An Acad Bras Cienc (2021) 93(2): e20190580 DOI 10.1590/0001-3765202120190580
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ECOSYSTEMS

Evaluation of different induced molting methods in *Callinectes ornatus* (Crustacea, Decapoda, Portunidae) as a tool for the commercial production of soft-shell crabs

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Abstract: We investigated the effects of eyestalk ablation and cheliped autotomy in inducing molting in *Callinectes ornatus*. The specimens in intermolt stage were divided into two size classes: 1 (30-50 mm) and 2 (51-70 mm) and were further divided into four experimental groups. In the CA group (Cheliped Autotomy, n=76), crabs were submitted to cheliped autotomy; in the UA group (Unilateral Ablation, n=66) and BA group (Bilateral Ablation, n=66) to unilateral and bilateral eyestalk ablation, respectively. The C group (Control, n=70) was used as control. The animals were individually kept in tanks interconnected to a recirculation system for 30 days. The highest frequency of premolt was recorded in the BA group (32%), followed by the CA group (16%). The premolt frequency of class 1 (28%) was significantly higher ($p<0.05$) than of class 2 (8%). The mean time until molt in the BA group was significantly lower ($p <0.05$) than other groups tested. The highest mortality rates were 55% and 25% in crabs from the BA and CA groups, respectively. The results indicate bilateral eyestalk ablation and cheliped autotomy are potentially capable of inducing molt in *C. ornatus*, but these techniques have limited efficiency for the commercial application of large-scale soft-shell crab.

Key words: Ablation, Autotomy, intermolt, ecdysis, portunidae.

INTRODUCTION

The Portunidae family, composed of crustaceans popularly known as swimming crab, is characterized by the presence of morphological adaptations that allow them to explore the water column. One of these adaptations, perhaps the most obvious one, is the modification of the last pair of pereiopods, in the shape of oars for swimming function. Swimming crabs are widely distributed geographically and are considered important fishing resources in many countries (Melo 1996, FAO 2018).

Callinectes ornatus is a species of swimming crab distributed from North Carolina, USA, to Rio Grande do Sul, Brazil (Williams & Williams 1984, Branco et al. 2002). It is a species of great abundance in the coastal region of several Brazilian states, where its biology and distribution have been much studied (Mantelatto & Christofoletti 2001, Baptista et al. 2003, Watanabe et al. 2014, Tudesco et al. 2012). Crabs of this species are often caught as bycatch in shrimp fishing, and individuals caught are often killed and simply discarded in the environment

(Rodrigues-Filho et al. 2015, Mário Cesar et al. 2013, Moreira et al. 2011).

Like all other crustaceans, the swimming crabs periodically exchange their exoskeleton in a process called ecdysis or molt. The molting cycle can be divided into four stages: intermolt, premolt, molt, and postmolt (Freeman et al. 1987). Among these, intermolt is the predominant stage, occupying up to 60% of this cycle (Freeman et al. 1987, Vega-Villasante et al. 2007). During molt stage, the rigid exoskeleton is replaced by a new, flexible and soft, with high water content and low level of calcification (Chang & Mykles 2011, Tagatz 1968). At this moment, the animal is called "soft-shell crab" and, if consumed within this period, all parts of the body are edible, presenting great gastronomic value and high values in the international market (Gaudé & Anderson 2011, Hungria et al. 2017, Perry et al. 2010). However, if the animals are not slaughtered soon after the molt, the product will eventually lose its market value, as the new exoskeleton hardens in a few hours (Wheatly 1999, Taylor & Kier 2003, Perry et al. 2001).

Soft-shell crab rearing is a multimillion-dollar aquaculture activity that has become popular in the eastern United States and Asia because it is one of the most profitable forms of marketing crabs, and it is currently an important source of employment and income (Tobias-Quinitio et al. 2015, Kennedy & Cronin 2007). The production is mainly based on the capture of animals in premolt stage in the environment, which are transferred to production systems until the occurrence of molt (Gaudé & Anderson 2011, Tavares et al. 2018).

However, as the world production of soft-shelled crabs depends directly on fishing, the main limiting factor is the absence of safe and stable sources of animals in premolt stage (Perry et al. 1982, Perry & Malone 1985). To solve this problem, molt induction and synchronization methods could be exploited as a way to increase the commercial-scale production of soft-shell crab in captivity (Ary Jr. et al. 1987).

Different techniques of induction of ecdysis in crustaceans have been studied, among them are: injection of hormones, which rapidly elevates the concentrations of the ecdysteroids in the hemolymph (Cheng & Chang 1991, Rao et al. 1973, Rao et al. 1972); eyestalk ablation, which reduces the production of the molt-inhibiting hormone (MIH) (Skinner & Graham 1972, Molyneaux & Shirley 1988, Stella et al. 2000, Techa & Chung 2015) and, cheliped autotomy, which stimulates ecdysis as a way to repair defense organs (Smith 1990, Ary Jr. et al. 1987, Amador Del ángel et al. 1999, Alvarez & Meruane 2009, Dvoretsky & Dvoretsky 2012, Quinitio & Estepa 2011).

The results of the experiments conducted with the injection of ecdysteroids showed a significant increase in mortality rates of the animals (Cheng & Chang 1991, Rao et al. 1973). Also, as the technique involves injecting large amounts of hormones into the body to obtain favorable results, it would compromise the acceptance of the final product (soft-shell crab) and the harmlessness of the product to the consumer (Rao et al. 1972).

The use of eyestalk ablation and autotomy techniques in crabs in intermolt stage, as a tool to obtain soft-shell crabs, has not yet been fully investigated by researchers. On the other hand, the cheliped removal is a common technique used in mud crab rearing in Asia (Quinitio & Estepa 2011, Triño et al. 1999), while the eyestalk ablation is widely used in commercial shrimps farming (Bray & Lawrence 1992, Lawrence et al. 2009, Pervaiz et al. 2011, Wen et al. 2015). As a way to fill this gap in the literature, this study aimed to test three techniques of molt induction (uni and bilateral eyestalk ablation and cheliped autotomy) in *Callinectes ornatus*, to evaluate their efficacy and effect on mortality rates of crabs kept under controlled environmental conditions.

MATERIALS AND METHODS

Collection and identification

Specimens of *Callinectes ornatus* were obtained by bottom trawling, using nets with an average length of 7.5 m and a 20 mm aperture mesh between knots. The trawls were carried out by fishermen in Ipanema beach (25°40’S/48°27’W) and Shangri-lá beach (25°37’S/48°25’W), over sandy bottoms at 250 meters from the shore, localized in the municipality of Pontal do Paraná, Paraná, Brazil. Immediately after berthing of the boats, the live crabs were separated from the other animals caught in the trawl net and transferred to polyethylene boxes containing a small volume of seawater taken from the catch site. In the boxes, the crabs were protected by plastic screens (mesh aperture - 20 mm), to minimize mortality during transport to the Centro de Aquicultura Marinha e Repovoamento (CAMAR), belonging to the Grupo Integrado de Aquicultura e Estudos Ambientais (GIA), from the Universidade Federal do Paraná (UFPR), located 10 km from the fishing site (25°41’29.94”S/48°27’57.09”W).

In the laboratory, the crabs were weighed (total weight) with precision digital balance (Marte AL 500c - 0,001g, Brazil), measured (distance between the base of the lateral spines) with the aid of a manual caliper, identified up to the specific level based on the identification manual developed by Melo (1996), and sexed by visualizing the morphology of the abdominal somites in the cephalothorax (Baptista et al. 2003). The macroscopic signals of the molting stage were identified according to Freeman et al. (1987) and Oesterling (1988).

Experimental system

A total of 278 specimens in the intermolt stage were randomly distributed in interconnected polyethylene tanks in a recirculation system (30 tanks of 70 L volume), composed of an ultraviolet light water sterilizer (Sibrape® UVC AG-Industrial, Brazil), mechanical and biological filtration system and artificial aeration. Each tank housed about 10 crabs of varying size classes and experimental group. The photoperiod was set to 12:12 h (light: dark), controlled with the aid of an analog timer. In each box, about 5 kg of sand, about 30 mm thick, was added, previously heated to approximately 65°C, to eliminate any unwanted organisms. The sand layer was added to serve as a substrate for fixing nitrifying bacteria (Malone & Burden 1988).

To avoid cannibalism during the experiments, the animals were individually placed in perforated polyethylene terephthalate (PET) bottles of varied volumes (0.6 and 1 L), identified with numbered seals. The crabs were acclimated for up to 10 days to the experimental conditions before the initiation of the experiments, and during this period were fed once a week with pieces of fish to satiety. The acclimation period was necessary to minimize mortality associated with stress during capture, as observed by Chaves & Eggleston (2003). After the acclimation period, all the animals were again visually inspected to ensure that they were the intermolt stage. The molt stage identification was done observing the membranous layers of the cuticle in the propodus and dactylus of the fifth pair of pereiopods, following the recommendations of Freeman et al. (1987). Only after this, the experiment and the daily monitoring of molt stages were initiated.

Experimental groups

For the evaluation of the effects of induction techniques on molt and survival rates in *C.*

ornatus, specimens were separated into two size classes: 151 belonging to size class 1 (30 - 50 mm) and 127 to class 2 (51 – 70 mm). Each group was further divided into four experimental groups (CA, UA, BA and C). In the CA group (n=76), the crabs were submitted to autotomy induced of one of the chelipeds. In the UA group (n=66), the animals were subjected to unilateral eyestalk ablation. In the BA group (n=66), were ablated bilaterally. Group C (n=70) was used as a control treatment. In this case, the animals were not submitted to any complementary intervention.

The autotomy was performed on the right cheliped of each animal and induced using excision in the merus region, using previously sterilized surgical scissors. After excision, in at most one minute, the animal performed the autotomy of the limb (methodology adapted from Quinitio & Estepa 2011), with the immediate onset of the healing process of the autotomized limb (Hopkins 1989). The ablation technique was performed by excision, using a hemostat and a scalpel. The constriction was made close to the base of the eyestalk. The site of the incision was immediately cauterized “hot”, avoiding the extravasation of hemolymph (methodology adapted from Primavera 1989). The entire procedure was performed as soon as possible, not lasting more than two minutes for each animal.

The experiment lasted 30 days, based on the average duration of the intermolt stage reported for the genus *Callinectes* (Freeman et al. 1987, Vega-Villasante et al. 2007), period considered sufficient to evaluate if the techniques presented or not results in the molt induction. Daily the observation of the crabs was made regarding the occurrence of macroscopic signs of the premolt or molt and death and record of each event. During the experimental period, each crab was fed once a week, with pieces of fish, to satiety. This methodology was defined based on previous studies which showed that there was no difference between feeding the crabs daily or once a week (Tavares et al. 2018).

The following physical and chemical variables of water were analyzed daily as routine during the experiment: salinity, measured by means of an optical refractometer (Instrutemp, Brazil); pH, through digital pH meter (AZ-86505, Taiwan); temperature (°C), dissolved oxygen (mg/L) and percentage of oxygen saturation (%), by means of digital oximeter (YSI, 550A, USA); alkalinity (mg/L), by titration with sulfuric acid 0,02N (APHA 2005); concentration of nitrogen in the form of total ammonia (mg/L de N-AT), obtained by the indophenol method (APHA 2005) and nitrite (mg/L de N-NO_2^-) (APHA 1995), by reading the samples by spectrophotometry (Spectronic 20 Genesys, USA). For analysis, 200 ml aliquots were drawn with a beaker and analyzed as single samples.

Data analysis

The data, both from biometric and water quality measurements, were analyzed through the normality test of Shapiro-Wilk ($p<0.05$). The homogeneity of the variances between the experimental groups was analyzed by the Fisher test. The results regarding the frequency of ecdysis were analyzed using the Cochran Q test for paired samples, followed by McNemar Test. The effect of treatments on time required for ecdysis was tested by analysis of variance ANOVA – One-way, followed by the Tukey post-hoc test, using the software Statsoft Statistica®, version 10.0. Mortality data were plotted using the Kepler-Meier survival curve and analyzed using the Mantel-Cox, Breslow and Tarone-Ware methods using software IBM - SPSS® version 22.

RESULTS

The physical and chemical variables of water remained relatively stable throughout the experimental period (Table I). During the period of the experiment, the highest frequency of individuals who passed from the intermolt stage to the premolt stage was observed in the crabs submitted to the technique of bilateral eyestalk ablation (32%), followed by induced cheliped autotomy (16%), while the lowest frequency (5%) was recorded in unilaterally ablated crabs (Figure 1). No animals were observed in premolt or molt stage in the control treatment. All treatments presented significant differences ($p<0.001$) among themselves. However, there was a higher occurrence of premolt and, later molt, in size class1 than in animals in class 2. Of the total of 36 animals registered in premolt stage, only 8% were crabs belonging to class 2 (Table II).

After the animals had been submitted to the respective surgical procedures, the period up to the beginning of the premolt stage ranged from 10.8 to 18.8 days in the crabs belonging to size class 1 and 17.0 to 23.0 days in the animals of class 2. The premolt stage lasted from 6.2 to 9.0 days in the crabs belonging to size class 1, while in the few animals of class 2 the premolt lasted 8.5 days. After this period, the animals performed the molting. The bilaterally ablated crabs passed from the intermolt stage to the molt stage in less time when compared to the crabs from the other experimental treatments ($p<0.05$). Animals of the control group remained at the intermolt stage throughout the experiment (Table III).

The highest mortality rate at the end of the experimental period was recorded in bilaterally ablated crabs (55%) and autotomized crabs (25%), and the lowest rate in unilaterally ablated crabs (18%) and the control group (10%). There were significant differences ($p <0.001$) in the analysis of survival curves using the Mantel-Cox, Breslow and Tarone-Ware methods, indicating that the mortality pattern was distinct throughout the experimental period (Figure 2). There was no significant difference in the occurrence of deaths among animals of size class 1 and 2.

Table I. Mean ± standard deviation of water quality variables of the experimental system and recommendations of optimal conditions for farming of swimming crabs by Malone & Burden (1988) and Hochheimer (1988).

Physicochemical variables	Mean ± standard deviation	Values recommended by Malone & Burden (1988) and Hochheimer (1988)
Temperature (°C)	26,2 ± 2,0	22 - 28
Dissolved oxygen (mg/L)	4,86 ± 0,5	>7,0
Dissolved oxygen (%)	72,8 ± 7,4	Nd*
pH	7,99 ± 0,1	6,5 - 8,5
Salinity	35,3 ± 0,9	5 ppm above/below of harvesting water
Total Ammonia (mg/L)	0,07 ± 0,02	<1,0
Nitrite (mg/L)	0,07 ± 0,05	<0,5
Alkalinity (mg/L)	46,58 ± 9,90	>100,0

***Nd: Not available.**

It was also observed that cheliped autotomy and unilateral eyestalk ablation techniques similarly affected the mortality of the crabs. The mean period between the first day of the experiment and the death of the animals tested was 6.5 ± 3.5 days for the control group, 13.7 ± 1.5 days for the bilaterally ablated crabs, 14.2 ± 2.1 days for autotomized crabs and 16 ± 2.7 for unilateral eyestalk ablated crabs. In addition, a moderate correlation was observed (r^2 = 0.63) between the time the animals took to reach the premolt stage (independent variable) and the time they took to die (dependent variable), and also between the duration of the premolt and time elapsed until the death of the animals (r^2 = 0.62) (Figure 3).

DISCUSSION

In soft-shell crab cultivation systems, monitoring of the physical (temperature) and chemical variables (salinity, dissolved oxygen, pH, ammonia, nitrite, carbon dioxide and alkalinity) of water is essential to keep crabs healthy and to ensure that the process molting occurs with

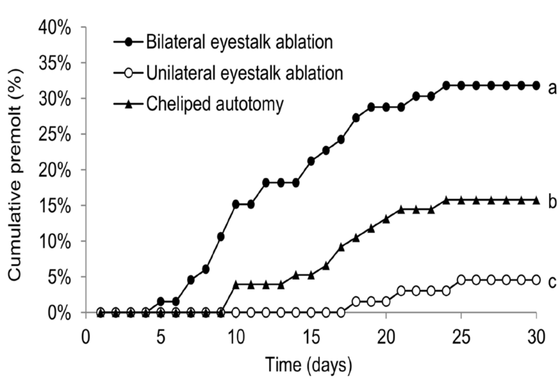

Figure 1. Cumulative premolt frequency in *Callinectes ornatus* subjected to different experimental treatments for 30 days. Different lowercase letters in rows indicate significant differences (p<0.001) among the experimental groups, calculated using the McNemar test.

minimal stress (Hochheimer 1988). During the studies, these variables remained relatively stable and within limits recommended by Hochheimer (1988) and Malone & Burden (1988), except for alkalinity and dissolved oxygen concentrations.

Alkalinity values remained below the recommended limit for the maintenance of swimming crabs in closed systems. However, no evidence was found that alkalinity had any influence on the pre-molt, molt or survival rates recorded in the present study. According to Malone & Burden (1988) maintenance of alkalinity above 100 mg/L has as main objective to avoid variation of pH and increase the efficiency of the nitrification process. However, neither the pH nor the concentrations of nitrogen compounds showed abnormal variations during the experiment.

The recorded concentrations of dissolved oxygen were also slightly below the recommended limit, but there were no indications of negative effects on the molting process. Although there are no records of the minimum concentrations supported by crabs, it is known that dissolved oxygen begins to be problematic only when its concentrations fall below 2.0 mg/L for more than one hour. Under these conditions, the molting process will be negatively affected, as well as the performance of the nitrifying bacteria present in the system, which can result in rapid accumulation of ammonia and nitrite and increase mortality rates (Malone & Burden 1988, Hochheimer 1988).

There is no information in the literature about the duration of each molting stage of *Callinectes ornatus*. Mantelatto & Christofoletti (2001) observed, after two years studying the molt cycle of *C. ornatus* in the region of Ubatuba (São Paulo - Brazil), the presence of animals in premolt stage in almost every month of the year, which suggests that the species present

Table II. Premolt and molt frequencies in *Callinectes ornatus* of different size classes subjected to different experimental treatments.

		PREMOLT		MOLT	
GROUPS	Size class	N° of observations	%	N° of observations	%
Control	1	0[a]	0	0[a]	0
	2	0[a]	0	0[a]	0
Cheliped autotomy	1	10[c]	26	10[c]	26
	2	2[b]	5	2[b]	5
Unilateral ablation	1	1[e]	3	1[e]	3
	2	2[d]	6	0[d]	0
Bilateral ablation	1	16[fc]	52	16[fc]	52
	2	5[f]	14	0[f]	0

Different lowercase letters in columns indicate significant differences (p<0.001) between classes, calculated using the McNemar test.

short periods between subsequent moltings. The same pattern was observed in laboratory studies with other *Callinectes* species. Freeman et al. (1987), working with *C. sapidus*, recorded a mean duration of the molting cycle of 30 and 37 days, for, respectively, 30-60 and 61-80 mm (carapace width) animals. Vega-Villasante et al. (2007) recorded similar patterns for *C. arcuatus* with 50-70 mm carapace width. In this last case, the total time of a molting cycle was, on average, 38 days. All the swimming crabs that passed from the intermolt stage to the premolt stage and that later performed the molt presented the same morphological characteristics (Figure 4) described in the determination of the molt stages of *C. sapidus* (Freeman et al. 1987) and *C. arcuatus* (Wehrtmann & Mena-Castaneda 2003). Using these references, it was also possible to identify specimens of *C. ornatus* that are close to the molt based on the presence of the white line along the distal borders of the pleopods, as well as predict the evolution of the ecdysis process observing the coloration this line passing from pink to reddish color as the time of ecdysis molt approaches.

For the three techniques tested, the highest frequency of ecdysis (28%) was observed in juveniles of *C. ornatus* smaller than 50 mm of carapace width (size class 1) and only 8% of ecdysis were recorded in crabs with carapace length between 51-70 mm (size class 2). Similar results have been reported for several species of crustaceans (Molyneaux & Shirley 1988, Smith 1940, Stella et al. 2000, Vega-Villasante et al. 2007). From a commercial perspective, the size class 1 crabs have not yet reached the minimum size for commercialization (appreciated only by the Japanese market), while size class 2 crabs, can be classified as “medium” size, with better commercial perspectives worldwide.

These results are expected as most decapods are characterized by indeterminate and continuous growth throughout life, but the relative increase in size (in percentage) decreases over time, as the intermolt periods tend to be larger with the age, thus limiting the

Table III. Time (days) until the premolt stage and, subsequently, until molting in *Callinectes ornatus* specimens subjected to different experimental treatments.

GROUPS	Size class	PREMOLT	MOLT
		Mean ± standard deviation	Mean ± standard deviation
Cheliped autotomy	1	16,2 ± 1,3[a]	6,7 ± 0,9[a]
	2	17,0 ± 2,2[a]	8,5 ± 0,7
Unilateral ablation	1	18,0 ± 4,31[a]	9,0 ± 2,8[a]
	2	23,0 ± 2,2[a]	-
Bilateral ablation	1	10,8 ± 1,0[b]	6,2 ± 0,7[a]
	2	19,6 ± 1,4[a]	-
Control	1	30 ± 0,0*	-
	2		

*During the 30 experimental days, all control group animals remained in the intermolt stage. Different lowercase letters, in columns, indicate significant differences ($p<0.05$), obtained by analysis of variance (ANOVA).

maximum size reached by the animals (Vogt 2012, Vega-Villasante et al. 2007). This can be understood as an evolutionary strategy related to the reduction of time in the life stages in which the animals are smaller, more vulnerable and more subject to predation/cannibalism (Marshall et al. 2005, Fernández 1999), and to the insignificance of the senescence of the reproductive tissues of species of crustaceans that present indeterminate growth (Vogt 2012, Zmora et al. 2009), enabling them to reproduce throughout virtually all of life (Goimier et al. 2006, Andrews et al. 1972, Catacutan 2002).

Both bilateral eyestalk ablation and induced cheliped autotomy methods reduced were successful in reducing the duration of the molting cycle in juveniles *C. ornatus*. The reduction observed in this study reached up to 46% (bilateral eyestalk ablation) and 20% (cheliped autotomy) of the mean intermolt period previously recorded for the genus *Callinectes* (Vega-Villasante et al. 2007, Freeman et al. 1987).

An increase in ecdysis rates was also observed in juveniles of bilaterally ablated *Paralithodes camtschatica*, with a significant reduction of 30% in the intermolt period (Molyneaux & Shirley 1988). Bilateral eyestalk ablation was also tested in megalopae of swimming crab *C. sapidus*, resulting in a reduction of approximately 15 to 28% of the intermolt period (Costlow Jr 1963). In shrimp *Palaemon elegans* and juveniles crayfish *Procambarus clarkii*, bilateral eyestalk elicited a reduction of the intermolt period between 15 and 24% (Webster 1985, Smith 1940). Moreover, bilateral eyestalk ablation was also a strong factor inducing ecdysis in natural populations of *Chasmagnathus granulata*, for which 65 to 100% molt were recorded in ablated animals (Stella et al. 2000).

The cheliped autotomy technique resulted in 26% ecdysis rate in juveniles *C. ornatus*, within a mean period of 22.9 (± 6.6) days. These

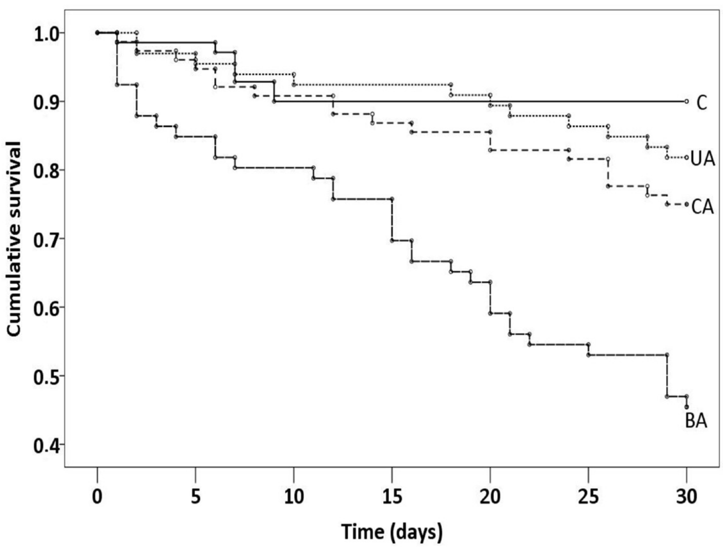

Figure 2. Survival curve of *Callinectes ornatus* specimens subjected to different experimental treatments for 30 days: C—Control group; CA—Cheliped autotomy; UA—Unilateral ablation; and BA—Bilateral ablation.

results corroborate Quinitio & Estepa (2011), who observed early molting in juveniles *Scylla serrata* autotomized, in that case with interval of 12 (± 1) days. Early ecdysis was also observed in adults *C. sapidus* in a study by Amador Del ángel et al. (1996), which recorded 60% of ecdysis in crabs between 50-75 mm in size in the mean period of 43.6 (± 3.8) days. These results suggest that autotomy of one of the chelipeds may reduce molting period in *C. ornatus*, although its effectiveness may vary according to the species and size class of the animal.

In the present case, unilateral eyestalk ablation also induced molting in juveniles *C. ornatus*, however, with much lower efficiency (only 3% of the crabs tested). Comparative studies related to unilateral eyestalk ablation are rare and show inconclusive results (Costlow Jr 1963, Molyneaux & Shirley 1988). According to Stella et al. (2000), the unilateral eyestalk ablation in *Chasmagnathus granulata* did not induce molt but caused the acceleration of the reproductive process and spawning. Similar results were observed in several studies with shrimps (Santiago Jr 1977, Santos & Pinheiro 2000, Pervaiz et al. 2011, Uawisetwathana et al. 2011).

Previous studies of unilateral eyestalk ablation were often criticized for not controlling important variables, such as the stage of the molting cycle, that would influence the results at the time of ablation (Sochasky 1973). In the present case, this variable was controlled by comparing macroscopic characteristics between treatment and control groups. The non-occurrence of premolt or molt signals in the control group reinforces the evidence that all crabs tested in this group were in the intermolt. Thus, there is enough evidence to conclude that the technique of unilateral eyestalk ablation is not an efficient tool for the induction of *C. ornatus* ecdysis during the large-scale soft-shell crab production process.

In addition to limited efficacy, in the case of larger animals, all the techniques investigated resulted in significant increases in *C. ornatus* mortality rates. In the case of unilateral eyestalk ablation, this increase was twice as high as in the control group, whereas the cheliped autotomy increased by three times the mortality rate, which was five times higher in bilaterally ablated crabs than in the group control.

Ecdysis is naturally a stressful period and usually results in increased mortality rates

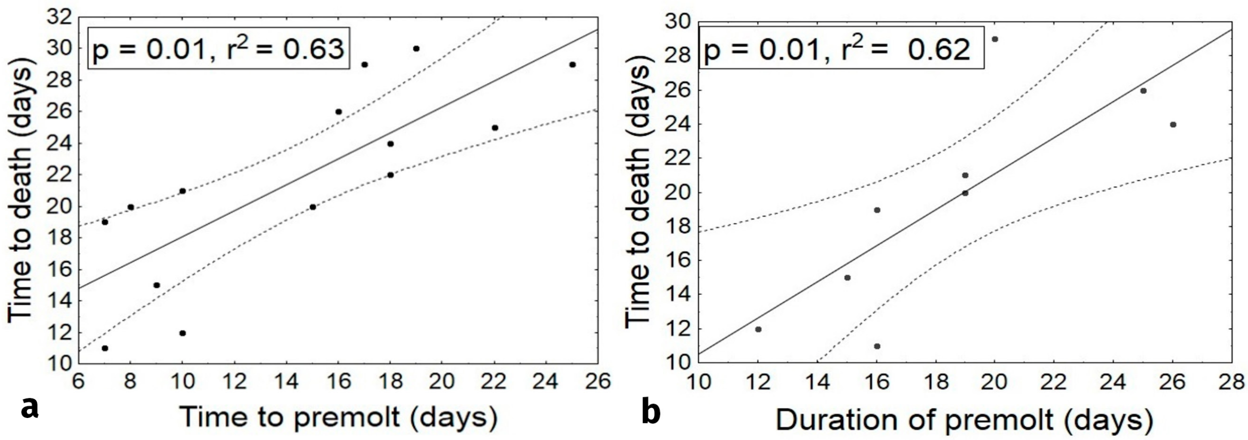

Figure 3. Relation between death and the premolt (a) and the duration of the premolt stage (b), the 95% confidence interval for *Callinectes ornatus* subjected to different experimental treatments for 30 days.

(Drach 1939). As evidenced in this and several other studies, crabs submitted to eyestalk ablation or cheliped autotomy are subject to additional stress (Snyder & Chang 1986, Mauviot & Castell 1976, Quinitio & Estepa 2011, Ary Jr. et al. 1987, Amador Del ángel et al. 1996).

The mortality rate recorded in juveniles *C. ornatus* submitted to bilateral eyestalk ablation in this study was similar to what was observed in juveniles of *Paralithodes camtschaticus* (Molyneaux & Shirley 1988). In crabs of the *C. sapidus* species, bilateral ablation resulted in mortality rate significantly higher than in unilateral ablation (Costlow Jr 1963). On the other hand, the mortality rate recorded in the autotomized crabs in this study was similar to the rate observed in *C. sapidus* submitted to the same technique (Amador Del ángel et al. 1996). According to Skinner & Graham (1972) and Juanes & Smith (1995) XRob3I+, autotomy is a stressful process, which can lead to death due to the breakage of several nerves located in the cheliped.

Mortality rates varied throughout the experimental period, and probably occurred as a function of the interventions performed on the animals during the experiment, and into even due to physiological factors related to ecdysis. It was observed that mortality rates were moderately correlated with both premolt and molt periods, this suggests that mortality was influenced by difficulties incurred close to the molting time. In bilaterally ablated crabs, about 30% of deaths occurred during premolt or molt. Abramowitz & Abramowitz (1940) suggested that the physiological changes associated with molting cause excessive stress, and the crab dies due to the absence of some factor from its eyestalks. Similar results were observed by Smith (1940), working with ablated juveniles *Procambarus clarkii*.

Bilateral eyestalk ablation and cheliped autotomy, in this order, are techniques that are potentially capable of inducing ecdysis in swimming crab *Callinectes ornatus* under controlled environmental conditions. However, there are many challenges to be overcome until such techniques can be efficiently used on a large scale for commercial soft-shell crab production. In addition to being of limited efficacy, especially when applied to commercial-sized animals, they greatly increase mortality losses. Thus, the results obtained here do not corroborate the application of eyestalk ablation (uni or bilateral) or cheliped autotomy in the commercial production of large-scale soft-shell

Figure 4. **Presence of morphological signs of molt stages in *Callinectes ornatus:* white line along the distal edges of the pleopod (a); reddish color indicating the proximity of molt (b).**

crab from the use of crabs from bycatch in shrimp fishing.

Acknowledgments

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the granting of the Research Productivity grant awarded to Dr. Antonio Ostrensky, for the project financing (process Nº 381091/2014-7) and the granting of a master's degree to Camila Tavares (process Nº 132835/2015-0).

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How to cite

TAVARES CPS, DA SILVA UAT, PEREIRA LA & OSTRENSKY A. 2021. Evaluation of different induced molting methods in *Callinectes Ornatus* (Crustacea, Decapoda, Portunidae) as a tool for the commercial production of soft-shell crabs. An Acad Bras Cienc 93: e20190580. DOI 10.1590/0001-3765202120190580.

Manuscript received on May 22, 2019; accepted for publication on October 23, 2019

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Author contributions

The study describes a 2-year study based on the master's project of Camila Prestes dos Santos Tavares, who was the main contributor to the conception and design of the work, data collection, data analysis and interpretation and drafting the article. The study was conducted under the supervision of Dr Antonio Ostrensky, who participated in the planning of the work, data analysis and critical revision of the article. The co-authors include two specialists in crustacean aquaculture. Dr Leandro Ângelo Pereira is an expert in environmental management and aquaculture, who contributed to the acquisition and interpretation of data, drafting the article and revising it critically for important intellectual content. Dr Ubiratã de Assis Teixeira da Silva is an expert in brachyuran crabs aquaculture and fishery extension, who participated in interpretation of data, drafting the article and revising it critically for important intelectual content. All authors participated in the final approval of the version to be published.