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Reducing Cannibalism of Narrow Clawed Crayfish *Astacus leptodactylus* Eschscholtz 1823 Through Management of Photoperiod and Stocking Density

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Abstract

In crayfish hatcheries, one of the main factors affecting reproduction operation is survival and cannibalism of brood stock. One of the main problems for expansion of *Astacus leptodactylus* Eschscholtz 1823 production is high mortality and cannibalism of broodstock during the reproduction operation. The effect of photoperiod and stocking density on cannibalism, moulting and survival of *A.leptodactylus* was examined in the context of broodstock husbandry. Cannibalism was lowest and survival highest when crayfish were housed at low density ($10 \cdot m^{-2}$) and with longer photoperiod. The longest photoperiod, 18 h light (L): 6 h dark (D) conditions resulted in high survival with minor cannibalism. Longest photoperiod also had the undesirable outcome of lower frequency of moulting, suggesting possible impacts on growth.

Introduction

The narrow clawed crayfish *Astacus leptodactylus* Eschscholtz 1823 is harvested from freshwater systems across Europe including in Turkey, Poland, Germany, England, Spain, Italy, France and Iran. Supply of crayfish in Europe has been reduced as a consequence of crayfish plague *Aphanomyces astaci* (Schikora 1906), overfishing, water pollution and agricultural irrigation (Holdich 1993). However, demand remains high because *A.leptodactylus* is one of the most popular freshwater crayfish species in Scandinavia, Germany and France (Kőksal 1988; Holdich 1993; Wickins et al. 2002). High price of *A.leptodactylus* creates an incentive for production. Production has been limited because of the needs to better understand methods for optimising culture production (Harlıoğlu 2004). One of the issues limiting expansion is examined here – the impact of cannibalism on survival, which affects both broodstock held for hatchery production and the grow-out phase.

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Cannibalism, survival and moulting tend to be linked in crustaceans because animals are less mobile and are soft-shelled during moulting (Sotelano et al. 2012; Daly et al. 2009; Marshall et al. 2005). Many studies have shown that cannibalism, survival and moulting are influenced by a complex range of interacting factors including temperature, salinity, feeding frequency, photoperiod and density with variation between species (Hartnoll1982; Elgar and Crespi 1992). Cannibalism in crustaceans generally increases when food is scarce because of intra- or inter-specific competition for food (Elgar and Crespi 1992). Studies showed that light intensity (Westin and Gydemo 1986; Gardner and Maguire 1998; Hoang et al. 2003) and photoperiod (Gardner and Maguire 1998; Hoang et al. 2003) had a significant effect on cannibalism.

Production of A.leptodactylus has the potential to provide industry and employment. However, there is a need for further research on production and culture (Harlıoğlu 2004), including on conditions promoting higher survival. There is no information currently available for effects of photoperiod and stocking density on cannibalism, survival and moulting of *A. leptodactylus* broodstock. There are rarely studies focused on effects of photoperiod or stocking density of decapod species broodstock. Most studies focused on larval or early juvenile stages. For example, Gardner and Maguire (1998) found that larvae of the Australian giant crab *Pseudocarcinus gigas* (Lamarck 1818) had shorter intermoult duration and development in treatments with longer photoperiods, and larvae reached rapidly to the megalopa stage in continuous light treatment. Survival was not significantly affected although sub-lethal cannibalism was strongly influenced by continuous lighting with greater damage to the dorsal spine (Gardner and Maguire 1998). Johnston et al. (2006) found that survival of post-puerulus and juvenile western rock lobster *Panulirus Cygnus* George 1962 was significantly reduced at lower densities. Tidwell et al. (2001) reported that continual light conditions have a positive impact on survival of freshwater prawn juveniles *Macrobrachium rosenbergii*(de Man 1879) during the nursery phase. Jones and Ruscoe (2000) reported that stocking density did not affect survival in *Cherax quadricarinatus* (von Martens 1868). The aim of the present study was to explore effects of photoperiod and stocking density on cannibalism, survival and moulting of *A. leptodactylus* broodstock.

Materials and Methods

Rearing conditions and experimental design

Three hundred and fifty *A. leptodactylus* (body weight 36.9-40.5 g; carapace length 5.69-5.93 cm) were collected from Aras Lake (Iran), randomly placed in five 1000 L tanks with 12 h light (L): 12 h dark (D) photoperiod and acclimated to this environment for 2 wk. The crayfish were then randomly assigned to thirty 90 L (90 cm× 40 cm×25 cm; 0.36 m^2 bottom area) fiberglass tanks with 20 cm water depth. Tanks were provided with flow-through non-chlorinated freshwater drawn from a bore at 1.5 $L \cdot min^{-1}$(1.7%) and aerated to maintain oxygen at 8.2±0.1 $mg \cdot L^{-1}$. Water temperature was 16.1±0.4°C during the experiment with this stability resulting from drawing the water from a bore. In order to reduce cannibalism and antagonist behaviour PVC tubes with 12 cm length and 5 cm diameter were provided (one PVC tube was provided for every crayfish). Crayfish were fed with a commercial diet (sturgeon diet from BioMar, France; 45.67 % protein, 16.8% lipid, and 9.7% ash) twice daily at 09:00 h and 19:00 h with any residual food removed prior to each feeding. Three photoperiod treatments (18 L: 6 D, 12 L: 12 D, 6 L: 18 D) and three stocking density treatments (10, 20 and 40 m^{-2}) were established to give nine treatment combinations with three replicates for each, except for the low-density treatment (10 m^{-2}) where four replicates were used. For 10, 20 and 40 individuals m^{-2} 4, 7 and 14 crayfish were put in each tank respectively.

Lighting was by 80 W fluorescent tubes providing a light intensity of250±50 lx at the water surface of the tanks. Photoperiods were manipulated using a 24 h time controller (Multi 9, Merlingerin, Germany). For 18 L: 6 D, 12 L: 12 D and 6 L: 18 D photoperiods, the lights came on at 0800 h and turned off at 1400 h, 1800 h and 0200 h respectively. External light was excluded from tanks with thick black polyvinyl sheeting on metal frames. Water quality was monitored daily using an OXI 45 PCRISON temperature and dissolved oxygen metre (Spain) and a CP-411 ELMETRON (Poland) for measuring pH.

The experiment ran for 40 days from July 20 to August 26, 2013. Tanks were monitored daily for moulting and mortalities with dead crayfish examined to determine the cause of death (cannibalism or disease). In the present study cause of death was divided into four categories; cannibalism (death of crayfish by cannibalism or antagonist behaviours), disease (usually caused by tail blisters), moulting (death of crayfish during the moulting process) and undetermined causes. In order to reach a better understanding for moulting efficiency we defined a parameter as moulting success (MS) where $MS\% = (M-D_m/M)*100$. In this formula M is number of moultings and D_m is crayfish deaths linked to moulting because we observed that many crayfish died due to cannibalism after moulting in high density tanks. A culture system with high MS% can provide potentially high moulting with low mortality and high growth rate.

Samples that showed some evidence of crayfish plague, such as melanism of the carapace, were cultured on general myces growth culture and IM (Isolation Media) for *A. astaci*.

Statistical Analyses

Results were analysed using computer software SPSS (version 16.0). The normality of data was tested by Kolmogorov–Smirnov. Two-way analysis of variance (ANOVA) was employed to evaluate the effects of photoperiod and stocking density, and their interaction on survival, cannibalism and moulting. Tukey's HSD post hoc test was employed to determine significant differences between treatments at $P<0.05$.

Results

Mortality and cannibalism

Mortality and cannibalism rates were both affected by photoperiod and stocking (Table. 1). There was a significant interaction between photoperiod and stocking density for cannibalism and mortality (Table. 1). Greatest cannibalism damage occurred to chelipeds (30%) and periopods (31.9%) and the lowest for pleopods (9.8%) (Fig.1). In general, disease (indicated by tail blisters; 28.8%) and cannibalism (37.8%) were the main causes identified for mortality (Fig. 2). Other causes were undetermined (22.9%) and moulting (10.4%).

Table 1. Result of two-way ANOVA for effects of photoperiod and stocking density.

	Density	**Photoperiod**	**D×P**
Mortality (%)	**0.01**	**0.012**	**0.026**
Cannibalism (%)	**0.017**	**0.022**	**0.037**
Mortality categorised by			
moulting stage (%)	0.251	0.313	0.424
moulting success (%)	**0.0219**	0.217	0.172
Moulting (%)	0.285	**0.035**	0.15

Data are P values with significant results highlighted in bold.

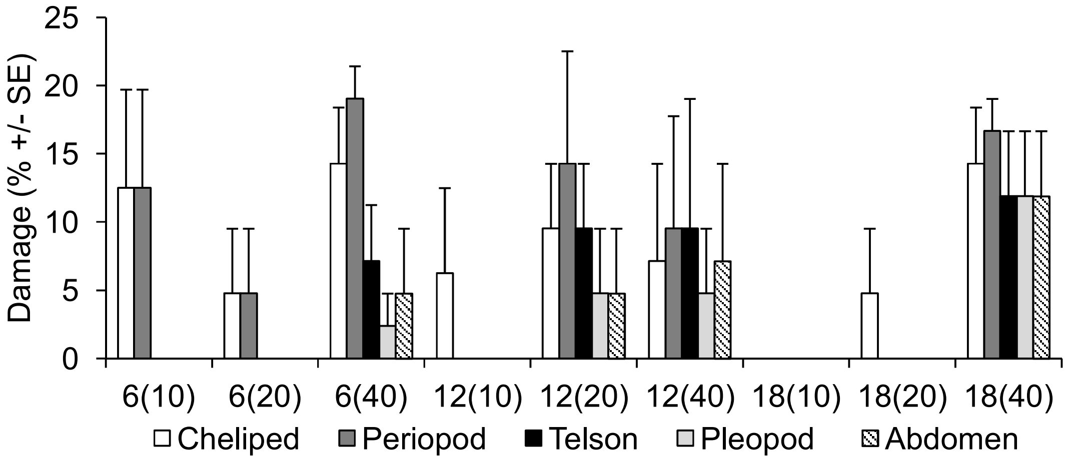

Fig. 1. Regions of the body of *A. leptodactylus* damaged through cannibalism under different photoperiods (6, 12 and 18 h L) and stocking density (10, 20, 40·m^{-2}). Three replicates per treatment except 10·m^{-2} treatments, which had four replicates.

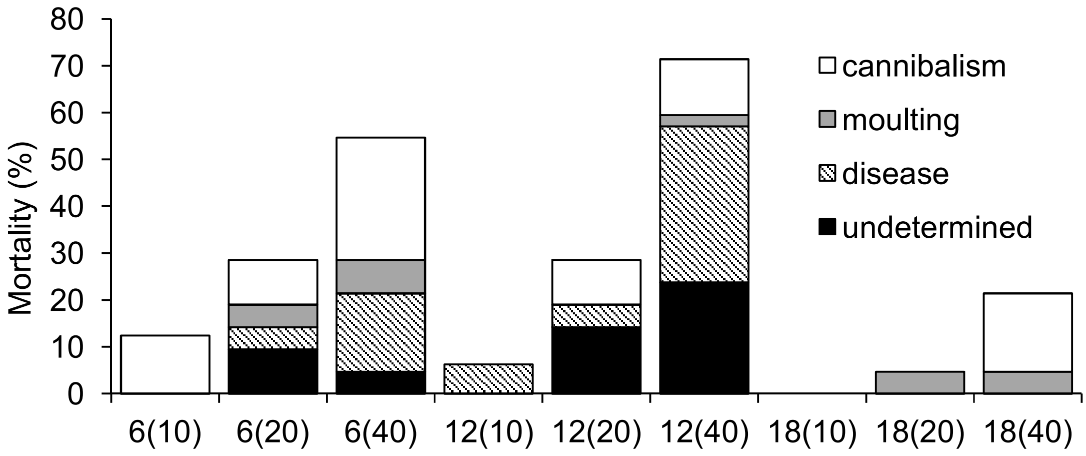

Fig. 2. Cause of mortality of *A. leptodactylus* held under different photoperiods (6, 12 and 18 h L) and stocking density (10, 20, 40 m^{-2}). Data were pooled across replicates (n=79).

Moulting

There was no significant interaction between photoperiod and stocking density for moulting success (%) and moulting (%) (Table.1). Photoperiod and stocking had a significant effect on moulting (%) and moulting success (%) (Table.1). Mortality categorised by moulting stage is shown in Fig. 3. Moulting success (%) and temporal patterns of moulting in different treatments are shown in Fig.4 and Fig.5 respectively.

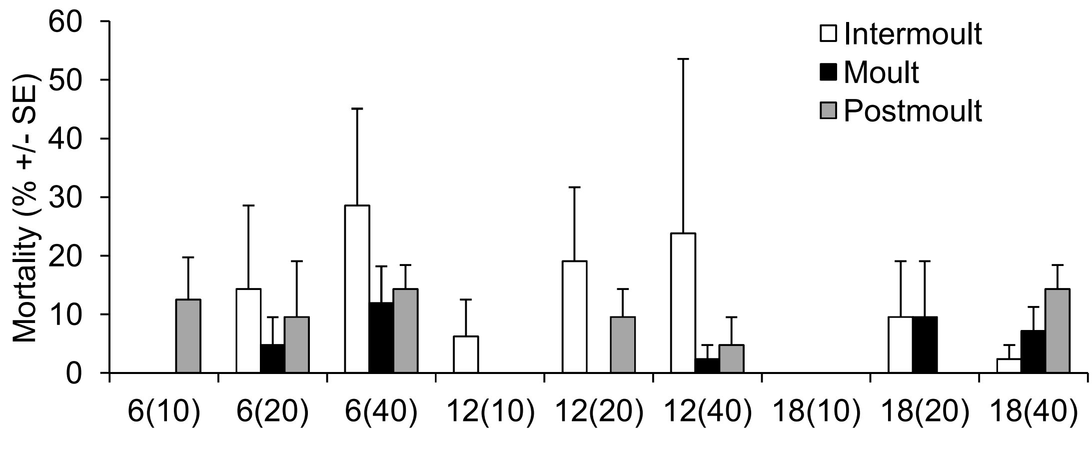

Fig. 3. Incidence of mortality relative to moult stage of *A. leptodactylus* held under different photoperiods (6, 12 and 18 h L) and stocking density (10, 20, 40 m^{-2}). Three replicates per treatment except 10 m^{-2} treatments, which had four replicates.

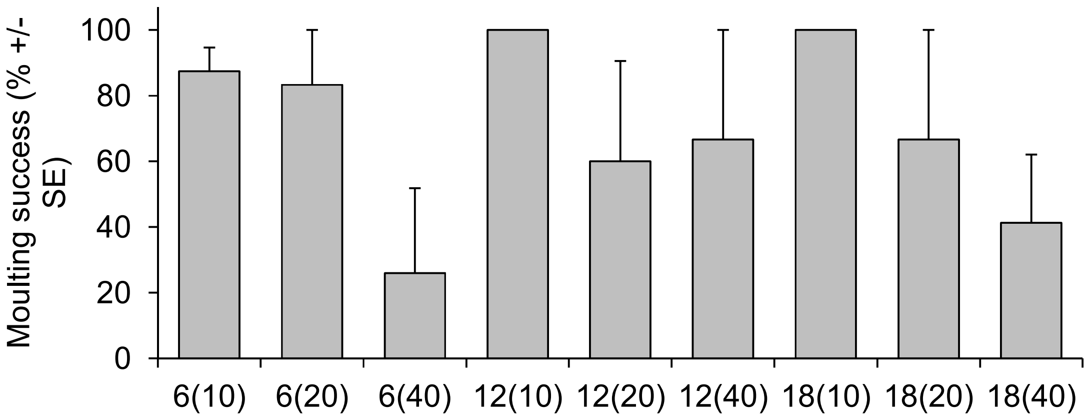

Fig. 4. Moulting success (% survived moults / total number moult attempts) of *A. leptodactylus* held under different photoperiods (6, 12 and 18 h L) and stocking density (10, 20, 40 m^{-2}). Three replicates per treatment except 10 m^{-2} treatments, which had four replicates.

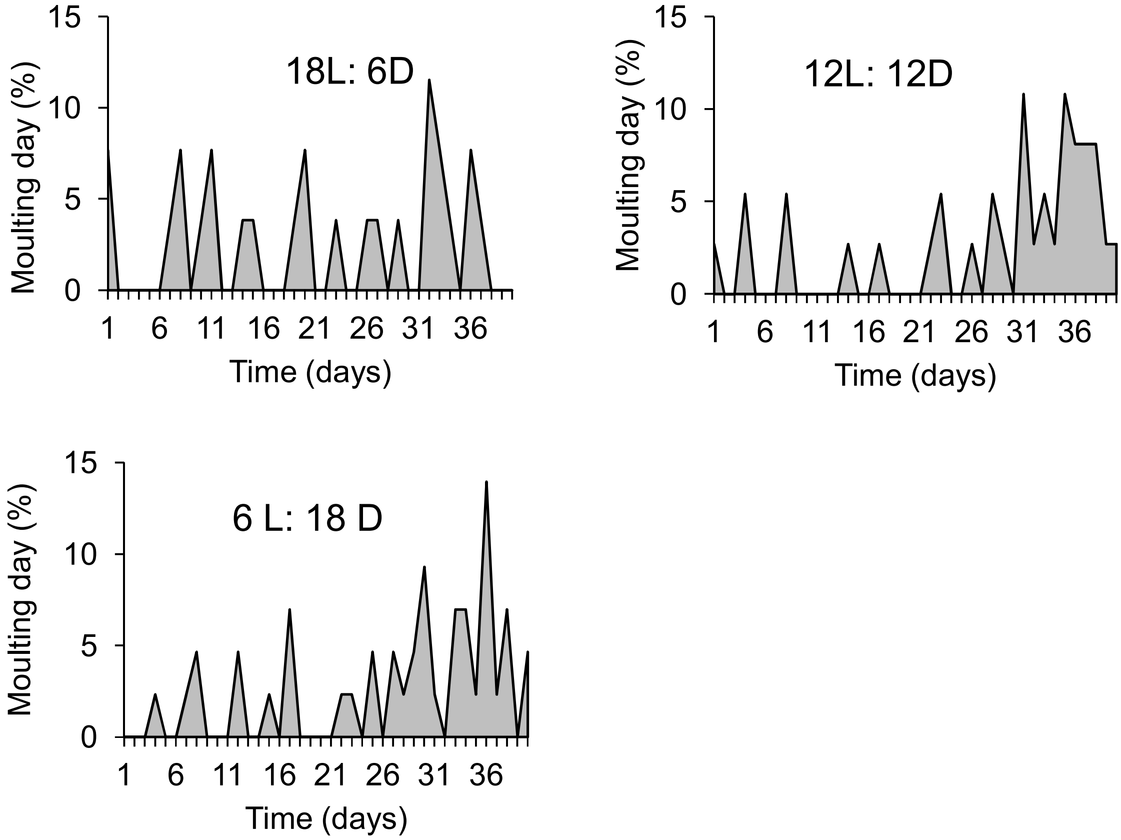

Fig. 5. Temporal patterns of moulting of *A. leptodactylus* held under different photoperiods. Data are pooled across replicates (n=79).

Discussion

This experiment showed that photoperiod and stocking density affect survival and cannibalism of *A. leptodactylus*. This information can improve management of broodstock during reproduction operation. It has been reported that high density increases cannibalism in crustaceans (Abdussamad and Thampy 1994). Management of density and grading of size categories is a common practice in aquaculture to reduce cannibalism (Daly et al. 2012). This study extends general knowledge on how cannibalism and survival can be moderated by photoperiod in high stocking density treatments.

Cannibalism

Cannibalism increases in many crustaceans when food is scarce and there is limited shelter availability (Elgar and Crespi 1992). Low availability of refuges not only increases cannibalism but also enhances sub-lethal effects of agonistic behaviour such as limb loss and reduced growth (Marshall et al. 2005). In the present study *A. leptodactylus* displayed cannibalistic behaviour even in the presence of high food supply and good refuge availability. Food supply and refuge availability are very important for controlling cannibalism in the culture of *A. leptodactylus*. Cannibalism of *A. leptodactylus* was observed not only through the vulnerable period of moulting. Cannibalism also has been observed during intermoult and postmoult periods when crayfish has a hard exoskeleton (Elgar and Crespi 1992). Cannibalism was higher during the first few days of the experiment, which may have been caused by agonistic interactions in the establishment of territories (Moore 2007). Cannibalism typically involved mortality of intermoult stage crayfish that were injured or killed but with limited consumption. In contrast, cannibalism during postmoult typically involved consumption of the entire body, with the exception of the carapace. These observations suggest that mortality and cannibalism during intermoult was primarily the result of aggressive or territorial behaviour.

Crayfish reared under short photoperiod and high density had higher cannibalism rate than other treatments. High rate of cannibalism may have been related to an increase in social interactions, because crayfish are most active in dark hours. High rate of cannibalism during short photoperiod can also be linked to the higher levels of moult frequency in this photo phase.

Damage tended to occur more to periopods than chelipeds, which has also been observed in southern king crab *Lithodes santolla* (Molina 1782) (Sotelano et al. 2012). Damage to periopods and chelipeds occurred more than pleopods or tail. Increase sub-lethal damage to periopods and chelae with higher stocking density has been observed previously on other crayfish species (Figiel and Miller 1995; Savolainen et al. 2004).

Moulting

Mortality was sometimes a result of failure of moulting that increased significantly in higher density treatments. The exact reason for this is unclear but may involve social interactions. Crayfish held in short light phase had shorter moult intervals and higher moult frequency than those held in long light phase treatments. This was possibly due to photoperiod effects on moult-stimulating hormone synthesis (MSH) activity or other metabolism-related hormones (e.g. the crustacean hyperglycemic family of hormones (CHH); mainly secreted from the eyestalk), which can be stimulated by strong light (Hoang et al. 2003). It can be suggested that culture should be conducted with short photoperiod, as faster moulting generally implies faster growth. However, this needs to be assessed with consideration of other factors such as mortality.

Effect of photoperiod on moulting and growth are variable indifferent species (Hoang et al. 2003). For example constant darkness or constant light can either stimulate or inhibit moulting (Hartnoll 1982; Skinner 1985). Also some studies have reported that photoperiod had no significant effect on moulting (Justo et al. 1991; Hoang et al. 2003)

Mortality

Mortality of *A. leptodactylus* was significantly lower in longer photoperiod and lower density treatments. The effect of longer light phase is presumably related to the nocturnal activity patterns of crayfish (Bojsen et al. 1998; Lozan 2000) with shorter dark periods resulting in less social interaction and fighting. Another factor contributing to higher survival in the 18 h L: 6 h D treatment may have been the lower moulting frequency in this treatment, and also because newly moulted animals are typically more vulnerable (James et al. 2001). However, in this case the mechanism was less clear because mortality was also common during intermoult.

In the present study stocking density had a significant effect on survival. Johnston et al. (2006) reported lower survival from high density ($19 \cdot m^{-2}$) treatments than low density treatments ($10 \cdot m^{-2}$) in *P. cygnus*. Mortality of *A. leptodactylus* occurred through a range of different causes including cannibalism, disease (indicated by tail blisters), and failed moulting. There was no obvious trend in the relative proportion of the mortality causes with increasing density.

However, disease (tail blisters) was higher at the highest density. Tail blisters are caused by an abiotic disease and are symptomatic of heightened stress (Holdich and Crandall 2002). In this trial, mortalities due to crayfish plague were not confirmed by culture on general myces growth culture and IM (Isolation Media).

Conclusion

The study demonstrated that optimum conditions for keeping broodstock of *A. Leptodactylus* involves longer light photoperiod (18 h L: 6 h D) and lower density ($10 \cdot m^{-2}$).

These conditions increase survival, lower cannibalism, and allow successful moulting. Although moulting frequency is lower under longer light photoperiod, the reduction in cannibalism and mortality is advantageous in the culture of *A. leptodactylus*.

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