Turkish Journal of Fisheries and Aquatic Sciences 8: 219-223 (2008)

Influence of Eyestalk Ablation and Temperature on Molting and Mortality of Narrow-clawed Crayfish (*Astacus leptodactylus*)

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Received 18 February 2008
Accepted 15 May 2008

Abstract

In this study, two experiments were conducted to investigate the effects of eyestalk ablation and temperature on molting and mortality of narrow-clawed crayfish (*Astacus leptodactylus*) in three replicates. In experiment 1, the percentage of molting in ablated crayfish was significantly higher than the percentage of non-ablated crayfish in control group ($P<0.05$). In experiment 2, the highest molting and mortality rate was found at 32˚C in ablated crayfish treated with four different levels of temperature (20, 24, 28 and 32˚C). At temperature of 20˚C, the animals had low mortality as well as reduced molting rate ($P<0.05$). Mortality was mostly occurred within the first days of the trial. Proficient and proper nourishment was found to be very important in all treatments in this study.

Key words: *Astacus leptodactylus*, Crayfish, Molting, Mortality, Temperature, Eyestalk ablation.

Introduction

Astacus leptodactylus or narrow-clawed crayfish are in economic terms important species in Western Asia and Eastern Europe e.g. Turkey, Poland, Italy, Germany, England, Spain, Austria and France (Holdich, 2002; Harlioglu, 2004). During the last decade, various species of crayfish have been placed into aquaculture programs. Crayfish farming in freshwater and brackish water can enhance production, which augments the export potential. A key factor in crayfish production is the molting cycle. Survival, molting and growth of decapods can be affected by a variety of environmental factors including temperature, oxygen concentration, salinity and light duration (Aiken *et al*., 1983; Chen *et al.*, 1995; Diaz *et al*., 2003; Hammond *et al*., 2005). A preliminary study on *Procambarus clarkii* suggested that temperature influential on the molting of the ablated crayfish (Chen *et al*., 1995).

Water temperature is a major limiting factor for poikilothermic aquatic organisms (Verhoef and Austin, 1999; Hammond *et al*., 2005) influencing body temperature and metabolic activity (Verhoef and Austin, 1999), food intake and growth. Molting and growth rates increase with increasing water temperature until the optimum is reached, and then with further increases decline, following a bell shaped curve model (Ponce-Palafox *et al*., 1997; Jobling, 2003).

The eyestalks of crustaceans contain neurosecretory cells that involve in the regulation of molting (Meade and Watts, 2001). Molting in crustaceans was thought to be regulated by two hormones; (1) molt-inhibiting hormone and (2) molting hormone. It is believed that the molt-inhibiting hormone is produced in the eyestalk and stored in the sinus gland whereas the molting hormone is produced in Y-organ. By eyestalk ablation, the molt inhibiting hormone is excluded that allows the molting hormone to act. Thus the removal of eyestalks causes an increase in ecdysteroid secretion from the Y-organ, which induces precocious molting (Nakatsuji and Sonobe, 2004; Venkitraman *et al*., 2004). Among all known stimuli to molting, eyestalk ablation is the most effective one in terms of time needed to response (Chen *et al*., 1995), which directly affects on the endocrine system of crayfish (Fingerman, 1987; Huner, 1990). Many biologists worked on the endocrine control of molting and growth in decapod crustacean (Smith, 1940; Scudamore, 1947; Bliss, 1966; Huner and Avault, 1977; Ponnuchamy *et al.*, 1981; Huner and Lindqvist, 1984; Chen *et al*., 1995; Venkitraman *et al*., 2004). In recent years, a few preliminary investigations have been accomplished on *Procambarus clarkii* (Chen *et al*., 1995), *P. zonangulus* (Hobbs and Hobbs, 1990) and *Cherax quadricarinatus* (Sagi *et al*., 1997). However, very little work has been done in astacidae crayfish of Caspian Sea.

Removal of eyestalk leads to weight increase and osmolality decrease of haemolymph (Meade and Watts, 2001; Venkitraman *et al*., 2004) and unilateral eyestalk ablation has been employed to induce ovarian maturation and spawning with varying success in many crustacean species (Zaib Un Nisa, 2001). It can also be used to shorten the molt interval and to stimulate gonad development in decapoda (Venkitraman *et al*., 2004).

Although the bilateral eyestalk ablation shortens

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the molt interval, it can also cause additional mortality (Bittner and Kopanda, 1973; Huner and Lindqvist, 1984), primarily because ablation simultaneously removes the four ganglia in each eyestalk, a considerable portion of the central nervous system (Chen *et al*., 1995). Therefore, ablation induced hormonal imbalance or stress, which often results in high mortality (Chen *et al*., 1993).

Materials and Methods

Animals and Holding Conditions

Individuals of both sexes (15 to 27 g in weight; 7.5 to 10 cm in total length and 3.8 to 5.1 cm in carapace length) were collected from ponds of the Research Center of Fisheries in Astaneh Ashrafiye, Iran, from May until October 2006, and transported to the laboratory in Guilan University. They were kept in water tanks contained freshwater (100 L), aerated with air pumps at temperature ranging from 16 to 19°C, with the oxygen concentration of 8 mg/L, pH of 7.8 to 8 and natural photoperiod for two weeks before experimentation. To feed the animals, fresh pieces of fish were used every day. Ionic composition of the freshwater in which animals were kept was 168.9, 78, 38.15, 220.1, 325 and 739 mg/L for Na^+, Ca^{2+}, Mg^{2+}, Cl^-, $CaCO_3$ and TDS, respectively.

Size and Weight Measurements

The length of the crayfish was measured by vernier calipers. Crayfish weighted by using an electronic balance after blotting the animal on a filter paper.

Eyestalk Ablation

Different procedures such as constriction of eyestalk with catgut, cauterization, enucleation and surgical removal of eyestalk are used for eyestalk extirpation of the X-organ sinus gland complex, which are all successful in unilateral eyestalk ablation (Venkitraman *et al*., 2004). In the present study, eyestalk was removed using sterile surgical blades, which reduced mortality to the minimum level. The crayfish used were in the intermolt stage. During ablation, in order to reduce heartbeat, loss of haemolymph and to control bleeding specimens held in pre-cooled water and Vitamin A-D ointment was applied on the sore.

Experiment 1: In this experiment, the water temperature influenced by the environment was ranging between 16 and 19°C, and the effect of eyestalk ablation was investigated in ablated and non-ablated groups with three replicates. There were 27 crayfish in intermolt stage in each treatment.

Experiment 2: In this experiment, water temperature in experimental and control groups were 20, 24, 28, 32, and 16 to 19°C (natural temperature), respectively. Nine ablated crayfish (in intermolt stage) per replicate were randomly assigned to each aquarium, which contained freshwater (50 L) with oxygen concentration of 8 mg/L and pH of 7.8 to 8. The crayfish were marked by cutting of telson or uropods to allow identification for individual (This method of marking is used when the crayfish molt. However, due to the small number of crayfish per aquarium, the recently molted crayfish were easily identified). Digital Multimeter (Consort, C535) was used to measure pH, temperature and the oxygen concentration.

Data Collection and Analysis

In both experiments at the present study, the water quality factors such as pH, oxygen concentration, salinity and temperature was closely monitored during the test and the number of live, dead and molted crayfish was recorded every day. The data obtained for survival and molting rates were analyzed by one-way analysis variance (ANOVA) of SPSS statistical software and differences were considered to be statistically significant to ($P<0.05$).

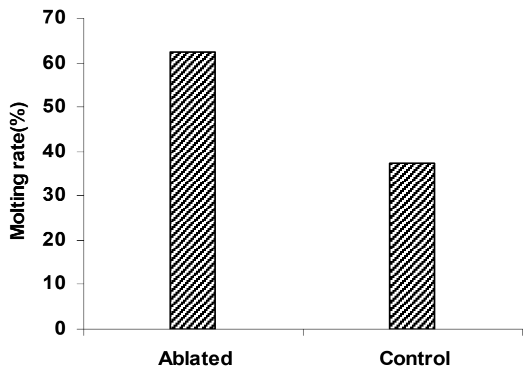

Figure 1. Comparison of molting rate in control group and ablated *A. leptodactylus*.

Results

Experiment 1

The ablation was found to have a significant effect on molting ($P<0.05$, Figure 1), while significant differences between eyestalk ablation and mortality were not observed ($P>0.05$).

Experiment 2

Molting Percentage: Mean molting rate tended to increase by increasing water temperature, which was twofold at the temperature 32°C in comparison with the temperature 20°C (Figure 2A) and significant relationship was observed between molting rates by increasing temperature ($P<0.05$, $R^2= 0.9089$). Total number of molting was increased to the maximum level in the second week and then declined during the rest of the experiment (Figure 2B; $R^2= 0.7952$).

Mortality Percentage: Survival of the crayfish declined significantly with increasing temperature ($P<0.05$). Mean mortality rate was the lowest in the first treatment and control group (20°C and 16 to 19°C respectively). Above 20°C, mean mortality rate increased progressively from 14.8% at 24˚C to 25.9% at 32°C and the highest mortality occurred during the first week of the experiment at 32°C (Figure 3A and 3B). There was a significant difference between mortality rates and temperature ($P<0.05$, $R^2= 0.9423$).

Discussion

In this study, eyestalk ablation had a significant effect on molting of *A. leptodactylus* ($P<0.05$, Figure 1), which is comparable with the results obtained from other studies conducted on other crustacean. Sanjeevraj *et al.* (1997) in a study showed that molting and growth rates in eyestalk-ablated prawns were higher than that of nonablated ones. In an experiment conducted on destalked *M. rosenbergii*, Okumura and Katsumi (2001) reported a rapid increase of ecdysteroid and a significant shorter molting interval in comparison with intact prawns. In other studies conducted on three species including, *A. astacus* (Huner and Lindqvist, 1984; Gydemon and Westin, 1988), *P. clarkii* (Chen *et al*., 1995) and *Metapenaeus dobsoni* (Venkitraman *et al*., 2004), a similar significant relationship was observed between eyestalk ablation and molting rate ($P<0.05$). In the first experiment of the present study, a low mortality was observed, whereas a high mortality rate in bilaterally eyestalk-ablated and a high survival rate in unilaterally ablated animals were reported in *Pamilirus ornatus* and *M. lankesteri* (Juinio Menez and Ruinata, 1996; Venkitraman *et al*., 2004).

As shown at the present study, water temperature is a key controlling factor for the growth and survival of cultured freshwater crustaceans (Holdich, 2002). Given interest in *A. leptodactylus* as an aquaculture species in Asia and Europe, we assessed the performance of this species under

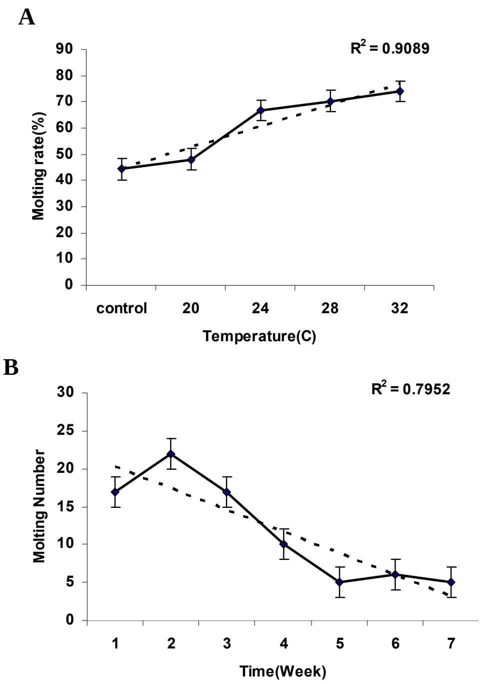

Figure 2. A: Effect of eyestalk ablation on molting rate of *A. leptodactylus* under different temperatures, B: Number of molting in during seven weeks.

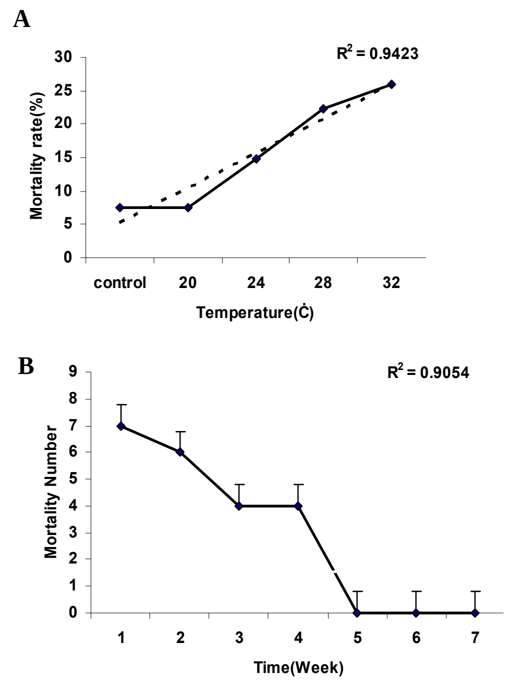

Figure 3. A: Effects of eyestalk ablation on mortality rate of *A. leptodactylus* under different temperature, B: Number of mortality in during of experiment.

different water temperature and eyestalk extirpation.

Temperature as well has been recognized as one of the main environmental factors affecting on the molting and growth of crayfish (Hammond *et al*., 2005; Holdich, 2002). This is verified by the results presented in Figure 2, which demonstrates that in this research, temperature had a pronounced impact on ablated crayfish molting.

The significant impact of temperature upon molting of ablated crayfish is consistent with other related studies ($P<0.05$; Figure 2). For example, ablated noble crayfish (*A. astacus*) and red swamp crayfish (*P. clarkii*) tended to molt during the warm water phase (Gydemon and Westin, 1988; Chen *et al*., 1995). The decreased molting in low temperature has also been observed on other crustacean species, such as lobster of *Homarus americanus* (Aiken *et al*., 1977) and fiddler crab or *Uca pugnax* (Passano, 1960).

The results can also be compared with other limited studies that have been conducted on red swamp crayfish production. Culley and Duobinis-Gray (1990) reported that soft-shell crayfish at 30±1°C had significantly higher molting rates (3 fold) than did crayfish at 20±1°C, due to shortening of molt intervals.

The impact of temperature upon crayfish growth and molting is of a complicated nature. For example, winter sub-freezing conditions can suspend the growth of crayfish such as *Orconectes virilis* for several months (Aiken and Waddy, 1987). However, high temperatures can also cause problems to *P. clarkii* by driving them deep into the mud, which almost suspends their growth (Chen *et al*., 1995).

Huner and Barr (1991) also stated that the intact *P. clarkii* had normal feeding and molting activities at temperatures above 12.8°C and growth inhibition occurred at temperatures above 32.2°C (Chen *et al*., 1995). In *Procambarus spiculifer*, growth was the highest at 23.4±0.4°C (Taylor, 1990) and in *Paranephrops zealandicus*, and mean molting interval was the shortest at 24°C (Hammond *et al*., 2005). Estimations based on the molting data presented in Figure 2 of the present study on *A. leptodactylus* suggest that the percentage of molting at 32°C under batch operation conditions was approximately twice higher than that at 20°C. Natural range of temperature tolerance for *A. leptodactylus* has been reported to be between 4 and 32°C (Koksal, 1988). However, results of the present study showed that the temperature 32°C resulted in the highest molting and a simultaneous mortality rate.

Survival of *A. leptodactylus* was strongly influenced by water temperature and feeding rate. In this study, mortality rate increased in high temperature and there was a significant relationship between temperature and mortality rate ($P<0.05$). Survival rates were comparable to those of other crayfish species (Mitchell and Collins, 1995; Verhoef and Austin, 1999; Hammond *et al*., 2005) at low water temperature, but decreased rapidly with increasing temperature. The negative relationship between survival and temperature was consistent with the findings on other crustaceans ($P<0.05$) (Ponce-Palafox *et al*., 1997; Verhoef and Austin, 1999).

Molting, as an important function, plays an important role for the growth and reproduction in crustacean, so by increasing water temperature and performing eyestalk ablation, as shown in this research, the rate of molting and then the growth and reproduction was improved. Since there were not any significant differences between the temperatures 24 and 32°C for the molting and a simultaneous lower mortality in 24°C, then the temperature 24°C determined a suitable temperature to induce molting in ablated crayfish *A. leptodactylus*.

The findings obtained at the present study are practically helpful to increase production by controlling molt and mortality in *A. leptodactylus* of Caspian Sea.

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