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Elevated water temperature impairs gamete production in male narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823)

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Abstract – Water temperature is one of the major environmental factors affecting the reproductive output of freshwater crayfish. The reproduction of freshwater crayfish only occurs in a limited water temperature range and minor changes in water temperature could have negative impacts on this biological process. Therefore, understanding the potential effects of temperature on the reproductive output of crayfish is important from both an ecological and aquaculture point of view. Spermatozoal production, hepatosomatic index (HSI), gonado-somatic index (GSI), testicular index (TI), and vas deferens index (VDI) were measured in *Pontastacus leptodactylus* maintained at 7.5, 11, and 19 °C during the reproductive season. It was found that the highest temperature significantly degrades the production of spermatozoa and VDI when compared to the crayfish maintained at lower temperatures ($P < 0.05$). On the other hand, water temperature did not significantly affect the values of HSI, GSI, and TI ($P > 0.05$). Furthermore, the highest temperature caused a negative impact on the vas deferens characterized by a softer and more adhesive texture. In conclusion, this study demonstrated that elevated water temperature has a negative impact on gamete production of male *P. leptodactylus*, as a cold water crayfish species, and may subsequently affect the whole reproduction process.

Keywords: sperm / Decapoda / global warming / Crustacean

Résumé – La température élevée de l'eau nuit à la production de gamètes chez l'écrevisse à pattes grêles mâle *Pontastacus leptodactylus* (Eschschscholtz, 1823). La température de l'eau est l'un des principaux facteurs environnementaux qui influent sur le rendement reproducteur des écrevisses d'eau douce. La reproduction des écrevisses ne se produit que dans une plage de température de l'eau limitée et des changements mineurs de la température de l'eau pourraient avoir des effets négatifs sur ce processus biologique. Par conséquent, il est important de comprendre les effets potentiels de la température sur le rendement reproducteur des écrevisses, tant du point de vue écologique que du point de vue de l'aquaculture. La production de spermatozoïdes, l'indice hépatosomatique (HSI), l'indice gonado-somatique (GSI), l'indice testiculaire (TI) et l'indice vas deferens (VDI) ont été mesurés chez *Pontastacus leptodactylus* maintenu à 7.5, 11 et 19 °C pendant la saison de reproduction. Il a été constaté que la température la plus élevée dégrade significativement la production de spermatozoïdes et le VDI par rapport aux écrevisses maintenues à des températures plus basses ($P < 0,05$). Par contre, la température de l'eau n'a pas eu d'effet significatif sur les valeurs de HSI, GSI et TI ($P > 0,05$). De plus, la température la plus élevée a eu un impact négatif sur le canal déférent caractérisé par une texture plus douce et plus adhésive. En conclusion, cette étude a démontré que la température élevée de l'eau a un impact négatif sur la production de gamètes de *P. leptodactylus* mâle, espèce d'écrevisse d'eau froide, et peut par la suite affecter l'ensemble du processus de reproduction.

Mots-clés : sperme / Décapode / réchauffement global / Crustacé

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1 Introduction

Pontastacus leptodactylus (Eschscholtz, 1823), also known as the narrow-clawed crayfish, Danube crayfish, Galician crayfish or Turkish crayfish, is widely distributed in Eastern Europe, and the Middle East (Köksal, 1988). This species is native to a vast area which extends from Iran in the east, Austria in the west, Russia in the north, and Greece in the south. In addition, *P. leptodactylus* has been introduced into many countries in Europe and Asia (Skurdal and Taugbøl, 2002; Harlioglu, 2004; Gherardi and Souty-Grosset, 2017). *Pontastacus leptodactylus* is also considered as an economic crayfish species that can be used for aquaculture purposes (Wickins and Lee, 2002).

Global warming is characterized by the increase in the surface temperatures in terrestrial and aquatic ecosystems. It alters the main ecological processes and the distribution of aquatic species (Poff *et al.*, 2002). Aquatic species of warm waters are well adapted to high temperatures, while aquatic animals of cold waters are more vulnerable to climate warming (Jeppesen *et al.*, 2010). The body temperature of cold-blooded aquatic animals is adjusted by water temperature. Therefore, the water temperature controls the main biological processes such as growth, behavior (Wittmann and Pörtner, 2013), moulting (Hammond *et al.*, 2006), immune response (Jiravanichpaisal *et al.*, 2004) and reproduction (Tropea *et al.*, 2010; Yazicioglu *et al.*, 2018) in aquatic animals.

The effects of temperature on crayfish reproduction depends on the species thermal tolerance, geographical distribution and response based on physiological and behavioral adaptations (Harlıoglu and Farhadi, 2017). It has been shown that water temperature affects ovarian development, spawning rate (Yeh and Rouse, 1995; Tropea *et al.*, 2010), mating time (Yazicioglu *et al.*, 2018) and sperm production (Bugnot and López Greco, 2009a, b) of crayfish species.

Reproduction in *P. leptodactylus* generally occurs in cold water at 7–12 °C (Skurdal and Taugbøl, 2002) and hence is likely to be affected by the incremental increase in water temperature caused by climate warming. However, the effect of higher water temperatures on the sperm quality of male freshwater crayfish is not known. Motility and fertilization success are two common methods for assessment of sperm quality in animals bearing flagellate spermatozoa (Hatef *et al.*, 2009). However, the spermatozoon in crayfish is aflagellate and immotile (Tudge *et al.*, 2009; Niksirat *et al.*, 2013a, b; Kouba *et al.*, 2015; Yazicioglu *et al.*, 2016) and the spermatozoal mass is covered by a spermatophore protective wall during the stay in the vas deference of the male or post-mating storage on the body of the female (Jamieson and Tudge, 2000; Niksirat *et al.*, 2014a; Niksirat and Kouba, 2016) rather than immersion in seminal plasma like other animals such as fish and mammals. Therefore, techniques such as sperm counting have been developed to evaluate gamete quality in the male decapod crustaceans (Farhadi *et al.*, 2018; Harlıoglu *et al.*, 2018). This study aims to investigate the effects of water temperature on the spermatozoal production, hepatosomatic index (HSI), gonado-somatic index (GSI), testicular index (TI), and vas deferens index (VDI) of the narrow-clawed crayfish *P. leptodactylus*.

2 Materials and methods

2.1 Animals and sampling process

Thirty-six mature males of *P. leptodactylus* (mean weight ± SE: 63.5 ± 3.7 g, range: 44–107 g, mean carapace length ± SE: 63.2 ± 1.1 mm, range: 55–74 mm) were captured from Keban Dam Lake (Elazıg, Turkey) in January, in the breeding season, 2018. The carapace length and weight of each crayfish was determined using a digital caliper and a digital balance, respectively.

2.2 Experimental design

Crayfish were held in three 1000 L fiberglass tanks containing artificial shelters constructed from 150 mm PVC pipes for 2 weeks for acclimation to the lab environment. Twelve crayfish for each treatment were weighted and randomly placed into nine 90 L aquaria and reared in the experimental conditions for 5 weeks at three different water temperatures (7.5, 11 and 19 °C). The mean weight and length of males was not significantly different between treatments. During the experiment, dissolved oxygen, and pH were determined daily. Mean dissolved oxygen, and pH were 7.8 ± 1 mg/L and 7.9 ± 0.2, respectively. Crayfish were fed a commercial feed containing 40.7% protein and 16.8% lipid two times per day and approximately 1% of body weight (Farhadi and Jensen, 2016). Cold water (7.5 °C) was provided from outdoor tanks and aquarium heaters were used to provide the other test temperatures (11 and 19 °C) (Skurdal and Taugbøl, 2002; Bugnot and López Greco, 2009a; Tropea *et al.*, 2010). No mortality was observed during the experiment in any treatments.

2.3 Reproductive parameters

At the end of the experiment, eight crayfish were dissected from each treatment to investigate the following reproductive parameters. The whole reproductive system, vas deferens, testes and hepatopancreas were weighted. GSI, VDI, TI, and HSI were calculated (Harlıoglu *et al.*, 2012, 2013) as follows:

- Gonado-somatic index (%): (reproductive system wet weight/body wet weight) × 100
- Vas deferens index (%): (vas deferens wet weight/body wet weight) × 100
- Testicular index (%): (testes wet weight/body wet weight) × 100
- Hepatosomatic index (%): (hepatopancreas wet weight/body wet weight) × 100

2.4 Spermatozoa extraction and counting

Equal amounts of the distal part of the vas deferens of each crayfish was cut into small pieces using sterile surgical blades in 1 mL of physiological solution (0.9% NaCl) to prepare the spermatophore solution (Harlıoglu *et al.*, 2012). The solution of each sample was subsequently transferred into 2 mL microtubes. Spermatozoa of crayfish were extracted according to Farhadi *et al.* (2018). Then, spermatozoal number was

counted under a light microscope using a hemocytometer (Bugnot and López Greco, 2009a, b; Harlıoglu *et al.*, 2012, 2013).

2.5 Statistical analysis

The normality and homoscedasticity of the data were confirmed using Kolmogorov–Smirnov and Leven's test, respectively. To compare differences among the treatments a one-way ANOVA was used. Significant differences between groups were determined using Tukey's HSD tests for post hoc comparisons. Data were analyzed using SPSS version 16.0 (2007 SPSS Inc.). The level of significance for all analyses was determined at $P < 0.05$.

3 Results

The number of crayfish spermatozoa and VDI were significantly affected by the water temperature ($P < 0.05$). Higher numbers of spermatozoa were obtained in the crayfish exposed to 7.5 °C ($2.65 \times 10^7 \pm 0.35$) and 11 °C ($3.22 \times 10^7 \pm 0.48$) compared to 19 °C ($1.25 \times 10^7 \pm 0.17$) ($P < 0.05$) (Fig. 1). The results revealed that HSI, GSI, and TI were not affected by the different water temperatures ($P > 0.05$). However, a significant ($P < 0.05$) reduction in VDI was observed at higher temperature (Figs. 2A–DA–D). It was found that 19 °C gave rise to anomalies in the vas deferens of crayfish characterized by a softer and more adhesive texture. The spermatozoa of crayfish exposed to 19 °C were mostly aggregated and a single separated spermatozoon rarely could be observed.

4 Discussion

The present study showed that the elevated water temperature impairs the spermatozoal production and increases abnormality in the vas deferens of crayfish, and subsequently reduces the spermatophores and spermatozoal number in *P. leptodactylus*. More than 100 proteins including respiratory, transport, cell defense, and cytoskeleton proteins have been identified in the spermatophores of crayfish. They are responsible for numerous vital functions necessary for the process of reproduction in crayfish such as capacitation and fertilization (Niksirat *et al.*, 2014b, 2015, 2016). These proteins need an optimum temperature to maintain their respective structure and activity. Therefore, the occurrence of more adhesive spermatozoa at 19 °C could be due to the denaturation of spermatozoal and spermatophore proteins and subsequent impairment of the whole gamete in crayfish; as observed in other animals (Thijssen *et al.*, 2014).

It has been shown that a short term *in vitro* incubation of male gametes at high temperatures (40 and 75 °C) could seriously damage spermatozoa in *P. leptodactylus*. Also, higher temperatures probably alter the biomechanical properties of the spermatophore layers and cause their subsequent adhesion to spermatozoa, which may reduce extraction of intact spermatozoa (Farhadi *et al.*, 2018). In crayfish, the highest gamete production and vasa deferentia weight occur during the peak of their reproductive season. Bugnot and López Greco (2009a) showed that spermatozoal production and weight of the vasa deferentia increased in *Cherax quadricarinatus* (von Martens, 1868) during the breeding season in summer, while the weight of the testes rose during the non-breeding season in winter. The narrow-clawed crayfish is a cold water freshwater species and the mating of this species takes place during the autumn when the water temperature is about 7–12 °C (Skurdal and Taugbøl, 2002). The observation of the highest spermatozoal production and VDI at lower temperatures (7.5 and 11 °C) compared to 19 °C indicates that water temperature is a critical factor for successful reproduction in *P. leptodactylus*.

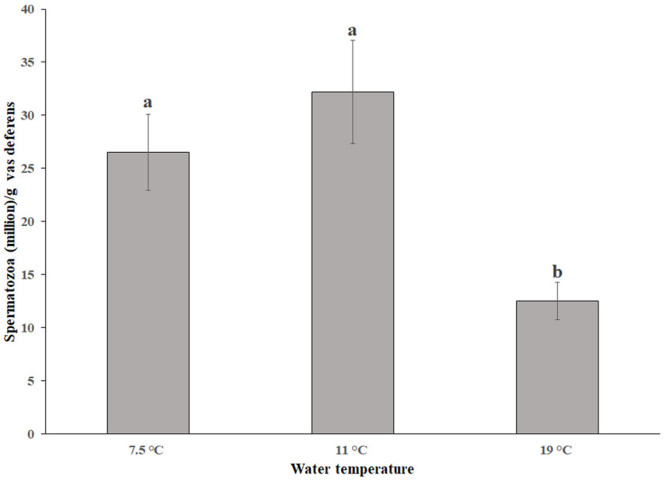

Fig. 1. Effect of water temperature on the number of spermatozoa in male narrow-clawed crayfish. Different letters on bars show significant difference at $P < 0.05$. Data are presented as mean ± SE.

Similarly, high temperatures (29–30 °C) showed a negative impact on the spermatozoal quality of the shrimp *Penaeus setiferus* (Linnaeus, 1767). On the other hand, lower temperature (25–27 °C) postponed spermatophore degradation and melanization in this species (Bray *et al.*, 1985; Pascual *et al.*, 1998). Perez-Velazquez *et al.* (2001) reported that high water temperature (29 °C) reduced spermatozoal number and increased abnormal spermatozoa compared to a lower water temperature (26 °C) in *Penaeus vannamei* (Boone, 1931). In addition, it has been reported that elevated water temperatures (30–33 °C) caused stressful situations in male penaeid shrimps (Pascual *et al.*, 1998; Sánchez *et al.*, 2001; Pascual *et al.*, 2003).

According to King *et al.* (2003) high water temperatures alter the concentration of reproductive hormones by inducing stress responses. Burt *et al.* (2011) stated that this situation probably affects offspring outcomes. On the other hand, there is not any information regarding the impact of high temperatures on the morphological and molecular characteristics of gametes in decapods. However, studies show that mammalian and fish spermatozoa are extremely sensitive to high temperature (Dadras *et al.*, 2017). For example, high temperatures increase the mortality and percentage of abnormal spermatozoa (Borg *et al.*, 1993), change acrosome and plasma membrane integrity (Borg *et al.*, 1993; Safaa *et al.*, 2008), and affect spermatozoon chromatin stability (Paul *et al.*, 2008; Pérez-Crespo *et al.*, 2008) in mammals.

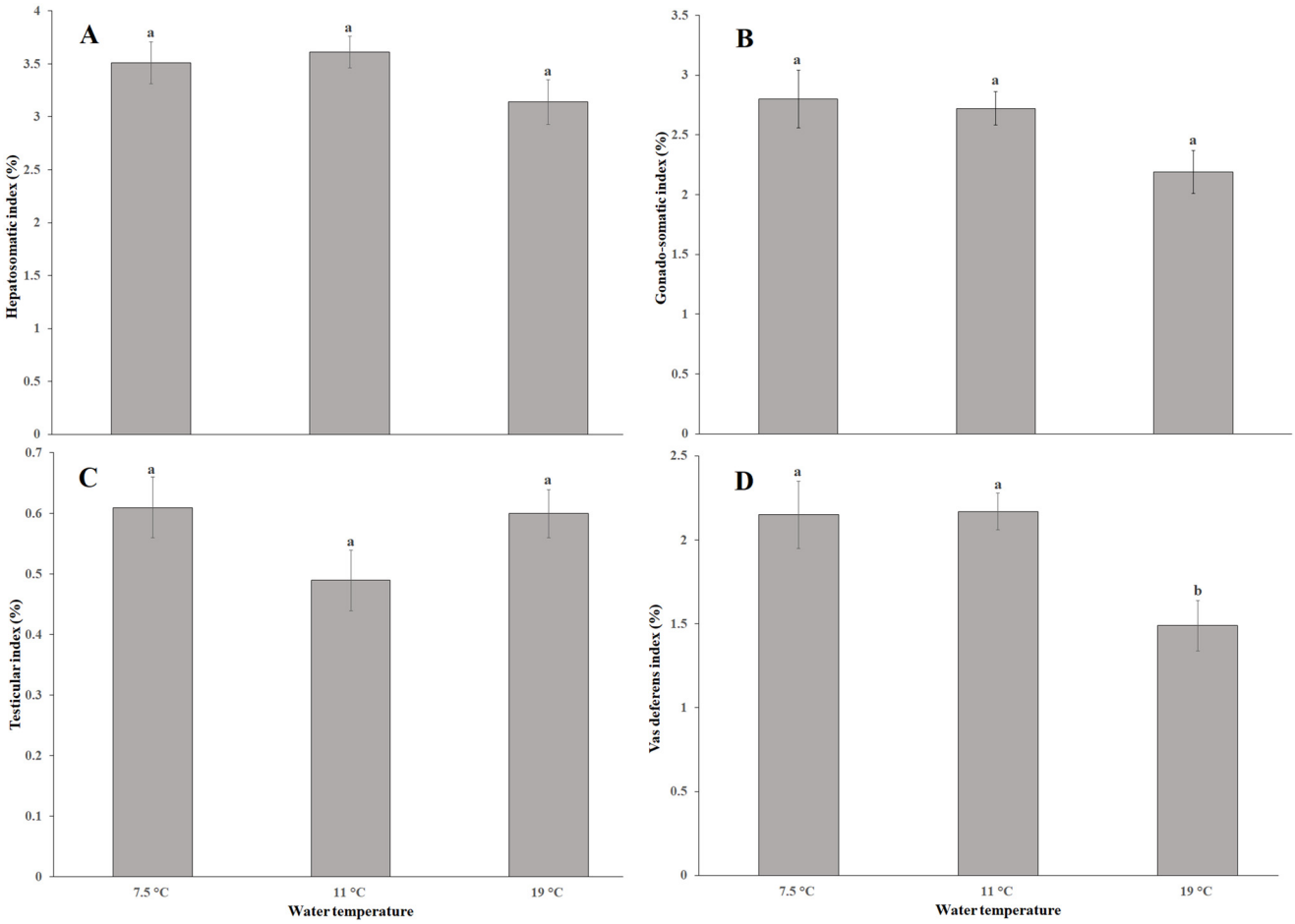

Fig. 2. Effect of water temperature on A: HSI, B: GSI, C: TI, and D: VDI of male narrow-clawed crayfish. Different letters on bars show significant difference at $P < 0.05$. Data are presented as mean ± SE.

The reproduction of crayfish could be affected by enhanced water temperatures arising from global warming. Seasonal changes in water temperature affect hormonal function and either induce or suppress gametogenesis. However, higher than normal temperatures have deleterious effects on gametogenesis (Pankhurst and King, 2010). King *et al.* (2003) found that exposure to high water temperature had deleterious impacts on the secretion of steroid hormones and hepatic vitellogenin synthesis and caused reduction in maternal investment and gamete viability in Atlantic salmon *Salmo salar* (Linnaeus, 1758). Similarly, high water temperature had an inhibitory effect on spermiogenesis in *S. salar* and rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Water temperature had significant effects on the blood concentration of gonadal steroids testosterone and 11-ketostestosterone in *O. mykiss* (Manning and Kime, 1985), showing that water temperature has a permissive role in male gametogenesis (Pankhurst and King, 2010).

5 Conclusion

Our results show that elevated water temperature negatively affects the reproductive output of the *P. leptodactylus*. Further studies are required to investigate the potential effects of elevated water temperatures on the reproductive output of native crayfish species. Also, future studies are needed to consider the effect of high temperatures on the morphological and molecular (*i.e.*, proteome and lipid profiles) characteristics of crayfish spermatozoa.

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