**Chapter 4:** Understanding early life hypoxia effects on the Atlantic silverside (*Menidia menidia*) through Dynamic Energy Budget theory

**Methods**

*DEB Model Description*

To model the stage-specific energy budget of *M. menidia* in a way that would allow us to explain early-life hypoxia effects with bioenergetic processes, we used DEBkiss, a simplified and widely applicable DEB model (Jager et al., 2013; Jager, 2018). The full set of assumptions and equations can be found in Jager (2018). Briefly, the flux of food (*JX*) or, for embryos, the egg buffer (*WB*) is immediately converted to assimilates which are allocated to a somatic fraction (*κ*) and a reproductive fraction (1-*κ*; Figure 1); these fractions are constant throughout the life cycle. The assimilation flux (*JA*) is the product of the scaled food level (*f*), the volumetric surface area (*L2*), and the parameter maximum area-specific assimilation rate (*JaAm*):

For embryos (*WB* > 0) and under *ad libitum* feeding *f* = 1. The differential equation for change in egg buffer over time is –*JA*. Within the somatic branch, which does not change with life stage, a flux to maintenance (*JM*) is prioritized while the remainder goes to the flux for structure (*JV*) with a conversion efficiency *yVA*. The maintenance flux is the product of volume and the parameter for the volume-specific cost for maintenance (*JvM*):

The differential equation for growth is equal to *JV*. For juveniles, the non-somatic fraction of assimilates is spent on maturation, or increasing complexity through gonad development. Once the mass at puberty is reached (*WVp*), reproductive flux (*JR*) toward egg production begins in adults with a conversion efficiency *yBA*. Although *M. menidia* have a distinct larval and juvenile stage, both are treated as the juvenile stage because the relevant aspects of their energy budget for DEBkiss are identical. DEBkiss also uses an optional flux to maturity maintenance (*JJ*) that comes from the 1-*κ* fraction of assimilates (Jager, 2018), which we chose to use in our model.

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where *WV* is the structural mass, *R* is the continuous reproduction rate, and *WB0* is the initial egg mass. The equation for continuous reproduction gives the differential equation for egg production over time. Because the model equations use dry weight for body size and our growth data is total length, we calculated a shape correction coefficient (*δM*) and dry weight density (*dV*) to allow the model to convert between the two.

To address the assumption of DEBkiss that all eggs hatch when buffer is depleted, regardless of body size or developmental progress (Jager et al., 2013), we added a survival variable. In addition to allowing an alternative outcome to hatching, this allowed us to examine survival as a consequence of hypoxia effects on the energy budget. We fitted mortality parameters for embryos and post-hatch fish (*μemb* and *μlar*) to data for survival to hatching and larval/juvenile survival (Figure 1). In our implementation of survival, the only DEB process influencing survival is egg buffer depletion, which determines the time to hatch and thus when the embryo mortality rate switches to the post-hatch mortality rate. This means survival is indirectly affected by the assimilation rate and conversion efficiency of assimilates into structure. The differential equation for proportion surviving over time is:

DEBkiss uses fewer parameters than the standard DEB model, which reduces data requirements and the risk of overfitting. While the standard DEB formulation uses a state variable for maturity that triggers changes between life stages, DEBkiss instead uses a constant size at puberty to specify when reproduction is initiated (Kooijman, 2010; Jager et al., 2013). It also has no reserve compartment between food assimilation and allocation, and for embryos this means that the egg buffer is assimilated into body structure and for maintenance, with hatching occurring when the egg buffer is fully depleted, instead of following reserve dynamics of the standard DEB model (Jager et al., 2013). The lack of reserve makes DEBkiss well-suited for animals with a small ultimate body size because reserve plays a smaller role in such species under DEB theory (Nisbet et al., 2000), but DEBkiss has been successfully applied to larger animals as well (e.g. Desforges et al., 2017).

Diagram

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**Figure 1.** The DEBkiss model (diagram adapted from Jager et al., 2013) with stage-specific survival parameters. The candidate parameters for hypoxia stress mechanisms are highlighted in red boxes.

*Base Model and Data*

For the base model we calculated and estimated parameters based on four types of data (state variables): total length over time, egg buffer mass over time (and through this, time to hatching), cumulative egg production over time, and proportion surviving since fertilization over time. We estimated three parameters by fitting them to data (*yVA*, *μemb*, and *μ­lar*) and fixed at suggested values parameters for which we had insufficient data to calculate or estimate. The primary parameters and their calculated or estimated values are found in Table 1. Fitting was done in Matlab with the packages BYOM v.6.4 (Jager, 2022) and DEBkiss v.2.3a (Jager, 2021). BYOM uses a Nelder-Mead simplex search to optimize the parameters for a set of ordinary differential equations (ODEs) by minimizing negative log-likelihood (NLL). The DEBkiss package works under BYOM to bring in the DEBkiss model parameters, variables, and equations so that the parameters can be estimated based on their effect on the DEBkiss equations and the ODEs derived from them. The ODEs give the predicted data for each type of observed data (length, egg production, egg buffer mass, and survival over time) the difference between which is used to calculate NLL.

BYOM allows users to turn fitting on and off for each parameter, and with fitting turned off for all parameters it runs a simulation that calculates predicted values over time for each state variable using the initial parameter values. Before estimating any parameters with the optimization described above, we ran simulations with fitting turned off using a set of recommended parameters (Jager, 2018) and parameters obtained from existing data on *M. menidia*. We visually assessed fit and checked NLL as we adjusted parameters to obtain a reasonable set of initial parameters before estimating any. This also helped us reduce the number of parameters being estimated to avoid overfitting and so that there were not multiple correlated parameters free at once, because we were able to obtain a reasonable fit using suggested default values for *yAV*, *yBA*, and *κ*. The default value for *yVA­* did not allow a realistic fit to the length data, but the length, reproduction, and egg buffer depletion data allowed it to be estimated with the BYOM optimization. Ultimate length was used to fix *JaAm* to a reasonable value before estimating *yVA* because both parameters affect growth and egg buffer depletion in the model and therefore can not be estimated simultaneously. Finally, we fixed all parameters except *μemb* and *μlar* to estimate these parameters, again using the visually best-fitting parameters from the simulations as initial values. The full-life and early-life predicted and observed data are shown in Figure 2.

**Table 1.** DEBkiss parameters, their abbreviations, and their fixed or estimated values. Units are given with the value unless the parameter is a unitless ratio.

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Symbol** | **Fixed or estimated** | **Value** |
| Max. area-specific assimilation rate | *JaAm* | Estimated | 0.333 mg mm-2 d-1 |
| Max. volume-specific maintenance rate | *JvM* | Fixed | 0.0214 mg mm-3 d-1 |
| Initial egg weight | *WB0* | Fixed | 0.15 mg |
| Total length at puberty | *LVp* | Fixed | 102 mm |
| Yield of assimilates on volume | *yAV* | Fixed | 0.8 |
| Yield of egg buffer on assimilates | *yBA* | Fixed | 0.95 |
| Yield of structure on assimilates | *yVA* | Estimated | 0.3646 |
| Fraction of assimilates allocated to soma | *κ* | Fixed | 0.8 |
| Scaled food level | *f* | Fixed | 1 |
| Scaled food level for embryo | *fB* | Fixed | 1 |
| Half-saturation total length | *Lf* | Fixed | 0 |
| Mortality rate for embryos | *μemb* | Estimated | 0.06393 |
| Mortality rate for larvae | *μlar* | Estimated | 0.02940 |

Diagram

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**Figure 2.** Predicted (lines) and observed data (dots) for the base DEBkiss model of *M. menidia*. The state variables are (A) total length (mm) over time (days), (B) cumulative reproduction (eggs) over time (days), (C) egg buffer mass (mg) over time (days), and (D) survival over time (days). Predicted data lines are calculated with the parameter values listed in Table 1.

The length and reproductive data allowed us to calculate length at puberty (*LVp*), which in this model is the length at which egg production begins. We obtained *WB0* from *M. menidia* egg dry weight data (Klahre, 1997) and calculated *δM* and *dV* from total length, egg diameter, and egg mass data (Cross et al., 2019; Klahre, 1997; Concannon et al., 2021). To calculate volume-specific maintenance costs (*JvM*), we used data on the rate of decrease in larval dry weight over a period of starvation in the congeneric species *M. beryllina* (Letcher and Bengtson, 1993). Borrowing from closely related species is a common practice in bioenergetic modeling when the species has similar habitat, life history, and physiology, as is the case here (Sibly et al., 2013; Bengtson, 1984). All *M. menidia* datasets came from experiments in which fish were fed *ad libitum* so *f* was set to 1. For experiments that exposed fish to different CO2 levels, we only used data from control groups to avoid potential stressor effects in the data.

Data for the state variable total length were sourced from three studies. Length at hatching and 15 days post-hatching (dph) came from a study that reared *M. menidia* offspring in different static oxygen levels across two experiments (Cross et al., 2019). This provided data for control oxygen levels used in the base model and three reduced oxygen treatments (Table 2). The study featured two additional experiments that exposed offspring to fluctuating oxygen and CO2 levels but the control conditions were static, so we used total length data from these treatments for the base model as well (Cross et al., 2019). We sourced additional length data for the base model from control levels of experiments that exposed *M. menidia* offspring to ambient and elevated CO2 levels (Murray and Baumann, 2018; Murray and Baumann, 2020; Concannon et al., 2021). All total length data were obtained from fish maintained in static laboratory conditions at 24°C.

The state variable cumulative egg production over time was also obtained from control groups in Concannon et al. (2021), a study in which wild-caught juveniles were held in the laboratory at 20°C in different CO2 treatments and strip-spawned once they reached reproductive maturity. Data for the state variables on egg buffer mass (i.e. time to hatching when egg buffer mass is zero) and survival to hatching and 15 dph under different oxygen levels were obtained from Cross et al. (2019). We also used survival data from the 24°C and control CO2 groups of a study on the effects of temperature and CO2 on *M. menidia* early life survival (Murray and Baumann, 2018). Four additional data points for long-term survival in laboratory conditions at 17°C were obtained from a study that exposed *M. menidia* offspring until 122 dph to two CO2 levels, of which we only used data from the control level (Murray et al., 2017).

**Table 2.** The mean survival to hatching, hatch time (at which egg buffer is zero), length at hatching, length at 15 dph, and survival to 15 dph from the different oxygen treatments in Cross et al. (2019). The control DO level means (7.7 mg l-1) also include data from Murray and Baumann (2018).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **7.7 mg L-1** | **4.2 mg L-1** | **3.1 mg L-1** | **2.7 mg L-1** |
| Survival to hatching | 74.3% | 70.6% | 85.8% | 30.2% |
| Hatch time (egg buffer mass = 0) | 6 days | 7 days | 8 days | 9 days |
| Length at hatching | 5.3 mm | 4.6 mm | 4.4 mm | 4.1 mm |
| Larval length at 15 dph | 15.8 mm | 12.2 mm | 9.2 mm | - |
| Larval survival to 15 dph | 44.0% | 22.2% | 20.9% | 0% |

*Hypoxia Stress*

We multiplied several DEBkiss parameters (Figure 1) by correction factors to attempt to explain observed differences in *M. menidia* length, hatching, and survival between experimental oxygen treatments (Cross et al., 2019). To summarize the experimental data on static hypoxia effects we are attempting to explain by altering these parameters, the mean values of data for each oxygen treatment are listed in Table 2. We used the parameter values from the base model, which contained full lifespan data, and altered one or more parameters at a time with oxygen-dependent correction factors, then fitted the model to data for only the first 136 days by estimating the best value of the parameter *K* that influences correction factor shape. We only used early life data to fit the hypoxia-altered parameters because we did not have late-life data for multiple oxygen treatments later in life to validate observed changes against and did not have any reproduction data for oxygen treatments. It did not make sense to include later life data in the calculations of NLL that influence the parameter estimates or to speculate about how well the predicted data match what we might expect to happen later in life if we not only lack late-life hypoxia data but also do not expect full life hypoxia to occur in nature.

A primary correction factor (*c*) that decreased exponentially with decreasing DO between (Figure 3) was calculated as:

where *K* is the shape parameter that affects the strength of the DO effect on predicted values of the state variables. DO is the treatment level of oxygen, and DOc is the critical oxygen level below which the *c* = 0. The value of *c* cannot exceed 1 with this function. A larger *K* value keeps *c* higher as oxygen decreases before a more abrupt drop, while a smaller *K* gives a more constant decline in *c* with hypoxia (Figure 3). Attempts to estimate DOc and *K* simultaneously showed that leaving DOc free did not improve the ability of the correction factor to fit the hypoxia data. Instead, DOc was fixed at a biologically relevant level of 2.044 mg L-1, which is the critical oxygen level below which embryonic routine metabolism becomes highly oxygen-dependent (Schwemmer, unpublished data). This correction factor was multiplied by *JaAm* and *yVA* because these parameters were hypothesized to decrease under hypoxia. To alter the parameters hypothesized to increase under hypoxia (*JvM*, *μemb*, and *μlar*) a secondary correction factor, *c1*, was calculated from *c*:

where *c1(max)* is the upper limit to the correction factor, or a maximum factor by which we are willing to multiply the parameters. We set *c1(max)* = 10 because the value doesn’t affect the shape of the curve below the limit and only very low *K* values would lead *c1* to reach this level at the DO treatments of the data. The correction factor *c1* was multiplied by *JvM*, *μemb*, and *μlar* to increase them with decreasing DO.

To find the best value of *K* for each DEBkiss parameter or combination of parameters, we added *K* as a model parameter and estimated it using the BYOM optimization to minimize NLL. We used initial criteria to identify the candidate parameters for inclusion in the best fitting model. The initial criteria for a given DEBkiss parameter were 1) that altering the parameter must lead to a change in at least one state variable in the same direction as the observed effect of hypoxia, and 2) that the final best model must include parameter(s) that account for the changes in all three state variables for which low oxygen data exist. For example, *yBA* does not meet the first criterion because applying *c* to it has no effect on any of the state variables. *μemb* meets the first criterion because applying *c1* to it changes the survival state variable, but it does not change total length or egg buffer mass over time so a model with a correction factor for *μemb* alone does not meet criterion 2. Once we narrowed down the list of candidate parameters that met criterion 1 (summarized in Table 3 with examples in Figure 4) we estimated *K* and calculated AIC with a correction factor applied to each individual parameter and every combination of two, three, or four parameters. We did not apply the correction factor to *JaAm* and *yVA* simultaneously because they are multiplied together to obtain *JV* and their individual contributions to the growth and egg buffer depletion can not be fully separated. Although *κ* met criterion 1, we did not include it as a candidate because we lacked the reproductive data needed to model any potential changes in relative energy allocation under hypoxia. We also did not include *f* despite it meeting criterion 1 because feeding was *ad libitum* across all experiments. We compared the AIC between each model to determine which combination of parameters best fit the data while also meeting criterion 2 and, therefore, which DEB processes best explain the hypoxia effects observed in experiments (Table 4).

Diagram

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**Figure 3.** The effect of DO on correction factor *c* (A) at three different values of shape parameter *K*, and correction factor *c1* (B) as a function of *c*.

**Table 3.** Summary of impacts of altering each DEBkiss parameter on predicted data for total length, time to hatching (egg buffer mass = 0), and survival over time. We used this information to choose which parameters to which to apply hypoxia-based correction factors by identifying those that best meet our requirement of accounting for hypoxia effects on all three state variables. The last column indicates whether the effect of changing the parameter matches the overall patterns observed in the data (i.e. an increase or decrease in at least one state variable).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Impact on predicted values of: | | |  |
| Parameter | Hypothesized hypoxia effect on parameter | Total length (mm) | Time to hatching | Survival proportion | Initial criteria met? |
| *JaAm* | ↓ | ↓ | ↑ | ↓ | Yes |
| *JvM* | ↑ | ↓ | ↑ (weak) | ↓ (weak) | Yes |
| *WB0* | ↓ | none | none | none | No |
| *LVp* | ↓↑ | none | none | none | No |
| *yAV* | ↓ | none | none | none | No |
| *yBA* | ↓ | none | none | none | No |
| *yVA* | ↓ | ↓ | ↑ | ↓ | Yes |
| *κ* | ↓ | ↓ | ↑ | ↓ | Yes |
| *f* | ↓ | ↓ | ↑ | ↓ | Yes |
| *fB* | ↓ | ↓ (prehatch only) | ↑ | ↓ | No |
| *Lf* | ↓↑ | none | none | none | No |
| *μemb* | ↑ | none | none | ↓ | Yes |
| *μlar* | ↑ | none | none | ↓ | Yes |

Diagram

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**Figure 4.** Predicted values of total length, egg buffer mass, and survival over time for each parameter’s base model value and two levels representing hypoxia effects on the parameter. These plots use assimilation (A, B, and C), maintenance (D, E, and F), and combined embryo and post-hatch mortality rates (G, H, and I) as examples to show how we selected DEBkiss parameters that would influence at least one of the state variables that was impacted by hypoxia in experiments. State variables are total length (A, D, and G), egg buffer mass (B, E, and H), and survival (C, F, and I). Reducing *yVA* with hypoxia affects the response variables similarly to *JaAm* so it is not shown in the figure.

**Results**

*Base Model*

We obtained realistic fits to all datasets. The only exception is late-life survival, for which the mortality was too high beyond the larval stage but could not be better fit due to lack of full-life survival data. However, this did not impair our ability to model the effects of hypoxia on early life survival. Estimating *yVA* returned a lower than typical value for conversion efficiency of assimilates to growth, but this gave a realistic fit to the length data and allowed a detailed and very close fit to egg buffer mass over time (hatch timing). The observed and predicted data for full life span and early life are plotted in Figure 2.

*Hypoxia Stress*

The best model of experimental hypoxia effects on *M. menidia* early life stages had correction factors applied to *yVA*, *μemb*, and *μlar*. The correction factor *c* was used to reduce *yVA* and *c1* was used to increase both *μemb* and *μlar*. This model met the initial criteria of affecting all three state variables (total length, egg buffer mass, and survival) in the same direction as hypoxia affected them in experimental data. Although adjusting *yVA* alone met the initial criteria of affecting all three state variables, also increasing both mortality parameters improved the fit to the data. It also had a lower AIC than all but one of the other models that met the initial criteria, with an AIC of 584.75. Adding a correction factor to *JvM* in addition to these three parameters reduced AIC slightly to 584.62 (AICmin). The relative likelihood of the model with correction factors for *yVA*, *μemb*, and *μlar* has a relative likelihood (Akaike weight) of 0.937, indicating it is 0.937 times as probable as the model that applies correction factors to *JvM*, *yVA*, *μemb*, and *μlar*. It was therefore not considered to have improved the fit, and in the interest of parsimony is not beneficial enough to justify the added complexity of applying the correction factor to a fourth parameter. The estimated *K* values and AIC for each version of the model, as well as the ΔAIC for models that fit the initial criteria, are listed in Table 4. The values of *yVA*, *μemb*, and *μlar* when their respective correction factors are applied for each DO level are listed in Table 5.

Reducing *JaAm* with hypoxia using correction factor *c* also resulted in a good fit to the data across oxygen levels and fulfilled the initial criteria. Combining the adjusted *JaAm* with correction factors to increase both mortality rates improved the fit as well, but this model fit slightly less well than the version that corrected *yVA*, *μemb*, and *μlar*, with an AIC value of 586.72 in the former model compared to 584.72 in the latter. The ΔAIC for this pair of models is 2, indicating that the model with *c* multiplied by *JaAm* performs similarly to the model with *c* multiplied by *yVA*, when correction factor *c1* is also included for both mortality parameters. The ΔAIC values relative to the AICmin for the models applying correction factors to *JaAm* + *μemb* and *yVA* + *μemb* are 5.95 and 4.64, respectively (ΔAIC for all models listed in Table 4). This suggests that although these are not the best fitting models, there is a moderate level of support for them, contrary to our hypothesis that adjusting *μlar* with oxygen would be required to get a good fit.

**Table 4.** The estimated *K* value and AIC when the correction factors were applied to each parameter or combination of parameters. ΔAIC is listed only for models that satisfied the initial criteria as the ones that do not fit the criteria are not eligible to be selected as the best model, and was calculated with AICmin = 584.62 for the *yVA* + *JvM* + *μemb* + *μlar* model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter(s)** | **Correction factor(s)** | **Estimated *K* [95% CI]** | **AIC** | **ΔAIC** |
| *JaAm* | *c* | 1.698 [1.694-2.702] | 600.70 | 16.08 |
| *yVA* | *c* | 1.475 [1.197-3.205] | 602.35 | 17.73 |
| *JvM* | *c1* | 0.3646 [0.3016-0.5179] | 599.49 | - |
| *μemb* | *c1* | 0.6257 [0.4351-0.9920] | 585.73 | - |
| *μlar* | *c1* | 0.3028 [0.2009-0.4918] | 575.03 | - |
| *JaAm* + *JvM* | *c* + *c1* | 1.720 [1.716-2.686] | 600.62 | 16.00 |
| *yVA* + *JvM* | *c* + *c1* | 1.468 [1.215-3.075] | 602.20 | 17.58 |
| *JvM* + *μemb* | *c1* + *c1* | 0.5200 [0.3740-0.8511] | 582.80 | - |
| *JaAm* + *μemb* | *c* + *c1* | 1.698 [1.694-2.041] | 590.27 | 5.95 |
| *yVA* + *μemb* | *c* + *c1* | 1.308 [1.198-1.777] | 589.26 | 4.64 |
| *JvM* + *μlar* | *c1* + *c1* | 0.3541 [0.2988-0.4479] | 568.10 | - |
| *JaAm* + *μlar* | *c* + *c1* | 1.698 [1.694-2.253] | 595.42 | 10.80 |
| *yVA* + *μlar* | *c* + *c1* | 1.340 [1.195-1.981] | 594.64 | 10.02 |
| *μemb* + *μlar* | *c1* + *c1* | 0.7659 [0.5434-1.145] | 580.08 | - |
| *JaAm* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.698 [1.694-2.023] | 586.72 | 2.10 |
| *yVA* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.315 [1.196-1.756] | 584.75 | 0.13 |
| *JvM* + *μemb* + *μlar* | *c1* + *c1* + *c1* | 0.7124 [0.4823-1.087] | 578.79 | - |
| *JaAm* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.720 [1.716-2.042] | 586.83 | 2.21 |
| *yVA* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.313 [1.216-1.753] | 584.62 | 0 |

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**Figure 5.** Best fit of DEBkiss model to experimental data from four DO levels, selected based on a combination of initial criteria that all three response variables’ predicted values are affected by the hypoxia correction factor and ΔAIC. (A) is total length (mm) over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time (days), with means rather than all data plotted for survival for ease of viewing patterns.

**Table 5.** The value of the DEBkiss parameters that best reproduce the hypoxia effects observed experimentally, calculated (along with 95% confidence intervals in brackets) for each DO treatment level using the correction factors *c* and *c1* and the estimated value of *K* = 1.315.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Product of correction factor and initial parameter value** | | | |
| **7.7 mg L-1** | **4.2 mg L-1** | **3.1 mg L-1** | **2.7 mg L-1** |
| ***yVA*** | 0.364  [0.364, 0.365] | 0.343  [0.337, 0.356] | 0.274  [0.261, 0.308] | 0.211  [0.198, 0.249] |
| ***μemb*** | 0.175  [0.175, 0.175] | 0.186  [0.179, 0.190] | 0.234  [0.207, 0.244] | 0.303  [0.256, 0.322] |
| ***μlar*** | 0.0807  [0.0806, 0.0807] | 0.0856  [0.0825, 0.0872] | 0.107  [0.0956, 0.112] | 0.139  [0.118, 0.148] |