アサヒガニ幼生の生残,摂餌および発生に及ぼす水温の影響

誌名	日本水産學會誌
ISSN	00215392
著者	皆川, 恵
巻/号	56巻5号
掲載ページ	p. 755-760
発行年月	1990年5月

Nippon Suisan Gakkaishi 56(5), 755-760 (1990)

Influence of Temperature on Survival, Feeding and Development of Larvae of the Red Frog Crab, *Ranina ranina* (Crustacea, Decapoda, Raninidae)*1,2

Megumi Minagawa*3

(Received November 16, 1989)

In order to examine temperature effects on several larval characters, zoeas of *Ranina ranina* were reared under 5 temperature levels ranging 17 to 33°C. Survival decreased at lower temperatures and optimum range for the survival seemed to become narrower in later instars. The molting depended significantly on the temperature, and molting intervals increased exponentially at lower temperatures. The optimum temperature was 25 to 29°C for growth and morphogenesis during all zoeal instars. The growth decreased and morphogenesis delayed at lower and higher temperatures out of optimum range. Food intake tended to increase at higher temperature at instars I to V but was the maximum at 25°C at instar VII. At higher temperature, reduction of growth and delay of morphogenesis were observed, in spite of a large amount of food intake in early instars. Unusual high temperature, which is not observed in nature, might affect the physiological condition of the larvae.

There are many projects to produce a large amount of seeds of the brachyuran crabs released to the open waters for sea farming in Japan.[1] Except for a few species, however, juvenile crabs have not been steadily produced, because of unclear factors of rearing conditions. To know them, we have begun to clarify the influence of biotic and abiotic environmental factors on survival and development of the decapod larvae.

Temperature is known to largely influence survival, molting intervals and total duration of larval development in many brachyuran species.[2-11] However, so limited is the information of the influence of temperature on growth, morphogenesis, feeding and variability of the number of larval instars in Brachyura, in comparison with Natantia,[12-15] Palinura[16] and Anomura.[17] Moreover, the relationship between feeding, which might be affected by temperature, and larval development has hardly been reported.

The rearing procedure of larvae of *R. ranina*, was reported in the previous papers,[18,19] but it is still difficult to produce a large number of juvenile crabs. To establish the mass-rearing system of this species, the effects of temperature on survival, feeding and development of the larvae were examined. Influence of feeding to the larval development and the suitable range of temperature for rearing the larvae are also discussed.

Materials and Methods

Ovigerous females were captured with traps off Hachijojima, an island located 290 km south of Tokyo. The 1st-instar zoeas hatched from a single female on July 6, 1988 were used for all experiments.

Newly hatched zoeas were introduced in experimental containers filled with filtered ambient sea water (salinity: 33–34‰). The containers were placed in temperature-controlled shallow bath without illumination controll. Zoeas were transferred to other clean containers with modified pipette every morning, and then prey was given to them. Dihydrostreptomycine sulfate was added to the rearing water at 10 ppm after giving them the prey. Zoeas at different instars were not reared in the same container. *Artemia* nauplii were given through all instars and short-necked clam *Ruditapess phillipinarum* were fed after instar V.

*1 Partly presented at the annual meeting of the Japanese Society of Scientific Fisheries, Tokyo, 1989.

*2 Studies on rearing larvae of the red frog crab *Ranina ranina*-IV.

*3 Hachijo Branch, the Tokyo Metropolitan Fisheries Experiment Station, Mitsune Hachijo, Tokyo 100-15, Japan (皆川 恵: 東京都水産試験場八丈分場).

Survival, molting intervals, growth and morphogenesis

Zoeas were reared at five temperature levels 17.2±0.30, 21.3±0.26, 25.5±0.31, 28.8±0.30 and 32.3±0.29°C, which were expressed as 17, 21, 25, 29 and 33°C for convenience, respectively. Two sets of zoeas at each temperature were cultured; 100 first-instar zoeas were prepared for each set. One set was examined for survival and molting intervals and the other was for growth and external morphology. Two-liter plastic containers were used during instars I-IV and 4-liter containers were for the later instars.

Five zoeas in one set at each temperature were fixed with 5% neutral formalin at instars III, V and VII to examine the growth of several characters and to count the setae on appendages. Carapace length (CL), rostrum length (RL), exopodal length of 2nd maxilliped (ML) and telson length (TL) were measured through a stereoscopic microscope.

Feeding

The number of *Artemia* nauplii fed per zoea・hour at 5 levels of temperatures: 17, 21, 25, 29 and 33°C, with 5 replicates each was examined at instars I, III, V and VII. The temperatures were 16.8±0.43, 21.2±0.43, 25.3±0.27, 29.2±0.35, 33.0±0.44°C. For acclimation to each temperature, zoeas reared at 28–29°C were transferred to 500 m*l* containers at each temperature 17 hours before the experiment. After acclimation zoeas were starved for 3 hours, and then they were transferred to 100 m*l* glass containers in which prey was already counted. Lastly filtered sea water was added at 50 m*l* to equal prey density at each temperature. One glass container contained 5 zoeas with 100 preys at instar I, 3 zoeas with 150 preys at instars III and V, and 1 zoea with 200 preys at instar VII. No antibiotic was used during the experiment. Prey taken by zoeas was calculated through the difference of the number of prey for 3-hour experiment.

Statistical procedures

The regression equation was calculated using the method of least squares. Logarithmic transformation was used in a regression curve. The correlation coefficient was tested with the F-test. Mean values were compared with Bartllett's test and Duncan's test. In the case of unequal varience, Kruscal-Wallis test and Mann-Whitney's U-test were used. All were tested with the personal computer software "Programs of statistical methods for biologists by N88-BASIC" (S. Ishii, Omori Maicon Co. Ltd.).

Results

Survival and molting intervals

Larvae reared at 25, 29 and 33°C reached megalopa. At 29°C, 41 megalopas, which were the maximum, were obtained. No larvae at 17 and 21°C survived beyond instars IV and VII, respectively. Survival decreased after instar V or VI at 21–33°C and tended to reduce steeper at instar VII at 33°C as compared with those at 25 and 29°C (Table 1).

Either the 7th- or the 8th-instar zoeas metamorphosed to meaglopas. Metamorphosis rate (MR), percentage of the number of megalopa metamorphosed from instar VII to the sum of the 8th-instar zoea and megalopa from instar VII, is one of the indications showing whether rearing conditions are better or not: higher MR shows that rearing conditions are better. MR was 76% at 33°C while it was 98% at 29°C.

Molting intervals prolonged in lower temperatures, but it was not significant between 29 and 33°C ($P<0.05$) (Table 2). The relationship between molting intervals and temperatures at instar I is shown as the following regression equa-

Table 1. Survival of *R. ranina* zoeas at five temperature levels, showing the number of individuals started at 100

Temperature (°C)	Instar										
	I	II	III	IV	V	VI	VII	VIII	M_7*1	M_8*1	MR (%)*2
33	98	92	85	81	58	45	42	4	13	1	76.5
29	100	98	95	85	71	50	49	1	40	1	97.6
25	99	97	96	91	75	55	51	5	31	4	86.1
21	100	95	91	82	57	14	0				
17	100	48	1	0							

*1 M_7: Megalopas molted from instar VII; M_8: Megalopas molted from instar VIII.
*2 Percentage of the number of megalopa metamorphosed from instar VII to the sum of the 8th-instar zoeas and megalopa from instar VII.

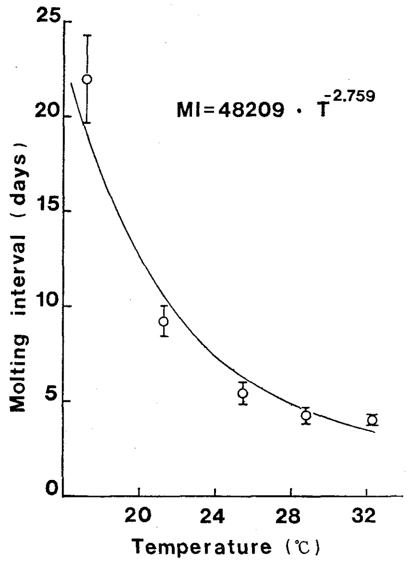

Fig. 1. Molting intervals at instar I of *R. ranina* at five temperature levels.
MI: Molting interval
T: Temperature

tion (Fig. 1).

$MI=48209 \cdot T^{-2.759}$ (MI: molting interval, T: temperature)

The correlation coefficient is -0.972 ($P<0.01$).

Accumulated temperatures (day·temperature) were the minimum at 29°C and those at 25 and 33°C were similar to each other. At 17 and 21°C, however, accumulated temperatures tended to be larger (Table 3).

Total zoeal duration

The relationship between the number of instars and the larval age at each temperature is shown in Fig. 2. The functions at 29 and 33°C were almost the same. The slopes of the functions decreased in lower temperatures, *i.e.*, zoeal duration was longer in lower temperatures. At 33°C, it took 32.9 days in M_7 (megalopa from instar VII) and 36.4 days in M_8 (megalopa from instar VIII). While at 25°C, 41.3 days in M_7 and 47.3 days in M_8 were needed (Table 2).

Feeding

At 33°C zoeas at instars I, III and V fed 1.4, 3.4 and 14.1 prey per zoea·hour, which were larger than those at 17, 21 and 25°C. Feeding at 25°C was not significantly different from the value at 29°C. At 25°C, however, the 7th-instar zoeas fed 53.7 prey per zoea·hour, which was 10 times and twice of those at 17 and 21°C, respectively (Fig. 3).

Table 2. Molting intervals (days) and duration (days in parentheses) of zoeas of *R. ranina* at five temperature levels

Temperature (°C)	Instar								
	I	II	III	IV	V	VI	VII_8	VII_M	VIII
33	4.1	3.8	4.4	4.4	3.9	4.7	5.1	7.6	6.0
		(7.9)	(12.3)	(16.7)	(20.6)	(25.3)	(30.4)	(32.9)	(36.4)
29	4.2	4.0	4.2	4.2	4.1	4.9	5.5	7.6	7.0
		(8.2)	(12.4)	(16.6)	(20.7)	(25.6)	(31.1)	(33.2)	(38.1)
25	5.4	5.0	5.3	5.4	5.2	6.1	5.8	8.9	9.1
		(10.4)	(15.7)	(21.1)	(26.3)	(32.4)	(38.2)	(41.3)	(47.3)
21	9.2	8.2	9.4	7.0	10.0	—			
		(17.4)	(26.8)	(33.8)	(43.8)				
17	20.5	17.5	—						
		(38.0)							

VII_8: Zoeas VII molted to instar VIII; VII_M: Zoeas VII metamorphosed.

Table 3. Accumulated temperature (temperature·day) from hatch to each instar of *R. ranina* zoeas at five temperature levels

Temperature (°C)	Instar								
	I	II	III	IV	V	VI	VII_8	VII_M	VIII
33	132	255	397	539	665	817	982	1063	1176
29	121	236	357	478	596	737	896	956	1097
25	138	265	400	538	671	826	974	1053	1206
21	196	371	571	720	933	—			
17	353	654	—						

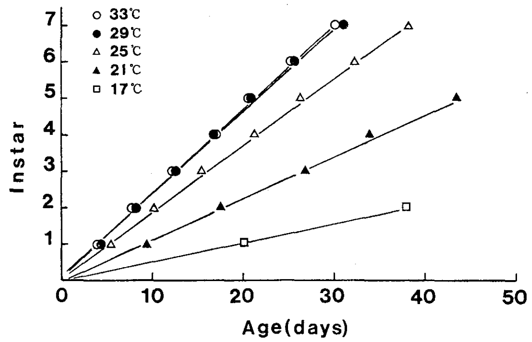

Fig. 2. Relationship between larval age and instar of *R. ranina* at five temperature levels.

Growth and morphogenesis

Growth and external morphology of zoeas at instars III, V and VII are shown in Table 4. Zoeas reared at 21, 25, 29 and 33°C proceeded to instar VII while only one zoea reached instar III at 17°C. CL, RL, ML and TL tended to be shorter both at lower and higher temperatures. At instar III, no apparent differences were observed in CL and TL excluding the fact that RL was shorter at 33°C and that ML was longer at 25°C compared to those at the other temperatures. At instar V, CL was longer at 29°C than those at the others. Growth in RL and ML reduced at 33°C. At instar VII, CL and TL at 29°C increased in comparison with those at other temperatures. RL and ML were shorter at 21 and 33°C than those at 25°C.

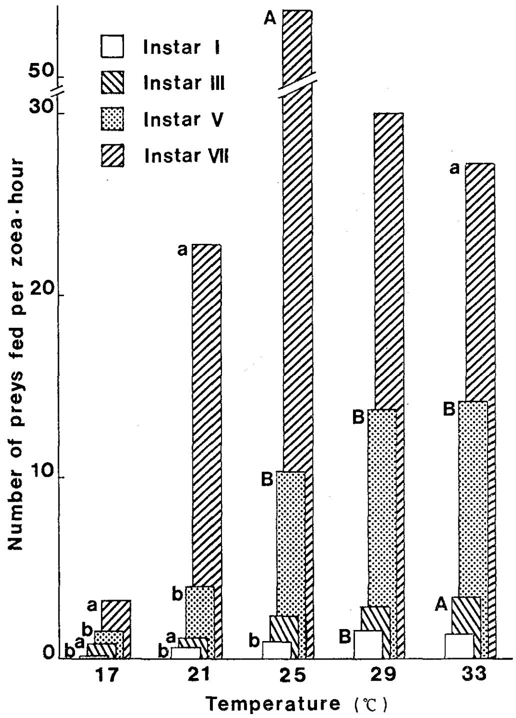

Fig. 3. Differences of preys taken by four different instar zoeas of *R. ranina* at five temperature levels.

There are the differences between capital and small letters in the same instar. (a: $P<0.05$; b: $P<0.01$)

Table 4. Variation of external morphology and growth of *R. ranina* zoeas at instars III, V and VII at five temperature levels

Instar	Temperature (°C)	the number of setae: ant	1mxp	2mxp	tel	uro	length in mm: car	ros	2mxp	tel
III	17*	19	13	13	24	3	1.63	—	0.55	0.45
	21	21.0^a	14.0	16.5	24.0	4.8^a	1.78	6.40^B	0.62^b	0.53
	25	22.4	14.0	17.0	25.4	5.6^A	1.86	6.56^B	0.68^B	0.56
	29	23.4^A	14.8	16.6	26.6	6.0^A	1.87	6.66^B	0.62^b	0.55
	33	23.2^A	14.8	16.8	23.8	4.6^a	1.82	5.64^b	0.58^b	0.53
V	21	35.0^b	23.2	24.8^b	37.0	19.8^b	2.96^a	9.56^A	1.00^B	0.86
	25	35.2^b	22.8^a	26.2^b	36.8	22.2^b	3.09	9.98^A	1.03^B	0.88
	29	38.2^B	24.2^A	28.0^B	36.2	24.8^B	3.15^A	9.46^A	1.02^B	0.93
	33	39.4^B	22.2^a	25.6^b	35.8	24.2^B	2.99^a	8.70^a	0.91^b	0.89
VII	21	46.2	28.0^a	31.0^a	44.3	33.0^b	4.35^a	13.33^a	1.46^a	1.38^a
	25	48.2	29.8^A	34.4^A	45.0^A	37.0^b	5.24	14.91^A	1.63^A	1.64
	29	51.0	31.4^A	32.8	41.5^a	39.8^B	5.45^A	13.26^a	1.59^A	1.66^A
	33	48.2	27.8^a	31.8^a	38.8^a	40.4^B	4.99^a	11.86^a	1.44^a	1.57^a

All figures show average values.
Significant differences between capital and small letters (a: $P<0.05$, b: $P<0.01$)
Abbreviation ant: antenna; car: carapace; ros: rostrum; tel: telson; uro: uropod; 1mxp: 1st maxilliped; 2mxp: 2nd maxilliped
* only one individual available.

The setae of the several appendages were also inclined to decrease at lower and higher temperatures (Table 4). At instar III, the number reduced in antennal exopod at 21°C. At instar V, setae on antennal exopod and uropod were more numerous at 29 and 33°C than those at 21 and 25°C. The setae on exopods of 1st and 2nd maxillipeds increased at 29°C. At instar VII, the setae in number on exopods of 1st and 2nd maxillipeds decreased at 21 and 33°C. On the other hand, the setae on uropod at 29 and 33°C increased as compared with those at 21 and 25°C.

Morphogenesis progressed simultaneously at each temperature until instar V: uropod and pleopod appeared at instars III and V, respectively in all temperatures. At instar VII, however, segment of the endopod of 2nd maxilliped was 4 in all individuals at 21°C and in 1 out of 5 individuals at 33°C.

Discussions

Temperature is one of the key factors in rearing crustacean larvae. Optimum range of temperature or temperature-salinity combination on larval survival was found or estimated in a several brachyuran species.[2-5,8,9] In *R. ranina*, the survival in all instars decreased at the lower temperature, such as 17 and 21°C, but the high temperature such as 33°C influenced the survival of only later-instar zoeas. The optimum range of temperature for survival, therefore, seems to become narrower in later instars.

Lucas[9] pointed out that acclimation to temperature during the embryoinc development influenced significantly the survival and the mean duration of zoeas. The surface temperature off Hachjojima is around 23–25°C in July when the females of *R. ranina* were ovigerous. Optimum temperature for zoeas of this species seems to be slightly higher than ambient water temperature during the embrionic development.

Molting intervals depended strictly on temperature. The relationship between molting intervals at instar I and temperatures was curvilinear. Similar results were reported on several decapod species.[8,12,14,20-23] Like the present results, Nakanishi[22] reported that integral temperatures were different under various rearing temperatures and tended to be the minimum at optimum temperature in *Paralithodes camtschaticus*.

The influence of temperature on food consumption has been examined on *Pandalus borealis*,[13] early stages of *Panulirus japonicus*[16] and *Paralithodes camtschaticus*.[21,22] Optimum temperatures were found in larvae of *P. japonicus* and later stages of *P. camtschaticus*. In *R. ranina*, optimum temperature for food consumption changed to be lower in later instar: zoeas fed more preys at higher temperatures than at lower temperatures at instars I to V, while at instar VII they took the maximum number of prey at 25°C.

The variability of the number of larval instar until metamorphosis in decapod crustaceans was reviewed by Gore[24] and Knowlton.[25] It is less common in Brachyura than in Anomura and Natantia, but several brachyuran cases were reported in my previous paper.[19] The causes of the variability were attributed to temperature[14,15,17,26,27], salinity,[14,26,28] zoeal density,[23] food,[11,26,29,30] population[27] and season.[31] Yatsuzuka[11] reported that the variablility tended to easily occur under insufficient food condition in four brachyuran species: *Portunus trituberculatus*, *P. pelagicus*, *Eriocheir japonicus*, *Holometopus dehaani*. Ewald[27] observed the similar tendency in *Tozeuma carolinense*. On the other hand, higher temperature caused the increase of the number of instars in two shrimps: *Crangon crangon*, *C. allmanni*.[14] While, Sandifer[15] revealed that the number of instars was least at intermediate (optimum) temperature in *Palaemonetes vulgaris*. In *R. ranina*, metamorphosis rate was the maximum at intermediate temperature but the relationship between the variability of the number of larval instar and food consumption was vague.

There are few reports on the influence of temperature on growth, morphogenesis and feeding, especially the relationship between feeding and growth and morphogenesis. Examining the influence of temperature on larval development of *Pandalus borealis*, Wienberg[13] reported that the same stage zoeas reached the same lengths at all temperatures until stage VII but insufficient number of specimens gave no clear conclusions after stage VIII. He also pointed out that there were no differences between the morphology and the number of stages at any temperatures. Crials and Anger[14] described a decreasing tendency in carapace length with increasing temperature on *Crangon crangon*. On the other hand, Rothlisberg[12] referred to the influence of temperature on larval growth of *Pandalus jordani* and concluded that optimum (intermediate) temperature exsisted for both survival and growth. In *R. ranina*, temperature exerted great influence on larval development. Better growth and more advanced morphogenesis were obtained at optimum temperatures (25–29°C). Growth and morphogenesis were closely related to each other: larger larvae showed advanced morphogenesis as pointed out in the previous paper.[19]

Wienberg[13] and Reeve[32] pointed out that growth seemed to be more influenced by feeding than molting intervals, based on the studies of *Pandalus borealis* and *Palaemon serratus*. This might be true to later-instar larvae of *R. ranina*, because at instar VII lower feeding did not cause longer molting intervals but affected the growth and morphogenesis at high temperature. At early instars, however, growth decreased at high temperature in spite of active food intake. Consequently, at high temperature food energy taken by larvae seems to be used insufficiently for growth as compared with molting, especially in early instars. The unusually high temperature might cause the reduction of assimilation efficiency or increasing oxygen consumption.

The mentioned data indicate that the optimum temperature for rearing larvae of *R. ranina* is 25 to 29°C.

Acknowledgements

I thank the following: Associate Professor K. Hayashi, Shimonoseki University of Fisheries, for his reading and criticizing the manuscript; Professor F. Takashima, Tokyo University of Fisheries, for his valuable advice; Ms. H. Iwasaki for her help with the rearing; Mr. K. Nishimura, ex-manager of Hachijo Branch in charge, for his giving me a chance to study; my colleagues at Hachijo Branch for their help with catching female crabs.

References

1) Japan Sea-Farming Association: Nihon Saibai Gyogyo Kyokai Jigyo Nenpo, Showa 63 Nendo, 241–258 (1990).
2) J. D. Costlow, Jr. and C. G. Bookhout: *Amer. Zool.*, **8**, 399–410 (1968).
3) J. D. Costlow, Jr., C. G. Bookhout, and R. Monroe: *Physiological Zoology*, **35**, 79–93 (1962).
4) J. D. Costlow, Jr., C. G. Bookhout, and R. Monroe: *Physiological Zoology*, **39**, 81–100 (1966).
5) J. D. Costlow, Jr., C. G. Bookhout, and R. Monroe: *Biol. Bull.*, **118**, 183–202 (1960).
6) M. E. Christiansen and J. D. Costlow, Jr.: *Mar. Biol.*, **32**, 215–221 (1975).
7) M. Sandoz and R. Rogers: *Ecology*, **25**, 217–228 (1944).
8) T. Kon: *Nippon Suisan Gakkaishi*, **36**, 219–224 (1970).
9) J. S. Lucas: *Bull. Mar. Sci.*, **22**, 824–840 (1972).
10) K. Ong and J. D. Costlow, Jr.: *Chesapeake Science*, **11**, 16–29 (1970).
11) T. Yatsuzuka: in "Suisangaku Shuusei", Tokyo Univ. Press, Tokyo, 1952, pp. 571–583.
12) P. C. Rothlisberg: *Mar. Biol.*, **54**, 125–134 (1979).
13) R. Wienberg: *Meeresforsch.*, **29**, 136–153 (1982).
14) M. M. Criales and K. Anger: *Helgoländer Meeresunters.*, **40**, 241–265 (1986).
15) P. A. Sandifer: *Fish. Bull.*, **71**, 115–123 (1973).
16) M. Inoue: *Special Rep. Kanagawa Pref. Fish. Exp. Stn.*, **1**, 1–91 (1981).
17) C. M. Boyd and M. W. Johnson: *Biol. Bull.*, **124**, 141–152 (1963).
18) M. Minagawa and M. Kudo: *Suisanzoshoku*, **36**, 221–225 (1988).
19) M. Minagawa: *Nippon Suisan Gakkaishi*, 56, 577–589 (1990).
20) W. Templeman: *J. Biol. Bd. Can.*, **2**, 485–497 (1936).
21) H. Kurata: *Bull. Hokkaido. Reg. Fish. Res. Lab.*, **21**, 9–14 (1960).
22) T. Nakanishi: *Bull. Japan Sea Reg. Fish. Res. Lab.*, **37**, 57–161 (1987).
23) C. H. Kwon: *Rep. Tonwi Univ.*, **5**, 1–136 (1981).
24) R. H. Gore: in "Crustacean issues 2. Larval growth" (ed. by A. M. Wenner), A. A. Balkema, Rotterdam, 1985, pp. 1–65.
25) R. E. Knowlton: *Thalassia Jugoslavica*, **10**, 138–158 (1974).
26) W. Templeman: *J. Biol. Bd. Can.*, **2**, 349–354 (1936).
27) A. C. Broad: *Biol. Bull.*, **112**, 144–161 (1957).
28) A. C. Broad: *Biol. Bull.*, **112**, 162–170 (1957).
29) A. B. Kircher: *Bull. Mar. Sci.*, **20**, 769–792 (1970).
30) J. J. Ewald: *Bull. Mar. Sci.*, **19**, 510–549 (1969).
31) H. Díaz and J. D. Costlow, Jr.: *J. Coast. Res.*, **3**, 169–177 (1987).
32) M. R. Reeve: *J. Mar. Biol. Ass. U. K.*, **49**, 77–96 (1969).