blooms have become ubiquitous phenomena (Purcell *et al.*, 2007),
67 anchovy and other pelagic fish populations may decline due to the increased predation
68 and competition.

69 Ecological aspects of pelagic fishes have been intensively studied by using otolith
70 analysis of wild collected specimens (*e.g.* Takahashi & Watanabe, 2005; Takasuka *et al.*,
71 2007). Survival in the wild, however, is the outcome of the combination of predation
72 pressure and growth, both dependent on temperature and other environmental factors.
73 However, an experimental approach using hatchery-reared fish may allow insights into
74 differences in biological performance such as growth and behavior, as have been
75 conducted on Atlantic herring *Clupea harengus* L. (Hunter, 1972), northern anchovy
76 *Engraulis mordax* Girard (Blaxter, 1985) and red drum *Sciaenops ocellatus* (L.)
77 (Fuiman *et al.*, 1999).

78 The goal of the present work was to collect basic information related to the
79 ontogeny of anti-predator behaviour such as swimming speed, responsiveness, jellyfish
80 avoidance and initiation of schooling behaviour in *Engraulis japonicus*. Masuda (2006)
81 reported that among common pelagic fishes *Scomber japonicus* is an exceptionally fast

82 grower with a faster cruise swimming speed compared to *Trachurus japonicus* whereas
83 *T. japonicus* has a fast burst swimming speed and has a high avoidance capability from
84 moon jellyfish *Aurelia aurita* (L.). In the present work, equivalent data for *E. japonicus*
85 were collected to conduct interspecific comparisons among these three species.

86

87 MATERIALS AND METHODS

88

89 HUSBANDRY OF BROODSTOCK AND HATCHERY PRODUCTION

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91 Candidates of broodstock were obtained at a commercial set net operated by local
92 fishermen in Tai, Maizuru, Kyoto (35° 56' N, 135° 45' E) on 15 May 2007. About 200
93 adult *E. japonicus* were collected from the catch, kept in two 70 l-containers,
94 transported to the Maizuru Fisheries Research Station (MFRS) of Kyoto University and
95 stocked in a broodstock tank (4 m in diameter, 30 m³ of sea water). Mean ± S.D.
96 standard length (L_S) of sub-sampled males (n = 17) and females (n = 13) were 100.8 ±
97 6.8 mm and 101.1 ± 8.9 mm, and their gonado-somatic index ($I_G = 100 \times (\text{gonad}$
98 $\text{weight}) \times (L_S)^{-1}$) were 1.50 ± 1.24 and 1.26 ± 0.99, respectively. Filtered seawater was
99 provided in the broodstock tank. They were fed with c. 100 g of defrosted krill twice
100 daily until satiation. First spawning was confirmed at 20.2° C on 13 June. Spawning
101 continued until mid August. The highest number of eggs spawned per day was 350,000
102 recorded on 25 July.

103 Eggs obtained in the morning of 10 July were used for the following hatchery

104 production: *c.* 44,000 eggs were spawned under natural temperature (21.8° C) and
105 sub-samples of *c.* 11,000 floating eggs were placed into each of two black 500-l
106 polyethylene tanks (Tank 1 and Tank 2). Water was exchanged at a rate of 70 l h⁻¹ with
107 filtered seawater at 22.5° C and air was provided at a rate of 600 ml min⁻¹. Hatching
108 was confirmed at 0700 hours on 11 July. Rotifers *Brachionus plicatilis* Müller were fed
109 with a density of 3 ind. ml⁻¹ in the afternoon of the 1st day post hatch (dph), and with a
110 density of 5 ind. ml⁻¹ at 0700 and 1400 hours thereafter until 20 dph. Defrosted green
111 algae *Nannochloropsis* sp. was added to each tank 20 ml at 0800 and 10 ml at 1500
112 hours to adjust the density to 40,000 cell ml⁻¹. *Artemia* sp. nauplii at 0.1-0.3 ind. ml⁻¹
113 enriched with nutritional supplement (Marine Gross, Nisshin Marinotech, Japan) were
114 fed from 20 dph until the end of experiment. Three to 10 individuals were sample from
115 both tanks daily until 15 dph, and at 2–5 days intervals thereafter for the measurement
116 of *L_S*. Fish were raised at natural temperature ranging from 21.3 to 25.1° C (23.2 ± 1.0°
117 C, mean ± S.D.) for the first 30 days, then 24.4 to 27.9° C (26.7 ± 1.1° C) up to 50 dph.

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119 MEASUREMENTS OF BEHAVIOURAL ONTOGENY

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121 Cruise and burst swimming speeds were measured every five days starting on 10
122 dph as previously described by Masuda *et al.* (2002). In this method, a single fish was
123 transferred to a glass container (10–30 cm diameter depending on the size of fish)
124 covered with a black vinyl sheet on its wall and the movement of fish was recorded
125 from above using a video camera (DCR-HC96, Sony, Tokyo, Japan). After recording for

126 90 s without disturbance, the fish was startled by sound stimuli to elicit burst response.
127 Consistent sound stimuli were produced using a pendulum (a 10 g of steel nut hung by a
128 string) that was released from a distance of 17 cm from the container wall. Preliminary
129 observation revealed that visual stimuli such as casting a shadow or flashing a camera
130 strobe did not induce consistent responses and that a larva did not seem to visually
131 recognize a predator such as jellyfish until sensing the water current made by a jellyfish.
132 Sound stimuli were provided three times at 10-s intervals. This procedure was repeated
133 for 10 individuals. Cruise swimming speed was measured from 10 s of recording before
134 providing the sound stimulus by tracing the movement of the fish using a flexible ruler
135 on a TV monitor. Burst swimming speed was measured only when the fish made a burst
136 start in response to the sound stimulus, and the largest value among the three trials was
137 used for further analysis. The burst swimming speed was defined as the movement in
138 0.13 s corresponding to four frames of the video recording. The distance was divided by
139 its standard length (L_S) to express speed as $L_S \text{ s}^{-1}$. Responsiveness, defined as the
140 percentage of obvious burst swimming responses against the provided stimuli, was also
141 measured at each date of measurement.

142 Escape performance exposed to jellyfish was visually observed using a method
143 previously reported by Nakayama *et al.* (2003) and Masuda (2006). Moon jellyfish
144 *Aurelia aurita* were collected off the MFRS by SCUBA diving. They were kept in two
145 500-l tanks and were used within 1 week after collection. Three actively pulsating *A.*
146 *aurita* (mean \pm S.D. bell diameter = 82.8 ± 7.5 mm) were put in a 10-l plastic container.
147 A larger tank made it impossible to follow the movement of the smallest larva and thus

148 this size of container was used. A larva of *E. japonicus* was gently put in this container,
149 and then observations were conducted to measure how long it took for the fish to be
150 captured by *A. aurita*. The observation was conducted for 5 min, and if the fish was not
151 eaten, it was removed and was replaced by another individual. Ten trials were conducted
152 every 5 days starting from 10 dph up to 40 dph (*i.e.* 70 individuals in total). Same
153 individuals of *A. aurita* were used throughout the replicates on each day, after which
154 they were released in the sea.

155 Behaviour in rearing tanks was also recorded via a video camera as described by
156 Masuda *et al.* (2003) starting on 20 dph with 3-5 days interval up to 50 dph. Two
157 semicircular reflective panels were put on the tank bottom *c.* 5 min prior to recording to
158 produce a clear image contrast. Focus of the video camera was locked at the layer with
159 highest density of larvae, and video analysis was conducted only on well-focused
160 individuals to capture behaviour of fish swimming in nearly two dimensions. Recording
161 was started at 1500 hours and lasted 5 min each day. Water and aeration was stopped
162 while video recording to minimize the effect of current on behaviour.

163 Three parameters of schooling were defined for video analysis: separation angle
164 (SA), distance to the nearest neighbour (D_{NN}) and separation swimming index (I_{SS}) (Fig.
165 1). SA and D_{NN} were criteria of parallel orientation and aggregation, respectively, and
166 were calculated on a still frame (Masuda *et al.*, 2003). A fish close to the centre of the
167 video frame was focused on (focal fish), and then the fish closest to the focal fish was
168 defined as the neighbouring fish. The angle between the body axis of the focal fish and
169 the neighbouring fish was measured on the TV monitor using a protractor and was

170 defined as the SA. On the same frame, three other individuals close to the focal fish
171 were selected then the distance between each fish to the closest individual from each
172 was measured by a ruler. The average of these five measurements was defined as the
173 D_{NN} of this frame. SA and D_{NN} were sampled from 30 frames each separated with a 20-s
174 interval. SA, ranging from 0° to 180° , was expected to be 90° when fish were located in
175 a random direction and was expected to decrease as they developed a parallel
176 orientation. D_{NN} was also expected to decrease as fish aggregated. D_{NN} was divided by
177 L_S to facilitate the comparison among different size groups.

178 I_{SS} was analysed from the same video frames as SA and D_{NN} measurements with
179 the addition of frames 1 s after these (Fig. 1b). I_{SS} reflects the tendency of parallel
180 swimming that is typical in the early stage of schooling (Masuda *et al.*, 2003). For the
181 measurement, vectors of the focal fish and the neighbouring fish were defined as
182 movement in a 1-s interval. Then the neighbouring fish vector was moved so that its
183 starting point overlaps with that of the focal fish, and the distance of the endings of two
184 vectors was divided by the average length of the two vectors. I_{SS} ranges from 0 to 2 and
185 it is expected to be 1.49 when two neighbouring individuals moved in random
186 directions at random speeds according to the simulation by Nakayama *et al.* (2007).

187 Routine swimming speed was also measured from video recordings in rearing tanks.
188 The movement of the focal fish in each video frame was traced for 1 s on a TV monitor.
189 The body length of the focal fish was measured on the TV monitor, and the routine
190 speed was expressed as $L_S \text{ s}^{-1}$.

191

192 STATISTICAL ANALYSIS

193

194 For the inter- and intraspecific comparisons, swimming speed data of *E. japonicus*
195 was divided into three size groups as pre-flexion (3.7–10 mm), post-flexion (10–21
196 mm) and metamorphosing (21–33 mm) larvae based on the criteria detailed in Fukuhara
197 (1983). Cruise and burst swimming speeds were compared among these three different
198 size groups using the Kruskal-Wallis test followed by the Dunn's test (Zar, 1996).
199 Swimming speeds of *E. japonicus* were then compared to those in *Trachurus japonicus*
200 and *Scomber japonicus* measured using the same methods (Masuda, 2006).
201 Responsiveness was compared among different size groups and species in the same
202 manner. In the analysis of survival against *A. aurita* predation, the Kruskal-Wallis test
203 followed by the Dunn's test were applied to compare different age (and size) of *E.*
204 *japonicus* larvae. The parameters of schooling behaviour were measured separately in
205 duplicated tanks, log transformed to improve the homogeneity of variance, and were
206 compared among different age groups in each tank using ANOVA followed by the
207 Tukey's test after a Bonferroni correction. SA and I_{SS} in each group were also compared
208 to 90° and 1.49, respectively, by t-test. All calculations for the statistical analysis were
209 conducted using JMP ver. 5.0.1J software (SAS Institute, Cary, NC, USA) except for the
210 Dunn's test (Zar, 1996).

211

212

RESULTS

213

214 Diameters of *E. japonicus* eggs were 1.25 ± 0.07 and 0.64 ± 0.03 mm in long and
215 short axis, respectively (n = 11) (Fig. 2). Mean notochord length of hatched larvae was
216 2.80 ± 0.06 mm. Pigmentation of the retina was first confirmed in the evening of 1 day
217 post hatch (dph) and all larvae had pigmented eyes on 2 dph. They attained 5.9 mm on
218 10 dph, 11.0 mm on 20 dph, 21.5 mm on 30 dph, 28.3 mm on 40 dph and 36.5 mm on
219 50 dph (Fig. 2). The growth of *E. japonicus* was fitted with an exponential curve as
220 follows: $L_S = 3.37 \times e^{0.0542\text{dph}}$ (R = 0.963).

221 Cruise swimming speed of *E. japonicus* showed allometric development showing
222 $0.5\text{--}3 L_S \text{ s}^{-1}$ in the larval (< 21 mm L_S) stage but they had a much higher value of $2\text{--}7$
223 $L_S \text{ s}^{-1}$ at the metamorphosing stage. Cruise speed in the metamorphosing stage of *E.*
224 *japonicus* was higher than values in the larval stage (Dunn's test, $P < 0.05$; Table I).
225 Cruise speed of *E. japonicus* in 10–21 mm L_S was significantly slower than that of
226 *Scomber japonicus* in the same size range. Burst swimming speed also showed an
227 allometric development. Burst speed of metamorphosing *E. japonicus* was significantly
228 faster than smaller (< 21 mm) individuals. Burst speeds of *E. japonicus* larvae were the
229 slowest among the three fish species, and were significantly slower than those of
230 *Trachurus japonicus* for both size ranges. Responsiveness to sound stimuli gradually
231 increased as fish grew, increasing from 43–50% in 6–11 mm L_S larvae to 87–93% in
232 17–26 mm L_S larvae. Responsiveness of *E. japonicus* was significantly inferior to that
233 of *T. japonicus* for both size ranges (Table I).

234 Larvae of *E. japonicus* smaller than 11 mm L_S were preyed upon by *A. aurita* in
235 150 s on average (Fig. 3). Anti-predator performance gradually improved, although

236 three out of ten individuals were eaten even when they attained 23 mm L_S . All ten
237 individuals survived 5-min predation trial in 26 mm or larger fish. Survival of *E.*
238 *japonicus* exposed to *A. aurita* was markedly inferior to that of *T. japonicus* and *S.*
239 *japonicus* (Masuda, 2006).

240 Separation angle (SA) gradually decreased in larvae from 15 to 25 mm L_S ,
241 indicating that parallel orientation developed during this period (Fig. 4a). Distance to
242 the nearest neighbour (D_{NN}) also decreased in this period, and values for 23 mm L_S fish
243 and larger showed an almost consistent value between 0.7 and 1.0 L_S (Fig. 4b).
244 Decrease of the separation swimming index (I_{SS}) was also remarkable between 15 and
245 25 mm L_S (Fig. 4c). SAs were significantly smaller than 90° for all fish in the 17 mm L_S
246 size group and larger (*t*-test, $P < 0.05$; Fig. 4a). I_{SS} were also smaller than 1.49 for all
247 groups at 17 mm L_S and larger (*t*-test, $P < 0.05$; Fig. 4c). Average routine swimming
248 speed in rearing tanks showed an allometric development from 1.6 $L_S s^{-1}$ on 20 dph (11
249 mm L_S) to 5.7 $L_S s^{-1}$ on 38 dph (28 mm L_S) (Fig. 4d).

250

251 DISCUSSION

252

253 In rearing tanks, the growth of *E. japonicus*, which attained 28.3 mm L_S in 40 dph,
254 was similar to that reported for wild specimens estimated from otolith growth
255 increments, i.e., attaining *c.* 30 mm L_S in 40 dph (Takahashi *et al.* 2001) or mean growth
256 rates of 0.58 mm d^{-1} (Takasuka *et al.* 2004) or 0.60 mm d^{-1} (Hwang *et al.* 2006). The
257 similarity of growth to wild specimens suggests a healthy condition for hatchery-reared

258 larvae in the present study. Interspecific comparison revealed that the daily specific
259 growth rate of *E. japonicus* was intermediate between *T. japonicus* and *S. japonicus* (Fig.
260 1). The body form of *E. japonicus* is relatively elongate compared to *T. japonicus* and *S.*
261 *japonicus*. Therefore growth of body mass in *E. japonicus* would be lower than was
262 expressed in L_S .

263 A single individual was observed in a limited space for the measurement of cruise
264 swimming, burst swimming, and predation by jellyfish in this study. This method can
265 make use of a limited number of materials and also facilitate comparison among
266 different species of fish larvae reared under different conditions. The drawback is the
267 potential stress on experimental animals. However, fish larvae usually accepted prey
268 items in the small container immediately after transfer, thus indicating that the impact of
269 stress on their behaviour may have been minimal.

270 Video analyses using single fish revealed that *E. japonicus* larvae are relatively
271 slow in both cruise and burst swimming compared to *T. japonicus* and *S. japonicus*.
272 Hunter (1972) measured the swimming speed of *Engraulis mordax* as $15 L_S s^{-1}$ of burst
273 speed and $1 L_S s^{-1}$ of intermittent swimming and concluded that they are relatively slow
274 swimmers. Fuiman *et al.* (1999) pointed out that slower swimming species such as *E.*
275 *mordax* and *Clupea harengus* are typically elongate larvae that transform at larger sizes
276 than fast swimmers such as *Sciaenops ocellatus*, *Scomber japonicus* and *Pagrus major*
277 (Temminck & Schlegel). A slow cruise swimming speed would reduce the chance of
278 encountering prey items and thus may induce a relatively slow growth of *E. japonicus*.
279 Although a slow cruise swimming speed may reduce the chance of encountering

280 predators, slow burst speed should be disadvantageous in avoiding predators once they
281 are encountered (Fuiman & Magurran, 1994).

282 Average routine swimming speed in rearing tanks gradually increased and the trend
283 corresponded well with that of the cruise swimming speed measured on a single
284 individual in an experimental container. O'Connell (1981) described that the number of
285 red fibers in trunk musculature increased from 2–3 layers at 16 mm L_S to 7–8 layers at
286 26 mm L_S in *Engraulis mordax*. Similar development of red muscle in *E. japonicus* is
287 likely to have enabled the improvement of cruise and routine swimming observed in the
288 present work. Swimming speed measured in still water, however, tends to be slower
289 than that measured in a chamber with current or in the open ocean (Clark *et al.*, 2005;
290 Leis *et al.*, 2006). Therefore caution should be used when applying absolute values of
291 swimming speeds obtained in laboratory systems to ecological contexts.

292 Average responsiveness to sound stimuli increased from 43–50% in 6–11 mm L_S to
293 87–93% in 17–26 mm L_S larvae of *E. japonicus*. Folkvard and Hunter (1986) studied
294 responsiveness of larval *E. mordax* to predation by adult *E. mordax* and juvenile
295 *Scomber japonicus*, and found that 18–48% of 6–13 mm L_S larvae and 70–100% of
296 15–50 mm L_S larvae responded to attacks by predators. Considering that their
297 measurement was based on the response to live predators and thus some larvae failed to
298 respond before being eaten, the tendency of increased responsiveness in *E. japonicus*
299 during ontogeny was similar to that of *E. mordax*.

300 *Engraulis japonicus* were highly vulnerable to jellyfish predation in the present
301 experiment; possible causes of this were low responsiveness, slow burst swimming, and

302 fragile body surface. Müller (1984) reported that *Clupea harengus* larvae suffer high
303 mortality during blooms of *Aurelia aurita*. Capelin *Mallotus villosus* (Müller) larvae
304 also suffer substantial mortality by *A. aurita* predation (Elliott & Leggett, 1997). Larvae
305 of *E. japonicus*, *C. harengus* and *M. villosus* have transparent and elongate bodies that
306 should be adapted to avoid visual predators, but may not be efficient in avoiding tactile
307 predators such as jellyfish. Baily & Batty (1984) compared predation by *A. aurita* on
308 the yolk-sac larvae of cod *Gadus morhua* L., flounder *Platichthys flesus* L., plaice
309 *Pleuronectes platesesa* L. and *C. harengus*, and found that species with smaller larvae
310 (*i.e.*, *P. flesus*) were more vulnerable to capture. They attributed the susceptibility of
311 small larvae to fewer reactions to encounter with *A. aurita*, which is in accordance with
312 the present work. They also suggested that smaller larvae received a higher dose of
313 toxin per unit of body weight. This high-dose susceptibility may also apply to
314 transparent and elongate-bodied larvae. Perhaps *E. japonicus* lack some protection from
315 nematocyst stings on their skin, which *T. japonicus* and *S. japonicus* possess. This may
316 explain why only small larvae of *T. japonicus* and *S. japonicus* were preyed upon,
317 whereas in the present work much larger *E. japonicus* larvae were preyed upon.

318 The first sign of schooling behavior was observed at 17 mm L_S and schooling was
319 completed at 25 mm L_S . Hunter & Coyne (1982) reported that the *E. mordax* begins
320 schooling at 11–15 mm L_S . This discrepancy in the timing of schooling onset between
321 the two species is unlikely to be due to interspecific differences in morphological
322 development, as both show similar morphological changes in sensory and swimming
323 organs. For example, rod cells in the retina appear at 14 mm L_T (= 12 L_S) and 10 mm L_S

324 (O'Connell, 1981; Uyan *et al.*, 2006), and guanine deposition is complete at 34 mm L_S
325 and 35 mm L_S in *E. japonicus* and *E. mordax*, respectively (Takahashi & Watanabe,
326 2004). Hunter & Coyne (1982) conducted behavioural observations in rearing tanks
327 whilst rearing water was being supplied, so that there should have been some water
328 current in their rearing tanks. Fish larvae often show rheotaxis so that they can form
329 school-like aggregations in moving water, which often collapses when the current is
330 stopped (Masuda unpubl. data). Indeed Hunter & Coyne (1982) suggested that the
331 disturbance of changing the water and the resulting fright response induced an early
332 onset of schooling. In any case, in contrast to most fish species that start to show
333 schooling behaviour when they attain the juvenile stage (Masuda & Tsukamoto, 1999),
334 both *Engraulis* species start to form schools prior to metamorphosis. This may imply
335 that the *Engraulis* life history strategy strongly depends on schooling behavior.

336 The basic formation of buccal lateral line nerves in *E. japonicus* is established by
337 20 mm SL (Ohshimo, 1999), and then the supraorbital canal walls are completely
338 ossified by 30.8 mm L_T (= *c.* 26 mm L_S) (Uyan *et al.*, 2006) corresponding with the
339 onset of schooling behaviour. This is in agreement with Cahn *et al.* (1968) who
340 suggested that canalization of buccal lateral line facilitates the capability of detecting
341 specific water movement and thus enables parallel orientation in schooling. Ogawa
342 (1967) described *E. japonicus* larvae smaller than 25 mm L_T (= *c.* 22 mm L_S) to have an
343 immature brain form with relatively small cerebelli; this implies that the development of
344 the brain may also be required for the onset of schooling.

345 Once schooling behavior was established, D_{NN} of *E. japonicus* was about 0.7–1.0

346 fold L_S . D_{NN} of Spanish mackerel *Scomberomorus niphonius* (Cuvier) measured using
347 the same method was about 1.2–1.5 L_S (Masuda et al., 2003) and 2.2 L_S in Pacific
348 bluefin tuna *Thunnus orientalis* (Temminck & Schlegel) (Torisawa et al. 2007). The
349 difference of D_{NN} is likely to reflect feeding strategy; piscivores like *S. niphonius* and *T.*
350 *orientalis* feed efficiently on highly mobile prey by expanding their search area,
351 whereas planktivores like *E. japonicus* larvae keep a low D_{NN} so that their school will
352 be less conspicuous without reducing feeding opportunities. Uyan et al. (2004)
353 suggested that the well-developed buccal lateral line system of this species is likely to
354 enable the high manoeuvrability in schooling.

355 Even though *E. japonicus* larvae are slow swimmers and ideal prey for various
356 piscine and gelatinous predators, their populations seem relatively stable in Japanese
357 coastal waters (Masuda, 2009). This is also the case for congeners in the Mediterranean
358 Sea, where *E. encrasicolus* is the most dominant species in coastal waters (Sabatés et al.,
359 2003). Somarakis et al. (2004) attributed the abundance of *E. encrasicolus* to their high
360 fecundity. *Engraulis japonicus* also have a high fecundity and spawn from early spring
361 to autumn, and they mature in as short as six months (Tsuruta, 1992), which
362 compensates for its short life span (Kawasaki, 1980). This reproductive advantage may
363 allow the *E. japonicus* population to persist even with high predation pressure by
364 jellyfish as well as fishing pressure. This is in contrast to the *Sardinops melanostictus* in
365 which maturation requires one or two years and spawning usually occurs during limited
366 time of year at specific sites (Morimoto, 2003).

367 *Engraulis japonicus* larvae are likely to be preyed upon by both piscine and

368 gelatinous predators. Reductions of piscine predators caused by overfishing may work
369 advantageously to the population of *E. japonicus* at least temporarily. Blooms of
370 jellyfish, however, threaten *E. japonicus* larvae. The threat is even more serious as the
371 trend of the period of jellyfish blooms extends due to the reduced quality of coastal
372 waters as well as the global warming trend (Purcell *et al.*, 2007). Survival of *E.*
373 *japonicus* larvae exposed to predators under various conditions such as elevated
374 temperature and turbidity requires further research.

375

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384

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Table I. Comparison of cruise swimming speed, burst swimming speed and responsiveness among different size groups, and among the three different fish species

	3.7–10 mm	10–21 mm	21 mm–33 mm
Cruise Speed ($L_S s^{-1}$)			
<i>Engraulis japonicus</i>	1.42 ± 0.74 (23) b	1.80 ± 0.99 (19) b	3.85 ± 1.24 (17) a
<i>Trachurus japonicus</i>	1.75 ± 1.15 (95)	1.65 ± 0.99 (55)	
<i>Scomber japonicus</i>	1.56 ± 0.72 (23)	3.20 ± 1.88 (47)*	
Burst Speed ($L_S s^{-1}$)			
<i>Engraulis japonicus</i>	6.08 ± 4.14 (17) b	9.50 ± 6.89 (16) b	28.4 ± 12.1 (17) a
<i>Trachurus japonicus</i>	18.7 ± 9.3 (87) b*	22.7 ± 7.10 (55) a*	
<i>Scomber japonicus</i>	8.03 ± 3.53 (12) b	15.7 ± 5.58 (35) a*	
Responsiveness (%)			
<i>Engraulis japonicus</i>	50.1 ± 40.0 (23) b	64.9 ± 37.6 (19) ab	88.2 ± 26.2 (17) a
<i>Trachurus japonicus</i>	79.3 ± 32.3 (95) b*	97.6 ± 8.7 (55) a*	
<i>Scomber japonicus</i>	27.5 ± 31.2 (23) b	66.7 ± 42.8 (47) a	

Values with different letters are significantly different among size groups in the same species, and values with an asterisk are significantly larger than that of *Engraulis japonicus* in the same size group (Dunn's test, $P < 0.05$).

Figure captions

Fig. 1. (a) Measurements of separation angle (SA) and the distance to the nearest neighbour (D_{NN}). l_1 is the D_{NN} for the individual F, and so is l_2 for the individuals N₁ and N₂. Average of l_1 , l_2 , l_2 , l_3 and l_4 will be the D_{NN} for this frame. (b) Measurement of the separation swimming index (I_{SS}). Swimming trajectories of neighbouring individuals were expressed as vectors, and the distance between the two vectors d divided by the average length of v_1 and v_2 was defined as I_{SS} .

Fig. 2. Growth (mean \pm S.D.) of *Engraulis japonicus* (black circles: present work) together with *Scomber japonicus* (triangles) and *Trachurus japonicus* (squares) (Masuda, 2006). Data were fitted to exponential curves.

Fig. 3. Developmental changes of avoidance of *Aurelia aurita* by *Engraulis japonicus* (present work), *Scomber japonicus* and *Trachurus japonicus* (Masuda, 2006). Symbols are the same as in Fig. 2. Values without sharing an alphabet letter differ significantly (Dunn's test, $P < 0.05$).

Fig. 4. Developmental changes of three schooling parameter as (a) separation angle, (b) distance to the nearest neighbour, and (c) separation swimming index and (d) development of routine swimming speed in rearing tanks. Open circles (lower case) and closed circles (upper case) represent tank 1 and tank 2, respectively. Values without sharing an alphabet letter differ significantly (Tukey's test, $P < 0.05$).

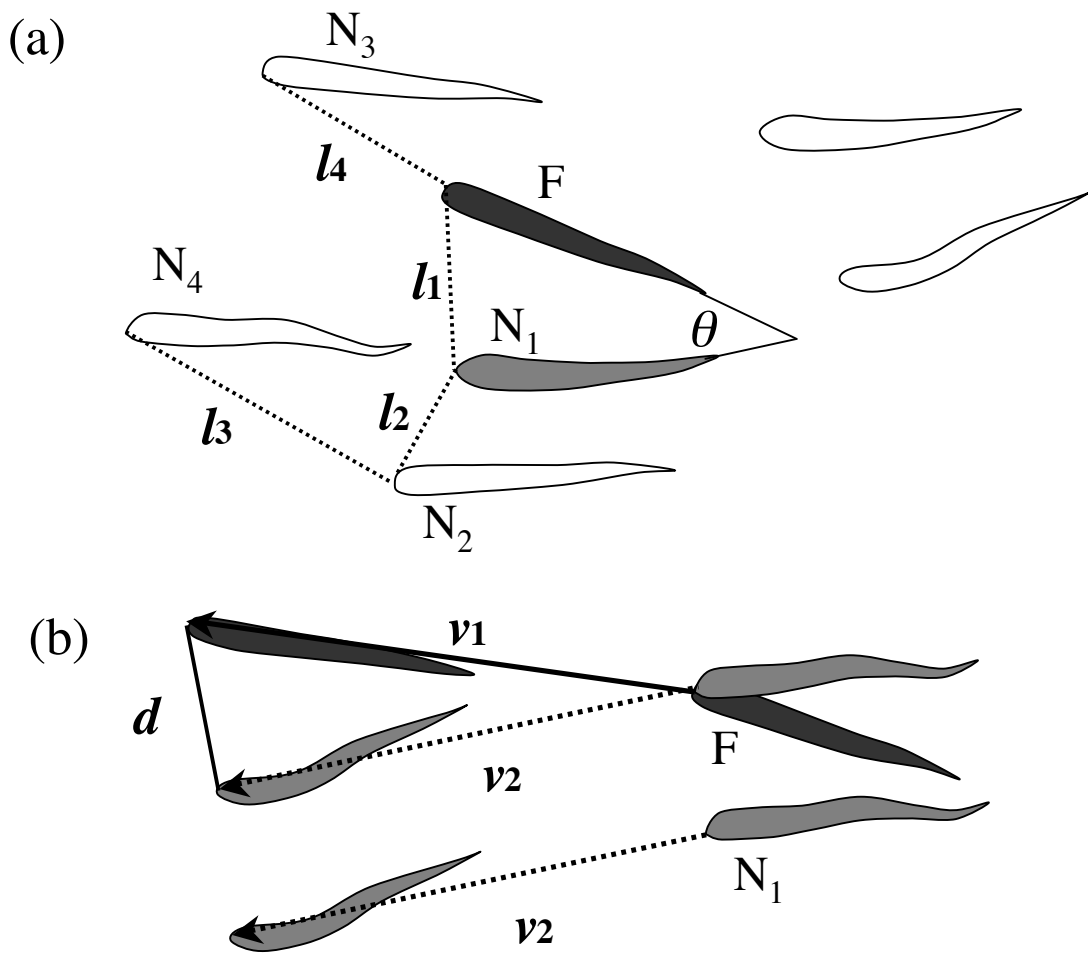


Fig. 1.

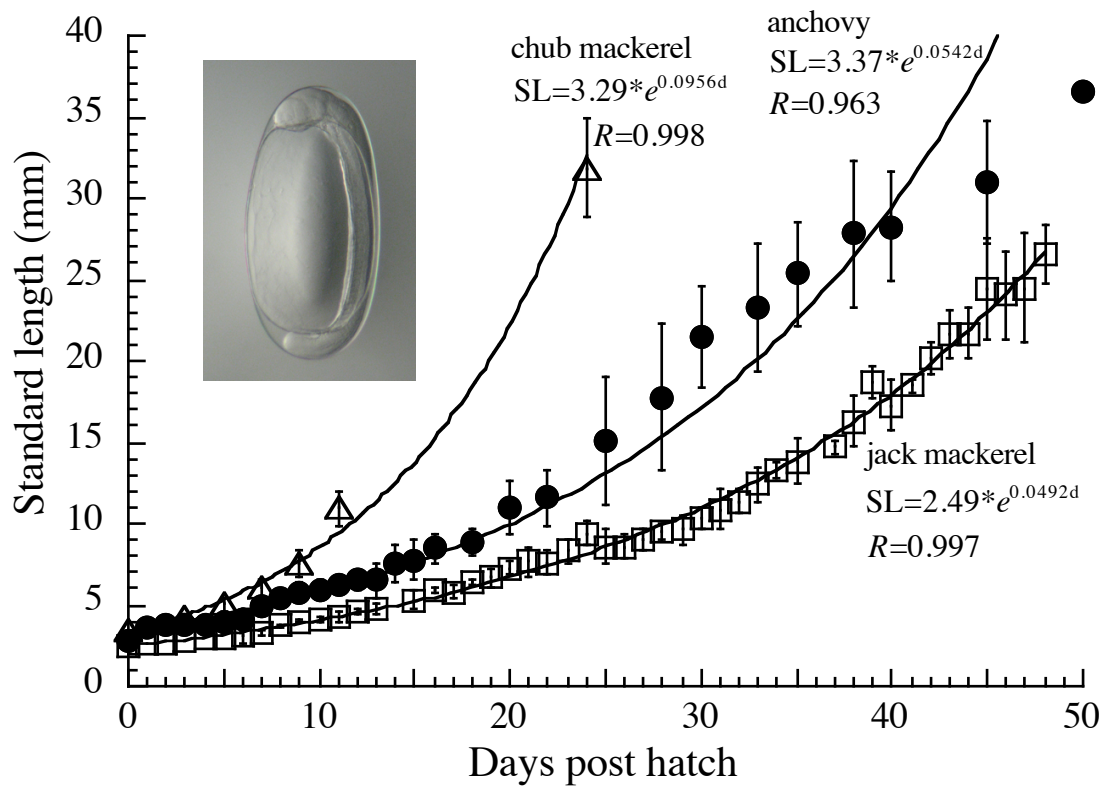


Fig. 2.

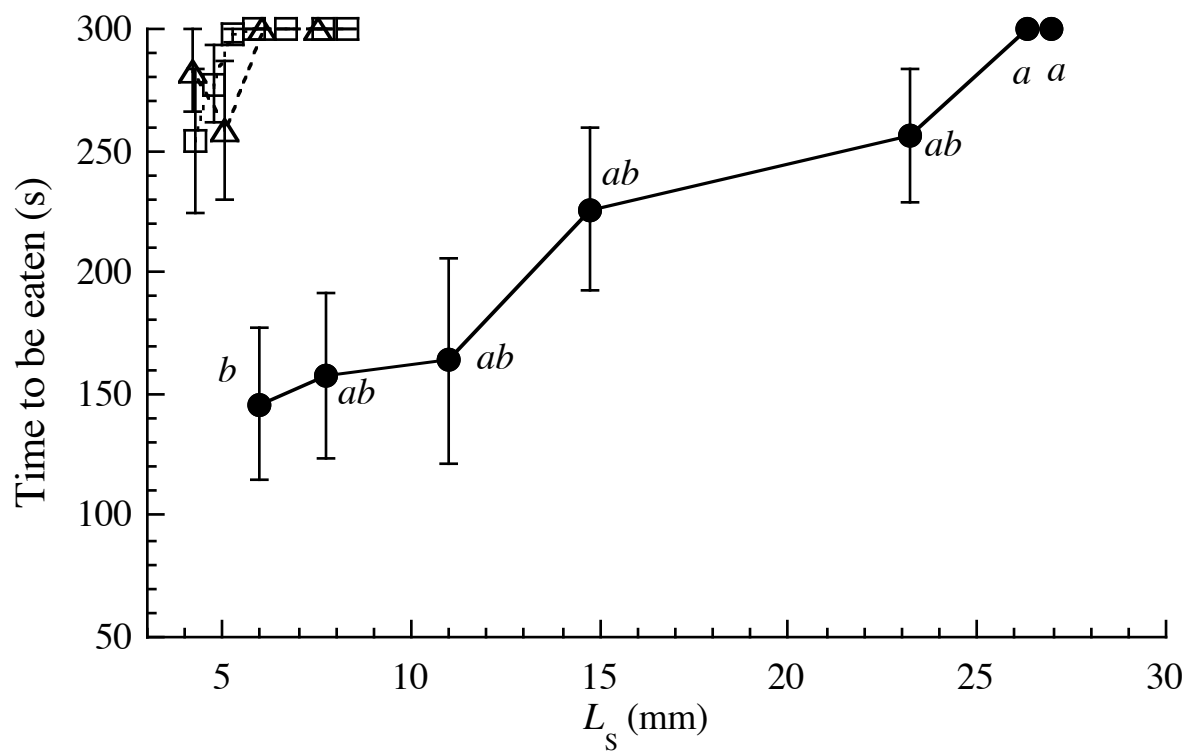


Fig. 3.

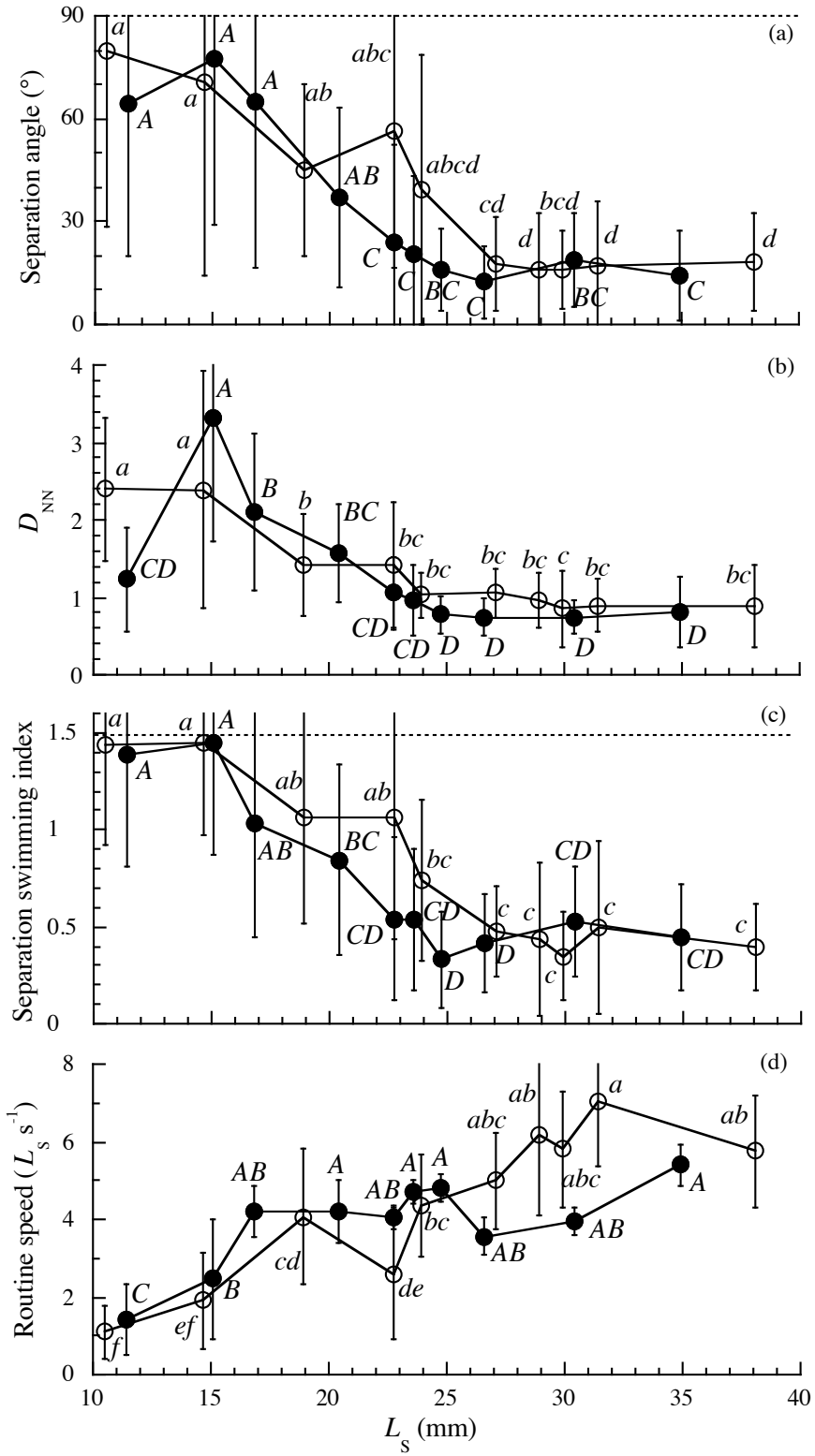


Fig. 4.