



Title	Comparison of low temperature adaptation ability in three native and two hybrid strains of the rotifer <i>Brachionus plicatilis</i> species complex
Author(s)	Kim, Hee-Jin; Iwabuchi, Mutsumi; Sakakura, Yoshitaka; Hagiwara, Atsushi
Citation	Fisheries Science, 83(1), pp.65-72; 2017
Issue Date	2017-01
URL	http://hdl.handle.net/10069/37768
Right	© Japanese Society of Fisheries Science 2016; The final publication is available at Springer via http://dx.doi.org/10.1007/s12562-016-1045-1

This document is downloaded at: 2019-03-26T04:46:18Z

1 **Comparison of low temperature adaptation ability in three native and two**
2 **hybrid strains of the rotifer *Brachionus plicatilis* species complex**

3
4
5 **HEE-JIN KIM*, MUTSUMI IWABUCHI, YOSHITAKA SAKAKURA, ATSUSHI HAGIWARA**

6
7
8 *Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo 1-14,*
9 *Nagasaki 852-8521, Japan.*

10
11
12 * Corresponding author: Tel/Fax: (+81) 95-819-2830. Email: heejin@nagasaki-u.ac.jp

26 **ABSTRACT:** Low temperature adaptation ability of five selected strains of *Brachionus plicatilis* species complex:
27 Japanese (NH1L), Australian, German, and two hybrid strains (♀NH1L and ♂Australian; N×A, and ♀NH1L and
28 ♂German; N×G) was investigated with life history, reproductive characteristics and mobility under different thermal
29 conditions at 12 and 25°C. The life history parameters at 12°C showed longer lifespans, reproduction periods and
30 generation times with reduced lifetime egg and offspring productions compared to those at 25°C. At 12°C, the
31 intrinsic rate of natural increase was higher in NH1L and N×A strains. Reproductive characteristics determined at
32 12°C with batch culture showed active population growth for NH1L and N×G strains, while no resting egg production
33 was observed in all the tested strains. The ratio of swimming rotifers at 12°C was monitored every hour for 6 hours
34 (short-term) and every day for 10 days (long-term). NH1L strain maintained over 81.3% of swimming, while other
35 strains exhibited low rates (< 60 %) in the short-term observations. In the long term observations, NH1L and two
36 hybrid strains showed over 75% of swimming rate from the initial day. The obtained results recommended that
37 outcrossing of rotifer strains is useful to obtain live food resources for cold water fish larviculture.

38

39

40 **KEYWORDS:** *Brachionus plicatilis* sp. complex; Low temperature adaptation; Life history; Swimming activity

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57 **INTRODUCTION**

58

59 The rotifer *Brachionus plicatilis* sp. complex including *B. plicatilis* sensu stricto and *B. manjavacas* is widely used in
60 aquaculture facilities as live food for the initial stage of larviculture [1]. For effective larviculture, live food species
61 should remain at a certain level in the water column of larval rearing tanks, while rotifers lose their availability in cold
62 water temperatures that are needed for the proper larval growth of cold water species. Temperature is known to be a
63 significant factor that controls rotifer physiological conditions related to life history [2], survival [3], reproduction [4]
64 and mobility [5]. The rotifer *Brachionus* has a wide range of temperature tolerance, but active population growth and
65 locomotion can occur temperatures ranging from 25 to 30°C [2, 3, 5]. Under low temperature conditions (typically
66 below 20°C), the rotifers showed inactivity in the aforementioned parameters [2, 5, 6], but larvae of cold water species
67 such as Japanese flounder *Paralichthys olivaceus* [7], Pacific cod *Gadus macrocephalus* [8] and Atlantic cod *Gadus*
68 *morhua* [9] must be cultured at 11-16°C during the initial feeding within 2 days after yolk exhaustion to obtain optimal
69 growth. To prevent cold shock by rapid thermal decrease, the rotifers should be cultured at the same temperature as
70 the larviculture [10]. The cold shock of rotifers results in a low survival rate and a decreased mobility of rotifers (*i.e.*,
71 sinking to the bottom of larval rearing tanks or sluggish movement) [10, 11]. These symptoms should make the
72 rotifers unavailable for cold water fish larvae in aquaculture facilities [3, 5, 12]. However, rotifers are difficult to
73 mass-culture at low temperature and show stagnation (or decreasing trends) of population growth [11, 13]. Given the
74 above factors, rotifer strains with strong tolerance to low temperature are required for an efficient larval rearing of cold
75 water fishes.

76 The present study was established in response to the requirement for appropriate live food for cold water fish larvae.
77 To date, the low temperature effects on physiological conditions of rotifers were considered for preservation and
78 explaining negative effects on population growth [3, 11, 14]. The low temperature tolerance appears to differ among
79 various species and strains of the rotifer *Brachionus* [2]. In addition, the hybrid strains obtained by cross-mating of
80 two strains of the rotifer *Brachionus* sp. showed distinct biological characteristics from their parental strains [4, 12].
81 Therefore, we employed three native strains (NH1L, Australian and German) and two hybrid strains (♀NH1L and
82 ♂Australian; $N \times A$, and ♀NH1L and ♂German; $N \times G$) for this study (Table 1). Rotifers can use alternative strategies
83 to maintain their normal metabolism under excessive stress conditions. Their metabolic activities under stressful
84 conditions are mainly assessed by life history parameters, reproductive characteristics and mobility [15-18]. This
85 study compared the low temperature adaptation ability of the employed rotifer strains in the *B. plicatilis* s. s., *B.*
86 *manjavacas*, and their hybrids in terms of their (1) life history parameters, (2) reproductive characteristics (*i.e.*, sexual
87 and asexual reproduction) and (3) mobility (swimming activity) that were investigated under the different thermal

88 conditions at 12 and 25°C.

89

90

91 **MATERIALS AND METHODS**

92

93 **Strain selection**

94

95 To determine potential strains from the rotifer collection in our laboratory, four strains of *B. plicatilis* s. s., three strains
96 of *B. manjavacas*, and two strains of their hybrid (Table 1) were pre-cultured at 20°C and a salinity of 17 ppt (parts per
97 thousand) under ad libitum food condition for one month. These strains were collected from different geographic
98 regions and were empirically known to have a strong tolerance to low temperature compared to other stock strains.
99 We tested the effect of low temperature on the population growth rate of these strains. The population growth at 10°C
100 was estimated through 14-day batch culture with or without thermal acclimation at 15°C under the optimal food
101 condition for population growth of these strains, at 7×10^6 *Nannochloropsis oculata* cells/ml [18]. The culture medium
102 was prepared by diluting natural seawater with Milli-Q water (Millipore 0.22µm) followed by GF/C filtration and
103 autoclave sterilization at 121°C for 20 min. The microalgae *N. oculata* cultured in modified Erd-Schreiber medium
104 [19] was centrifuged with cell grown medium at 5000 rpm for 10 min, and only precipitated algal cells were re-
105 suspended daily into the rotifer culture medium. To compare asexual reproductive activity at low temperature, the
106 population growth rate (r) on the last day of culture was calculated using the following formula: $r = \ln(N_t/N_0)/t$, where
107 N_0 =initial density of rotifers, N_t =the number of individuals on day t , t = culture days. Based on these results (Fig. 1 in
108 the results of *Rotifer strain selection*), the low temperature adaptation ability evaluated by the life history, reproductive
109 characteristics and mobility at 12°C (described below) were compared among the following five selected strains: three
110 native strains *i.e.*, Japanese (NH1L), Australian, German, and two hybrid strains *i.e.*, $N \times A$ (♀NH1L and ♂Australian),
111 $N \times G$ (♀NH1L and ♂German).

112

113 **Individual culture**

114

115 The individual cultures of the five selected rotifer strains were carried out to determine the effects of low temperature
116 on the following life history parameters: (1) lifespan, (2) reproduction period, (3) generation time, (4) lifetime egg
117 production and (5) lifetime offspring production. With these data, (6) the intrinsic rate of natural increase (r_m) was
118 calculated by the following formula: $\sum l_x \cdot m_x \cdot e^{-r_m x} = 1$, where x =age interval, e =natural logarithm, l_x =the

119 probability of surviving to age x , m_x =the number of female offspring per female of age x born during the interval x to
120 $x+1$.

121 The rotifers were cultured at 25°C and 17 ppt with daily feeding of *N. oculata* at 7×10^6 cells/ml. From these
122 cultures, a certain number of rotifers carrying amictic eggs were transferred into a screw capped bottle containing 15 ml
123 of fresh culture medium, and then agitated to shake off their eggs. About 200 of the separated eggs were each
124 incubated in a well of a 12-well microplate under the same conditions as the stock cultures. Hatchlings (F_1) carrying
125 an amictic egg were individually inoculated into a 48-well microplate, and F_2 (< 4 h) hatched from these eggs were
126 acclimatized to the experimental temperature. To initiate experimental culture, F_2 individuals were individually
127 transferred into a well of 24-well microplate containing 1 ml of fresh medium suspending *N. oculata* at 7×10^6 cells/ml.
128 The experimental set-up was established with the same methods as described in the *Strain selection* section. The
129 inoculated rotifers ($n=3$) of each strain were cultured at 25 or 12°C under complete darkness and were transferred daily
130 into a new well containing fresh medium. The observation of the life history parameters was consecutively made with
131 the rotifer transference.

132

133 **Batch culture**

134

135 Batch cultures were initiated with five F_2 individuals carrying amictic eggs in 5 ml of food suspension which were
136 prepared by the same methods as described in the part of *Individual culture*. These cultures were maintained in the
137 microalgae *N. oculata* suspension at the constant concentration of 7×10^6 cells/ml without changing the medium for 14
138 days. A culture trial was maintained in a well of a 6-well microplate and was incubated at 25 or 12°C under complete
139 darkness ($n=6$). The number of female rotifers in each culture was counted daily with the following categories based
140 on the types of eggs carried [14]: female carrying no eggs, amictic female carrying female eggs (FF); mictic female
141 carrying male eggs (MF); mictic female carrying resting eggs (RF). The r was calculated by the same formula as
142 mentioned in the *Strain selection* ($r = \ln(N_t/N_0)/t$), and mixis and fertilization rates were calculated using the following
143 formula:

$$144 \text{ Mixis (\%)} = \{(MF+RF)/(FF+MF+RF)\} \times 100$$

$$145 \text{ Fertilization (\%)} = \{(RF)/(MF+RF)\} \times 100.$$

146

147 **Swimming activity**

148

149 The cultures of the five selected strains were maintained under the same culture conditions as the previous tests. From
150 the culture of each strain, 50 individuals were randomly selected and transferred into a well of 12-well microplate
151 containing 3 ml of fresh medium at 17 ppt and 12°C. The microplates containing the rotifers were wrapped around
152 with foamed styrol to prevent thermal fluctuation of medium. These were adapted to complete darkness for 5 min and
153 then the swimming rotifers were immediately counted. After the counting, the plates were maintained at 12°C until
154 the next observation. As a short term observation for 6 hours, the number of swimming rotifers was monitored every
155 hour. For a long term observation for 10 days, the whole rotifer cultures (at 25°C) were transferred to 12°C and were
156 monitored every day with the same methods as the short term observation. Swimming rate (%), which is the
157 proportion of swimming individuals among the total individuals, was estimated by the average of 5 independent
158 replicates ($n=5$).

159

160 **Statistical analysis**

161

162 For the selection of rotifer strain, the population growth rate of the nine rotifer strains at low temperature associated
163 with a treatment *i.e.*, with or without the acclimation were compared by Tukey-Kramer *post hoc* test, when a one-way
164 ANOVA detected a significant difference. The indices of life history parameters, reproductive characteristics, and
165 swimming rate of the 5 selected strains between the set-up temperatures (at 12 and 25°C) was compared using Tukey-
166 Kramer *post hoc* test after one-way ANOVA. All statistical analyses were performed by StatView version 5.0
167 software (SAS Institute, Inc., USA).

168

169

170 **RESULTS**

171

172 **Rotifer strain selection**

173

174 The population growth rate (r) with or without the thermal acclimation is shown in Figure 1. For the 14-days cultures
175 at 10°C without the acclimation, the Shizuoka and the Australian strains showed negative growth rates compared to the
176 7 other strains ($P<0.05$, Fig. 1a). In the culture trials with the acclimation at 15°C, the Shizuoka strain was extinct
177 during the acclimation. The other strains cultured at 10°C showed no significant differences in population growth rate
178 (Fig. 1b). The NH1L, Amami, and Russian strains maintained their population growth rate at low temperature, but the
179 five other strains: Makishima, Australian, German, $N\times A$, and $N\times G$, needed the acclimation ($P<0.05$) to maintain their

180 population growth rate. Through the comparison of population growth rate, the rotifer strains which showed active
181 propagation at 10°C with acclimation (Australian, German, $N \times A$, and $N \times G$) and without acclimation (*i.e.*, NH1L) were
182 selected to evaluate low temperature tolerance.

183

184 **Individual culture**

185

186 Table 2 shows life history parameters of the rotifer strains at different culture temperatures (12 and 25°C). There were
187 no significant differences in the lifespan and the generation time among the all strains cultured at 25°C. On the other
188 hand, other parameters showed differences at 25°C. The reproduction period was the longest in the German strain
189 ($P < 0.05$). The lifetime egg production ($P < 0.05$) and lifetime offspring production ($P < 0.05$) were the highest in the
190 Australian strain. The intrinsic rate of natural increase (r_m) at 25°C was the highest in the Australian strain ($P < 0.0001$).
191 When rotifers were cultured at 12°C, there were no significant differences in the reproduction period, lifetime egg
192 production, and lifetime offspring production among the tested strains. For the other parameters at 12°C, the longest
193 lifespan and generation time were observed in the German strain ($P < 0.0001$), and the highest r_m was in the NH1L and
194 the $N \times A$ strains ($P < 0.0001$).

195

196 **Batch culture**

197

198 The characteristics of sexual and asexual reproductions determined by batch cultures under different water temperature
199 (12 and 25°C) are shown in Table 3. For asexual reproduction, the $N \times A$ strain most actively propagated for 14 days at
200 25°C ($P < 0.0001$), while the NH1L and $N \times G$ strains exhibited higher population growth rate at 12°C ($P < 0.05$).
201 Activity of sexual reproduction was defined by the following 4 parameters: mixis rate, male density, fertilization rate,
202 and the total number of produced resting eggs. These parameters were higher in the Australian strain at 25°C
203 ($P < 0.001$). For the two hybrid strains at 25°C, the $N \times A$ showed the abnormality of un-hatched mictic eggs (male eggs)
204 and no resting egg production in the $N \times G$ strain even males being presented. At 12°C, the 5 tested strains showed no
205 resting egg production. Higher mixis rate and male density at 12°C were observed in the NH1L and $N \times A$ ($P < 0.001$),
206 and in the Australian culture ($P < 0.05$), respectively.

207

208 **Swimming activity**

209

210 The swimming activity of the tested rotifer strains at 12°C is shown in Figure 2. In the short-term observations (for 6

211 h), a higher proportion of swimming individuals (81.3 ± 6.7 to $91.3\pm 5.3\%$) was observed in the NH1L strain ($P<0.05$).
212 The other strains showed $< 60\%$ swimming rates during the observation period ($P<0.0001$). In the long-term
213 observations (for 10 d), NH1L, $N\times A$, and $N\times G$ showed higher swimming rates (79.2 ± 4.6 to $93.2\pm 5.2\%$) than the other
214 strains ($P<0.0001$) after one day from the beginning of observation at 12°C . The five tested strains showed over 75%
215 of swimming rate on the last day of long-term observation.

216

217

218 **DISCUSSION**

219

220 This study compared the low temperature adaptation ability of various rotifer strains of *B. plicatilis* s. s., *B. manjavacas*
221 and their hybrids by testing life history parameters, reproductive characteristics and mobility at 12°C , which can
222 provide information to help supplying a suitable live food for effective larviculture of cold-water fishes. The
223 outcrossing between two different species: *B. plicatilis* s. s. (NH1L) and *B. manjavacas* (Australian and German), was
224 possible by a close phylogenetic distance [20]. Such hybrid strains show different morphological and physiological
225 characteristics from their parents [4, 12]. In this study, the strains even in the same species showed different low
226 temperature adaptation ability. For strain selection, the population growth of nine rotifer strains (Table 1) was
227 determined at 10°C with or without thermal acclimation at 15°C . Acclimation is important for survival at low
228 temperature [6], and thus most strains showed higher growth rate after acclimation (Fig. 1). However, the Shizuoka
229 strain did not survive during the acclimation, and experienced mortality at 10°C without acclimation. It may imply
230 that the critical temperature for survival of the Shizuoka strain is higher than for the other strains, which may be around
231 20°C . No differences associated with the acclimation were observed with the NH1L, Amami and Russian strains.
232 These differences might reflect the geographic features of their habitats, because organisms are adapted to the ambient
233 conditions where they live [21, 22]. Miracle and Serra [2] reported that temperature ranged as rotifer habitat had
234 negligible influences on their physiological conditions, while low temperature induces species- and strain-related
235 properties because of the restricted temperature tolerances of rotifers. The results of this study showed that there are
236 strain-related properties (Table 2) and these may be affected by the temperature ranges of their original habitats.

237 Life history parameters, a tool for diagnosing the physiological condition of rotifers [23, 24], were evaluated by the
238 individual culture of each strain. The tested rotifers at 12°C (Table 2) had similar propensities when they were
239 exposed to extreme stresses [17, 25]. In the rotifer *Brachionus* sp., low temperature prevented normal metabolism as
240 shown by the expression of the *hsp-20* at 10°C , which is for cellular defense under environmental stress conditions [26].
241 The embryonic development period of rotifers is elongated by decreasing temperature [27], and thus it probably retards

242 reproduction. The calculated r_m evaluates the reproductivity of animals [28]. In this study, the r_m value at 25°C was
243 similar to that reported by King and Miracle [29] under favorable conditions (0.32-0.38), while that at 12°C was below
244 0.11. This implies that the physiological status of the rotifers in our study was kept at good condition at 25°C, which
245 resulted in the active reproduction. The highest value of r_m at 12°C was shown by the NH1L and $N \times A$ strains (Table 2)
246 and shows the possibility of mass-culture of rotifers at low temperatures [30].

247 Batch cultures of the selected rotifer strains were tested to evaluate the possibility of mass culture at low temperature.
248 The intrinsic feature (e.g., thermal tolerance) derived from the temperature of maternal culture, determines the sexual
249 reproduction characteristics of rotifer descendants [31]. Therefore, this study employed F₂ rotifers from mothers
250 cultured under the same environmental conditions to reduce the hereditary effects. Thermal fluctuation to low
251 temperature was reported to negatively affect sexual [31] and asexual reproduction [3] of rotifers. This study showed
252 that sexual reproduction of rotifers was negatively affected by low temperature that caused an inactive mixis induction
253 and no resting egg production, compared to asexual reproduction (Table 3). The asexual reproduction of the five
254 tested rotifer strains maintained a certain level of population growth. The required level of food can be varied with
255 culture temperature to maintain stable population growth of rotifers [13]. It is expected that populations of the selected
256 rotifer strains could maintain steady growth at the low temperature because of being sufficiently fed. Active
257 propagation was observed in the batch culture of NH1L associated with the determined r_m at the low temperature (Table
258 2). The hybrid strains, $N \times A$, and $N \times G$ strains also showed high population growth rates, perhaps due to the inherited
259 genetic background from the NH1L strain [12].

260 The frequency and duration of immobility after thermal fluctuation determines the efficiency as live foods for fish
261 larvae [5, 32]. The short term observation for 6 h was set up to compare with the study by Fielder et al. [32]. They
262 reported that *B. plicatilis* should be cultured at lower temperature or acclimated for at least 6 h to larval rearing
263 conditions before transfer to larval rearing tanks. In this study, the NH1L strain showed the highest swimming activity
264 during the short term observation (Fig. 2a), suggesting its potential to be used as live food for cold water species.
265 Moreover, in the long term observation, the two hybrid strains originated from the NH1L mothers also showed over 60%
266 of mobility on the first day (Fig. 2b). This phenomenon may also indicate that two hybrid strains are better adapted to
267 low temperatures by inherited characters from their mother compared to the other strains [12].

268 Recently, the application of rotifer strains acclimated to low temperatures has been used to acquire economic benefits
269 in the hatchery production of the cold water fish, Pacific cod *Gadus macrocephalus* [8]. The present study
270 demonstrated that the low temperature adaptation ability of the rotifer *B. plicatilis* sp. complex is indeed strain-related.
271 Among the five tested strains in this study, the NH1L strain has the highest ability to adapt at low temperature and is
272 expected to maintain their availability in the larval rearing tanks for the optimal growth of cold water aquaculture

273 species [5, 10, 32]. In addition, the outcrossing of rotifer strains is useful to obtain live food resources for cold water
274 fish larval rearing.

275

276

277 **ACKNOWLEDGEMENT**

278 This research was supported by a Ministry of Education, Science, Sports and Culture Grant-in-Aid for Scientific
279 Research (B) (2012-2014, No. 24380108) to A. Hagiwara. The authors deeply appreciate Prof. Michael J. Miller in
280 Nihon University and the anonymous reviewers for the valuable comments to improve the manuscript.

281

282

283 **REFERENCES**

284

- 285 1. Fontaneto D, Giordani I, Melone G, Serra M (2007) Disentangling the morphological stasis in two rotifer species of
286 the *Brachionus plicatilis* species complex. *Hydrobiologia* 583: 297-307
- 287 2. Miracle MR, Serra M (1989) Salinity and temperature influence in rotifer life history characteristics. *Hydrobiologia*
288 186/187: 81-102
- 289 3. Assavaaree M, Hagiwara A, Lubzens E (2001) Factors affecting low temperature preservation of the marine rotifer
290 *Brachionus rotundiformis* Tschugunoff. *Hydrobiologia* 446/447: 355-361
- 291 4. Hagiwara A, Suga K, Akazawa A, Kotani T, Sakakura Y (2007) Development of rotifer strains with useful traits for
292 rearing fish larvae. *Aquaculture* 268: 44-52
- 293 5. Øie G, Olsen Y (1993) Influence of rapid changes in salinity and temperature on the mobility of the rotifer
294 *Brachionus plicatilis*. *Hydrobiologia* 255/256: 81-86
- 295 6. Lubzens E, Rankevich D, Kolodny G, Gibson O, Cohen A, Khayat M (1995) Physiological adaptations in the
296 survival of rotifer (*Brachionus plicatilis*, O. F. Müller) at low temperatures. *Hydrobiologia* 313/314: 175-183
- 297 7. Dou SZ, Masuda R, Tanaka M, Tsukamoto K (2005) Effects of temperature and delayed initial feeding on the
298 survival and growth of Japanese flounder larvae. *J Fish Biol* 66: 362-377
- 299 8. Tomoda T, Dan S (2014) Stagnant water larviculture using the rotifer *Brachionus plicatilis* acclimated at low
300 temperature in Pacific cod *Gadus macrocephalus*. *Aquaculture Sci.* 62: 307-318.
- 301 9. Otterlei E, Nyhammer G, Folkvord A, Stefansson SO (1999) Temperature- and size-dependent growth of larval and
302 early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic
303 cod. *Can J Fish Aquat Sci* 56: 2099-2111

- 304 10. Tomoda T, Koiso M, Shima Y (2008) Temperature effects of rotifer *Brachionus plicatilis* mass culture on rearing
305 efficiency of larval Japanese flounder *Paralichthys olivaceus*. Nippon Suisan Gakkaishi 74: 625-635
- 306 11. Assavaaree M, Hagiwara A, Ide K, Maruyama K, Lubzens E (2001) Low-temperature preservation (at 4°C) of
307 marine rotifer *Brachionus*. Aquac Res 32: 29-39
- 308 12. Kotani T, Ihara K, Hagiwara A (2006) Cross-mating of euryhaline rotifer *Brachionus plicatilis* strains as a means to
309 develop useful strains for larval fish food. Aquaculture 261: 495-500
- 310 13. Stelzer C-P (1998) Population growth in planktonic rotifers. Does temperature shift the competitive advantage for
311 different species? Hydrobiologia 387/388: 349-353
- 312 14. Hagiwara A, Hino A, Hirano R (1988) Effects of temperature and chlorinity on resting egg formation in the rotifer
313 *Brachionus plicatilis*. Nippon Suisan Gakkaishi 54: 569-575
- 314 15. Munro PD, Henderson RJ, Barbour A, Birkbeck TH (1999) Partial decontamination of rotifers with ultraviolet
315 radiation: the effect of changes in the bacterial load and flora of rotifers on mortalities in start-feeding larval turbot.
316 Aquaculture 170: 229-244
- 317 16. Yin XW, Niu CJ (2008) Effect of pH on survival, reproduction, egg viability and growth rate of five closely related
318 rotifer species. Aquat Ecol 42: 607-616
- 319 17. Yoshinaga T, Hagiwara A, Tsukamoto K (2000) Effect of periodical starvation on the life history of *Brachionus*
320 *plicatilis* O. F. Müller (Rotifera): a possible strategy for population stability. J Exp Mar Biol Ecol 253: 253-260
- 321 18. Yúfera M, Navarro N (1995) Population growth dynamics of the rotifer *Brachionus plicatilis* cultured in non-
322 limiting food condition. Hydrobiologia 313/314: 399-405
- 323 19. Hagiwara A, Hamada K, Hori S, Hirayama K (1994) Increased sexual reproduction in *Brachionus plicatilis*
324 (Rotifera) with the addition of bacterial and rotifer extracts. J Exp Mar Biol Ecol 181: 1-8
- 325 20. Yoshinaga T, Minesishi Y, Rumengan IFM, Kaneko G, Furukawa S, Yanagawa Y, Tsukamoto K, Watabe S (2004)
326 Molecular phylogeny of the rotifers with two Indonesian *Brachionus* lineages. Coast Mar Sci 29: 45-56
- 327 21. Quetin LB, Ross RM (1991) Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*.
328 Amer Zool 31: 49-63
- 329 22. Vanhaecke P, Sorgeloos P (1980) International Study on *Artemia*. IV. The biometrics of *Artemia* strains from
330 different geographical origin. In: Persoone G, van Sorgeloos P, Roels O and Jaspers E (eds) The Brine Shrimp
331 *Artemia*, Universa Press, Wetteren, Belgium pp. 393-405
- 332 23. Korstad J, Olsen Y, Vadstein O (1989) Life history characteristics of *Brachionus plicatilis* (rotifera) fed different
333 algae. Hydrobiologia 52: 43-50
- 334 24. Yoshinaga T, Hagiwara A, Tsukamoto K (2003) Life history response and age-specific tolerance to starvation in

- 335 *Brachionus plicatilis* O. F. Müller (Rotifera). J Exp Mar Biol Ecol 287: 261-271
- 336 25. Zha C-W, Xi Y-L, Huang L, Zhao L-L (2007) Effect of sublethal exposure to chlordecone on life history
337 characteristics of freshwater rotifer *Brachionus calyciflorus* Pallas. Bull Environ Contam Toxicol 78: 79-83
- 338 26. Rhee J-S, Kim R-O, Choi H-G, Lee J, Lee Y-M, Lee J-S (2011) Molecular and biochemical modulation of heat
339 shock protein 20 (*Hsp* 20) gene by temperature stress and hydrogen peroxide (H₂O₂) in the monogonont rotifer,
340 *Brachionus* sp. Comp Biochem Physiol Part C 154: 19-27
- 341 27. Herzing A (1983) The ecological significance of the relationship between temperature and duration of embryonic
342 development of rotifers. Hydrobiologia 104: 237-246
- 343 28. Birch LC (1948) The intrinsic rate of natural increase of an insect population. J Anim Ecol 17: 15-26
- 344 29. King CE, Miracle MR (1980) A perspective on aging in rotifers. Hydrobiologia 73: 13-19
- 345 30. Hirayama K, Kusano T (1972) Fundamental studies on physiology of rotifer for its mass culture-II. Influence of
346 water temperature on population growth of rotifer. Nippon Suisan Gakkaishi 38: 1357-1363
- 347 31. Hino A, Hirano R (1985) Relationship between the temperature given at the time of fertilized egg formation and
348 bisexual reproduction pattern in the deriving strain of the rotifer *Brachionus plicatilis*. Nippon Suisan Gakkaishi 51:
349 511-514
- 350 32. Fielder DS, Purser GJ, Battaglione SC (2000) Effect of rapid changes in temperature and salinity on availability of
351 the rotifers *Brachionus rotundiformis* and *Brachionus plicatilis*. Aquaculture 189: 85-99

352

353

354

355

356

357

358

359

360

361

362

363

364 Tables

365

366 **Table 1** Origin of the nine strains of *Brachionus plicatilis* sp. complex from the rotifer collection of Nagasaki

367 University that were used in this study

Strain (Species)		Origin
Japanese (<i>Bp</i>)	NH1L	Japanese eel culture pond in 1970s (Hagiwara et al., 1994)
	Amami	National Center for Stock Enhancement, Fisheries Research Agency in Kagoshima, Japan
	Makishima	Fisheries Research Agency in Makishima, Japan
	Shizuoka	National Center for Stock Enhancement, Fisheries Research Agency in Shizuoka, Japan
Russian (<i>Bm</i>)		Russia (Snell et al., 1995)
Australian (<i>Bm</i>)		Marchland, Albany in Australia
German (<i>Bm</i>)		Schlei-Fjord in Germany (Kotani et al., 2006)
Hybrid	<i>N</i> × <i>A</i>	Hybrid between the NH1L (♀) and the Australian (♂)
	<i>N</i> × <i>G</i>	Hybrid between the NH1L (♀) and the German (♂)

368 *Bp*: *Brachionus plicatilis* sensu stricto

369 *Bm*: *Brachionus manjavacas*

370

371

Table 2 Life history parameters of the five selected strains of rotifer *Brachionus plicatilis* sp. complex at 25 and 12°C

	°C	NHIL	Australian	German	<i>N</i> × <i>A</i>	<i>N</i> × <i>G</i>
Lifespan (d)	25	14.6±5.5	14.3±4.4	12.4±2.5	12.2±5.4	14.5±4.8
	12	28.6±11.1 ^b	35.4±11.8 ^b	46.8±5.1 ^a	32.0±6.1 ^b	39.0±11.7 ^{ab}
Reproduction period (d)	25	7.0±2.0 ^{ab}	7.6±0.6 ^{ab}	8.0±1.8 ^a	5.9±1.0 ^b	7.0±1.0 ^{ab}
	12	21.3±10.1	23.7±10.6	28.7±5.2	23.3±5.2	30.8±10.9
Generation time (d)	25	1.8±0.5	1.5±0.2	1.7±0.3	1.7±0.1	1.6±0.2
	12	4.9±0.4 ^c	7.3±0.8 ^b	11.0±1.6 ^a	5.6±1.1 ^c	7.0±0.1 ^b
Lifetime egg production (eggs)	25	20.1±4.4 ^c	26.6±1.7 ^a	22.4±2.1 ^{abc}	21.4±4.4 ^{bc}	23.4±1.7 ^{ab}
	12	14.9±6.1	15.3±5.8	15.7±2.9	16.5±2.6	18.8±4.9
Lifetime offspring production (ind.)	25	19.6±4.9 ^c	26.3±1.5 ^a	22.1±2.4 ^{abc}	21.2±4.2 ^{bc}	23.1±1.7 ^{ab}
	12	14.4±5.9	13.5±5.5	15.2±3.0	15.0±3.5	18.0±4.6
Intrinsic rate of natural increase (r_m)	25	0.30±0.01 ^c	0.36±0.01 ^a	0.34±0.00 ^b	0.34±0.01 ^b	0.32±0.01 ^{bc}
	12	0.11±0.00 ^a	0.08±0.01 ^b	0.06±0.00 ^c	0.10±0.00 ^a	0.08±0.00 ^b

Values are means ± SD ($n=3$). Superscripts denote significant differences ($a>b>c$) in each life history parameter at a certain temperature ($P<0.05$).

Table 3 Reproduction parameters of the five selected strains of rotifer *Brachionus plicatilis* sp. complex at 25 and 12°C for 14 days

	°C	NH1L	Australian	German	N×A	N×G
Population growth (<i>r</i>)	25	0.36±0.01 ^c	0.37±0.01 ^{bc}	0.36±0.00 ^{bc}	0.38±0.00 ^a	0.37±0.00 ^b
	12	0.27±0.01 ^a	0.26±0.02 ^{ab}	0.24±0.01 ^b	0.25±0.01 ^{ab}	0.26±0.01 ^a
Mixis (%)	25	4.4±0.9 ^{bc}	14.6±3.4 ^a	11.7±3.7 ^a	7.3±1.2 ^b	0.6±0.8 ^c
	12	16.0±3.0 ^a	3.8±2.3 ^b	7.2±2.3 ^b	15.2±4.4 ^a	6.9±1.2 ^b
Male (ind./ml)	25	1.1±0.2 ^b	3.9±1.1 ^a	0.4±0.3 ^{bc}	0 ^c	0.01±0.01 ^c
	12	0.2±0.1 ^{ab}	0.3±0.3 ^a	0.1±0.2 ^{ab}	0 ^b	0.02±0.02 ^{ab}
Fertilization (%)	25	17.6±10.3 ^b	34.9±9.1 ^a	12.1±12.7 ^{bc}	0 ^c	0 ^c
	12	0	0	0	0	0
Resting egg (total No.)	25	1.7±2.0 ^b	52.3±17.3 ^a	5.3±7.8 ^b	0 ^b	0 ^b
	12	0	0	0	0	0

Values are means ± SD (*n*=6). Superscripts denote significant differences (a>b) in each reproduction parameter at a certain temperature (*P*<0.05).

Figure legends

Fig. 1 Population growth rate of the nine rotifer strains of *Brachionus plicatilis* sp. complex cultured at 10°C for 14 days without (a) or with acclimation at 15°C (b) for 14 days. Columns and error bars describe means and standard deviations of replication ($n=5-6$), respectively. Small alphabetic letters (a>b>c in the panel A) indicate significant differences among the rotifer strains ($P<0.05$) and asterisks (in the panel B) are differences between two treatments without and with acclimation ($P<0.05$).

Fig. 2 Swimming rate (% of 50 individuals) of the five selected rotifer strains of *Brachionus plicatilis* sp. complex at 12°C with 6 hours of short (a) and 10 days of long (b) term observation. Plots and error bars describe means and standard deviations of replications ($n=5$), respectively. Small alphabetic letters (a>b>c>d) indicate significant differences among the rotifer strains ($P<0.05$).

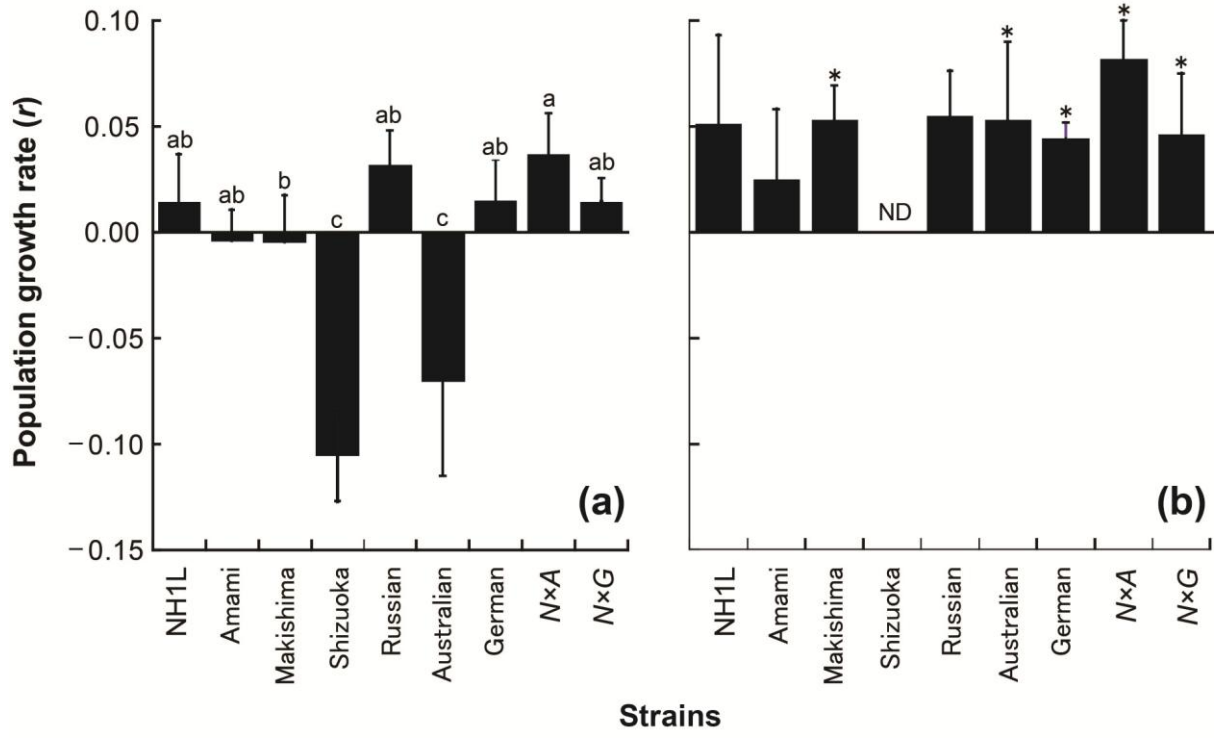


Fig. 1

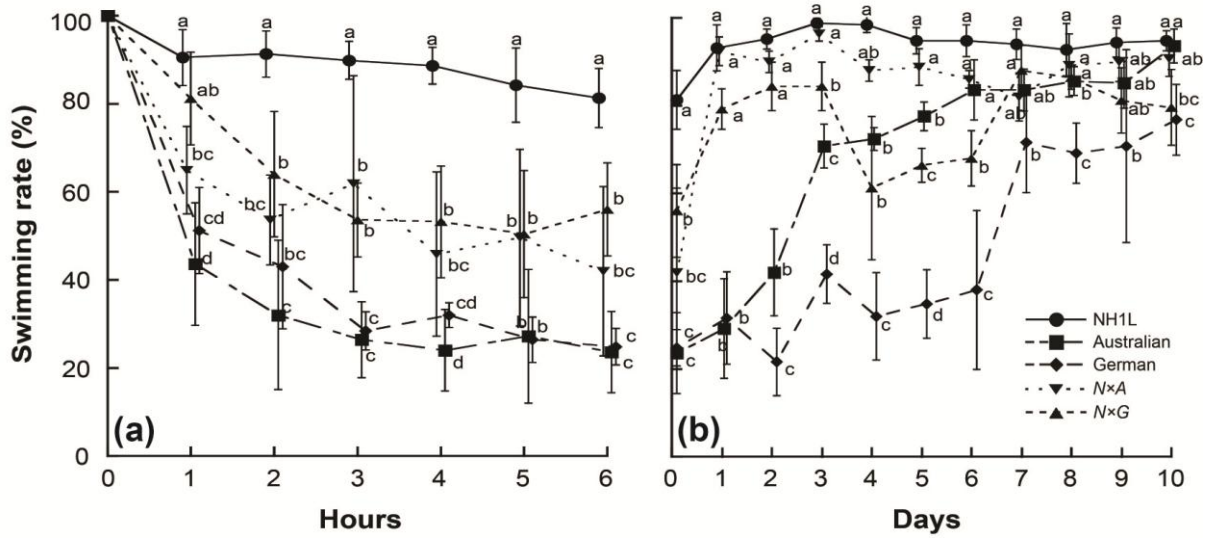


Fig. 2