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Reproductive performance of *Octopus maya* males conditioned by thermal stress

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ABSTRACT

Observations of wild male *O. maya* suggest that temperatures below 27 °C favour their reproductive performance. From these observations we hypothesize that, as in females, the temperature modulates the reproductive performance of adult *O. maya* males. The study aimed to evaluate the physiological condition, reproductive success, and histological damage in testis of male *O. maya* exposed to thermal stress, to determine the implications of ocean warming over their reproductive performance. High temperatures (28–30 °C) negatively affect the growth and health of male *O. maya*. In octopuses maintained at 30 °C, as a consequence of the thermal stress we observed an increment in the haemocytes number, a reduction in the oxygen consumption rate, and an inflammatory process in the testis. The number of spermatozoa per spermatophore was not affected by temperature, but higher spermatophores production was observed at 30 °C. The paternity analysis showed that the offspring had multiple paternity with an average of 10 males contributing in a single spawn. The paternal contribution was affected by temperature with high, medium, or no paternal contribution in animals maintained at 24 °C (control group), 28 °C and 30 °C, respectively. The temperatures from 28 °C to 30 °C deeply affected the reproductive performance of *Octopus maya* males, suggesting that, as embryos, reproductive performance of adult males of this octopus species can be used as a tool for monitoring thermal changes in Yucatán Peninsula, located at the entrance of Gulf of Mexico.

1. Introduction

Aquatic environments are thermally heterogeneous in time and space. Organisms inhabiting these environments, specifically ectotherm organisms, show morphological, behavioural and physiological mechanisms (phenotypic plasticity) that give them adaptive capabilities to cope with environmental changes (Bozinovic and Pörtner, 2015; Deutsch et al., 2015; Piasečná et al., 2015; Pigliucci, 1996; Somero, 2010) Animal physiology, ecology, and evolution are affected by temperature and it is also expected that community structure will be

Abbreviations: ASP, Percentage of alive spermatozoa; B, Breeders; BW, Octopus total body weight; DF, Dilution factor; DGI, Digestive gland index; DGW, Digestive gland weight; DO, Dissolved oxygen; DS, Differentiation stratum; Ɛ, Extinction coefficient; F, Water flow rate; F_{IS}, Inbreeding coefficient; GSI, Gonadosomatic index; Hc, Hemocyanin concentration; H_o/H_e, Observed/Expected heterozygosity; hOp, Hemolymph osmotic pressure; H_{W-E}, Hardy–Weinberg equilibrium; L/D, Light/Dark; Na, Allele number; O, Offsprings; *O. maya*, *Octopus maya*; $O2_i/O_{2o}$, Oxygen concentration of the water inlet/outlet; OP, Osmotic pressure; OsmC, Osmoregulatory capacity; PS, Proliferative stratum; PVC, Polyvinyl carbonate; SCI, Spermatophoric complex index; SCW, Spermatophoric complex weight; SGC, Strata of germ cells; SGR, Specific growth rate; ST, Seminiferous tubules; STN, Spermatophores total number; TASC, Total number of alive spermatozoa; THC, Total haemocytes count; TSC, Total Number of spermatozoa; TW, Testis weight; VO_2, Oxygen consumption; WG, Weight gain; Wi/Wf, Initial/Final weight; wOp, Water osmotic pressure; ww, Wet weight; YP, Yucatan Peninsula

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strongly influenced by global warming (Nguyen et al., 2011). For example, temperature seemed to play the most important role in structuring the distribution of cephalopod body size along the continental shelves of the Atlantic Ocean (Rosa et al., 2012).

In the eastern region of the continental shelf of Yucatan Peninsula (YP), Mexico, a summer upwelling allows sub-superficial subtropical water from the Caribbean (between 150 and 200 m deep) to enter the shelf with temperatures between 16 °C and 22 °C (Enriquez et al., 2013). This cold water mass, besides functioning as an external temperature control for the shelf, transports nutrients which are used by primary producers (Enriquez et al., 2010). This upwelling affects only the eastern portion of the YP continental shelf provoking a summer thermal gradient that runs from the western to the eastern shelf from high to low temperatures, offering different environments to aquatic species of the zone (Zavala-Hidalgo et al., 2006, 2003; Ciencias de la atmósfera, http://uniatmos.atmosfera.unam.mx/ACDM/).

Octopus maya is endemic to the YP continental shelf and the most important octopus fishery in the American continent, with an annual production fluctuating between 8000 and 20,000 Tons (Galindo-Cortés et al., 2014; Gamboa-Álvarez et al., 2015; Markaida et al., 2016; SAGARPA, 2013). *O. maya* as an ectotherm organism is particularly temperature-sensitive (Noyola et al., 2013a,b) that can be affected in its morphology, behaviour, physiology and reproduction by changes in ambient temperature with spatio-temporal fluctuations (Avila-Poveda et al., 2015). Predictions of the thermal processes on the YP shelf indicate that sea temperatures may rise between 2.5 and 3 °C in the zone where upwelling has no effect (Enriquez et al., 2013; Saldívar-Lucio et al., 2015). Gamboa-Álvarez et al. (2015) observed that during the August-December fishing season, the greatest abundances of *O. maya* was found along the Campeche coast (western zone, without upwelling influence), where small octopus were fished; whereas, in the eastern zone, less abundances were recorded, but octopus with higher biomass were caught.

In laboratory conditions, at 31 °C the spawning of female *O. maya* was significantly reduced and only 13% of the total females (n = 32) spawned, while the few fertilized eggs (embryos) were not developed or died after two weeks (Juárez et al., 2015). It was observed that females exposed to a temperature decrease of 1 °C every 5 days and starting at 31 °C, only 87% spawned after temperatures reached less than 27 °C, and of these only 50% of the eggs laid (mean 530 eggs per spawn) were fertilized (Juárez et al., 2015). Those results suggested that temperature could be deleterious to sperm stored in the spermathecae of the oviductal glands, which play a crucial role in octopus reproduction (Olivares et al., 2017). At a later date, Juárez et al. (2016) found that juveniles performance from stressed females had lower growth rate and twice the metabolic rate than hatchlings coming from unstressed females, providing evidence that temperature stress experienced by females has consequences on the performance of hatchlings. Taking into consideration that *O. maya* wild population could be affected in summer when the benthic temperatures reach 30 °C, Angeles-Gonzalez et al. (2017) pustuled the hypothesis that that thermal condition causes migration of octopuses from western to eastern zone of the Yucatan Peninsula (YP) where upwelling events limit temperature increase. That migration was used to explain why reproduction occurs all the year in the eastern zone while in the western zone of YP only occurs in winter when temperatures are low (22–25 °C). When was analysed the multilocus microsatellite genotypes of wild *O. maya* across its distribution area to find out if the population is structured, and if the structure matches the mentioned thermal zones, was find that there is significant genic differentiation in the *O. maya* population that match with the two different thermal zones where *O. maya* is distributed. That results suggested that thermal differences between zones is the responsible of such genic structure differences (Juárez et al., 2018), suggesting that if these two subpopulations differ in features such as reproductive season, it is necessary to adjust management policies to the different population dynamics in each region to improve fishery productivity (Juárez et al., 2018).

To date, a small number of studies have investigated multiple paternity within cephalopods by using microsatellite markers demonstrating that multiple paternity could be a common characteristic in octopus species. Diverse studies have found at least two to four genetically distinct sires involved in the contribution to the progeny in *Graneledone boreopacifica*, *O. vulgaris* and *Euprymna tasmanica* (Voight and Feldheim, 2009; Quinteiro et al., 2011; Squires et al., 2014).

There is enough evidence demonstrating that temperatures higher than 27 °C have serious consequences on the reproductive performance and success of female *O. maya*. In this sense, new questions arise: As was observed in females, is 27 °C a thermal threshold for reproductive performance of *O. maya* males? Do *O. maya* males have the physiological mechanisms that allow them to compensate possible damages at temperatures higher than 27 °C? To address these questions, we designed a series of experiments to evaluate the effects of fixed temperatures (24 °C, 28 °C and 30 °C) on adult males of *O. maya* through assessment of their: i) Physiological condition, evaluating the specific growth rate, weight gain, digestive gland index, blood haemocytes and hemocyanin concentration, osmotic capacity and oxygen consumption; ii) Reproductive performance, evaluated through sperm quality and its relationship with histological characteristics of the testis, and iii) Reproductive success, estimated through the proportion of hatchlings generated by each male in each spawning. Wild adult females were mated with laboratory stressed males. Considering that multiple paternity can be present in *O. maya,* a paternity analysis implementing specific microsatellite markers was performed to assess the reproductive success of the experimental males.

To our knowledge, this is the first work that investigates the chronic thermal effect in the reproductive performance and success of male octopuses.

2. Material and methods

2.1. Ethics statement

In this study, octopuses were anesthetized with ethanol 3% in seawater at experimental temperatures (Estefanell et al., 2011; Gleadall, 2013) to induce narcotisation to enable humane killing (Andrews et al., 2013) in consideration of ethical protocols (Mather and Anderson, 2007), and the animaĺs welfare during manipulations (Moltschaniwskyj et al., 2007). Our protocols were approved by the experimental Animal Ethics Committee of the Faculty of Chemistry at Universidad Nacional Autónoma de México (Permit number: Oficio/FQ/CICUAL/099/15). We encouraged the effort to minimize animals stress and the killing of the minimum necessary number of animals for this study.

2.2. Animal capture and laboratory conditioning

Seventy-two wild *O. maya* adult males with body weight above 400 g were captured in the Sisal coast of the Yucatan Peninsula (21°9′55″N, 90°1′50″W), by using the local drift-fishing method known as "Gareteo" (Pascual et al., 2011; Solís-Ramírez, 1967). Male octopuses were caught during three collection trips from June to September of 2015. All males were anatomically mature with a well-developed reproductive system (Avila-Poveda et al., 2016). Octopuses were maintained in a 400-L black circular tank with seawater recirculation and exchange during the capture and then transported to the Experimental Cephalopod Production Unit at the Multidisciplinary Unit for Teaching and Research (UMDI-UNAM), Sisal, Yucatan, Mexico. Octopuses were acclimated for 10 d in 6 m diameter outdoor ponds provided with aerated natural seawater (26 ± 1 °C). The ponds were covered with black mesh reducing direct sunlight to 70%, and connected to seawater recirculation systems coupled to protein skimmers and 50 µmb bag filters. PVC 50 mm diameter open tubes were offered as refuges in proportion 2:1 per animal. Octopuses were fed individually