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Post-mating spermatophore storage strategies in two species of crayfish: implications for broodstock management

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Female crayfish stores male gametes after mating until the beginning of egg laying and fertilization. The aim of the present study was to investigate the duration of post-mating spermatophore storage as well as the timing and temperature of spawning in two crayfish species of economic importance, namely the signal crayfish Pacifastacus leniusculus *and the noble crayfish* Astacus astacus. *Results showed that the average duration of the post-mating spermatophore storage is significantly (*$P < 0.05$*) longer in the noble crayfish (34.6 ± 1.7 days, range: 19 to 60 days) than the signal crayfish (3.9 ± 0.5 days, range: 1 to 18 days). The highest percentages of the post-mating spermatophore storage duration in the signal crayfish (46.5%) and the noble crayfish (44.5%) were 1 and 31 to 40 days, respectively. While there is an overlap in the timings of mating and egg laying in the signal crayfish, these two reproductive processes were not observed at the same days in the noble crayfish and there was at least 2 weeks interval between last mating and first egg laying individuals. Average mating and egg laying temperatures were significantly (*$P < 0.05$*) higher in the signal crayfish than the noble crayfish. The average temperatures for mating in both species were significantly (*$P < 0.05$*) higher than the temperatures that they utilized for egg laying. In conclusion, female noble crayfish stores post-mating spermatophores a longer duration compared with the signal crayfish. Also, the signal crayfish mates and lays egg in temperatures that are higher than the noble crayfish. Spawning season is shorter in the signal crayfish compared with the noble crayfish. The results of present study provide information contributing to the crayfish broodstock management in aquaculture.*

Keywords: crustacean, decapoda, noble crayfish, signal crayfish, sperm storage

Implications

Crayfish is a delicious and healthy food item for human. An increasing market demand for this aquatic organism encourages development of techniques for artificial reproduction and farming of crayfish during recent decade. Here we studied the time interval between mating and egg laying in broodstock of two widespread freshwater crayfish species including signal and noble crayfish. Results of present study can directly be used by crayfish farmers for management of broodstocks across Europe and America. Also, this study provides basic information for biologists to further study of biology of reproduction in crayfish.

Introduction

The post-mating sperm storage is well known in a wide range of animals including vertebrates (Holt and Lloyd, 2010; Holt, 2011) and invertebrates (Bauer, 1986; Wolcott *et al*., 2005; Niksirat *et al*., 2014). Crayfish as members of decapod crustaceans make up a large group of invertebrates including three families, 33 genera, and over 640 known species (Crandall and Buhay, 2008). Crayfish male produces immotile spermatozoa (Tudge, 2009; Niksirat *et al*., 2013a and 2013b; Kouba *et al*., 2015; Yazicioglu *et al*., 2016) that are packed into spermatophore and transferred to the female body surface in Astacidae and Parastacidae, or into the *annulus ventralis* which is known as a spermatophore storage segment in Cambaridae (Hamr, 2002), during mating. Crayfish male gametes undergo post-mating morphological and molecular changes that are necessary for them to acquire fertilization ability (Niksirat *et al*., 2015a and 2016; Niksirat and Kouba, 2016). Spermatozoa are released from spermatophore during fertilization and after fertilization eggs are attached to the body of female crayfish until hatching (Niksirat *et al*., 2015b).

Noble crayfish *Astacus astacus* occurs in the open waters and it is widely distributed mainly in the northern and central Europe (Holdich *et al*., 2009). The signal crayfish *Pacifastacus leniusculus* is a North American species with a wide native

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range between the Pacific Ocean and Rocky Mountains (Taylor *et al*., 2007). Although, there are some reports regarding the timing of spawning in crayfish (Lewis, 2002), the duration of post-mating spermatophore storage is not fully investigated in crayfish, yet. Because of high market demand of crayfish, there is an increasing interest in crayfish culture (Skurdal and Taugbøl, 2002). Production of juveniles is an important step for development of sustainable crayfish farms. Knowledge such as the duration of post-mating spermatophore storage in relation with environmental factors (e.g. temperature and season) can facilitate management of crayfish broodstock with aim of juvenile production.

The goal of present study was to investigate the duration of post-mating spermatophore storage as well as the timing and temperature of spawning in the signal crayfish and the noble crayfish. The results of present study can provide basic data regarding the time interval between mating and the onset of egg laying and subsequent fertilization in two commercially and ecologically important crayfish species that can be used for the management of broodstock in farms within their native ranges and/or natural habitats.

Material and methods

Adult signal crayfish (*Pacifastacus leniusculus* Dana, 1852; $n = 142$) and noble crayfish (*Astacus astacus* Linnaeus, 1758, $n = 72$) were collected from the Babačka Brook (Sklené nad Oslavou, Czech Republic) and Kramata Reservoir (Hrabice, Czech Republic), respectively. The experiment was started on September 26, 2012. A total of 36 and 71 pairs of noble and signal crayfish were used for experiment, respectively. Each pair consisted of one male and one female. The pairs of crayfish were placed in plastic mesh boxes which were divided into four chambers to keep each pair separately. Plastic mesh boxes were placed in two 850 l outdoor tanks. Animals were fed during experiment. All animals were kept under the same natural temperature and photoperiod. Water temperature was recorded using data loggers (Minikin; Environmental Measuring Systems, Brno, Czech Republic). All pairs were checked for mating and egg laying twice a day by observing presence of spermatophore and eggs in the abdominal part of female, respectively.

Statistical analysis

The non-parametric Mann–Whitney test was carried out to compare the post-mating spermatophore storage duration as well as suitable temprature for the mating and egg laying between two studied species using SPSS statistical package version 16.0. For all statistical tests, $P < 0.05$ was considered significant. Data are presented as the mean ± SEM.

Results

Results showed that the average duration of the post-mating spermatophore storage is significantly ($P < 0.05$) longer in the noble crayfish (34.6 ± 1.7 days, range: 19 to 60 days) than the

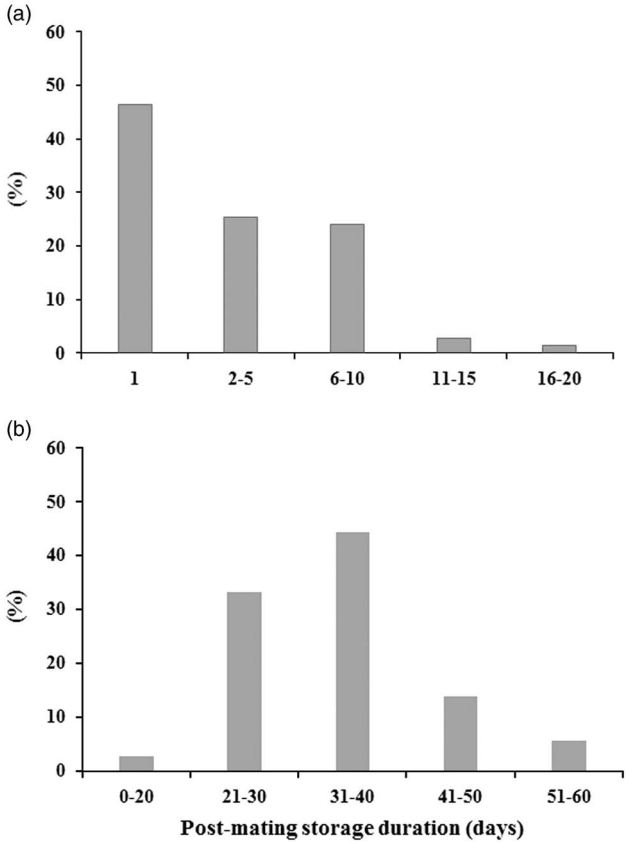

Figure 1 Bars show classification of the post-mating spermatophore storage duration in the signal (a) and the noble crayfish (b).

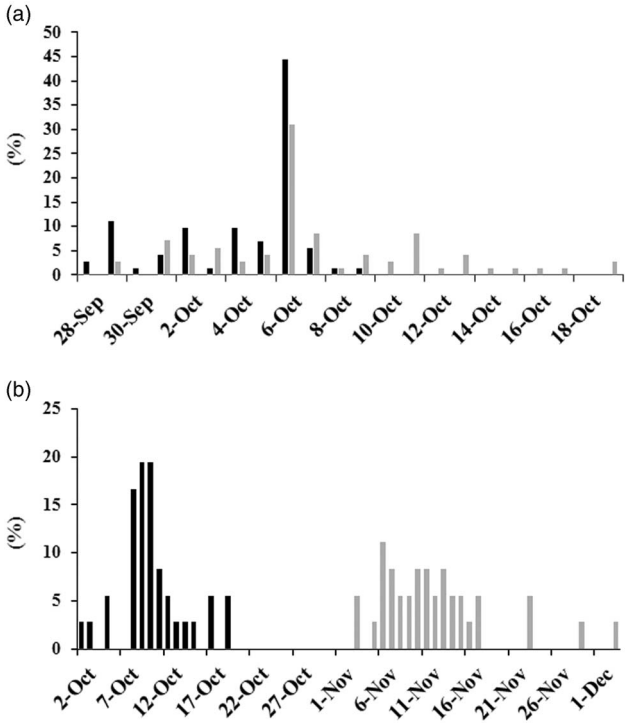

Figure 2 Mating and egg laying dates in the signal (a) and the noble crayfish (b) during spawning season. Black and gray bars show mating and egg laying percentages, respectively.

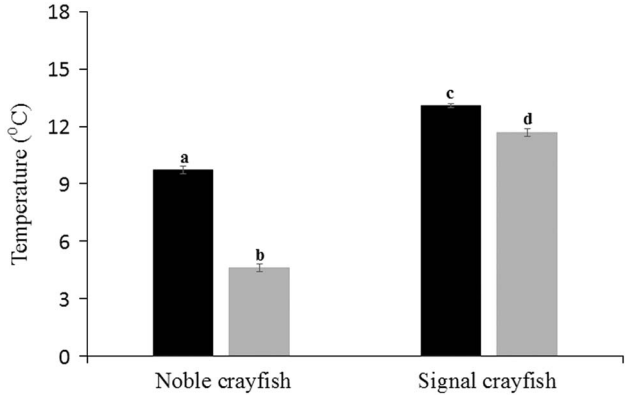

Figure 3 Average water temperatures during mating and egg laying in the noble crayfish (36 pairs) and the signal crayfish (71 pairs). Values marked with different superscripts differ significantly from each other at $P < 0.05$. Data of temperature are shown as mean ± SEM. Black and gray bars show the mating and egg laying, respectively.

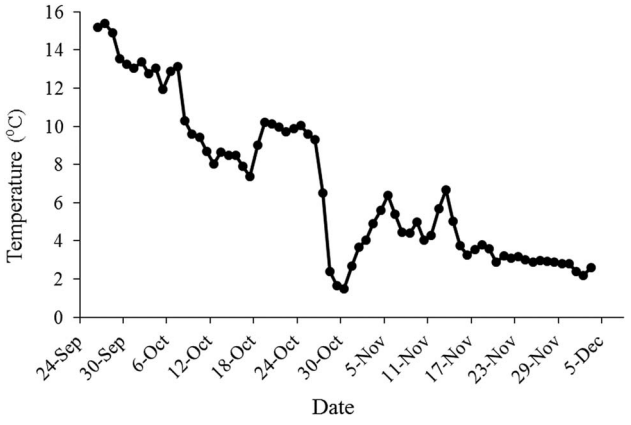

Figure 4 Water temperature during the experiment.

signal crayfish (3.9 ± 0.5 days, range: 1 to 18 days). The highest percentages of the post-mating spermatophore storage duration in the signal crayfish (46.5%) and the noble crayfish (44.5%) were 1 and 31 to 40 days, respectively (Figure 1a and b). The first and last matings and egg layings in the signal crayfish were observed on September 28, October 9, September 29 and October 19, respectively. The first mating in the noble crayfish occured on October 2. Mating in this species ended on 19th of the same month. However, first egg laying female was not observed before November 3. Egg laying terminated on December 3 in the noble crayfish. While there was an overlap in the mating and egg laying timings in the signal crayfish, these two reproductive processes were not observed at the same days in the noble crayfish and there was at least 2 weeks interval between the last mating and first egg laying individuals (Figure 2a and b).

Average mating and egg laying temperatures were significantly ($P < 0.05$) higher in the signal crayfish than the noble crayfish. The average temperatures for mating in the both species were significantly ($P < 0.05$) higher than the temperatures that they utilized for egg laying (Figure 3). Temperature trend during experiment is shown in Figure 4.

Discussion

Results of the present study showed that the female noble crayfish stores the post-mating spermatophores for longer periods compared with the signal crayfish. In addition, we managed to show that the signal crayfish preferes warmer waters for mating and laying of eggs in comparison with the noble crayfish. Buřič *et al.* (2013) observed that the female spiny-cheek crayfish, *Orconectes limosus*, can successfully store spermatophore from their autumn mating for more than half a year. They stated that this trait allows the female to increase its chance for multiple successful matings and also finding the best mate.

Post-mating spermatophore storage has been reported in many other species of decapod crustaceans including crabs, shrimps and lobsters (Bauer, 1986; Moyano *et al.*, 2009). In some decapods such as clawed lobsters and some crabs with a long life-span, post-mating male gametes can be saved across consecutive molts by storage in some parts of the seminal receptacles of females that are not cast out during molting. This strategy allows post-mating spermatophores to be utilized successfully for next years for fertilization of eggs (Factor, 1995; Becker *et al.*, 2011). This strategy is well observed in the mated females Tanner crab, *Chionoecetes bairdi*, that produced 100% of fertilized eggs in the same year after mating but continued to produce 97% and 71% fertilized eggs in the next 2 years even without any contact by males (Paul, 1984). Also, Jensen and Bentzen (2012) reported that female Dungeness crab, *Metacarcinus magister* can fertilize eggs using stored sperm as old as 2.5 years. Female blue crab *Callinectes sapidus* that mates in summer and fall must store spermatophore for 7 to 11 months before using them in following summer for fertilization. This post-mating spermatophore storage strategy is very important for the blue crab because female mates only once during whole lifetime (Millikin and Williams, 1984; Hines *et al.*, 2003). Long-term post-mating spermatophore storage strategy by female decapods has an important role in the population biology and sustainable fishery, because it may compensate negative effects of fishing to some extent by allowing posthumous paternity to the largest males that are caught by fishing activities (Gosselin *et al.*, 2005; Sainte-Marie *et al.*, 2008; Taylor *et al.*, 2014; Ellis *et al.*, 2015; Vogt, 2016).

In addition, it has been proved that decapod spermatozoa undergo some morphological and molecular modifications providing them fertilizing ability, also known as spermatozoon capacitation (Alfaro *et al.*, 2007). Vanichviriyakit *et al.* (2004) proved that an extensive molecular modification of the spermozoon including protein tyrosine phosphorylation takes place during post-mating storage in the female thelycum of giant tiger prawn *Penaeus monodon*. It takes 6 to 7 h for spermatophore of Pacific white shrimp *Litopenaeus vannamei* to be capacitated in the thelycum of female at 28°C. During post-mating interval, spermatozoa undergo a morphological change including further development of a region in the spermatozoon so called filamentous meshwork,

located between the nucleus and the hemispherical cap (Alfaro *et al*., 2007). In addition, observation of some biochemical changes confirm that capacitation of *L. vannamei* spermatozoa happens following 4 to 6 h of post-mating storage in thelycum of female at 28°C (Aungsuchawan *et al*., 2011). It is well-known that higher temperatures accelerate biological processes (Cossins, 2012). Therefore, shorter post-mating spermatophore storage duration in the female signal crayfish could be attributed to higher water temperature compared with the noble crayfish that preferes colder temperatures and stores post-mating spermatophore for longer periods.

Post-mating spermatophore storage duration was poorly documented in decapods in general and crayfish in particular. Here we managed to demonstrate the patterns of spermatophore storage in relation with environmental factors such as temperature in broodstocks of two ecologically and economically important crayfish species that were kept under equal outdoor conditions. Our results can provide information for farmers to predict reproductive behaviors of studied crayfish species and therefore can facilitate broodstock management. For example, we have frequently observed that males eat eggs from female crayfish. If farmers are aware of the egg laying timings for those two species, they can separate mated females from males and transfer them from the mating to spawning facilities where females are allowed to incubate their fertilized eggs in safer conditions. To sum up, female noble crayfish stores post-mating spermatophores for longer periods compared with the signal crayfish. Also, signal crayfish mates and lays egg in temperatures that are significantly higher than noble crayfish with spawning season shorter in signal crayfish (September 28 to October 19) compared with noble crayfish (October 2 to December 3). The results of present study provide information contributing to the crayfish broodstock management in farms.

Acknowledgments

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