Lethal limits of high temperature for two crayfishes, the native species *Cambaroides japonicus* and the alien species *Pacifastacus leniusculus* in Japan

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ABSTRACT: Lethal limits of high temperature were studied to clarify the effects on the survival of the endangered Japanese crayfish species *Cambaroides japonicus* and the alien species *Pacifastacus leniusculus*. After the acclimation period for 2 weeks at 16°C, the temperature was raised at a rate of 1°C per week. As a result, the ultimate upper lethal temperatures of *C. japonicus* and *P. leniusculus* were 27.0 and 31.1°C, respectively, and the lethal temperature for *P. leniusculus* was significantly higher than for *C. japonicus*. The natural distributions of these two species are discussed in terms of the temperature tolerance.

KEY WORDS: *Cambaroides japonicus*, crayfish, distribution, lethal temperature, *Pacifastacus leniusculus*, tolerance.

INTRODUCTION

Three crayfish species are distributed in Japan. The Japanese crayfish *Cambaroides japonicus* (de Haan 1841) is the only native representative and the others, *Pacifastacus leniusculus* (Dana, 1852) and *Procambarus clarkii* (Girard, 1852), are alien species introduced from North America during a period from 1926 to 1930.1 Miyake described two species of *Pacifastacus* naturalized in Japan, *P. leniusculus* and *Pacifastacus troubridgei*.1 However, we have accepted the theory that they are the same species, the latter being an intraspecific variation of the former.2,3

The Japanese crayfish lives in cold, clear brooks and lakes in northern Japan, including Hokkaido, Aomori, Akita, and Iwate Prefectures.1 Natural populations of *C. japonicus* have drastically declined, prompting the Japanese authorities (Fisheries Agency in 1998 and Environment Agency in 2000) to designate it as an endangered species.

The signal crayfish *P. leniusculus* is native to permanent water bodies in cool temperate regions of north-western USA and south-western Canada.4 It occupies a fairly wide range of habitats and is found in large rivers, swift to sluggish streams, and lakes, and usually occurs under large rocks and decaying vegetation.2 This species was first introduced into Japan for use as food in 1928. At first, it had been distributed only in Lake Mashu in Hokkaido; however, the range of their distribution has drastically increased, especially in eastern Hokkaido.5 In Obihiro City in Hokkaido, the distribution of *P. leniusculus* was newly recorded in a river within the city in 2000.6 At Lake Shikaribetsu in Hokkaido, *P. leniusculus* is now established and has drastically increased in number while *C. japonicus* has disappeared.7

In general, temperature is a major limiting factor for aquatic poikilothermic animals. Temperature influences the geographic and local distribution, growth, metabolism, reproduction and life histories, and has indirect effects through its influence on water chemistry.8 *Cambaroides japonicus* and *P. leniusculus* survive in low water temperature at near-freezing point.6,7 Furthermore, the range of the water temperature of habitats of *C. japonicus* is relatively narrow, up to only about 20°C in summer.9 Thus, knowledge of the ultimate upper lethal temperature (UULT) is important for the survival of *C. japonicus* in relation to restocking areas that have been depopulated and affecting the possible distribution range of *C. japonicus* and *P. leniusculus* in Japan.
MATERIALS AND METHODS

Experiments on the UULT, which is the highest temperature that can be tolerated for a long period of time, obtained by gradually raising the acclimation temperature,\textsuperscript{10} of \textit{C. japonicus} and \textit{P. leniusculus} were carried out at the laboratory in the National Fisheries University.

Size measurements

In the present study, the total length (TL) from the apex of the rostrum to the caudo-mesial margin of the telson and carapace length (CL) from the posterior margin of the orbit to the mid-dorsal posterior margin of the carapace were used for size measurements.

Samples

Crayfish were obtained during seasons when actual temperatures approximated the desired acclimation level. For \textit{C. japonicus}, 20 individuals (nine males and 11 females; 10.6–27.8 mm CL) were collected from Hokkaido on 14 May 2000. Details of the sampling station are omitted here because of conservation measures for this population. For \textit{P. leniusculus}, 20 individuals (12 males and eight females; 14.7–37.8 mm CL) were collected on 30 September 2000 at the Kikanko River in Obihiro City, Hokkaido, where \textit{C. japonicus} is no longer observed.\textsuperscript{6} The water temperatures in the sampling stations were 15.6°C for \textit{C. japonicus} and 16.3°C for \textit{P. leniusculus}.

Temperature tolerance test

Prior to the experiment, the test animals were placed individually in aquaria that were prepared in a biotron and were acclimated for 2 weeks. The acclimation temperature was 16°C as \textit{C. japonicus} grows well and has been shown to actively forage at 14–18°C in natural conditions.\textsuperscript{9} \textit{Pacifastacus leniusculus} can live in the wild at these water temperatures.\textsuperscript{6,7,11} Each aquarium was provided with gentle aeration, and an artificial burrow made of a gray-colored straight PVC pipe with suitable sizes was placed in each aquarium. The sizes of artificial burrows were determined based on a study on burrows of \textit{C. japonicus}\textsuperscript{12} and similarly proportioned artificial burrows were used for \textit{P. leniusculus}. Thus, the internal diameters of the burrows were selected from 13 mm, 20 mm, 30 mm, 40 mm and 50 mm, with lengths of TL×3.

After the acclimation period, the temperature of the aquaria was raised by 1°C over a 24-h period and this temperature was maintained for the following 6 days (i.e. an increase of 1°C per week) by controlling the biotron temperature system.

The photoperiod was 12 h light : 12 h dark. Observations were made four times a day, twice each during the day and night. During the day, white fluorescent lamps were used with a low light intensity of 100–200 lx on the water surface, because \textit{C. japonicus} normally live in shaded areas.\textsuperscript{13} Night-time observations were made under a red light, which has been shown to minimally influence the behavioral pattern for a freshwater shrimp \textit{Macrobrachium} sp.\textsuperscript{14} The water temperature was measured at every observation. All individuals were fed with an artificial diet (Hayashikane Sangyo Co., Ltd, Shimonoseki, Japan) once a day.

\textit{Cambaroides japonicus} normally moulted and grew with the diet (Nakata \textit{et al.}, unpubl. data, 2001). One-third of the rearing water was changed every 5 days.

The thermal tolerance test was continued until all of the animals were dead. The criterion of death in the present study was the cessation of the beating of the scaphognathites according to Gladwell \textit{et al.}\textsuperscript{15} From the present experiment, the relationship between the test temperatures and percent survival (%) can be determined. Based on the method of Tsuchida and Setoguma,\textsuperscript{16} the UULT value was determined graphically at 50% mortality. The present experiments were conducted during the period from 22 May 2000 to 5 February 2001.

Observations on behavior

We observed the behavior of the crayfish during the experimental period based on stress responses to high temperature in fish.\textsuperscript{17} As temperature increases within the upper critical range, the stress response in fish was classified into three progressive phases.\textsuperscript{17} The first external indications of abnormal behavior are a reluctance to feed, sudden bursts of activity with frequent collisions with the side walls of the tank, rolling and pitching and rapid ventilatory movements. In the second phase, the fish becomes quiescent with short bursts of weak swimming, often floating on its side or back and may rapidly change color and increase its ventilatory movements. In the third phase, movements are restricted to the opercula, pectoral fins and eyes, and cease with the death of the fish.\textsuperscript{17} Further, in some crayfish species, sluggish movements were observed when prodded, a phenomenon that has been used as evidence of high
temperature stress.\textsuperscript{18,19} These stress responses were discerned in the present study.

RESULTS

Lethal limit of high temperature for \textit{C. japonicus}

At 21°C after 46 days from the start of the experiment, the first individual died from an abnormal moult, in which a part of the new carapace did not regenerate (Fig. 1). At 25°C after 70 days, some individuals moved sluggishly on prodding. Above 26°C, the number of dead individuals increased to three, six, six and three at 26, 27, 28 and 29°C, respectively. An individual died from an abnormal moult at 26°C. At 30°C, the last individual died (Table 1). The percent survival (%) drastically reduced above 26°C (Fig. 2). The UULT was estimated to be 27.0°C. During the experiment, seven and four individuals moulted at 16–19°C and 20–25°C, respectively. Food intake remained unchanged up to the day the animals died.

Lethal limit of high temperature for \textit{P. leniusculus}

The behavior of animals did not change up to 104 days at the temperature of 29°C. At 30°C, some individuals moved sluggishly on prodding and the food intake decreased. At this temperature five individuals died. The numbers of dead individuals were four and nine at 31 and 32°C, respectively. At 33°C, the remaining two crayfish died (Table 1). The lethal temperature of \textit{P. leniusculus} was significantly higher than that of \textit{C. japonicus} (Jonckheere’s test, $P < 0.001$) (Fig. 2). The UULT of \textit{P. leniusculus} was estimated at 31.1°C. Six individuals moulted at 18–27°C.

DISCUSSION

For \textit{C. japonicus}, two individuals died with abnormal moults at 21 and 26°C (Fig. 1). We previously observed more than 100 moults of \textit{C. japonicus} at 16°C under the same rearing conditions as in the present study; however, no crayfish died with such abnormal moults (Nakata \textit{et al.}, unpubl. data, 2001). In a previous thermal study of \textit{P. leniusculus} in the USA, four test animals died due to abnormal moulting by abrupt transfer to high temperature of 29–31°C.\textsuperscript{20} It was concluded that they were unable to tolerate the stress of moulting imposed by the stress of severe thermal shock.\textsuperscript{20} The dead \textit{C. japonicus} with abnormal moulting may have been affected by thermal shock.

As the maximum water temperatures in summer in the natural habitats of \textit{C. japonicus} are about 20°C,\textsuperscript{3} the distribution of \textit{P. leniusculus} can extend to the habitat of \textit{C. japonicus} regarding temperature, judging from the present study. Recently, \textit{P. leniusculus} has been extending its range, especially in eastern Hokkaido.\textsuperscript{3} In Lake Shikaribetsu, Hokkaido, where the maximum water temperature

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig1.png}
\caption{\textit{Cambaroides japonicus} male, which died during an abnormal moult at 21°C. Upper, the moulted exuvia of the crayfish (10.9 mm in carapace length); lower, the body from the moult. A part of the new carapace did not regenerate.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Percent survival of the two crayfishes, \textit{Cambaroides japonicus} and \textit{Pacifastacus leniusculus} at 16–33°C after acclimation to 16°C for 2 weeks. The test temperatures were raised at a rate of 1°C per week.}
\end{figure}
Table 1  Upper lethal temperature of two crayfish species, Cambaroides japonicus and Pacifastacus leniusculus

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<th>Cambaroides japonicus</th>
<th>Pacificastacus leniusculus</th>
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<tr>
<td>Lethal temperature (°C)</td>
<td>Carapace length* (mm)</td>
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<tr>
<td>21</td>
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*Carapace length was measured after the crayfish had died. †These crayfish died during molting.

is about 20°C, P. leniusculus is now established while C. japonicus has disappeared. Both C. japonicus and P. leniusculus often hide under and/or between rocks and in burrows, and feed on similar detrital materials. Thus, the fundamental niche of these two species may overlap. Pacificastacus leniusculus grows faster and is more fecund than C. japonicus; therefore, the populations of P. leniusculus will dominate compared with C. japonicus when P. leniusculus invades to a local population of C. japonicus.

In other experiments using a different experimental method, the incipient lethal temperature (ILT) of P. leniusculus, which was measured by maintaining at an acclimation temperature and then an abrupt transfer to a higher temperature, was reported to be approximately 32–33°C. The critical thermal maximum (CTM) of P. leniusculus, which was determined by raising the temperature from the ambient acclimation level at a constant rate so that there was no significant time lag between the water temperature and the internal temperature of the animal was near 31°C after an acclimation at 20°C. These values are similar to the UULT value of 31.1°C in the present study. As gradual changes in temperature in the present study are a more natural phenomenon than rapid changes of several degrees, the UULT provides a useful criterion for the thermal tolerance in studies on natural distribution and suitability of aquacultural environments.

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REFERENCES


