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Hemolymph profiles of pond-reared and lake pen-cultured adult Chinese mitten crab, *Eriocheir sinensis* H. Milne Edwards, 1853

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ABSTRACT

Levels of seven hemolymph parameters (considered as indicators of physiological and immune status of organisms) in pond-reared (PR) and lake pen-cultured (PC) adult Chinese mitten crabs sampled from three experimental sites *viz.*, a pond at Huangjin Lake area, a net-pen in the Huangjin Lake and a pond at Lu Lake area were analysed. Two sites in the Huangjin Lake area where the pond meets the lake, possessed good water quality whereas at Lu Lake area where the pond was not connected to the Lu Lake, the water quality was relatively poor. Hemocyanin content and total hemocytes count in PR crabs from Lu Lake area were significantly lower than those of PR and PC crabs from Huangjin Lake area, indicating PR crabs from Lu Lake area had relatively poor physiological and immune status. There were no significant differences in hemolymph profiles between PR and PC crabs from Huangjin Lake area. These results indicate that water quality had a significant effect on the physiological and immune status of cultured Chinese mitten crabs. The resuls indicate that pond-rearing is better for culture of Chinese mitten crabs, especially in ponds which are connected to natural water resources.

Keywords: Chinese mitten crab; Hemolymph profile; Lake pen-cultured; Pond-reared; Water quality

Introduction

Chinese mitten crab *Eriocheir sinensis* is a euryhaline crustacean with a catadromous life cycle (Hanfling and Weetman, 2003; Wu *et al.,* 2007). This species inhabits temperate and tropical waters ranging from East Russia (Vladivostock) to South China, with its center of occurrence in the Yellow Sea, where the crab is considered a delicacy, particularly at the right stage of breeding season (Rainbow, 1998; Chang *et al.,* 2006; FAO, 2006). In its native regions (primarily in China), this crab used to be an important wild caught species. Due to depleted natural production and increasing market demand, Chinese mitten crab is being cultured in China since 1980s (Du and Zhao, 1988; Hymanson *et al.,* 1999). With success in the artificial propagation of this crab with natural as well as artificial saline water, the Chinese government, as part of the efforts to diversify aquaculture species, has provided stimulus for expansion of Chinese mitten crab farming (Zhao, 1980; FAO, 2006). During the last two decades, Chinese mitten crab culture in China has developed very rapidly, and its aquaculture is now practised all over China except in Tibet (Hymanson *et al.,* 1999; Lu, 2002; Cheng *et al.,* 2006; Qiu *et al.,* 2011).

Various aquaculture environments like earthen ponds, pens in shallow lakes and reservoirs, paddy fields, lakes as well as reservoirs are used for culture of Chinese mitten crabs (Wu *et al.,* 2007). Pond-rearing (PR), pen-culture (PC), paddy field-raising (PFR) and extensive culture in lakes/reservoirs (ELR) are the four most common culture methods in China (FAO, 2006). PR has an obviously high stocking density (22,500-37,500 nos. ha^{-1}) as compared to 10,000-15,000 nos. ha^{-1} for PC, 6,000-9,000 nos. ha^{-1} for PFR and 200-600 nos. ha^{-1} for ELR (FAO, 2006). In general, high stocking density largely implies great benefits, however, it could induce a number of stressors that in turn could compromise the immune system of aquatic animals and increase their susceptibility to disease (Lee and Wickins, 1992; Rosenberry, 1995). There is a point of view that pond-reared Chinese mitten crabs are more susceptible to diseases compared to those raised by other methods. There have been no studies so far to compare the physiological and immune status of Chinese mitten crabs cultured by various methods. Many authors have demonstrated that certain hemolymph parameters in crustaceans could be used as effective indicators of physiological as well as immune status (Defur *et al.,* 1980; Morris *et al.,* 1986; Hall and van Ham, 1998; Matsumasa and Murai, 2005; Radford *et al.,* 2005; Patterson *et al.,* 2007). Perez-Jar *et al.* (2006) measured hemolymph phenoloxidase activity (PO), total hemocytes count (THC), hemagglutinating activity (HA), total proteins (TP), and glucose (Glu) concentrations to evaluate the immune and metabolic status of wild and pond-reared southern white shrimp *Litopenaeus schmitti* adult males during continuous reproductive activity. Lorenzon *et al.* (2007) analyzed the physiological profiles of transported American lobster *Homarus americanus* and observed that glucose, lactate, total protein and cholesterol were significantly higher in the group with high body temperature compared to those with low temperature until 96 h after immersion in the recovery tank. The physiological profiles of transported crab *Cancer pagurus* were investigated by hemolymph bleeding and the authors reported that post-transport, the glucose, total protein and hemolymph density were lower in the crabs transported in water compared to those transported in boxes with high humidity (Lorenzon *et al.,* 2008).

In the present study, seven hemolymph parameters of two groups of adult Chinese mitten crabs (pond-reared and lake pen-cultured) from the same water body (Huangjin Lake area) and one group of pond-reared adult crabs from another water body (Lu Lake area) were investigated to compare the physiological and immune status between the three groups. In addition, the effect of sex on hemolymph parameters of pond-reared and pen-cultured adult crabs was also analyzed. The results obtained here could provide important information for Chinese mitten crab culture.

Materials and methods

Experimental crabs

The present study was conducted in China at two lake areas *viz.*, Huangjin Lake area and Lu Lake area (Hubei). In mid-April 2007, Chinese mitten crab fry (200 nos. per kg) were procured from Chongmin Island (Shanghai) and reared at three experimental sites: a 3 ha net-pen in the Huangjin Lake at a stocking density of 12,000 nos. ha^{-1}; a 0.3 ha pond connected to the Huangjin lake, with a stocking density of 30,000 nos. ha^{-1}; and a 0.3 ha pond located at the Lu Lake area but not directly connected to it, with a stocking density of 30,000 nos. ha^{-1}. At Huangjin Lake area, there was water exchange between the pond and the lake. The pond-reared crabs were fed with a commercial diet (Zhengda Feed Company Wuhan), whereas aquatic plants, algae and zoobenthos formed the natural food for the pen-cultured crabs. Water samples were collected with a horizontal water sampler (General Oceanics, Miami, USA) at 20-30 cm above the pond bottom. At each experimental site, water was sampled from five locations and pooled together into a 2 l clean plastic bottle. Water samples were placed in ice and sent for analysis.

In November 2007, the pond-reared and pen-cultured adult crabs from the three experimental sites were harvested. Twenty adult crabs (females and males equally represented) were randomly sampled at each site and transported to College of Fisheries, Huazhong Agricultural University, Hubei, China.

Water quality parameters

Water samples were analyzed for pH, ammonium nitrogen (NH_4^+-N), nitrite-N (NO_2^--N), nitrate-N (NO_3^--N), and total phosphorus (tp) following standard methods (APHA, 1985).

Morphology of crabs and determination of hemolymph parameters

The crabs were anaesthetized in ice and body weight, shell length as well as shell width were recorded. About 0.1 ml of hemolymph was collected by puncturing the first abdominal segment with a 1 ml ice-chilled syringe and 0.1 ml fixative (10% formaldehyde solution, pH 6.5) was added, and thoroughly mixed. A drop of hemolymph-fixative mixture from each adult crab was placed on Neubauer hemocytometer and hemocytes were counted using an Olympus light microscope, CX 21 (Olympus, Tokyo, Japan) for estimation of total hemocytes count (THC).

Approximately 2 ml of hemolymph was collected with a 2.5 ml ice-chilled syringe in EDTA in isotonic solution as an anticoagulant (Lu *et al.,* 2007). One part of the hemolymph was diluted with an equal part of anticoagulant. The anticoagulant-hemolymph mixture was centrifuged for 20 min at 5000 g at 4 ^{o}C to separate plasma. The plasma was immediately frozen and stored at -80 ^{o}C for biochemical analysis. The glucose (Glu, mmol l^{-1}), total protein (TP, g l^{-1}), triglyceride (TG, mmol l^{-1}), total cholesterol (TC, mmol l^{-1}), and calcium (Ca, mmol l^{-1}) concentrations in the hemolymph were measured with commercially available kits (Olympus, Japan) based on colorimetric reaction in the automatic analyzer (Olympus ®AU 2700, Japan), according to the instructions of the manufacturer. The hemocyanin (Hc, mmol l^{-1}) concentration was determined at 335 nm with an ultraviolet spectrophotometer (Thermo Electron Corporation Biomate 5, USA), according to the method of Chen and Cheng (1995).

Statistical analysis

The statistical program (SPSS Statistics 17.0, IBM SPSS, 2008) was used for analyses. Means and standard deviations/errors were computed. The morphological traits and hemolymph parameters across the three experimental sites were analysed using one-way ANOVA. Differences in levels of hemolymph parameters between female and male adult crabs from experimental sites were examined using the Students' t-test ($p < 0.05$).

Results and discussion

Water quality characteristics of the three experimental sites

The water quality parameters from the three experimental sites are summarized in Table 1. Total phosphorus in water from the pond located at Lu Lake area was very high, (0.21368 ± 0.01256 mg l^{-1}), indicating that the pond water was eutrophic (Xia *et al.,* 2004). The concentration of total phosphorus in the other two experimental sites were normal and within permissible limits. A similar trend was observed in the values of water pH, NH_4^+-N, NO_2^--N and NO_3^--N from all the three experimental sites. Water from Huangjin Lake area and Lu Lake area were slightly acidic in nature, with water pH values below 7. Comparison of water quality parameters across the locations indicated that the two sites at Huangjin Lake area were more suitable for aquaculture which could be attributed to the water exchange between the pond and the Huangjin Lake.

Comparison of hemolymph parameters and morphology of adult crabs across the three experimental sites

In this study, none of the adult crabs had missing pereiopod. The body color was normal and the crabs appeared healthy with no signs of disease. Fig. 1 shows the variation in the levels of seven hemolymph parameters (*viz.,* Glu, TP, TG, TC, Ca, Hc and THC) in crabs across three experimental sites and two culture methods. No significant differences were observed in levels of all the hemolymph parameters between pond-reared and pen-cultured crabs, from Huangjin Lake area implying similar physiological and immune status among them (Tilden *et al.,* 2001; Pascual *et al.,* 2003; Oliveira *et al.,* 2007; Lorenzon *et al.,* 2008). This is contrary to the view expressed by aquaculturists that pond-reared crabs are highly susceptible to diseases.

The values of hemolymph Hc and THC of pen-cultured crabs from Huangjin Lake area were found to be significantly higher than those of pond-reared crabs from Lu Lake area (Fig. 1f and 1g). As is known, Hc plays a predominant role in oxygen transport in many crustacean species (Taylor and Whiteley, 1989; Sanchez *et al.,* 1991), suggesting its importance in physiological activities. Pascual *et al.* (2003) while experimenting on the white shrimp *Litopenaeus vannamei*, indicated that the oxyhemocyanin (OxyHc) concentration could reflect the nutritional status of an organism. In crustaceans, hemocytes are of paramount importance for diverse immune responses such as blood coagulation, phagocytosis, encapsulation, nodule formation and wound healing (Omori *et al.,* 1989; Hose *et al.,* 1990; Johansson and Soderhall, 1992). Therefore, pen-cultured crabs with higher Hc and THC levels could be considered to possess stronger metabolic and immune abilities compared to pond-reared crabs.

Pascual *et al.* (2003) also reported that the hemolymph TP value could reflect the nutritional condition of *L. vannamei*. Rodríguez *et al.* (2000) opined that hemolymph TP level could be used as a reference tool for monitoring shrimp health status, as a high correlation was observed between protein level and the immunological state of *L. vannamei* (Pascual *et al.,* 2003). Thus, hemolymph TP could be used as an effective indicator of physiological status. Values pertaining to nutritional and physiological conditions (namely, hemolymph TP and Hc) and an indicator of immune ability (THC) in pond-reared crabs from Huangjin Lake area were significantly higher than those of pond-reared crabs from Lu Lake area where the water quality was comparatively poor (Fig. 1b, 1f and 1g). This indicated that pond-reared crabs from Huangjin Lake area possessed better physiological and immune status compared to their counterparts from Lu Lake area (Taylor and Whiteley, 1989; Johansson and Soderhall, 1992; Pascual *et al.,* 2003; Perez-Jar *et al.,* 2006).

From the results, it could be inferred that the physiological and immune status of pond-reared crabs were not different from those of pen-cultured crabs. The pond-reared crabs cultured in good quality water performed equally well as pen-cultured crabs, irrespective of the high stocking density. Further, water quality has significant effect on the physiological and immune status of crabs. Table 2 shows the average body weight, shell length, and shell width of adult crabs from the three experimental sites. Although the pond-reared crabs were observed to be smaller and lighter than pen-cultured crabs, which is in agreement with a report by Food and Agriculture Organization (FAO, 2006), pond-culture was considered better than pen culture from the economic viewpoint because the stocking density in the pond was much higher compared to that in the pen. Pond culture at Huangjin Lake area yielded encouraging results. Pond-rearing method can therefore be recommended in ponds connected to natural water resources such as lakes

Table 1. Water quality parameters (Mean ± SD) at the experimental sites

Experimental sites	pH	Ammonium nitrogen (mg l^{-1})	Nitrite-N (mg l^{-1})	Nitrate-N (mg l^{-1})	Total phosphorus (mg l^{-1})
PNWH*	6.66±0.054	0.53±0.021	0.0047±0.00010	0.10±0.0090	0.0934±0.0026
PDWH	6.75±0.036	0.39±0.032	0.0083±0.00021	0.093±0.0030	0.05271±0.0019
PDWL	6.70±0.041	0.40±0.047	0.0056±0.00016	0.059±0.0042	0.21368±0.01256

*PNWH: pen water from Huangjin Lake; PDWH: pond water from Huangjin Lake; PDWL: pond water from Lu Lake

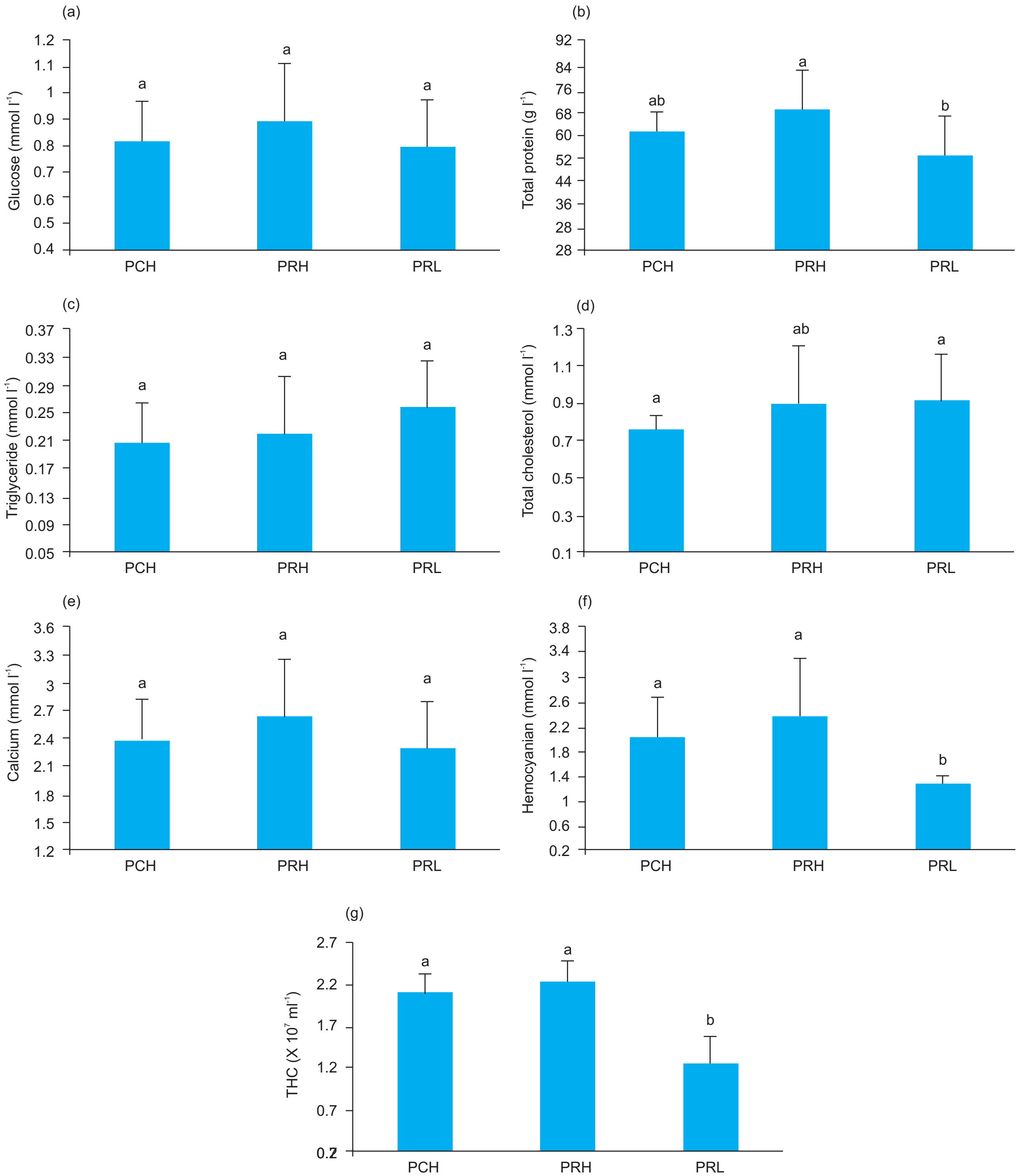

Fig. 1. Variations in the levels of hemolymph parameters (glucose, total protein, triglyceride, total cholesterol, calcium, hemocyanin and total hemocytes count,THC) in adult Chinese mitten crabs among three experimental sites and two culture methods.
PCH: pen-cultured adult crabs from Huangjin Lake area; PRH: pond-reared adult crabs from Huangjin Lake area; PRL: pond-reared adult crabs from Lu Lake area.
(a) Hemolymph glucose (b) Hemolymph total protein (c) Hemolymph triglyceride (d) Hemolymph total cholesterol (e) Hemolymph calcium (f) Hemolymph hemocyanin (g) Hemolymph total hemocytes count (THC).

Table 2. Morphological traits (Mean ± SE (n)) of Chinese mitten adult crabs from different experimental sites

Morphological traits	Females			Males		
	PCH	PRH	PRL	PCH	PRH	PRL
Body weight (g)	125.52^{a}±3.98 (10)	107.62^{b}±2.09 (10)	110.55^{b}±3.57 (10)	157.43^{a}±4.32 (10)	137.10^{b}±7.76 (10)	135.58^{b}±4.22 (10)
Shell length (cm)	6.63^{a}±0.093 (10)	6.49^{a}±0.049 (10)	6.43^{a}±0.079 (10)	7.02^{a}±0.11 (10)	6.70^{a}±0.13 (10)	6.73^{a}±0.091 (10)
Shell width (cm)	6.053^{a}±0.034 (10)	5.80^{b}±0.053 (10)	5.86^{b}±0.069 (10)	6.48^{a}±0.088 (10)	6.04^{b}±0.11 (10)	6.08^{b}±0.080 (10)

Within a trait, means followed by different superscripts differ significantly ($p<0.05$)

PCH: pen-cultured adult crabs from Huangjin Lake area; PRH: pond-reared adult crabs from Huangjin Lake area; PRL: pond-reared adult crabs from Lu Lake area

or rivers assuring good water quality, ultimately resulting in good physiological and immune status.

Effects of sex on the values of hemolymph parameters of PC and PR adult crabs

Hemolymph parameters of the experimental crabs are presented in Table 3 and 4 . The hemolymph TG value in female crabs was higher than that of male crabs from each culture method. Levels of blood TG for humans and animals are related to activity, and individuals that exhibit low activity often had high concentrations of blood TG (Baidupedia, 2011). Female crabs are less active than the male counterparts. This reduced activity in females could partly be attributed to high TG concentrations. In each culture method, levels of hemolymph Glu and THC in male crabs were higher than those of females, indicating male crabs are more active, resulting in better nutritional and immune status, compared to their female counterparts (Pascual *et al.,* 2003; Perez-Jar *et al.,* 2006).

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Table 3. Hemolymph parameters (Mean ± SE (n)) of pen-cultured adult crabs from Huangjin Lake area

Hemolymph parameters	Female	Male
Glucose (mmol l^{-1})	0.70^{a} ±0.035 (10)	0.91^{a} ±0.044 (10)
Total protein (g l^{-1})	63.20^{a} ±2.62 (10)	59.85^{a} ±1.65 (10)
Triglyceride (mmol l^{-1})	0.24^{a} ±0.015 (10)	0.19^{a} ±0.011 (10)
Total cholesterol (mmol l^{-1})	0.79^{a} ±0.038 (10)	0.73^{a} ±0.015 (10)
Calcium (mmol l^{-1}),	2.13^{a} ±0.12 (10)	2.62^{a} ±0.16 (10)
Hemocyanin (mmol l^{-1})	2.21^{a} ±0.31 (10)	1.83^{a} ±0.051 (10)
THC ($\times 10^{7}$ cells ml^{-1})	1.99^{a} ±0.041 (10)	2.24^{b} ±0.060 (10)

Means followed by same superscript do not differ significantly.

THC: total hemocytes count

Table 4. Hemolymph parameters (Mean ± SE (n)) in pond-reared adult crabs from two lakes

Hemolymph parameters	Huangjin lake area		Lu lake area	
	Female	Male	Female	Male
Glucose (mmol l^{-1})	0.77^{a} ±0.044 (10)	1.01^{a} ±0.073 (10)	0.68^{a} ±0.044 (10)	0.89^{b} ±0.054 (10)
Total protein (g l^{-1})	74.88^{a} ±3.42 (10)	62.94^{a} ±4.66 (10)	58.78^{a} ±5.38 (10)	46.89^{a}±3.50 (10)
Triglyceride (mmol l^{-1})	0.27^{a} ±0.032 (10)	0.18^{b} ±0.014 (10)	0.31^{a} ±0.016 (10)	0.21^{b} ±0.021 (10)
Total cholesterol (mmol l^{-1})	0.82^{a} ±0.079 (10)	1.01^{a} ±0.085 (10)	0.88^{a} ±0.060 (10)	0.95^{a} ±0.079 (10)
Calcium (mmol l^{-1})	2.55^{a} ±0.19 (10)	2.68^{a} ±0.27 (10)	2.53^{a} ±0.21 (10)	2.03^{a} ±0.12 (10)
Hemocyanin (mmol l^{-1})	2.47^{a} ±0.34 (10)	2.26^{a} ±0.34 (10)	1.30^{a} ±0.041 (10)	1.26^{a} ±0.057 (10)
THC ($\times 10^{7}$ cells ml^{-1})	2.12^{a} ±0.054 (10)	2.33^{b} ±0.063 (10)	1.17^{a} ±0.092 (10)	1.35^{a} ±0.098 (10)

Means followed by same superscript do not differ significantly

THC, total hemocytes count

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