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Effects of Salinity and Temperature on the Larval Development of the Semiterrestrial Sesarmid Crab *Neoepisesarma lafondi* (Jaquinot and Lucas, 1853) from a Mangrove Swamp in Okinawa Island, Japan

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Abstract: The effects of different salinities and temperatures on the larval development of the semiterrestrial sesarmid mangrove crab *Neoepisesarma lafondi* (Jaquinot and Lucas, 1853) were examined under laboratory conditions. Its development comprised of four zoeal stages and the megalopa. Successful larval development was observed in 15-30 and 10-35% at 25 and 30°C, respectively. In freshwater and 5% salinity at both temperatures the larvae died within 12-18 hours without moulting to subsequent stages. The larvae reached the first crab stage for salinities between 10 and 35% at 30°C only. The highest survival rate for each larval stage was recorded at 20% for both temperatures. Total development to the first crab stage in 15-30% required 17-20 and 15-19 days at 25 and 30°C, respectively. Significant differences were detected only for megalopal duration at the different salinities and temperatures tested. Results of the combined effects of salinity and temperature suggest that the larvae of *N. lafondi* develop in estuarine water and recruit to the mangrove swamp at the megalopa stage, where they spend the rest of their lives.

Key words: Sesarmid crab, *Neoepisesarma lafondi*, larval development, mangrove swamp

Introduction

The majority of sesarmid crabs (Sesarmidae) live in coastal habitats such as estuaries, mangrove swamps, salt marshes, brackish water ponds, rocky coasts, stony beaches, and the banks of rivers (Anger, 1995; Schuh and Diesel 1995a and Schubart *et al.*, 2000). They are important components of mangrove ecosystems in the Indo-Pacific region, and over forty species have been reported to be predominantly associated with those mangrove ecosystems (Jones, 1984 and Frusher *et al.*, 1994). Many species of sesarmid crab have acquired physiological adaptations permitting extended activity outside water or dwelling in fresh water, but most of these have retained a conservative mode of development, producing planktotrophic larvae that develop in the sea (Burggren and McMahon, 1988).

Neoepisesarma lafondi is found in Japan (Iriomote Island), in Malaysia (Malay Peninsula), and in Indonesia (Sumatera and Nias Islands) (Sakai, 1976). This species inhibits mud-flats of mangrove swamps or river mouths at mangrove estuaries (Sakai, 1976), and mangrove forests. *Neoepisesarma lafondi* also occurs abundantly in the mangrove swamps of Iriomote Island, Japan.

Salinity is an important environmental factor, which has many physiological and ecological effects on the larval development of estuarine crabs (Anger, 1991; Minagawa, 1992 and Frusher *et al.*, 1994). Salinity changes always have stressful effects on aquatic organisms, especially crustaceans, but tolerance to such changes is frequently variable during ontogeny (Kinne, 1971). Salinity tolerance and response to temperature variations are important ecophysiological traits of decapod larvae and other aquatic invertebrates living in coastal and estuarine waters (Browne and Wanigasekera, 2000). There is often a complex co-relationship between the two factors, where temperature can also modify the effects of salinity, thereby changing the salinity tolerance of an organism, and salinity can modify the effects of temperature (Kinne, 1963; Browne and Wanigasekera, 2000).

A number of studies have investigated the combined effects of salinity and temperature on the larval development of marine invertebrates inhabiting estuarine or coastal areas (Costlow *et al.*, 1960; Davis and Calabrese, 1964; Kinne, 1964; Anger, 1991; Zimmerman and Pechenik, 1991; Schuh and Diesel 1995a; Anil *et al.*, 1995, Anil and Kurian, 1996; Qiu and Qian, 1997; Kashenko, 1998 and 2000; Kashenko *et al.*, 2002). Although there are several papers dealing with the effects of a variety of salinity levels on the larval development of different species of brachyuran crabs (Foskett., 1977; Chen and Cheng, 1980; Lago, 1989; Anger *et al.*, 1990; Schuh and Diesel, 1995b; Anger, 1996; Mia and Shokita, 1997; Kannupandi *et al.*, 1997; Diesel and Schuh, 1998; Islam *et al.*, 2000a,b,c; Islam *et al.*, 2002; Islam and

Shokita, 2003), the combined effects of salinity and temperature have been poorly studied. Within the genus *Neoepisesarma*, larval morphology is only known for *N. lafondi* (Higa, 1997), and the combined effects of salinity and temperature on its larval development have never been examined. Considering the importance of this species in mangrove ecosystems, we examined the influence of salinity and temperature on its larval development.

Materials and Methods

The study was carried out at the Laboratory of Fisheries Biology of the University of the Ryukyus, Okinawa, Japan. An ovigerous female of *Neoepisesarma lafondi* measuring 39mm in carapace length and 41mm in carapace width was captured by hand from the Urauchi River mangrove swamp of Iriomote Island, southern Okinawa (24°20'N~24°30'N, 123°45'E~123°60'E), Japan, on 6 August 2001. The female was brought to the laboratory and kept in a plastic container (32x18x22 cm) with seawater of 20±1% salinity, ambient temperature of 29.5±0.01°C and with moderate aeration. The female was fed with small dry fish (*Clupea pallasii*) and aged mangrove leaves (*Bruguiera gymnorrhiza*). Seawater was changed daily until the eggs hatched. Hatching occurred on the morning of 20 August 2001. The larvae were reared for mass culture under the same conditions as indicated for the ovigerous female.

Within one day after hatching, the most photopositive and active larvae were selected from the mass culture. A 5-ml glass pipette was used to retrieve the active larvae from the rearing container and to place them into one-liter plastic test containers (20 individuals per container). The larvae were subjected to eight different salinity levels (0-35%, by steps of 5%), measured with an Atago Hand Refractometer (S/Mill-E, 0.100%, Japan) to the nearest 1%, at two different temperatures (25 and 30°C) and two replicates were made for each treatment. The above salinities were obtained by diluting filtered seawater with dechlorinated tap water. The temperature was controlled by thermostat (IC Thermostat, Five Plan, Ex-003, Japan). Half of the aerated test water in each container was replaced daily. The larval stages were identified based on the setal number observed on the maxillipedal exopods under a binocular stereomicroscope (NIKON SMZ-10).

Newly hatched nauplii of *Artemia* sp. were added daily to each bowl as larval food. In addition, finely chopped meat of the short-necked clam (*Ruditapes philippinarum*) was fed to the megalopa. Moulting, survival rate and development durations were checked daily for each larval stage between 7:00 am and 7:00 pm, while the dead larvae were preserved in 50% ethylene glycol for later re-identification of stages. The experiment was terminated when all the larvae had moulted to the first crab stage or died. The female of *N. lafondi* scarified and deposited in the University Museum "Fujukan" of the University of the Ryukyus, Okinawa, Japan, under the registration number RUMF-ZC-00018. Percentages of larval survival for each salinity and temperature treatment at the end of each stage were calculated. Durations were determined based on the daily observation of 4 larvae for each stage of zoeae and 1-3 larvae for megalopae. Development duration at each salinity and temperature level was analyzed using a two-factor ANOVA on the statistical package Minitab 11.12 for Windows.

Results

The development durations for each larval stage of *Neoepisesarma lafondi* at different salinity levels with different temperature conditions are presented in Table 1. At the lower salinity (0-5%), they did not moult to the subsequent stages and died within 12-18 hours of exposure. The shortest development duration was recorded for zoeae to megalopa at 20% for both temperatures. The development was not completed in 10% or at the full strength seawater (35%) at 25°C, while it was completed at 30°C (Table 1, Fig. 1). A two-factor analysis of variance (ANOVA) detected no significant differences ($P>0.01$) at different salinities and temperatures tested for zoeae, while it detected significant differences ($P<0.01$) only for megalopae stage (Table 2).

The highest survival rate was recorded for first to fourth zoeae (100%) at 20% salinity at 25 and 30°C, while highest survival rate (41%) was recorded for megalopae stage only at 15% salinity at 30°C (Fig. 1). Survival of megalopa was decreased gradually with increasing salinity from 15-35% at 30°C (Fig. 1). When compared with temperature levels, the first zoeal survival rate was always higher at 30°C than at 25°C in all salinity levels tested. At 25°C, the fourth zoeae did not moult to the megalopae stage at 10% and full strength seawater.

The two-factor analysis of variance (ANOVA) detected significant differences ($P<0.05$, $F=130$, $df=5$) among the total duration required to complete larval development at the different salinities and temperatures tested (Fig. 2). Total duration required for first zoeae to moult to the first crab stage ranged from 15 to 21 days and was minimum at 20% salinity. The optimum range of salinity was 15-20 and 20-25% for zoeae and 15-20 and 15% for megalopae at 25 and 30°C respectively (Fig. 3). Lower temperature (25°C) extended the duration of larval development in *N. lafondi*, whereas higher temperature (30°C) promoted faster development. The shortest duration required for complete larval development was 15 days in 20% salinity at 30°C. Complete mortality was observed for all larval stages and

Table 1: Development duration (days) of *Neoepisesarma lafondi* from hatching to first crab stage reared under six different salinity (10-35%) conditions at two constant temperatures (25-30°C). Salinities 0 and 5% were not included, since no larvae survived these treatments. Durations were determined based on the daily observation of 4 larvae for each stage of zoeae and 1-3 larvae for megalopae. A two-factor ANOVA detected no significant differences (P>0.01) among the salinity levels tested for zoeae, but significant differences (P<0.01) were only detected for megalopae stage. Abbreviations: Z, zoea; M, megalopae; ns, no survive

		Salinity (%)					
Temp. (°C.)	Larval stages	10	15	20	25	30	35
25	Z-I	3.5±0.35	2.8±0.56	3.0±0.61	3.0±0.71	4.0±0.71	3.0±0.71
25	Z-II	3.0±0.71	3.0±0.71	2.8±0.56	3.0±0.35	3.0±0.71	3.5±0.50
25	Z-III	3.0±0.71	3.2±0.41	3.0±0.61	3.0±0.35	3.3±0.83	3.5±0.50
25	Z-IV	2.5±0.61	3.3±0.75	2.5±0.80	3.0±0.35	3.0±0.71	3.0±1.12
25	M	ns	6.5±0.50	5.7±0.47	5.5±0.00	7.0±0.00	ns
30	Z-I	3.0±1.12	3.4±1.08	2.5±0.35	3.0±0.71	3.0±0.00	3.5±1.12
30	Z-II	3.0±0.71	2.5±0.50	2.5±0.00	2.5±0.35	2.5±0.35	3.0±0.00
30	Z-III	3.0±0.61	3.0±0.00	2.5±0.71	2.5±0.00	2.5±0.50	3.0±0.35
30	Z-IV	2.5±0.35	2.6±0.96	2.0±0.00	2.6±0.54	3.0±0.71	3.0±0.35
30	M	7.5±0.00	6.4±0.96	5.5±0.41	6.3±0.25	8.0±0.00	9.0±0.00

Table 2: Summery from the analyses of variance of larval development duration under different salinity and temperature conditions. Statistical analyses were carried out on percentage data and treatment means were tested by two-factor ANOVA using Minitab 11.12 for Windows. Abbreviations: S=significant, NS=non-significant, DF=degrees of freedom, SS=sum of squares, MS=mean squares

Larval stages	Source	DF	SS	MS	F-value	P-value
First zoea	Salinity	5	2.222	0.444	0.7616^{NS}	13.84
	Temperature	1	0.444	0.444	0.7616^{NS}	99.47
	Interaction	5	2.222	0.444	0.7616^{NS}	5.2
	Error	24	14	0.583		
	Total	35	18.889			
Second zoea	Salinity	5	1.472	0.294	0.8144^{NS}	13.84
	Temperature	1	0.25	0.25	0.6925^{NS}	99.47
	Interaction	5	0.833	0.167	0.4626^{NS}	5.2
	Error	24	8.667	0.361		
	Total	35	11.222			
Third zoea	Salinity	5	1.229	0.246	0.5290^{NS}	13.84
	Temperature	1	2.507	2.507	5.3914^{NS}	99.47
	Interaction	5	1.285	0.257	0.5527^{NS}	5.2
	Error	24	11.167	0.465		
	Total	35	16.188			
Fourth zoea	Salinity	5	2.806	0.651	0.7211^{NS}	13.84
	Temperature	1	1	1	1.2853^{NS}	99.47
	Interaction	5	0.833	0.167	0.2147^{NS}	5.2
	Error	24	18.667	0.778		
	Total	35	23.306			
Megalopa	Salinity	5	52.583	10.517	52.3234^{S}	13.84
	Temperature	1	78.028	78.028	388.199^{S}	99.47
	Interaction	5	130.806	26.161	130.1542^{S}	5.2
	Error	24	4.833	0.201		
	Total	35	266.25			

temperatures at 0-5% salinity. Compared with the optimum condition (20-25%), the mortality was significantly higher at 10 and 35% salinity, with an increase in development duration. Development to the first crab stage was achieved at salinities ranging from 15-30% at 25°C and 10-35% at 30°C.

Discussion

A comparison of salinity ranges for successful larval development in *N. lafondi* with other species of brachyuran crab shows certain differences (Table 3). The portunid crab *Charybdis natator* Herbst and the varunid crab *Chasmagnathus convaxus* (De Haan) complete their larval development at 25-35 and 21-28%, respectively (Islam *et al.*, 2000a,b). Many sesarmid crabs show a narrow range of salinity tolerance including the present one *N. lafondi*, *Perisesarma bidens* De Haan (Islam *et al.*, 2000c), *Neosarmatium trispinosum* Davie (Islam and Shokita, 2003), *Armases miersii* Rathbun (Schuh and Diesel, 1995a) and *Sesarma curacaoense* De Man (Schuh and Diesel, 1995b). The salinity tolerances of *Neosarmatium indicum* larvae decrease during development (Islam *et al.*, 2002). In a previous study on a related species, the authors speculated that the first zoeae are released into brackish nearshore waters, and that subsequent stages occur in the relatively stable conditions of lower estuaries (Islam *et al.*, 2000c and 2002). In contrast, *A. miersii* passes its entire development in supra-tidal rockpools (Schuh and Diesel, 1995a), and *S. curacaoense* completes its development at a wider range of salinity condition (Diesel and Schuh, 1998; Schuh and Diesel, 1995b).

The hyper-osmoregulatory capacity increases gradually from hatching to adult and the hypo-osmoregulatory capacity increase from the megalopa to the adult

Table 3: Comparison of larval stages, development durations, and ranges of salinity and temperature tolerance of brachyuran crabs under laboratory conditions. Abbreviations: z, zoeae; m, megalopae; *, range of optimal salinity; no data

Brachyuran species	Stages (z, m)	Duration (days)	Salinity* (%)	Temp. (°C)	References
Portunid crabs					
Charybdis natator	6z, m	28-45	25-35	22-25	Islam *et al.* (2000a)
Scylla serrata	3z, m	.	25-30	26-30	Chen and Cheng (1980)
Thalamita crenata	3z, m	25-31	25-35	26-28	Kannupandi *et al.* (1997)
Varunid crabs					
Chasmagnathus convexus	4z, m	28-36	21-28	17-20	Islam *et al.* (2000b)
Helice formosensis	5z, m	.	20-30	20-22	Mia and Shokita (1997)
Helice leachi	5z, m	.	15-20	21-24	Mia and Shokita (1997)
Sesarmid crabs					
Armases angustipes	4z, m	15-20	20-32	22-25	Anger *et al.* (1990)
Armases cinereum	4z, m	24-27	20-30	23-30	Costlow *et al.* (1960)
Armases miersii	3z, m	14-19	20-30	25	Schuh and Diesel (1995a)
Armases miersii	3z, m	15-20	15-25	24	Anger (1996)
Armases miersii	3z, m	12-14	15-25	25	Anger *et al.* (2000)
Armases ricordi	4z, m	19-25	30-35	25.4	Diesel and Schuh (1998)
Armases roberti	4z, m	17-28	25-35	25.4	Diesel and Schuh (1998)
Neoepisesarma lafondi	4z, m	15-21	20-30	25-30	Present study
Neosarmatium indicum	5z, m	28-35	20-30	25-28	Islam *et al.* (2002)
Neosarmatium meinerti	5z, m	28-35	30-35	23-27	Lago (1989)
Neosarmatium trispinosum	5z, m	24-28	20-25	25-30	Islam and Shokita (2003)
Perisesarma bidens	4z, m	18-32	20-30	23-25	Islam *et al.* (2000c)
Sesarma curacaoense	2z, m	14-17	20-30	25.4	Schuh and Diesel (1995b)
Sesarma curacaoense	2z, m	15-19	20-25	24	Anger and Charmantier (2000)
Sesarma reticulatum	3z, m	.	25-30	25	Foskett (1977)

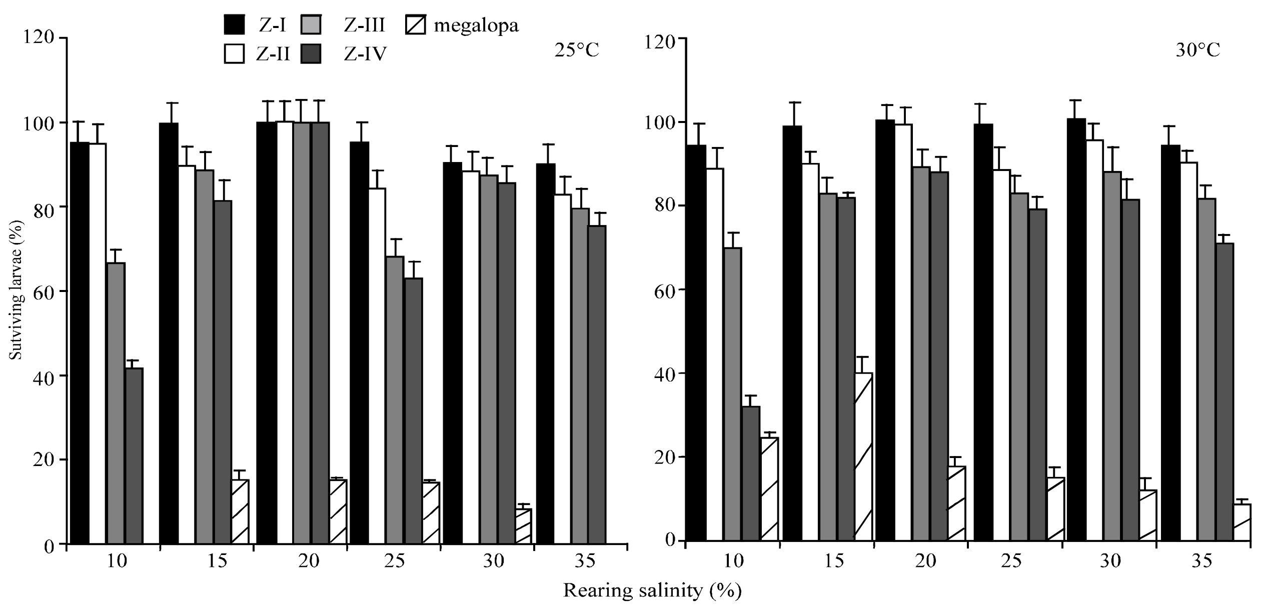

Fig. 1: Survival to the first crab stage of *Neoepisesarma lafondi* under six different salinities and two different temperatures. Initial *N*=20 larvae per treatment. Vertical bars indicate standard deviation. Salinities 0 and 5% were not included here, since no larvae survived at these treatments

(Charmantier *et al.*, 1998). The type of osmoregulation did not change during development from larval hatching through the adult phase (Charmantier and Anger, 1999). Anger and Charmantier (2000) studied the salinity tolerance of *S. curacaoense* during development from hatching to the end of the first crab stage. They found that successful development through metamorphosis occurred in the full salinity range tested (15-32%), although mortality increased significantly and development delayed was at 15% salinity. Our results are similar with those of Anger and Charmantier (2000). Larval survival of *A. miersii* was frequently higher at 15-25% than in seawater (Anger 1996), while higher mortality occurred at the extremes of 10 and 55%. This is again similar to the present results. Lowest mortality and shortest time of development occurred generally at 15-25%, indicating an optimum at moderately reduced salinities (Anger *et al.*, 2000). Larval mortality in lower

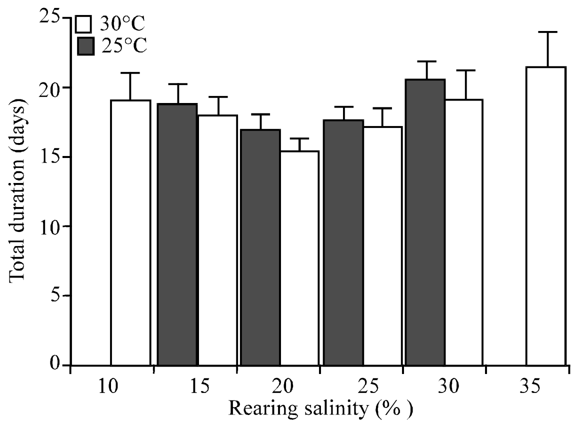

Fig. 2: Development duration to the first crab stage of *Neoepisesarma lafondi* under six different salinities and two different temperatures. Durations were determined based on the daily observation of 4 larvae for each stage of zoeae and 1-3 larvae for megalopae. Vertical bars indicate standard deviation. Significant differences ($P<0.01$, two-factor ANOVA) were detected among the salinities and temperatures tested

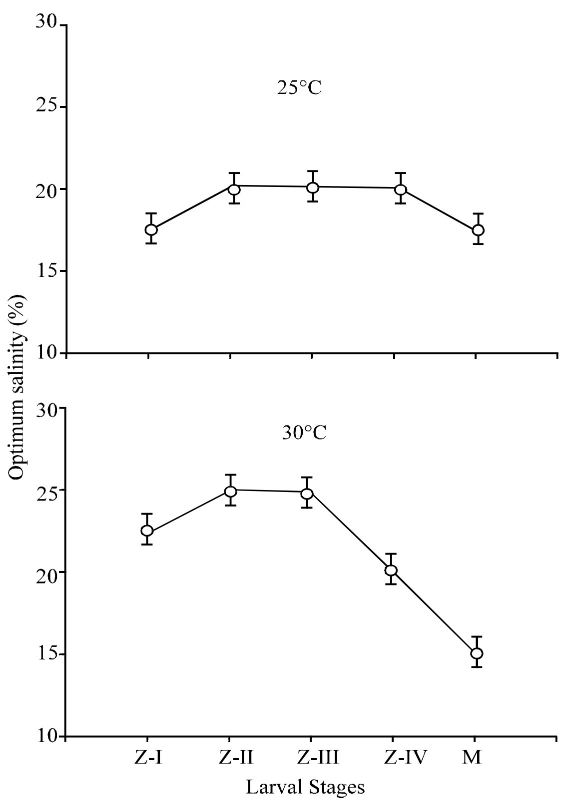

Fig. 3: Optimum salinity required for the complete larval development of *Neoepisesarma lafondi* at 25 and 30°C. Vertical bars indicate standard deviation

salinity (0-10%) is possibly due to imbalance in osmoregulatory mechanism, and susceptibility to lower salinity that can be a major limiting factor in the distribution of a species (Kannupandi *et al.*, 1997; Charmantier *et al.*, 1998; Charmantier and Anger, 1999; Anger *et al.*, 2000; Anger and Charmantier 2000).

The optimum salinity level required for complete development of each larval stage of *N. lafondi* varied at ca. 20-30% at 25-30°C. The results of the present study indicate that the late zoeae and megalopae prefer a lower salinity than the early zoeae. This suggests that the larvae of *N. lafondi* begin to migrate toward the river when they are in the fourth zoeal stage. The salinity of the Urauchi River mangrove swamp is nearly 15-25%, which is lower than seawater and similar to the rearing conditions. Hence, the present results suggest that the larvae of *N. lafondi* develop in estuarine water and recruit to the mangrove swamp at the megalopal stage, where they spend the rest of their life.

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