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Effects of Temperature and Salinity on the Survival and Metabolism of *Tresus keenae* (Mollusca: Bivalvia)

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We examined the variation in survival and the respiration and filtration rates of *Tresus keenae* in response to changes in water temperature and salinity. The survivorship of animals exposed to temperatures below 25°C for 7 days was 80%; however, all test animals died on the fourth day at 28°C. The upper lethal temperature over 7 days was 25.9°C. After exposure to lower temperatures, 93% of the animals survived at temperatures over 5°C for 10 days. Survivorship rapidly decreased below 4°C with all test animals dying at 2°C on the eighth day. The LT_{50} over 10 days was 4.8°C. The respiration and filtration rates of *T. keenae* increased as temperature increased. It is believed that energy consumption increases as a result of the increased respiration rate at temperatures above the upper lethal temperature. At temperatures below the lower lethal temperature, the metabolic rate of *T. keenae* was substantially lowered. In response to changes in salinity, the survivorship of *T. keenae* was 90% at 30.2 psu after exposure for 5 days; at below 26.8 psu, all test animals died by the fifth day. The LS_{50} was 29.1 psu. As salinity decreased, both the respiration rate and the filtration rate decreased. At 23.5 psu, the respiration and filtration rates decreased by 48 and 34%, respectively. These data have implications for increasing efficiency in the production and management of shellfish aquaculture farms.

Key words: *Tresus keenae*, Survival, Temperature, Salinity, Respiration, Filtration

Introduction

Temperature influences the health of aquatic animals through its effects on metabolic rates and subsequent oxygen demand, and by assisting the proliferation, invasiveness, and virulence of bacteria and other pathogens that cause a variety of pathologies in the host (Wedemeyer et al., 1999). Temperature controls the metabolic activity of bivalves and influences virtually all biochemical and physiological activities of these organisms (Magnuson et al., 1979). The salinity of coastal littoral habitats varies annually and seasonally; in response, aquatic organisms have evolved a range of adaptations to cope with the changing salinity. Marine species are essentially isosmotic over the range 50-150% seawater (Tucker, 1970). Knowledge of the roles played by temperature and salinity is important in aquaculture, because a decrease in salinity causes various physiological changes in heartbeat, oxygen consumption, and excretion of ammonia and uric acid (Pierce and Greenberg, 1972). *Tresus keenae* is a large nonadhesive shellfish of the family Mactridae that inhabits the Geoje, Sacheon, Namhae, and Yeosu regions on the southern coast of Korea. Fishing is the exclusive means by which this highly prized shellfish is caught and most of the catch is exported to Japan. Excessive fishing has reduced the productivity of the fishery and this situation has been exacerbated by the slow growth of *T. keenae*, which takes 3-4 years to reach reproductive age. There have been several studies investigating the use of food (Bayne and Widdows, 1978; Widdows et al., 1979) and dissolved oxygen (Davenport and Wong, 1986) in relation to temperature (Read and Cumming, 1967; Kennedy, 1976) and salinity (Tucker, 1970; Shumway, 1977) for a range of shellfish species. However, aside from research related to artificial seedling production, there appears to be no reports on *T. keenae*. The present study investigated the physiological responses of *T. keenae*, such as survivorship and changes in metabolism, to variations in temperature and salinity.

Materials and Methods

The *T. keenae* used in this research were individuals

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with a shell length of 2 to 3 cm cultivated at the Shellfish Research Center (Namhae, Korea) in 2003. They were held in cages for interim culture and then cultured in the outdoor aquafarm until the shell length reached 15 ± 1 cm. *T. keenae* were transferred to the experimental lab, cultivated in a 0.5-ton capacity water tank, and used in experiments after acclimating to various test temperatures for 10 days. *Tetraselmis* sp. was used as feed. Salinity was adjusted to 33.5 psu (ordinary seawater) and the illumination to a 12 h light:12 h dark photoperiod. Stagnant and circulating water methods were used concurrently. Survivorship was computed from the number of survivors over the duration of each experiment.

The temperature change experiments were conducted using an automatic temperature control and thermostat circulation tank. The experimental temperatures were 24, 25, 26, 27, and 28°C for the upper limit and 2, 3, 4, 5, 6, and 7°C for the lower limit. Ranges of salinity used were 0, 16.8, 20.1, 23.5, 26.8, 28.5, 30.2, and 33.5 psu. An automatic salinity gauge (PR-100SA; Atago Co., Tokyo, Japan) was used to measure salinity.

The respiration rate was determined with an oxygen measurement apparatus (YSI 5000; SonTek, San Diego, CA, USA), and the filtration rate was measured with the Cole and Hepper (1954) method using 0.001% neutral red. The LT_{50} and LS_{50} were analyzed by the probit method (Finney, 1971).

Results

Temperature

Survivorship and the respiration and filtration rates of *T. keenae* in response to increases in temperature are shown in Figures 1 and 2. Survivorship at 24°C for 7 days was 97%, but decreased to 80% at 25°C. However, upon exposure to 27°C, survivorship fell to 63.4% by the fifth day and was 0% on the seventh day of exposure. At 28°C, survivorship on the third day was 40% and all were dead by the fourth day. The LT_{50} of *T. keenae* over 7 days, computed by the probit method was 25.9°C and the temperature range with a 95% confidence interval was 23.6-29.7°C. The respiration and filtration rates were measured on animals that survived on the third day of exposure to the range of test temperatures, 24-28°C, for 7 days (Fig. 2). Although the respiration rate showed a general tendency to increase with increasing temperature, the rates at 27 and 28°C were substantially higher than those at 24 and 25°C. Furthermore, the standard deviation in respiration rates at 28°C was very large, suggesting that the respiratory rhythm was irregular at high temperatures (Fig. 2). There was a similar increase in the filtration rate with increasing temperature, with substantial increases at temperatures that were 50% of the lethal temperature. The effects of reduced temperatures on survivorship, the filtration rate, and the respiration rate are shown in Figures 3 and 4. Survivorship of *T. keenae* at 6°C was 93% over 10 days of exposure, and decreased at temperatures lower than 4°C. The survivorship at 4°C over 10 days was 26.7%, 13.3% at 3°C, and all died at 2°C. The lower lethal temperature of *T. keenae* computed by the probit method for 10 days was 4.8°C (confidence interval of 4.5-5.2°C; Fig. 3). The respiration rate also decreased with decreasing temperatures with the most rapid decrease at 5°C (Fig. 4). The filtration rate showed a similar response to decreasing temperatures (Fig. 4).

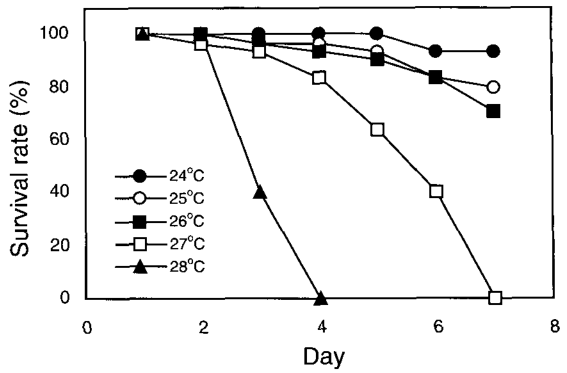

Fig. 1. Survival rate of juvenile *Tresus keenae* at high temperature during 7days-culture.

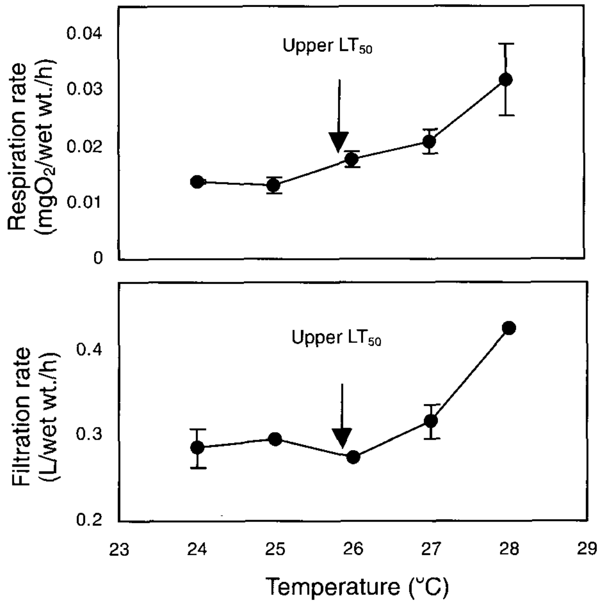

Fig. 2. Changes of the respiration and filtration rate of *Tresus keenae* in response to increase in temperature from 24℃ to 28℃.

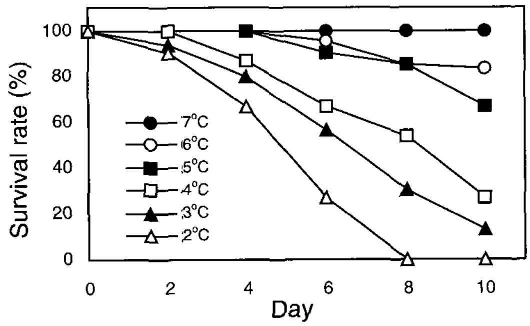

Fig. 3. Survival rate of *Tresus keenae* at low temperature during 10days-culture.

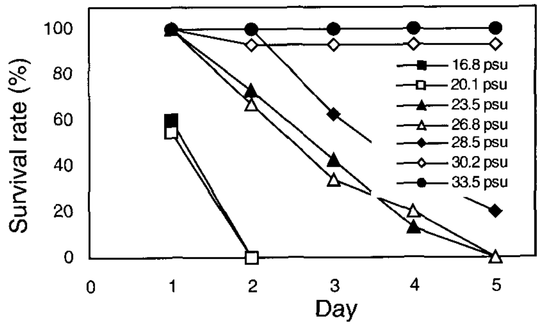

Fig. 5. Survival rate of *Tresus keenae* with decreasing salinity from 33.5 psu to 16.8 psu.

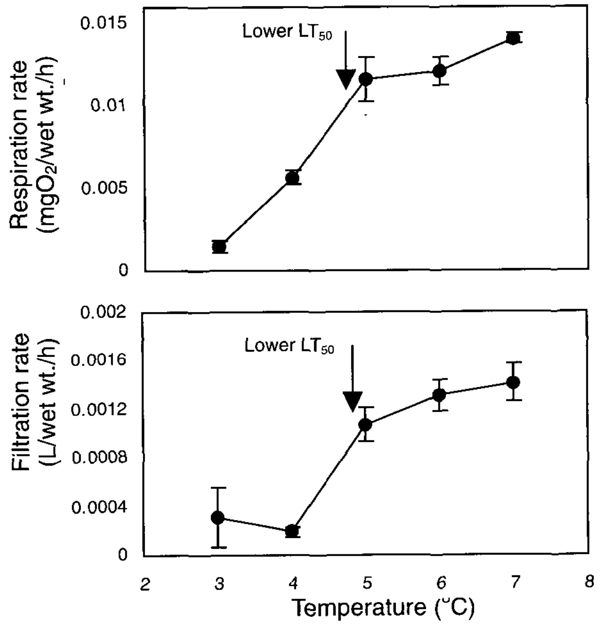

Fig. 4. Changes of the respiration and filtration rate of *Tresus keenae* in response to decrease in temperature from 7℃ to 2℃.

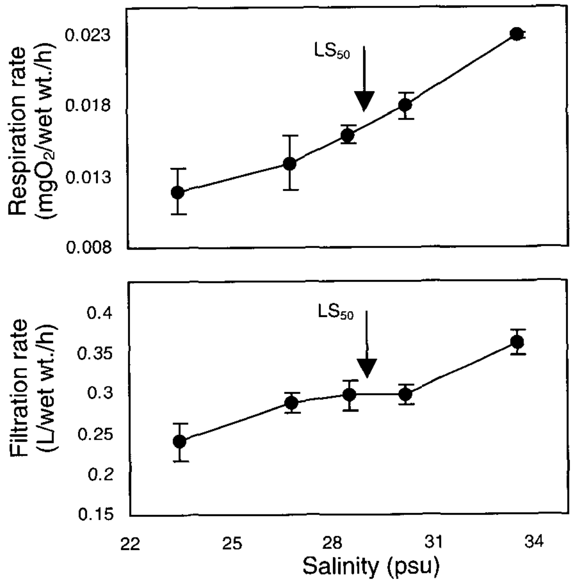

Fig. 6. Changes of the respiration and filtration rate of *Tresus keenae* with decreasing salinity from 33.5 psu to 23.5 psu

Salinity

The effects of salinity on survivorship and the respiration and filtration rates of *T. keenae* are shown in Figures 5 and 6. Survivorship following the reduction in salinity was 90% at salinities above 30.2 psu, but decreased rapidly to 60% by the third day at a salinity of 28.5 psu, and down to 50% on the fifth day of exposure. At salinities of 23.5 and 26.8 psu, all test animals died by the fifth day of exposure, while all died by the second day at a salinity of less than 20.1 psu. The LS_{50} as analyzed by the probit method was 29.1 psu (confidence interval of 28.0-30.5 psu; Fig.5).

The respiration rate decreased with a decrease in salinity. At a salinity of 23.5 psu, the respiration rate showed a reduction of about 48% in comparison to that at 33.5 psu (Fig. 6). The filtration rate also decreased with a reduction in salinity, and at a salinity of 23.5 psu, the filtration rate was about 34% lower than that at 33.5 psu (Fig. 6).

Discussion

The physiological responses of marine organisms represent the sum of all cellular and biochemical reactions to the influence of environment variables. For this reason, organisms are capable of reflecting environmental deterioration even before the effects are manifest in the population or community as a whole (Bayne et al., 1985). Among the external environmental factors that affect the shellfish, temperature has perhaps the most profound effect, affecting not only metabolic rate, but also the activity level and energy balance (Newell and Kofoed, 1977). Changes in salinity upset the delicate balance in the salt and water content of cells. Rapid changes in salinity will cause the shellfish to seal its shell immediately in an attempt to limit the increase in metabolic rate as the animal attempts to accommodate the changes in the salt and water balance of its cells (Lange, 1972). The physiological range of the temperature at which survival of *T. keenae* is possible was found to be 4.8-25.9°C. The respiration and filtration rates increased with an increase in temperature, and these rates rapidly increased at temperatures above the upper lethal temperature of 25.9°C. However, the rates of respiration and filtration substantially decreased at temperatures below 5°C, indicating clearly defined responses to higher and lower temperatures. The metabolic rate increased with an increase in temperature, which was similar to results reported for the gastropod Australorbis glabratus, in which the respiration rate increased with increases in temperature up to 37°C (von Brand et al., 1948). Both these results were consistent with the typical relationship between temperature and metabolic rate in poikilotherms. If the duration of exposure to temperatures in the range of the upper lethal temperature is continued, oxygen consumption is reduced and the energy available for physiological activities becomes limited (Rosas et al., 1997), eventually causing death.

The upper lethal temperature for shellfish varies depending on the temperature to which the species has been acclimated. In the case of *Mytilus coruscus*, which was acclimated to 20°C, the LT_{50} was 26.6-27.5°C (Shin and Wi, 2004), while *Ruditapes philippinarum* and *Haliotis diversicolor*, which were both acclimated to 25°C had LT_{50}s of 30.1 to 33.7°C (Shin et al., 2000) and 32.7°C (Chen and Chen, 1999), respectively. In *Venus antiqua* (Urban, 1994), which was acclimated to 13°C, the critical temperature was 25.6°C, slightly lower than that of other shellfishes (Table 1). In contrast, *T. keenae* was acclimated to 20°C but had an upper lethal temperature of 25.6°C, which is relatively lower than that of other shellfish species in Table 1. It has been suggested that because there is little variation in temperature in the deepwater habitat of *T. keenae*, this species is not able to tolerate as wide a temperature range as other species whose habitats experience more variation in temperature. The lower lethal temperature of *T. keenae* was 4.8°C. The tolerance of *T. keenae* was substantially lower than that of the ark shell *Tegillarca granosa*, which lives in the intertidal zone and has a lower lethal temperature of -1°C (Yin et al., 1994). Although it is well-known that the metabolism of organisms decreases at low temperatures, reports on the changes in related physiological processes are scarce.

Many intertidal invertebrates show changes in seasonal tolerance to low temperature due to endogenous circa-annual rhythms. Aarset (1982) suggested that the tolerance of low temperatures in shellfish may increase in organisms that have acclimated to higher salinities. It can therefore be deduced that the tolerance of *T. keenae* to low temperatures would be weak because their deepwater environment (1,520 m) is more stable with regard to temperature and salinity than the intertidal environment. However, investigations into the cellular processes that mediate tolerance to low temperatures are necessary to support this hypothesis.

The influence of salinity on the physiological processes of marine and coastal organisms is one of the factors that determine species distributions (Widdows, 1985). Typical responses at low salinity include reduced food intake, lower growth rate (Bohle, 1972; Widdows, 1985), and closure of the shell (Hand and

Table 1. Limiting critical ranges of environmental factors on several shellfishes. Value in the parenthesis is lower LT_{50}

Items	Species	Acclimation Temperature and Salinity	LT_{50} and LS_{50}	References
Temperature (°C)	*Mytilus coruscus*	20°C (33.5 psu) 18°C (32.0 psu) 20°C (35.0 psu) 13°C (34.0 psu) 20°C (33.5 psu)	26.6-27.5	Shin and Wi (2004)
	Mytilus edulis		28	Read and Cumming (1967)
	Ruditapes philippinarum		30.1-33.7	Shin et al (2000)
	Haliotis diversicolor		32.7	Chen and Chen (1999)
	Venus antiqua		25.6	Urban (1994)
	Tresus keenae		25.9 (4.8)	The present study
Salinity (psu)	*Mytilus coruscus*	20°C (33.5 psu)	17-21.8	Shin and Wi (2004)
	Ruditapes philippinarum	18°C (32.0 psu)	23-24.9	Shin e al. (2000)
	Haliotis diversicolor	20°C (32.0 psu)	20-45	Chen and Chen (2000)
	Argopecten purpuratus	12°C (30.0 psu)	27	Navarro and Gonzalez (1998)
	Anadara granosa	28-30°C (32.0 psu)	19	Davenport and Wong (1986)
	Tresus keenae	20°C (33.5 psu)	29.1	this present study

Stickle, 1977; Shumway, 1977). The LS_{50}s for salinity tolerance in shellfish as presented in Table 1 are diverse. The LS_{50} of *T. keenae* in this study was 29.1 psu, which is higher in comparison to the values for *M. coruscus* (Shin and Wi, 2004) and *R. philippinarum* (Shin et al., 2000), which inhabit the intertidal zone. This finding is assumed to be a consequence of the weak tolerance toward salinity changes of a species that lives in a deep and relatively stable environment compared to an organism living in the less stable intertidal zone. In addition, the fact that there are different ranges of tolerance in different species is believed to be attributable to the generic range of the organism (Otto, 1973).

We found that the tolerance of *T. keenae* to temperature and salinity changes was low in comparison to other shellfish, which appears to be attributable to the habitat that *T. keenae* has already experienced and the characteristics of the animal. These data have implications for increasing efficiency in the production and management of shellfish aquaculture farms.

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