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Research paper

Temperature-driven changes in the neuroendocrine axis of the blue crab *Callinectes sapidus* during the molt cycle

Daniela Dantas David [a], Giovanna Zanetti [a], José Araújo Souto-Neto [a], Cristhian David Sua-Cespedes [a], José Thalles Lacerda [a], Ana Maria de Lauro Castrucci [a b]

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Highlights

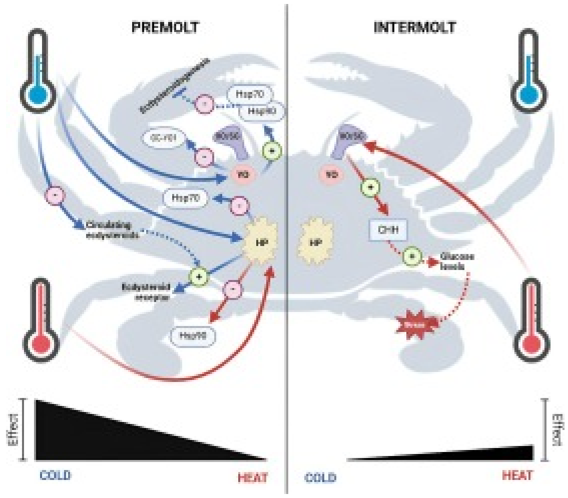
- Premolt blue crabs exhibited a larger response (reduction) in ecdysteroid concentrations at 19°C than the intermolt animals;
- The premolt Y-organ showed an expressive reduction of the ecdysteroid receptor *CasGC-YO1* transcripts compared to the intermolt animals at 19°C;
- The XO-SG displayed higher ecdysteroid receptor expression at all three temperatures in the intermolt animals compared to premolt crabs, whereas in the hepatopancreas there was an increase in the receptor transcripts only at 19°C.

- Both heat shock proteins show increased gene expression in the Y-organ of premolt crabs subjected to 19 °C.
- In the XO/SG, *CasMIH* transcripts were not affected by molt stage or temperature while *CasCHH* expression increased in the XO/SG of intermolt animals subjected to the warmer temperature compared to 19 °C and to premolt crabs at the same temperature.

Abstract

Environmental cues such as temperature induce macroscopic changes in the molting cycle of crustaceans, however, the physiological mechanisms behind these changes remain unclearWe aimed to investigate the regulatory mechanisms in the intermolt and premolt stages of the *Callinectes sapidus* molt cycle in response to thermal stimuli. The concentration of ecdysteroids and lipids in the hemolymph, and the expression of heat shock proteins (HSPs) and molt key genes were assessed at 19 °C, 24 °C and 29 °C. The premolt animals exhibited a much larger response to the colder temperature than intermolt animals. Ecdysteroids decreased drastically in premolt animals, whereas the expression of their hepatopancreas receptor (*CasEcR*) increased, possibly compensating for the low hemolymphatic levels at 19 °C. This decrease might be due to increased HSPs and inhibited ecdysteroidogenesis in the Y-organ. In addition, the molting-inhibiting hormone expression in the X-organ/sinus gland (XO/SG) remained constant between temperatures and stages, suggesting it is constitutive in this species. Lipid concentration in the hemolymph, and the expression of *CasEcR* and *CasHSP90* in the XO/SG were influenced by the molting stage, not temperature. On the other hand, the expression of HSPs in the hepatopancreas is the result of the interaction between the two factors evaluated in the study. Our results demonstrated that temperature is an effective modulator of responses related to the molting cycle at the endocrine level and that temperature below the control condition caused a greater effect on the evaluated responses compared to the thermostable condition, especially when the animal was in the premolt stage.

Graphical abstract



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Introduction

The blue crab, *Callinectes sapidus*, plays a pivotal role in estuarine ecosystems and significantly contributes to the economy, with the crab fisheries producing over 73 thousand tons and yielding approximately 220 million dollars in 2015 (NMFS, 2017). This species has been extensively studied to understand the molt cycle and the neuroendocrine regulation of these and other physiological processes (Freeman et al., 1987, Hines et al., 1987, Havens and Mcconaugha, 1990, Chung, 2010, Chen and Watson, 2011, Chen et al., 2012, Techa and Chung, 2013, Techa and Chung, 2015, David et al., 2022).

Central to the life cycle of the blue crab is its complex molt cycle, which is essential for growth and reproduction. The molt cycle is subdivided into five distinct stages: premolt, ecdysis, early postmolt, late postmolt, and intermolt, each characterized by specific physiological and morphological changes. After completing the reproductive cycle, mature females enter the anecdysis, absence of molt (Diez & Lovrich, 2013), because of Y-organ (YO) degeneration, thus ceasing the molt-inducing hormone, ecdysteroids, synthesis and increasing the molt inhibiting hormone production (Jivoff et al., 2007).

At the molecular level, ecdysteroids produced by the Y-organ (YO) are pivotal in controlling the molting cycle, as documented by Skinner, 1985, Lachaise et al., 1993, and Mykles (2011). The function of these steroid hormones is to induce the ecdysis through the interaction with the ecdysteroid receptor EcR which has 4 isoforms (*CasEcR1, CasEcR1a, CasEcR2* e *CasEcR2a* in *C. sapidus*. This process is intricately coordinated with the molt inhibiting hormone (MIH), which is produced by a cluster of neurons in the eyestalk known as the X-organ (XO).

The axons from the X-organ terminate in the sinus gland (SG), a neuroendocrine depot, thus meticulously regulating the molting cycle and inhibiting the ecdysis (Techa & Chung, 2013). One of the candidates for the MIH receptor in the Y-organ of *C. sapidus* is a membrane guanylyl cyclase, GC-YO1, which is highly expressed in the intermolt (Zeng et al., 2008), although other types of receptors for this hormone have been studied Das et al., 2018, Mykles and Chang, 2020, Mykles, 2021).

The biosynthetic pathway of ecdysteroids initiates with the conversion of diet cholesterol in 5-beta-diketol which is then converted into several ecdysteroid precursors, among which ecdysone and 3-dehydroecdysone are the major YO products in decapod crustaceans (Mykles, 2011). The hemolymph ecdysteroid concentration and type may vary according to the species (Mykles, 2011), sex (Cuzin-Roudy et al., 1989, Tamone et al., 2005), life stages (Wilder et al., 1990, Chung, 2010), and stages and sub-stages of the molt cycle (Snyder and Chang, 1991b, Snyder and Chang, 1991a, Martin-Creuzburg et al., 2007). YO becomes active at the beginning of the premolt, with hypertrophy and an increase in its synthesizing capacity, and the cycle closes at the end of the premolt with the repression of YO, and consequent reduction of ecdysteroid synthesis (Mykles & Chang, 2020).

In addition to MIH, XO synthesizes other peptides related to chromatophore and retina pigment migration, carbohydrate metabolism, reproduction, molt and growth, osmotic and ionic balance, and heart activity (Kleinholz, 1976). Among these peptides, the crustacean hyperglycemic hormone, CHH, regulates carbohydrate metabolism (Santos & Keller, 1993), stress-induced hyperglycemia (Webster, 1996, Chang et al., 1998, Lorenzon et al., 2004), and inhibits ecdysteroid synthesis (Webster and Keller, 1986, Chung and Webster, 2003).

In fact, metabolic processes and the molting cycle share a complex interplay. Throughout the molt cycle, during periods of food deprivation or vitellogenesis, energy reserves, including lipids and glycogen, are metabolized by the predominant hepatopancreatic cell type, the R cells (Al-Mohanna and Nott, 1989, Vogt et al., 1989, Cervellione et al., 2017). Furthermore, the hepatopancreas plays a vital role in reproduction by secreting vitellogenin, a precursor to yolk in certain species (Jayasankar et al., 2002, Jayasankar et al., 2020).

Environmental factors, notably temperature, significantly impact the frequency, period, and increment of the molt cycle (Leffler, 1972, Cunningham and Darnell, 2015). Both ecdysteroid-related molecules as well as CHH and MIH signaling are sensitive to temperature variations, influencing the molting cycle's regulatory mechanisms (Wittmann et al., 2018, Andersen et al., 2022, Gong et al., 2015). Another group of molecules that are thermosensitive are the heat shock proteins (HSP) and the constitutive or cognate heat shock family (HSC). They have been related to gene expression changes in response to thermal stress since they interact with elements in the promoter regions of a variety of genes (Roberts et al., 2010). In crustaceans, in addition to being responsive to temperature variations, these proteins have been reported to respond to hypoxia (Guan et al., 2021) and to osmotic stress (Chang, 2005), to regulate vitellogenin transcription (Liang et al., 2020), in and to associate with the molting cycle (Spees et al., 2003, Cesar and Yang, 2007).

The critical temperature range for the blue crab suggests a delicate balance between physiological needs and environmental constraints. García-Rueda and coworkers (2021) demonstrated that the critical temperature (CT) for the species is 42 °C as maximal and 11 °C as minimal, and that its thermal preference range lies between 26 °C and 33 °C in the Southern hemisphere, although its global optimal thermal window is narrowed to 22 °C–23 °C (Marchessaux et al., 2022).

Accordingly, elucidating the physiological adaptations of *C. sapidus* to varying temperatures is critical for advancing our comprehension of temperature's role in the pivotal stages of the molting cycle. This understanding also sheds light on environmental factors essential for the efficient cultivation of blue crabs. The impact that temperature has on the macroscopic parameters of the molting cycle is evident and well-studied. This study aimed to understand the underlying mechanisms responsible for these changes. To achieve this goal, we measured ecdysteroid and lipid hemolymph levels (specifically cholesterol and triglycerides) and examined gene expression related to molt pathways within the neuroendocrine axis (*CasCHH*, *CasMIH*, *CasEcRs*, *CasCG-YO1*), as well as heat shock proteins (*CasHSP70* and *CasHSP90*), in the hepatopancreas, XO/SG tissue, and YO tissue of *C. sapidus* throughout the intermolt and premolt periods under a variety of thermal environments.

Section snippets

Animals and sample collection

Male specimens of *C. sapidus* (average weight 61.58 ± 19.68 g and average carapace width 9.15 ± 0.77 cm) were collected in Iguape, São Paulo, transported to the Laboratory of

Comparative Physiology in the Institute of Biosciences of the University of São Paulo, and maintained in 10 L tanks with artificial seawater, salinity of 10‰, 24°C, photoperiod regimen 12h:12h (LD, lights on at 8 AM). Light intensity (White LED 3528, 420–750nm, waterproof, 88 lights, Brazil) was kept at 100lx,…

Ecdysteroid and metabolite hemolymph levels

The ecdysteroid, the major hormone responsible for ecdysis, and its precursor, cholesterol, as well as the triglycerides, were compared between molt stages and temperatures. Considering the difference between initial and final concentration after the temperature stimuli, in intermolt crabs, the effect was significant only at 24°C, where there was a reduction of hormone level after the experimental period. In the premolt animals, the lower temperature elicited a reduction whereas at 24°C and…

Discussion

We investigated the effect of temperature on the neuroendocrine system components – XO-SG, Y-organ, and hepatopancreas – associated with the molt cycle of male blue crabs, *Callinectes sapidus*. It is well established in the literature that the circulating ecdysteroid levels throughout the molt cycle are powerful markers of the ecdysis timing (Quackenbush, 1986, Chang, 1995, Chang and Mykles, 2011), and for some species even of the stage and substage of the cycle (Skinner, 1962, Spindler and…

Conclusions

This study brought new insights into the thermal effects on the molt neuroendocrine axis of the blue crab C*allinectes sapidus*. Our findings reveal distinct temperature-dependent patterns in the circulating levels of ecdysteroids, cholesterol, triglycerides, and expression of molt-related genes in two stages. The delay usually seen in ecdysis at cold temperatures may be explained by the reduction in ecdysteroid concentration observed in our results. As to heat shock proteins, their expression is …

Author contributions

DD David, JA Souto-Neto and AML Castrucci conceived and designed the study; DD David, G Zanetti collected the data; D David, G Zanetti, CD Sua-Cespedes, JT Lacerda analyzed the data; DD David contributed new methods; AML Castrucci acquired funds and supervised the study; DD David wrote the paper and all coauthors contributed with criticisms and reviewed the manuscript.…

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CRediT authorship contribution statement

Daniela Dantas David: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giovanna Zanetti:** Writing – review & editing, Formal analysis, Data curation. **José Araújo Souto-Neto:** Writing – review & editing, Conceptualization. **Cristhian David Sua-Cespedes:** Writing – review & editing, Formal analysis. **José Thalles Lacerda:** Writing – review & editing, Formal analysis. **Ana Maria de Lauro Castrucci:** Writing –…

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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