湿度对黄喉拟水龟胚胎发育与新生幼体特征的影响

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摘要:黄喉拟水龟(*Mauremys mutica* Cantor)为东亚常见的淡水水生龟类之一,主要分布于中国、日本和越南。在中国,黄喉拟水龟已成为潜在的水产养殖品种,研究了湿度对黄喉拟水龟胚胎发育和新生幼体的影响。恒温 29℃时,用未受精卵做对比,在 -12、-150kPa 和 -300kPa 3 种孵化湿度下,观察并记录了黄喉拟水龟胚胎发育过程中卵重的变化、孵化周期、孵化成功率、卵壳龟裂率、卵壳受精斑变化规律及新生幼体的特征。在 -12kPa 和 -150kPa 处理组,受精卵在孵化中期开始持续增重;而在 -300kPa处理组,受精卵在孵化中期开始持续减轻;孵化湿度显著影响卵壳受精斑的变化,-12kPa 处理组的受精斑绕卵短径合拢的时间显著长于 -150kPa 和 -300kPa 处理组;受精卵的卵壳龟裂率随着孵化湿度的增加而增加;湿度显著影响新生幼体体重和背甲宽,但对体高、背甲长、腹甲长、腹甲宽以及尾长无明显影响;孵化湿度对孵化周期及新生幼体的运动能力影响不明显。

关键词:黄喉拟水龟;湿度;胚胎发育;新生幼体

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Effects of different hydric environment on embryonic development and hatchling traits of yellow pond turtle (*Mauremys mutica* Cantor)

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Abstract: The Asian yellow pond turtle, *Mauremys mutica* Cantor, is a member of the family *Bataguridae*, native in China, Japan and Vietnam. It has become a potential aquaculture species and its husbandry and management have been a focus of aquaculture practice recently in China. One mature female turtle lays only 2.8 eggs on average per clutch and has only 2.1 nests on average per year. Therefore, it is very important to determine how to artificially incubate those eggs under optimal incubation conditions to achieve best results for aquaculture. Yellow pond turtle does not protect its eggs and leaves the embryo development of the turtles to the mercy of environment where eggs are laid. Hydric environment is an important ecological factor, and incubation under hydric environment has been proved to have profound effects on embryonic development and husbandry outcomes. In this paper, the effects of different hydric environment on embryonic development of fertilized eggs and hatchling traits of yellow pond turtle have been studied in comparison to unfertilized eggs. The eggs were incubated in wet (-12kPa), humid (-150kPa) and dry (-300kPa) conditions at constant temperature of 29℃, data on the egg mass change, embryonic development duration, hatching success ratio, egg shell crack ratio, egg-shell white ring change and hatchling morphological traits were collected and analyzed. Eggs mass increased from the middle to the end of incubation period under the wet (-12kPa) and humid (-150kPa) conditions, whereas eggs mass for those

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incubated under the dry (−300kPa) condition decreased. The hydric environment significantly affected white ring change on the eggshell. Under the wet (−12kPa) condition, white ring folding was significantly slower than that under the humid (−150kPa) and dry (−300kPa) conditions. The ratio of fertilized egg shell crack was positively correlated to the hydric environment. The effects of different hydric environment on body weight and carapace width of hatchling were significant, but were not significant on body height, carapace length, plastron length, plastron width, and trail length of hatchling. The effects of different hydric environment on duration of embryonic development and hatchling moving were not significant. The results confirm that appropriate moisture is important for the incubation management and the optimal incubation conditions for eggs of Asian yellow pond turtle are from −12kPa to −150kPa for aquaculture.

Key Words: *Mauremys mutica*; humidity; embryonic development; hatchling

黄喉拟水龟(*Mauremys mutica* Cantor)隶属龟鳖目(Testudinate),龟科(Bataguridae),拟水龟属(*Mauremys*),为东亚常见的淡水水生龟类之一,主要分布于中国、越南和日本。在中国,黄喉拟水龟已成为潜在的水产养殖品种之一,但黄喉拟水龟的繁殖力较低,其性成熟需要 5a,而 1 只成熟母龟平均 1a 仅产 2.1 窝卵,每窝 2.8 枚卵[1,2],这些特性限制了该养殖产业的发展。因此如何在有限产卵量的基础上提高孵化成功率及提高新生幼体的质量,是生产中急需解决的问题。由于黄喉拟水龟没有护卵及孵卵行为,所以其胚胎发育是否正常与外界生态因子有着密切的联系。温度显著影响黄喉拟水龟胚胎发育速度、成活率和性别[3,4],湿度对黄喉拟水龟胚胎发育和新生幼体的影响如何,迄今尚未见报道。在其他龟鳖物种中,关于湿度的影响已有一些研究。龟鳖卵在孵化过程中重量会有变化,这取决于卵与孵化环境间的水分交换,从而会影响胚胎发育的卵内水环境[5]。卵与孵化环境间水分交换受卵自身的性质,如卵表面积大小、卵内溶质含量、卵内渗透活性物质含量、卵类型和卵壳通透性等,以及胚胎发育阶段与代谢速率和外部孵化环境性质,如孵化温度、孵化介质湿度和卵与介质的接触程度等因素的影响[6~9]。然而卵内水环境对胚胎发育速率、孵化成活率和新生幼体特征是否有精细影响,迄今为止并无统一的看法。以往孵化湿度对龟鳖类卵孵化影响的研究表明,不同物种的卵对湿度做出的反应不同,同物种卵在不同实验条件下对湿度做出的反应也不一样[8,10~13]。因此,我们研究了湿度对黄喉拟水龟胚胎发育和新生幼体的影响,以期为龟鳖的繁殖生态学积累科学数据,同时为黄喉拟水龟的生产实践提供借鉴。

1　材料和方法

试验用黄喉拟水龟卵取自位于广州的中国水产科学研究院珠江水产研究所。所有试验卵均于产卵当日收集,经可孵性鉴别(有无乳白色受精斑)后,称重、测量和编号,移入长 × 宽 × 高规格为 315 mm × 240mm × 125mm 内含蛭石的塑料孵化盒中。受精斑朝上,卵埋深度 2 ~ 3cm。蛭石湿度设置 3 个梯度,分别为 −300、−150kPa和 −12kPa,由干蛭石∶水以 1∶0.44、1∶0.89 和 1∶2 配成。每个湿度处理设一平行组,共 6 个孵化盒,每个孵化盒内埋入 20 枚受精卵和 5 枚未受精卵。孵化盒口覆盖穿孔塑料薄膜,并放于(29 ± 0.5)℃生化培养箱内孵化。孵化过程由专人负责管理,每天定时称量每个孵化盒的总重量,依据原始总重量确定添水量,每隔 5d 称卵重并彻底换一次与原始湿度相同的孵化介质,确保孵化湿度基本恒定。

孵化期为卵入孵时间与孵出时间的间隔。在幼体破壳前统计卵壳龟裂率,本文中卵壳龟裂是指在孵化中后期,卵吸水膨胀,导致卵壳钙质层产生细丝状裂纹,而卵膜并不破裂。幼体孵出后即被收集,用 JJ100 型精密电子天平(常熟双杰测试仪器厂)称重,用游标卡尺(上海台海工量具有限公司)测量背甲长、背甲宽、腹甲长、腹甲宽、体高、尾长,隔日测定幼体的运动表现。测定幼体的运动表现前预先将幼体置于室温下的水体中适应 0.5h,在总长为 1500mm 的直泳道内进行测定,泳道内摆放刻度为 1000mm 的塑料直尺(华杰文具),水深 50mm 左右。测定时由专人用手指轻触幼体尾尖驱赶幼体,另一人用数码摄像机(SONY DSCT-100)记录幼体运动过程,随后用 Media Player Classic 软件分析行为录象。用静止不动个体数和游完 1000mm 的时间这两项指标衡量幼体的运动表现。

所有数据在作进一步统计分析前,用 Kolmogorov-Smirnov 和 Levene 分别检验正态性和方差的同质性。用 X^2 检验比较不同孵化湿度条件下的孵化成功率和卵壳龟裂率。用线性相关、方差分析(ANOVA)、协方差分析(ANCOVA)和 Post-hoc 比较(Tukey's 检验)等处理分析相应的数据。文中涉及的 ANCOVA 分析均以初始卵重为协变量,描述性统计值用平均值 ± 标准误表示,显著水平设置为 $a = 0.05$。

2 结果

2.1 孵化湿度对受精卵与未受精卵卵重的影响

本试验中黄喉拟水龟受精卵在 3 个湿度组中的初始重量分别是:(17.34 ± 0.34) g(−12kPa),(17.28 ± 0.32) g(−150 kPa),(17.30 ± 0.36) g(−300kPa);未受精卵是(17.18 ± 0.42) g(−12kPa),(17.17 ± 0.39) g(−150 kPa),(17.21 ± 0.34) g(−300kPa)。经统计分析无显著差异($F_{2,117} = 0.07$, $P = 0.93$; $F_{2,27} = 0.003$, $P = 0.997$)。孵化过程中,受精卵及未受精卵在 3 个湿度组中的重量变化见图 1 和图 2。−12kPa 条件下受精卵终末重量与初始重量差异极显著($F_{1,70} = 29.56$, $P < 0.001$),未受精卵则无显著差异($F_{1,17}$, $P = 0.705$);−150kPa 条件下受精卵及未受精卵的终末重量均与其初始重量的差异不显著($F_{1,74} = 3.879$, $P = 0.053$; $F_{1,17} = 2.908$, $P = 0.106$);−300kPa 条件下受精卵终末重量与初始重量无显著差异($F_{1,60} = 0.0797$, $P = 0.376$),未受精卵则差异极显著($F_{1,17} = 36.725$, $P < 0.001$)。因此,在孵化末期,−12kPa 条件下孵化的受精卵要显著重于 −150kPa 和 −300kPa 条件下的卵($F_{2,102} = 22.34$, $P < 0.001$);−300kPa 条件下孵化的未受精卵要显著轻于 −12kPa 和 −150kPa 的卵($F_{2,27} = 18.11$, $P < 0.001$)。

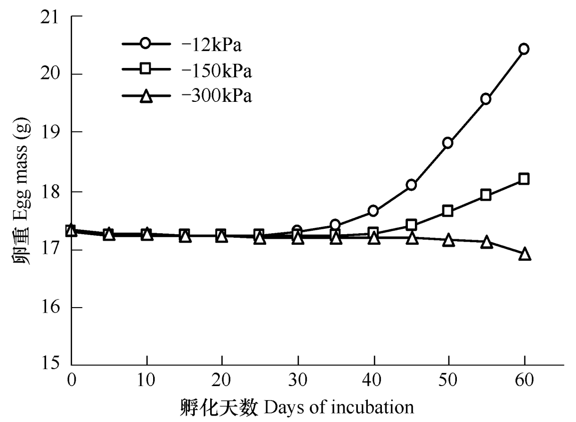

图 1 不同湿度黄喉拟水龟受精卵孵化期重量动态变化

Fig. 1 The variations of fertilized egg mass incubated in different humidity medium

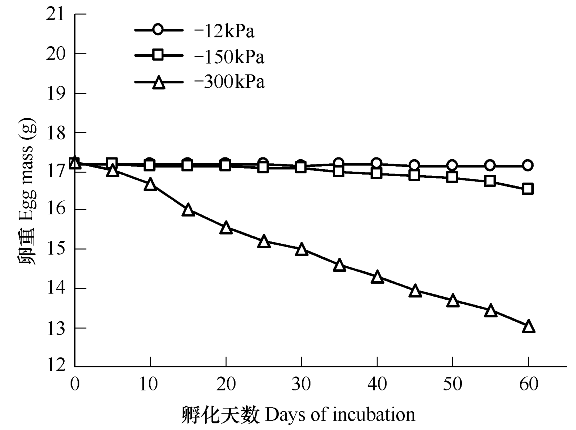

图 2 不同湿度黄喉拟水龟未受精卵孵化期重量动态变化

Fig. 2 The variations of unfertilized egg mass incubated in different humidity medium

2.2 湿度对孵化期、孵化成功率、卵壳龟裂以及受精斑变化的影响

黄喉拟水龟受精卵在 3 种孵化湿度下的孵化期、孵化成功率以及卵壳龟裂率的统计结果见表 1。黄喉拟水龟孵化期与卵初始重量无关($F_{1,103} = 0.93$, $P = 0.34$)。孵化湿度不影响黄喉拟水龟孵化期($F_{2,102} = 0.10$, $P = 0.90$),也不影响孵化成功率($X^2 = 1.977$, d$f = 2$, $P = 0.372$),但显著影响卵壳龟裂率($X^2 = 26.394$, d$f = 2$,

表 1 不同湿度下黄喉拟水龟卵的孵化期、孵化成功率以及卵壳龟裂率

Table 1 Incubation length, hatching success and shell crack ratio of *M. mutica* eggs incubated at different humidity medium

介质湿度(kPa) Medium moisture	卵 Eggs	孵化期 Incubation length (d)	卵壳龟裂率 Shell crack ratio (%)	孵化成功率 Hatching success (%)
−12	40	66.08 ± 0.40 ($n = 36$)	100 (36/36)	90 (36/40)
−150	40	65.87 ± 0.34 ($n = 38$)	73.68 (28/38)	95 (38/40)
−300	40	66.03 ± 0.31 ($n = 31$)	38.71 (12/31)	77.5 (31/40)

$P<0.001$)。3 种孵化湿度下受精卵的初始相对受精斑长度(白色环带长度/卵短径一周的长度)无差异($F_{2,27}=0.80, P=0.46$),但在 －12kPa 条件下,相对受精斑环带的宽度变化在孵化 1 ~ 13d 内明显受到抑制,孵化 13d 后其增长速率加快,在 －150kPa 和 －300kPa条件下未出现这种情况(图 3)。受精斑环带绕卵短径合拢的时间随着孵化湿度的增加而延长, －12kPa条件下受精斑环带合拢的时间显著长于 －150kPa和 －300kPa 条件下的卵($F_{2,27}=219.27, P<0.001$)。

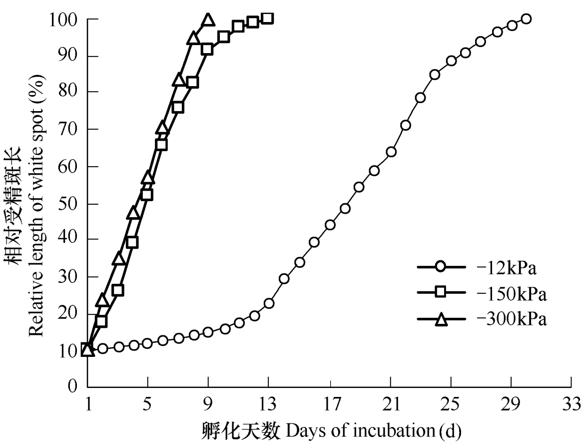

图 3　不同湿度下黄喉拟水龟卵相对白斑环带宽度的动态变化

Fig. 3　The variations of relative width white ring on *M. mutica* eggs incubated in different humidity medium

2.3　湿度对黄喉拟水龟新生幼体形态特征的影响

湿度对黄喉拟水龟新生幼体形态特征影响的结果见表 2。协方差分析表明,湿度显著影响新生幼体体重和背甲宽, －150kPa 条件下孵出的幼体较重、背甲宽较大; －12kPa 条件下孵出的幼体较轻、背甲宽较小;而 －300kPa条件下孵出的幼体最轻、背甲宽最小。但湿度对新生幼体体高、背甲长、腹甲长、腹甲宽以及尾长无明显影响。

表 2　湿度对黄喉拟水龟新生幼体形态特征的影响

Table 2　Effects of medium humidity on morphology of *M. Mutica* hatchlings

项目 Items	介质湿度 Medium humidity			
	－12kPa	－150kPa	－300kPa	ANCOVA
样本数 Samples	36	38	31	
体重 Body mass (g)	10.78 ± 0.17[ab] 7.42 ~ 12.28	11.06 ± 0.16[a] 8.63 ~ 12.80	10.42 ± 0.18[b] 7.77 ~ 12.09	$F_{2,101}=3.298$ $P<0.05$
体高 Body height (mm)	17.72 ± 0.12 16.16 ~ 19.30	17.88 ± 0.11 16.57 ~ 19.19	17.58 ± 0.15 16.21 ~ 19.54	$F_{2,101}=1.471$ $P=0.234$
背甲长 Carapace length (mm)	38.93 ± 0.23 36.29 ~ 41.56	39.32 ± 0.25 35.09 ~ 42.58	39.09 ± 0.29 36.28 ~ 42.56	$F_{2,101}=0.633$ $P=0.533$
背甲宽 Carapace width (mm)	32.11 ± 0.27[ab] 26.97 ~ 35.79	32.96 ± 0.22[a] 30.40 ~ 35.96	31.90 ± 0.35[b] 28.04 ~ 36.11	$F_{2,101}=4.158$ $P<0.05$
腹甲长 Plastron length (mm)	32.06 ± 0.22 27.29 ~ 34.24	32.24 ± 0.25 27.29 ~ 35.67	32.20 ± 0.27 27.88 ~ 35.74	$F_{2,101}=0.141$ $P=0.868$
腹甲宽 Plastron width (mm)	21.58 ± 0.24 18.15 ~ 24.24	21.81 ± 0.25 19.42 ~ 24.82	21.15 ± 0.23 17.88 ~ 24.58	$F_{2,101}=1.828$ $P=0.66$
尾长 Tail length (mm)	23.08 ± 0.36 18.53 ~ 27.66	23.27 ± 0.36 18.22 ~ 26.95	23.49 ± 0.28 19.55.41 ~ 27.52	$F_{2,101}=0.33$ $P=0.72$

2.4　湿度对黄喉拟水龟新生幼体运动表现的影响

在黄喉拟水龟新生幼体运动表现测定中, －12kPa 实验组有 22.2% (8/36)新生幼体、－150kPa 组有 13.2% (5/38)新生幼体、－300kPa 组有 22.6% (7/31)新生幼体在直泳道上静止不动,随后的运动表现统计分析未包含这些个体。方差分析表明:在孵化湿度为 －12、－150kPa 和 －300kPa 条件下孵出的黄喉拟水龟新生幼体游完 1000mm 所需时间为(23.21 ±1.31)s、(22.70 ±1.40)s 和(27.08 ±1.78)s,无显著差异($F_{2,82}=2.41, P=0.096$)。

3　讨论

黄喉拟水龟受精卵在高湿度环境下孵化会增重,在孵化中后期增重尤其明显,在低湿度环境下孵化卵重会减轻,这与其它一些龟鳖卵的研究结果是相似的[14~16]。在孵化过程中受精卵与环境间以水气或液态水的形式进行水分交换,而以液态形式交换的先决条件是卵必须和孵化介质直接接触[5]。本试验将整个卵埋入

介质中孵化,卵与介质有效接触面积大,有利于水分以液态的形式进行交换,这可能正是同在低湿度 -300kPa 孵化条件下,导致卵全埋介质孵化的黄喉拟水龟卵孵化后期减轻的重量比卵半埋介质孵化的乌龟(*Chinemys reevesii*)卵[16]孵化后期减轻的重量少得多的原因之一。

在孵化周期内,未受精卵在高湿度介质里,卵重相对稳定,在低湿度下卵重减轻明显。受精卵在同等低湿度下重量的减轻要比未受精卵少很多。受精卵与未受精卵表现的差异预示黄喉拟水龟的胚胎发育在卵重的保持及水分的吸收中起到一定的作用。Booth[13]指出卵从孵化介质中吸收水分是由胚胎作用诱导的,并从原肠胚后期起诱导作用。随着胚胎的发育,诱导作用增强,水分吸收增加,同时胚胎新陈代谢增强,热量产生增多,卵内水气压增加,从而使卵内水分以水气形式散失的量也增加[16]。正是在这两种作用力下,受精卵在孵化的过程中保持着胚胎发育所需水分的动态平衡。因而,在高湿度环境下孵化吸收水分的量大于散失的量时,表现为卵增重;在低湿度环境下孵化,吸收的量小于散失的量时,表现为卵减轻。

在高和低湿度环境下孵化,胚胎发育所处卵内水环境是不相同的。本研究结果显示,湿度对黄喉拟水龟孵化期、孵化成功率及运动表现影响微弱,与其它一些刚性卵的研究结果相似[5,13,14,16]。但湿度显著影响卵壳的龟裂率,湿度越高卵壳的龟裂率越高。卵壳龟裂一般发生在胚胎发育的中后期,卵吸水相对增加,导致卵膨胀,从而在卵的钙质层产生裂纹,而卵膜并不破裂,卵内液体不会流出,胚胎得以正常发育。从卵裂发生的部位来说,一般首先发生在卵的两侧,然后裂纹布满整个卵。卵裂程度与新生幼体的背甲宽和体重存在一定的相关关系,即卵壳龟裂程度大孵出的新生幼体背甲相对较宽体重相对较重[3]。

在龟鳖繁殖中,卵壳表面的乳白色受精斑不但可以作为判断卵是否受精的依据[18],受精斑的变化还可以作为预测胚胎发育进程的依据[3]。在特定环境条件下孵化,受精斑的变化会停止,这预示着胚胎发育进程被阻断。阻断的形式按胚胎发育进程阶段可以分为两种:(1)原肠胚期阻断,即受精卵在受精斑出现以前,将其置于特定环境下,比如在低温环境下孵化,受精斑不出现,胚胎发育阻断,去处这种特定环境后,受精斑出现,胚胎继续发育[19~21];(2)原肠胚后阻断,即受精斑已经出现,在孵化过程中遇到不良孵化条件时,比如长时间的暴雨或温度骤降,受精斑不再变化,胚胎发育阻断,当环境条件转好时,受精斑重新出现变化,胚胎继续发育[22]。本试验中,在 -12kPa 条件下相对受精斑环带的长度增长速率在孵化 1 ~ 13d 内明显受到抑制,可能原因是 -12kPa 这种湿度,对早期的胚胎发育有一定的影响,所以也导致其孵化成功率要低于 -150kPa 组。从本研究结果看,黄喉拟水龟卵孵化对湿度的适应范围还是挺大的,除了 -300kPa 条件下稍差一点外,当湿度范围为 -12kPa 到 -150kPa 时,胚胎发育,孵化期、成功率及幼体没有太大差异,均适宜在生产实践中应用。

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