



Title	Vertical position, specific gravity and swimming ability of Pacific cod <i>Gadus macrocephalus</i> yolk-sac larvae reared at four temperatures
Author(s)	Li, Zhe; Yamamoto, Jun; Sakurai, Yasunori
Citation	Fisheries science, 81(5), 883-889 <a href="https://doi.org/10.1007/s12562-015-0911-6">https://doi.org/10.1007/s12562-015-0911-6</a>
Issue Date	2015-09
Doc URL	<a href="http://hdl.handle.net/2115/62748">http://hdl.handle.net/2115/62748</a>
Rights	The final publication is available at Springer via <a href="http://dx.doi.org/10.1007/s12562-015-0911-6">http://dx.doi.org/10.1007/s12562-015-0911-6</a>
Type	article (author version)
File Information	FS-Li Zhe.pdf



[Instructions for use](#)

1 **Title:**

2 Vertical position, specific gravity and swimming ability of Pacific cod *Gadus*  
3 *macrocephalus* yolk-sac larvae reared at four temperatures

4

5 **Authors:**

6 Zhe Li<sup>1</sup>, Jun Yamamoto<sup>2</sup>, Yasunori Sakurai<sup>1\*</sup>

7

8 **Author Affiliations :**

9 <sup>1</sup> Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato,  
10 Hakodate, Hokkaido 041-8611, Japan

11 <sup>2</sup> Field Science Center for Northern Biosphere, Hokkaido University, 3-1-1 Minato,  
12 Hakodate, Hokkaido 041-8611, Japan

13

14

15 **\*Corresponding author:** Yasunori Sakurai (sakurai@fish.hokudai.ac.jp )

16 Tel.: +81 0138 40 8861 / Fax: +81 138 8863

17

18 **Authors E-mail:** Zhe Li ; lz880526@live.cn

19 Jun Yamamoto ; yamaj@fish.hokudai.ac.jp

20

21

22

23

24 **Abstract**

25 Vertical position and movement rate were measured in the yolk-sac larvae of the  
26 Pacific cod *Gadus macrocephalus* at different rearing temperatures (3, 5, 7 and 9 °C)  
27 and an identical temperature of 5 °C in experimental columns during the initial 3 days  
28 post hatch (dph). Larval specific gravity was also measured through sinking velocities.  
29 Yolk-sac larvae swam upward and initially (0 dph) remained in the upper section of  
30 the experimental column through intermittent upward swimming and gradually  
31 moved to near-neutral buoyancy at 3 dph at corresponding rearing temperatures.  
32 Rearing temperature significantly affected the swimming ability: at 0 dph, vertical  
33 movement rates measured at 5 °C were low in the yolk-sac larvae reared at warm  
34 temperatures. However, no such difference was observed at 3 dph. The difference in  
35 vertical movement rate may have resulted from the temperature-mediated body sizes  
36 (standard length, SL) and yolk-sac sizes (yolk-sac volume, YSV) at hatching. Specific  
37 gravity did not change for the varying temperatures during the initial 3 days, but  
38 decreased as ontogeny progressed. Therefore, we suggest that Pacific cod yolk-sac  
39 larvae achieve a demersal-pelagic habitat transition through upward swimming and  
40 increasing buoyancy.

41

42 **Key words:** Pacific cod, yolk-sac larvae, various temperatures, vertical position,  
43 specific gravity, vertical movement rate

44

45 **Introduction**

46 Pacific cod *Gadus macrocephalus* is a commercially and ecologically important  
47 marine fish species that is widely distributed around the rim of the North Pacific  
48 Ocean [1, 2]. The females spawn demersal eggs from December to February [3, 4].  
49 Pacific cod eggs remain strictly demersal during hatching [5]. After hatching, yolk-sac  
50 larvae actively swim upward as early as 1 day post hatch (dph) and tend to be  
51 near-neutrally buoyant until 3 dph, at which they appear to be negatively buoyant [6,  
52 7]. In the environment of Mutsu Bay in Japan, yolk-sac larvae develop into pelagic  
53 larvae alongside a demersal-pelagic habitat transition [4, 8], which appears to be  
54 relatively rapid and associated with lipid/fatty acid synthesis [9]. Pacific cod larvae  
55 are thereby assumed to achieve vertical migration as a result of increased buoyancy  
56 just prior to hatching, rather than swimming [9]. However, the assumption is uncertain  
57 because the swimming ability of yolk-sac larvae may overcome negative buoyancy  
58 and migrate to surface layers [10]. On the other hand, yolk-sac larvae cannot swim  
59 continuously [11]; therefore, negatively buoyant larvae require a high swimming  
60 frequency to achieve the habitat transition and maintain their vertical position in  
61 surface layers [12]. In other words, both buoyancy and swimming behavior appear to  
62 play important roles in the habitat transition process.

63 Temperature potentially impacts larval size and endogenous reserves at hatching:  
64 warmer temperatures result in smaller body size but larger yolk-sac size [13, 14]. In  
65 addition, temperature affects physics of swimming conditions (e.g., dynamic  
66 viscosity) [15] and metabolic rates of fish larvae [16], both of which affect larval

67 swimming speed. Eventually, temperature has direct implications for larval survival  
68 by regulating growth rate which is also related to body size [17-19], as well as  
69 mortality through swimming performance that develops with increasing body size  
70 [20-22].

71 The buoyancy of yolk-sac larvae is suggested to be highly related to egg density  
72 and influences their vertical distributions [23]. Variation in specific gravity of  
73 yolk-sac larvae may be due to asynchronous spawning and thus hatchings. Generally,  
74 specific gravity changes with ontogeny, involving yolk absorption [23]; however,  
75 such change differs among species: it increases in herring yolk-sac larvae with yolk  
76 absorption, but decreases in Atlantic cod yolk-sac larvae [24].

77 Previous studies have mostly focused on horizontal swimming performance of fish  
78 larvae [22, 25-28], while limited studies have described the vertical swimming  
79 performance of fish larvae. Moreover, little is known regarding the demersal-pelagic  
80 habitat transition of Pacific cod yolk-sac larvae.

81 The objectives of this study were to determine: (1) the vertical position of Pacific  
82 cod yolk-sac larvae in an experimental column; (2) the effect of rearing temperature  
83 on vertical movement rate at an identical temperature; and (3) change in specific  
84 gravity of the yolk-sac larvae reared at various temperatures with age. Specific gravity  
85 of warmer-reared Pacific cod larvae was expected to be higher due to larger yolk-sac  
86 size at hatching [14, 24]. The relevance of these results is discussed in an attempt to  
87 describe the physical process of the vertical migration of Pacific cod yolk-sac larvae.

## 88 **Materials and methods**

89 **Egg collection and rearing**

90 Adult Pacific cod were collected with bottom-set nets in Mutsu Bay, Aomori,  
91 Japan during the spawning season in February 2014. Gametes from one female and  
92 three males were mixed and rinsed at 4 °C. The fertilized eggs were shipped in an  
93 insulated container to the laboratory at the Faculty of Fisheries, Hokkaido University  
94 in Hakodate, Hokkaido, Japan. At about 5 h post-fertilization, the demersal eggs were  
95 scattered in a thin layer at the bottom of plastic containers (i.e., approximately 3,600  
96 eggs/container,  $N = 8$ ) within filtered seawater (4 °C, 34 in salinity) and two replicate  
97 containers were used for each of four rearing temperatures: 3, 5, 7 and 9 °C.  
98 Seawaters were aerated with air-stones to increase oxygen concentration and  
99 circulation to the demersal eggs in stagnant water. Time to hatch was defined as the  
100 day on which 50 % of the eggs had hatched at each temperature. At 0 dph of each  
101 rearing temperature, the yolk-sac larvae were transferred to polyethylene containers  
102 (400 ml) with air-bleed holes on the lids and reared at their incubation temperature for  
103 the initial 3 days, unfed but dependent on the yolk reserves. The rearing temperatures  
104 were  $3.2 \pm 0.4$ ,  $5.2 \pm 0.3$ ,  $7.1 \pm 0.2$  and  $8.9 \pm 0.2$  °C (mean  $\pm$  SD) during the  
105 embryonic and larval period. Light regime was maintained at 12L:12D. Half of the  
106 seawater was replaced with fresh seawater at the corresponding temperature daily and  
107 every other day during the embryonic and larval period, respectively. Dead eggs or  
108 larvae were removed with a pipette.

109 **Experimental design**

110 *Vertical position*

111 Vertical positions of yolk-sac larvae were measured at their rearing temperatures  
112 at 0–3 dph (i.e., larvae reared at 3 °C were measured at 3 °C). An acrylic column (400  
113 mm height, 60 mm diameter) was marked with 4-mm intervals on the outside and  
114 filled with chilled seawater at a specific temperature (i.e., 3, 5, 7 or 9 °C) to a depth of  
115 320 mm. The trials were conducted in a temperature-controlled incubator with a  
116 transparent viewing window under a light intensity of about  $6.8 \mu\text{E m}^{-2} \text{s}^{-1}$  at the water  
117 surface. Ten larvae at each temperature were gently and slowly introduced into the  
118 bottom of the acrylic column using a small column (40 mm height, 32 mm diameter)  
119 which was attached to a net lid and a slender tube. The net lid was gently opened by  
120 drawing a fastening string. After being allowed to freely swim for 10 min, vertical  
121 positions of the larvae were recorded through the transparent viewing window.  
122 Movement direction and angle of body orientation were additionally observed and  
123 estimated during swimming and resting bouts to determine whether the vertical  
124 position was maintained through buoyancy or swimming behavior [7, 10]. The  
125 seawater temperatures were measured before and after the observations with a  
126 thermometer ( $\pm 0.1$  °C). Eight replicates were conducted in each rearing  
127 temperature/age combination.

### 128 ***Vertical movement rate***

129 Vertical movement rates of yolk-sac larvae reared at the four temperatures were  
130 measured at an identical temperature of 5 °C at 0–3 dph. The identical temperature  
131 was used to eliminate the influence of ambient temperature on vertical movement rate,  
132 such as viscosity [15]. Prior to each replicate, twenty larvae were acclimated at 5 °C

133 for 15 min except those reared at 5 °C. The larvae were then transferred into the  
134 bottom of the acrylic column in the same way as in the vertical position trials. To  
135 minimize repeated recordings as far as possible, only ten of the twenty larvae were  
136 randomly selected and recorded using a video camera (*Sony* HDR-CX370) through  
137 the transparent viewing window. In addition, movement distance less than 4 mm was  
138 not considered since the behavior may be due to twisting, rather than swimming [29].  
139 The seawater temperatures were also measured before and after the recordings with  
140 the same thermometer. A total of 13–36 larvae from eight replicates were subsampled  
141 and photographed for measuring the standard length (SL) and yolk-sac volume (YSV)  
142 in each rearing temperature/age combination.

#### 143 *Specific gravity*

144 The experimental method generally followed that of Neilson et al. [30] on larval  
145 Atlantic cod *Gadus morhua*. Specific gravities of Pacific cod yolk-sac larvae were  
146 determined by measuring sinking velocities in seawaters of four density types. Four  
147 acrylic columns (400 mm height, 65 mm diameter) were marked at 5-cm intervals and  
148 filled with filtered seawater of known salinities to a depth of 350 mm. The seawater  
149 salinities were measured with a YSI EC300 (with a resolution of 0.002) and adjusted  
150 to 10, 15, 20 and 25 with artificial sea salt (Rohto marine, Rei-Sea, Tokyo, Japan). An  
151 Onset HOBO® Pendant Temperature logger UA-001-08 (Onset Computer,  
152 Bourne, HOBO, Massachusetts, USA) was used to note the temperatures during the  
153 measurement period. Seawater densities were calculated with the known salinities and  
154 noted temperatures [31].

155 At a fixed time, twenty larvae in each rearing temperature/age combination were  
156 individually anesthetized in FA100 (Dainippon Sumitomo Pharma, Japan) for  
157 approximately 1 min and after rinsed in filtered seawater with a certain test salinity ( $N$   
158 = 4), they were gently released at the surface of the seawater with the corresponding  
159 salinity. The time to sink 10 cm was measured with a resolution of 0.01 s after sinking  
160 5 cm to obtain a stable speed. The anesthetic time was short and the larvae were  
161 seemingly not influenced by the anesthetic, since all of them revived after the trials.

## 162 **Data analysis**

163 In each rearing temperature/age combination, the median of vertical positions was  
164 calculated in eight replicates and regarded as the level of vertical position. The mean  
165 25th and 75th percentiles of vertical positions were also calculated to describe the  
166 vertical position range of the yolk-sac larvae in the experimental column. Two-way  
167 ANOVA was employed to examine the difference in vertical positions in the  
168 experimental column with rearing temperature ( $N = 4$ ) and age ( $N = 4$ ) as the main  
169 factors.

170 The vertical movement rate was defined as vertical movement distance divided by  
171 time (mm/s). Video analysis software (MEPG video Wizard DVD) was used to  
172 calculate vertical movement rates at 1/30 s accuracy. At each age, the Kruskal-Wallis  
173 rank-sum test was used to determine the difference in vertical movement rate across  
174 the various rearing temperatures. Pairwise multiple comparisons were conducted  
175 using the Kruskal-Wallis test (function 'Kruskalmc' in the R package 'pgirmess')  
176 within R version 3.1.2. A linear regression model ( $y = ax + b$ ) was used to clarify the

177 relationships between vertical movement rate and temperature-specific morphometric  
178 characteristics: body size (SL) and yolk-sac size (YSV) measured from all the  
179 sampled larvae [32].

180 At each salinity used for testing, 20 sinking velocity measurements were grouped  
181 into four sections (i.e., 5 measurements/section). The mean sinking velocity in each  
182 section was treated as one replicate to a particular salinity (i.e., four  
183 replicates/salinity). A linear regression ( $\rho = a + b\omega$ ) or an exponential regression  
184 model ( $\rho = a\omega^b$ ) was used to describe the relationship between sinking velocities ( $\omega$ )  
185 and seawater densities ( $\rho$ ) (i.e., one replicate sinking velocity/salinity). The chosen  
186 regression model was based on the higher regression coefficient. The point at which  $\omega$   
187 = 0, which was referred as neutral buoyancy, was assumed to represent larval specific  
188 gravity to this section and back-calculated from the determined regression model. The  
189 mean specific gravity from four sections was regarded as the specific gravity in a  
190 rearing temperature/age combination. The change in the larval specific gravity was  
191 analyzed with the linear regression model ( $y = ax + b$ ). The differences in specific  
192 gravity for various temperatures were examined using the Kruskal-Wallis rank-sum  
193 test across the ages. Statistical significance was set at  $p < 0.05$ .

## 194 **Results**

195 The yolk-sac larvae swam upward and maintained position at the upper half of the  
196 experimental column as early as 0 dph at all the rearing temperatures (Table 1; Fig. 1).  
197 Neither rearing temperature nor age significantly affected vertical position during the  
198 initial 3 days (Fig. 1;  $p > 0.05$ ).

199 Swimming behavior markedly changed during the initial 3 days. At 0 dph, yolk-sac  
200 larvae swam upward in nearly straight routes and sank quickly at an angle of almost  
201 90° (degree from horizontal) during swimming and resting bouts, respectively. The  
202 swimming behavior utilized can be described as burst swimming. At 3 dph, yolk-sac  
203 larvae swam upward in sinuous routes and sank relatively slowly at an angle of 30° –  
204 45° during swimming and resting bouts, respectively. The swimming behavior utilized  
205 was similar to the routine swimming. In general, yolk-sac larvae tended to be  
206 negatively buoyant and maintained vertical positions by intermittent swimming at 0  
207 dph. As ontogeny progressed, yolk-sac larvae maintained vertical positions by their  
208 near-neutral buoyancy instead of swimming at 3 dph. Some larvae stayed at the  
209 bottom during the observation period (Supplementary video). All the mentioned  
210 swimming behaviors were consistently observed at all the rearing temperatures.

211 At an identical temperature of 5 °C (Table 1), vertical movement rate remained  
212 lowest in the larvae reared at 9 °C at 0–2 dph, and was lower in the larvae reared at  
213 7 °C than that at 3 °C only at 0 dph (Fig. 2;  $p < 0.05$ ). At 3 dph, there was no  
214 significant difference in vertical movement rates of larvae reared at the various  
215 temperatures (Fig. 2;  $p > 0.05$ ). In the current study, vertical movement rate was  
216 positively correlated with body size (Fig. 3;  $p < 0.001$ ) and negatively correlated with  
217 yolk-sac size (Fig. 4;  $p < 0.01$ ) across the rearing temperatures during the initial 3  
218 days. Subsampled 21–79 individuals were taken into account in each rearing  
219 temperature/age combination (Table 2).

220 In the current study, Pacific cod yolk-sac larvae were negatively buoyant relative to

221 the densities of the seawaters (1.0258 (B)–1.0265 (A) g/cm<sup>3</sup> at 3–9 °C, Fig. 5) at 0  
222 dph. The specific gravity of yolk-sac larvae decreased with age at all the rearing  
223 temperatures (Fig. 5; all  $p < 0.05$ ). No consistent differences in specific gravities were  
224 observed for the temperatures across the initial 3 days. At 3 dph, however, all specific  
225 gravities decreased to the level close to the seawater densities (Fig. 5), indicating that  
226 the yolk-sac larvae were near-neutrally buoyant at this stage.

227

## 228 **Discussion**

229 Three laboratory experiments were conducted to determine the method that Pacific  
230 cod yolk-sac larvae utilize to migrate upward and whether rearing temperature affects  
231 the vertical movement ability of yolk-sac larvae. In the current study, the yolk-sac  
232 larvae swam upward as early as 0 dph and the vertical position did not change across  
233 the rearing temperatures or ages. These results are similar to the previous study by  
234 Hurst et al. [7] reporting that yolk-sac larvae swim upward and remain at an upper  
235 position as early as 1 dph at 4 °C. Since first feeding occurs at depletion of  
236 approximately 50 % yolk reserves [33], vertical migration of pre-feeding larvae  
237 appears to coincide with first feeding. In the Baltic Sea, *Gadus morhua* yolk-sac  
238 larvae have been thought to reach the upper water layers where prey availability is  
239 high as soon as possible, at least before the yolk reserves are completely depleted [12,  
240 33]. In the environment of Mutsu Bay, Pacific cod yolk-sac and yolk-absorbed larvae  
241 mainly inhabit depths at 8–45 m where their favorable prey, copepod nauplii, is  
242 abundant [4].

243 Compared with the field depth, the experimental column depth in the current study  
244 was extremely small (i.e., 32 cm), possibly allowing the yolk-sac larvae to reach the  
245 upper half of the experiment column in 10 min. Therefore, there was no difference in  
246 the vertical positions within the temperatures or ages in the current study. However,  
247 the different strategies in which larvae maintain vertical position suggest that larval  
248 specific gravity decreases with age. As a result, larvae do not require high swimming  
249 activity to maintain vertical position, but depend rather on neutral buoyancy. The  
250 observation of some individuals remaining at the bottom of the acrylic column may be  
251 due to their periodically resting there [7] or swimming upward after the observations.

252 Vertical movement rate of larvae reared under warmer conditions was initially  
253 low at an identical temperature of 5 °C. This might have resulted from the  
254 temperature-mediated body sizes and yolk-sac sizes at hatching [14, 32]. At 3 dph, the  
255 convergence in vertical movement rate may be due to the same ambient temperature  
256 (5 °C) rather than the morphometric differences.

257 The specific gravity of the yolk-sac larvae generally decreased with age during the  
258 initial 3 days (Fig. 5). The result was affirmed by the observations that the larvae sank  
259 quickly during resting bouts at 0 dph, but slowly at 3 dph. Moreover, Laurel et al. [9]  
260 proposed that Pacific cod larvae achieve vertical migration through a rapid decrease in  
261 specific gravity. Similarly, North Sea herring larvae are also negatively buoyant at  
262 hatching and migrate upward, involving yolk absorption and decreasing specific  
263 gravity. Decreased specific gravity appears to be a physiological adjustment to  
264 facilitate the attainment of surface layers for first feeding and energetic conservation

265 because of low first feeding success [24]. Although specific gravity was expected to  
266 be positively related with temperature at hatching, the homogenous specific gravities  
267 might have resulted from temperature-specific development rates during time delays.  
268 Specific gravities were not measured until the point of 50 % hatching, during which  
269 the yolk-sac larvae underwent temperature-specific decreases in specific gravity. After  
270 measurements, all the larvae possibly achieved a similar specific gravity.

271 From the above considerations, vertical migration of Pacific cod yolk-sac larvae  
272 appears to be associated with upward swimming and increasing buoyancy. Upward  
273 swimming ensures that the yolk-sac larvae depart from the bottom water layers. They  
274 exhibited active and intermittent swimming behavior which initially offsets the  
275 negative buoyancy. However, yolk-sac larvae may immediately descend during  
276 resting bouts due to negative buoyancy and possibly hover within a specific depth in  
277 the field. Thus initial net upward movement was hardly or limitedly achieved. In this  
278 case, since both swimming ability and buoyancy increase as ontogeny progresses,  
279 larvae swim upward rather easier or even float without swimming [9]. Increased  
280 buoyancy may gradually replace swimming behavior as the dominant factor to  
281 maintain vertical position in this species. Thereafter, larvae might be capable of  
282 selecting vertical positions to some extent by swimming behavior and maintain their  
283 positions by neutral buoyancy.

284 Interestingly, the result of change in buoyancy contrasts with the previous study of  
285 Hurst et al. [7] demonstrating that Pacific cod larvae are neutrally to negatively  
286 buoyant over the period from hatching to 3 dph. The divergence between the results

287 may be due to population-specific physiological adaptations to different habitats. The  
288 different depths of spawning locations, for example, may lead to different strategies of  
289 vertical migration to surface layers. The spawning location is deeper in the Gulf of  
290 Alaska (> 100 m) than that in Mutsu Bay (about 60 m) [4, 9]. Yolk-sac larvae thus  
291 have to migrate upward further in the Gulf of Alaska [4, 34]. Consequently, the larvae  
292 may complete vertical migration sooner by neutral buoyancy at hatching in the Gulf  
293 of Alaska, and maintain vertical position by intermittent swimming as a decrease in  
294 buoyancy occurs. On the other hand, larvae in Mutsu Bay may not need to migrate  
295 upward so early as those in the Gulf of Alaska. The larvae may hover for a period due  
296 to weak swimming ability and high specific gravity, and become capable of ascending  
297 to surface layers in a short time as an increase in buoyancy occurs [9].

### 298 **Ecological implications**

299 The demersal-pelagic habitat transition is an essential physiological process for  
300 Pacific cod yolk-sac larvae after hatching, inducing yolk-sac larvae to depart from the  
301 low oxygen zone at hatching depths and initiate first feeding at depths where  
302 favorable prey availability is expected [12] in the method involving upward  
303 swimming and increasing buoyancy. If some larvae fail to achieve the habitat  
304 transition before first feeding or yolk depletion, they are likely to suffer high mortality  
305 due to low oxygen concentration and prey availability [12]. Thus, this process appears  
306 to play an important role in larval survival and even recruitment during their early life  
307 stages [35].

308 The temperature-mediated swimming ability is likely to be an adaptive strategy to

309 various spawning environments. Larvae hatching in a low temperature obtain high  
310 swimming ability that might offset the effects of low metabolic rate and high viscosity  
311 in the low temperature [15]. However, it is unclear that how far larval swimming  
312 ability offsets the impacts of a low water temperature. Furthermore, whether (and how)  
313 water temperature impacts the habitat transition speed of this species is still unknown.  
314 If water temperature impacts more than larval swimming ability, larvae hatching in a  
315 higher temperature are likely to complete the habitat transition and start first feeding  
316 earlier than those hatching in a lower temperature. Meanwhile, an earlier habitat  
317 transition might be harmful to yolk-sac larvae because they may be exposed to  
318 predators earlier and high mortality is consequently expected. In this case, water  
319 temperature, influencing the habitat transition speed, appears to become an indicator  
320 of survival of Pacific cod yolk-sac larvae. Therefore, further studies are required to  
321 clarify the role of water temperature during the demersal-pelagic habitat transition of  
322 Pacific cod yolk-sac larvae.

323

324

### 325 **Acknowledgments**

326 We thank to the Wakinosawa Fishery Association for offering great quality  
327 Pacific cod eggs. Thanks are also extended to two anonymous reviewers for detailed  
328 revision and helpful comments on this manuscript.

329 **References**

- 330 1. Alderdice D, Forrester C (1971) Effects of salinity, temperature, and dissolved  
331 oxygen on early development of the Pacific cod (*Gadus macrocephalus*). J  
332 Fish Res Board Can 28: 883-902
- 333 2. Laurel B, Stoner A, Ryer C, Hurst T, Abookire A (2007) Comparative habitat  
334 associations in juvenile Pacific cod and other gadids using seines, baited  
335 cameras and laboratory techniques. J Exp Mar Biol Ecol 351: 42-55
- 336 3. Sakurai Y, Hattori T (1996) Reproductive behavior of Pacific cod in captivity.  
337 Fish Sci 62: 222-228
- 338 4. Takatsu T, Nakatani T, Miyamoto T, Kooka K, Takahashi T (2002) Spatial  
339 distribution and feeding habits of Pacific cod (*Gadus macrocephalus*) larvae in  
340 Mutsu Bay, Japan. Fish Oceanogr 11: 90-101
- 341 5. Bian X, Zhang X, Sakurai Y, Jin X, Gao T, Wan R, Yamamoto J (2014)  
342 Envelope surface ultrastructure and specific gravity of artificially fertilized  
343 Pacific cod *Gadus macrocephalus* eggs. J Fish Biol 84: 403-421
- 344 6. Forrester C (1964) Laboratory observations on embryonic development and  
345 larvae of the Pacific cod (*Gadus macrocephalus* Tilesius). J Fish Res Board  
346 Can 21: 9-16
- 347 7. Hurst TP, Cooper DW, Scheingross JS, Seale EM, Laurel BJ, Spencer ML  
348 (2009) Effects of ontogeny, temperature, and light on vertical movements of  
349 larval Pacific cod (*Gadus macrocephalus*). Fish Oceanogr 18: 301-311
- 350 8. Sakurai Y (2007) An overview of the Oyashio ecosystem. Deep-Sea Res Pt II  
351 54: 2526-2542
- 352 9. Laurel BJ, Copeman LA, Hurst TP, Parrish CC (2010) The ecological  
353 significance of lipid/fatty acid synthesis in developing eggs and newly hatched  
354 larvae of Pacific cod (*Gadus macrocephalus*). Mar Biol 157: 1713-1724
- 355 10. Davis MW, Olla BL (1994) Ontogenetic shift in geotaxis for walleye pollock,  
356 *Theragra chalcogramma* free embryos and larvae: potential role in controlling  
357 vertical distribution. Environ Biol Fishes 39: 313-318
- 358 11. Webb PW, Weihs D (1986) Functional locomotor morphology of early  
359 life-history stages of fishes. Trans Am Fish Soc 115: 115-127
- 360 12. Grønkvær P, Wieland K (1997) Ontogenetic and environmental effects on  
361 vertical distribution of cod larvae in the Bornholm Basin, Baltic Sea. Mar Ecol  
362 Prog Ser 154: 91-105
- 363 13. Methven DA, Brown JA (1991) Time of hatching affects development, size,  
364 yolk volume, and mortality of newly hatched *Macrozoarces americanus*  
365 (Pisces: Zoarcidae). Can J Zool 69: 2161-2167
- 366 14. Laurel BJ, Hurst TP, Copeman LA, Davis MW (2008) The role of temperature  
367 on the growth and survival of early and late hatching Pacific cod larvae  
368 (*Gadus macrocephalus*). J Plankton Res 30: 1051-1060
- 369 15. Hunt von Herbing I (2002) Effects of temperature on larval fish swimming  
370 performance: the importance of physics to physiology. J Fish Biol 61: 865-876
- 371 16. Beveridge OS, Petchey OL, Humphries S (2010) Mechanisms of  
372 temperature-dependent swimming: the importance of physics, physiology and

- 373 body size in determining protist swimming speed. J Exp Biol 213: 4223-4231
- 374 17. Pepin P (1991) Effect of temperature and size on development, mortality, and
- 375 survival rates of the pelagic early life history stages of marine fish. Can J Fish
- 376 Aquat Sci 48: 503-518
- 377 18. Otterlei E, Nyhammer G, Folkvord A, Stefansson SO (1999) Temperature-
- 378 and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus*
- 379 *morhua*): a comparative study of Norwegian coastal cod and northeast Arctic
- 380 cod. Can J Fish Aquat Sci 56: 2099-2111
- 381 19. Folkvord A (2005) Comparison of size-at-age of larval Atlantic cod (*Gadus*
- 382 *morhua*) from different populations based on size-and temperature-dependent
- 383 growth models. Can J Fish Aquat Sci 62: 1037-1052
- 384 20. Litvak MK, Leggett WC (1992) Age and size-selective predation on larval
- 385 fishes - the bigger-is-better hypothesis revisited. Mar Ecol Prog Ser 81: 13-24
- 386 21. Leggett W, Deblois E (1994) Recruitment in marine fishes: is it regulated by
- 387 starvation and predation in the egg and larval stages? Neth J Sea Res 32:
- 388 119-134
- 389 22. Shepherd T, Costain K, Litvak M (2000) Effect of development rate on the
- 390 swimming, escape responses, and morphology of yolk-sac stage larval
- 391 American plaice, *Hippoglossoides platessoides*. Mar Biol 137: 737-745
- 392 23. Saborido-Rey F, Kjesbu OS, Thorsen A (2003) Buoyancy of Atlantic cod
- 393 larvae in relation to developmental stage and maternal influences. J Plankton
- 394 Res 25: 291-307
- 395 24. Yin M, Blaxter J (1987) Temperature, salinity tolerance, and buoyancy during
- 396 early development and starvation of Clyde and North Sea herring, cod, and
- 397 flounder larvae. J Exp Mar Biol Ecol 107: 279-290
- 398 25. Batty R, Blaxter J (1992) The effect of temperature on the burst swimming
- 399 performance of fish larvae. J Exp Biol 170: 187-201
- 400 26. Childs MR, Clarkson RW (1996) Temperature effects on swimming
- 401 performance of larval and juvenile Colorado squawfish: implications for
- 402 survival and species recovery. Trans Am Fish Soc 125: 940-947
- 403 27. Green BS, Fisher R (2004) Temperature influences swimming speed, growth
- 404 and larval duration in coral reef fish larvae. J Exp Mar Biol Ecol 299: 115-132
- 405 28. Peck MA, Buckley LJ, Bengtson DA (2006) Effects of temperature and body
- 406 size on the swimming speed of larval and juvenile Atlantic cod (*Gadus*
- 407 *morhua*): implications for individual-based modelling. Environ Biol Fishes 75:
- 408 419-429
- 409 29. Døving K, Mørstøl M, Andersen J, Knutsen J (1994) Experimental evidence of
- 410 chemokinesis in newly hatched cod larvae (*Gadus morhua* L.). Mar Biol 120:
- 411 351-358
- 412 30. Neilson JD, Perry RI, Valerio P, Waiwood KG (1986) Condition of Atlantic
- 413 cod *Gadus morhua* larvae after the transition to exogenous feeding -
- 414 morphometrics, buoyancy and predator avoidance. Mar Ecol Prog Ser 32:
- 415 229-235
- 416 31. Millero FJ, Poisson A (1981) International one-atmosphere equation of state of

417 seawater. Deep-Sea Res 28: 625-629  
418 32. Bian X, Zhang X, Sakrai Y, Jin X, Gao T, Wan R, Yamamoto J (2014)  
419 Temperature - mediated survival, development and hatching variation of  
420 Pacific cod *Gadus macrocephalus* eggs. J Fish Biol 84: 85-105  
421 33. Hunt von Herbing I, Boutilier R, Miyake T, Hall B (1996) Effects of  
422 temperature on morphological landmarks critical to growth and survival in  
423 larval Atlantic cod (*Gadus morhua*). Mar Biol 124: 593-606  
424 34. Boehlert GW, Gadomski DM, Mundy BC (1985) Vertical distribution of  
425 ichthyoplankton off the Oregon coast in spring and summer months. Fish Bull  
426 83: 611-621  
427 35. Houde E, Hoyt R (1987) Fish early life dynamics and recruitment variability.  
428 Am Fish Soc Symp 2: 17-29  
429  
430  
431

432 **Figure captions and Table titles**

433

434 Fig. 1 Vertical position of Pacific cod larvae in the experimental column  
435 during the initial 3 days after hatching at 3 (a), 5 (b), 7 (c) and 9 °C (d). Data  
436 points represent the average of median position in eight replicates and error  
437 bars present average 25th and 75th percentiles of larval positions

438

439 Fig. 2 Mean vertical movement rate of Pacific cod larvae at 5 °C during the  
440 initial 3 days after hatching. The larvae were reared at 3, 5, 7 and 9 °C,  
441 respectively. Data points and error bars represent means  $\pm$  SE calculated  
442 from 21–79 individuals in eight replicates

443

444 Fig. 3 Relationship between vertical movement rate (V) and body size  
445 (standard length, SL) of larvae reared at 3, 5, 7 and 9 °C during the initial 3  
446 days after hatching

447

448 Fig. 4 Relationship between vertical movement rate (V) and yolk-sac size  
449 (volume, YSV) of the larvae reared at 3, 5, 7 and 9 °C during the initial 3  
450 days after hatching. The data were multiplied by 1,000 at the x-axis

451

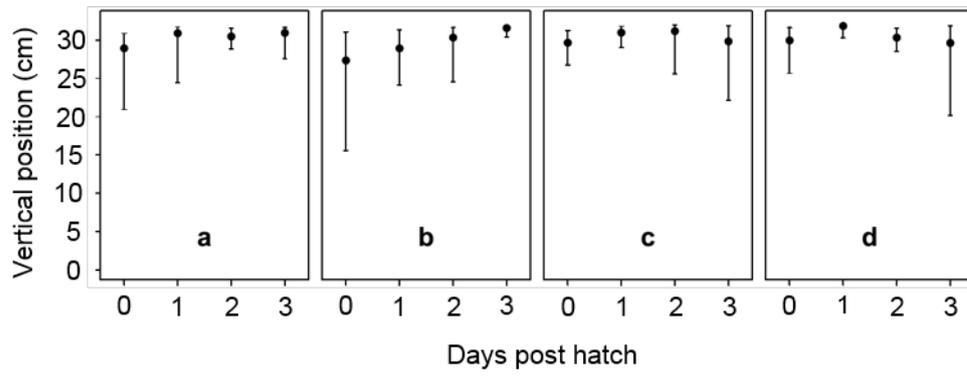
452 Fig. 5 Specific gravity of Pacific cod larvae reared 3, 5, 7 and 9 °C during the  
453 initial 3 days after hatching. Line A and B stand for seawater densities at 3  
454 and 9 °C, respectively. Data points and error bars represent means  $\pm$  SD

455

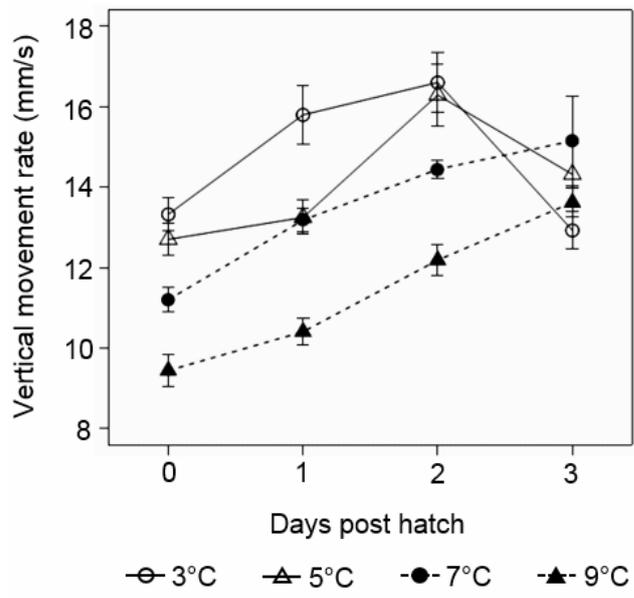
456 Table 1 Actual temperatures (mean  $\pm$  SD, °C) in trials of vertical position (A)  
457 and vertical movement rate (B)

458

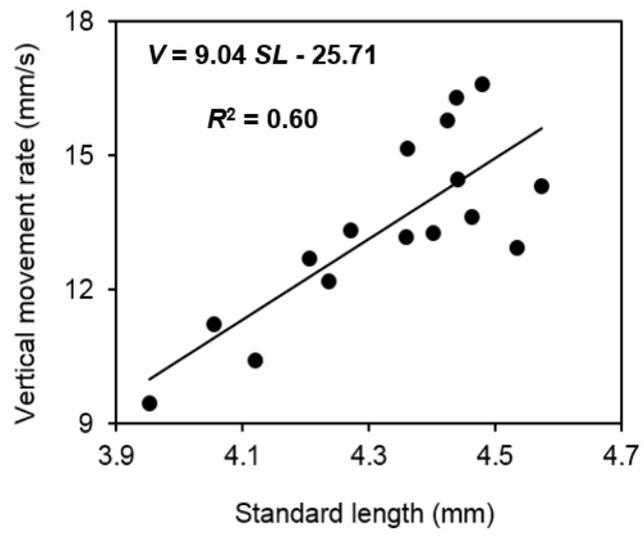
459 Table 2 Standard length (SL, mean  $\pm$  SD) and yolk-sac volume (YSV, mean  $\pm$  SD) of  
460 larvae reared at 3, 5, 7 and 9 °C during the initial 3 days after hatching, based on 13–  
461 36 subsample individuals at each temperature/age combination



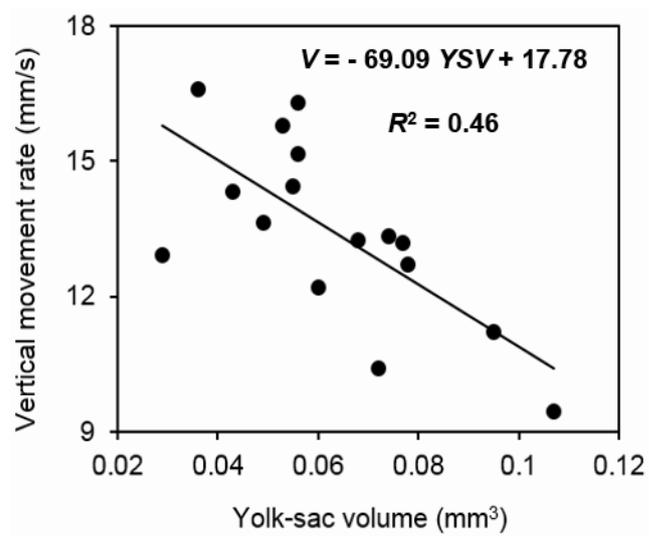
**Fig. 1**



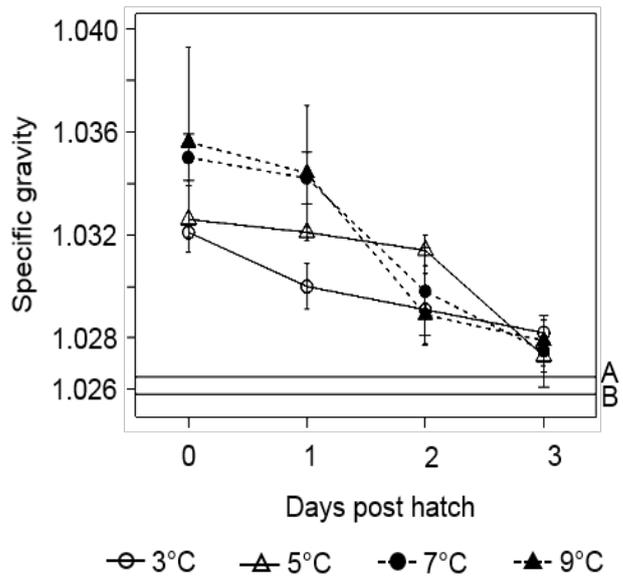
**Fig. 2**



**Fig. 3**



**Fig. 4**



**Fig. 5**

**Table 1**

---

Temperature treatment (°C)	Actual temperature (mean $\pm$ SD, °C)	
	A	B
3	3.2 $\pm$ 0.2	5.1 $\pm$ 0.2
5	5.1 $\pm$ 0.1	5.1 $\pm$ 0.1
7	7.1 $\pm$ 0.2	5.2 $\pm$ 0.2
9	9.0 $\pm$ 0.3	5.3 $\pm$ 0.1

---

