

Des carences en oméga 3 peuvent endommager la fonction cardiaque – Illustration avec un modèle poisson



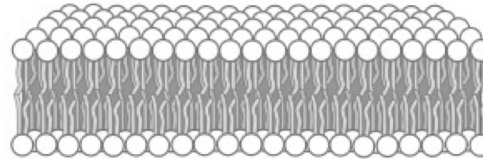
Marie VAGNER

E. Pante, A. Viricel, T. Lacoue-Labarthe, J.L. Zambonino, P. Quazuguel, E. Dubillot, V. Huet, H. Le Delliou, C. Lefrançois, N. Imbert-Auvray

N-3 long chain polyunsaturated fatty acids (HUFA n-3 ou omega 3):

Structural components of the cell membrane

- **EPA** (ecosapentaenoic acid, 20:5n-3)
- **DHA** (docosahexaenoic acid, 22:6n-3)

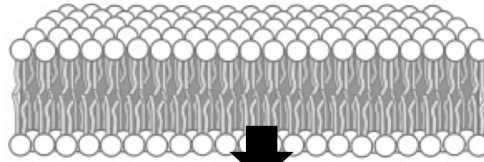


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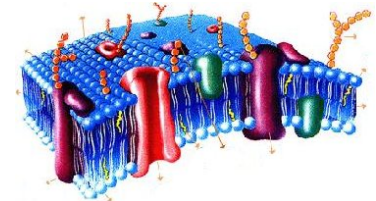
- **DHA** (docosahexaenoic acid, 22:6n-3)



Fluidity and permeability of membranes

Conformation & activity of embedded proteins

Development & functions of cells and tissues

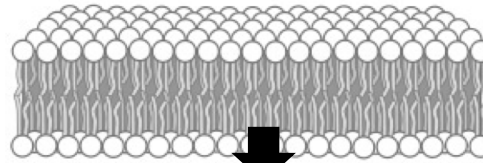


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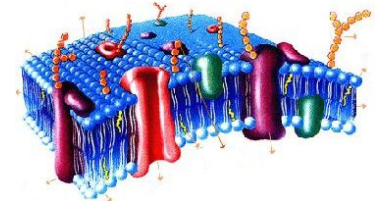


Cnumb.: DB numb n-DB position

Fluidity and permeability of membranes

Conformation & activity of embedded proteins

Development & functions of cells and tissues



Individu (fonct. Physio.: e.g. croissance, reproduction, métabolisme)

Population

Ecosystème (fitness, selection habitat, migration, trophic web,...)

While necessary to maintain vital functions, HUFA n-3 weakly synthesized *de novo* by marine fishes

→ Must be supplied by food

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→ **Must be supplied by food**

Low enzymatic activities in marine fishes

Delta 6 désaturase

C18-C20 élongase

Delta 5 désaturase

C20-C22 élongase

C22-C24 élongase

Delta 6 désaturase

β -oxydation

18:3 n-3

18:4 n-3

20:4 n-3

20:5 n-3

22:5 n-3

24:5 n-3

24:6 n-3

22:6 n-3

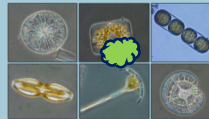
EPA

DHA

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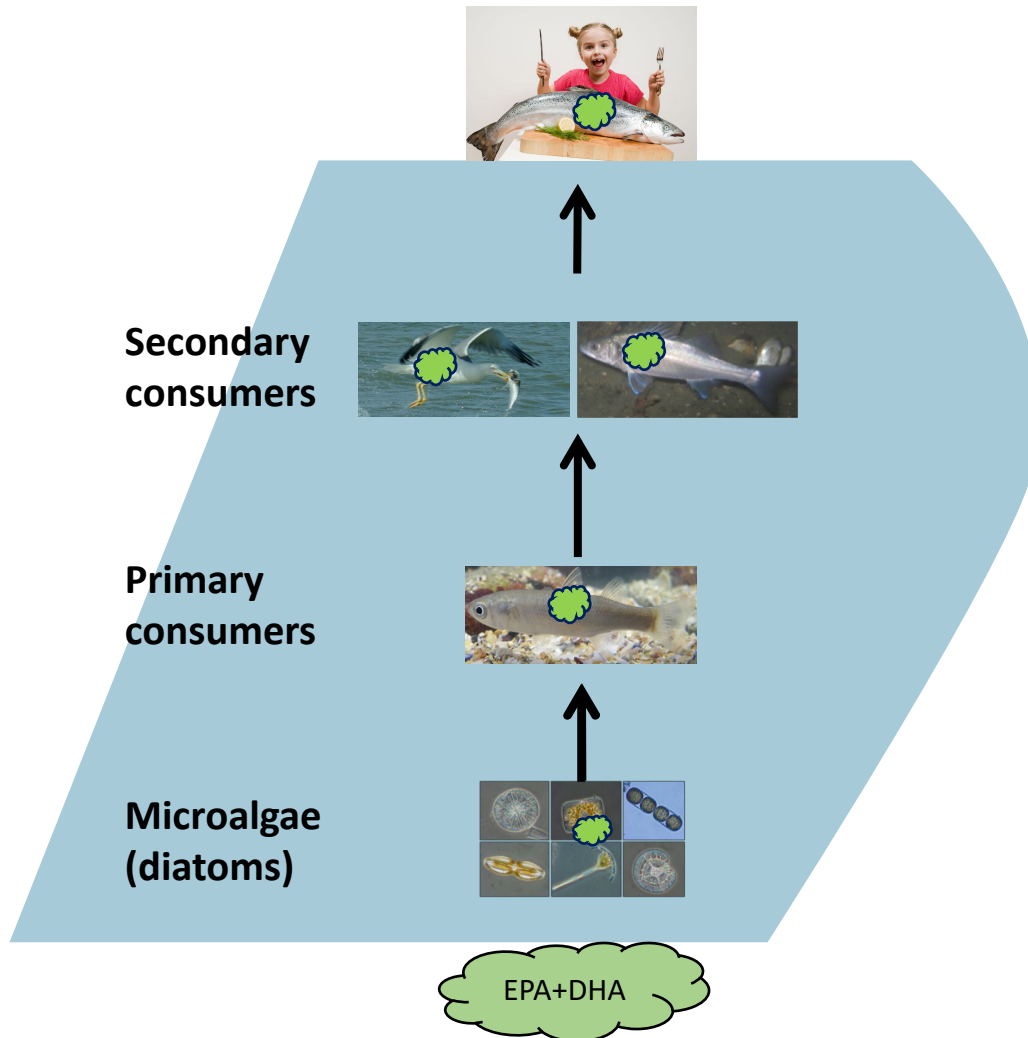
Microalgae
(diatoms)



EPA+DHA

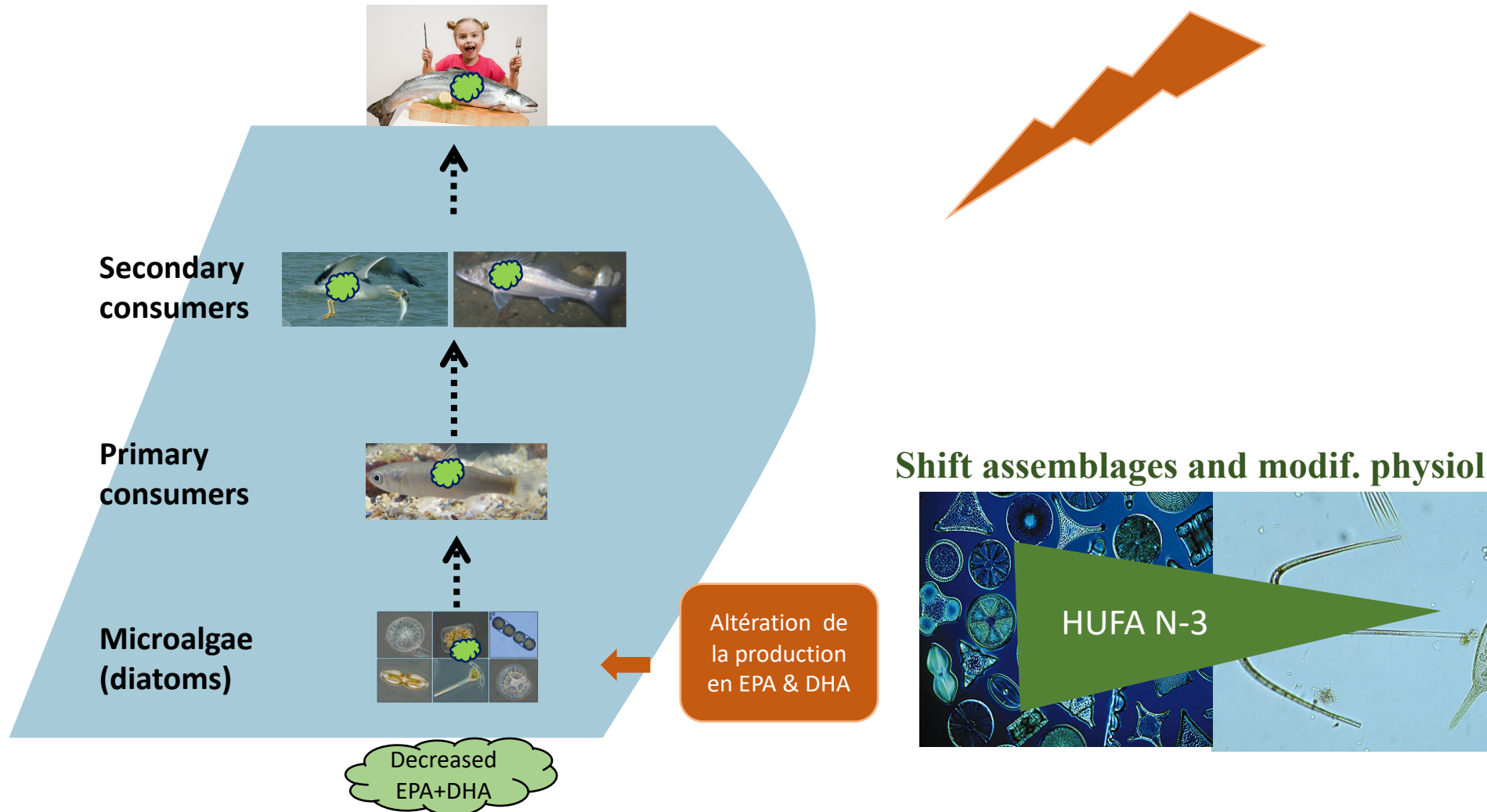
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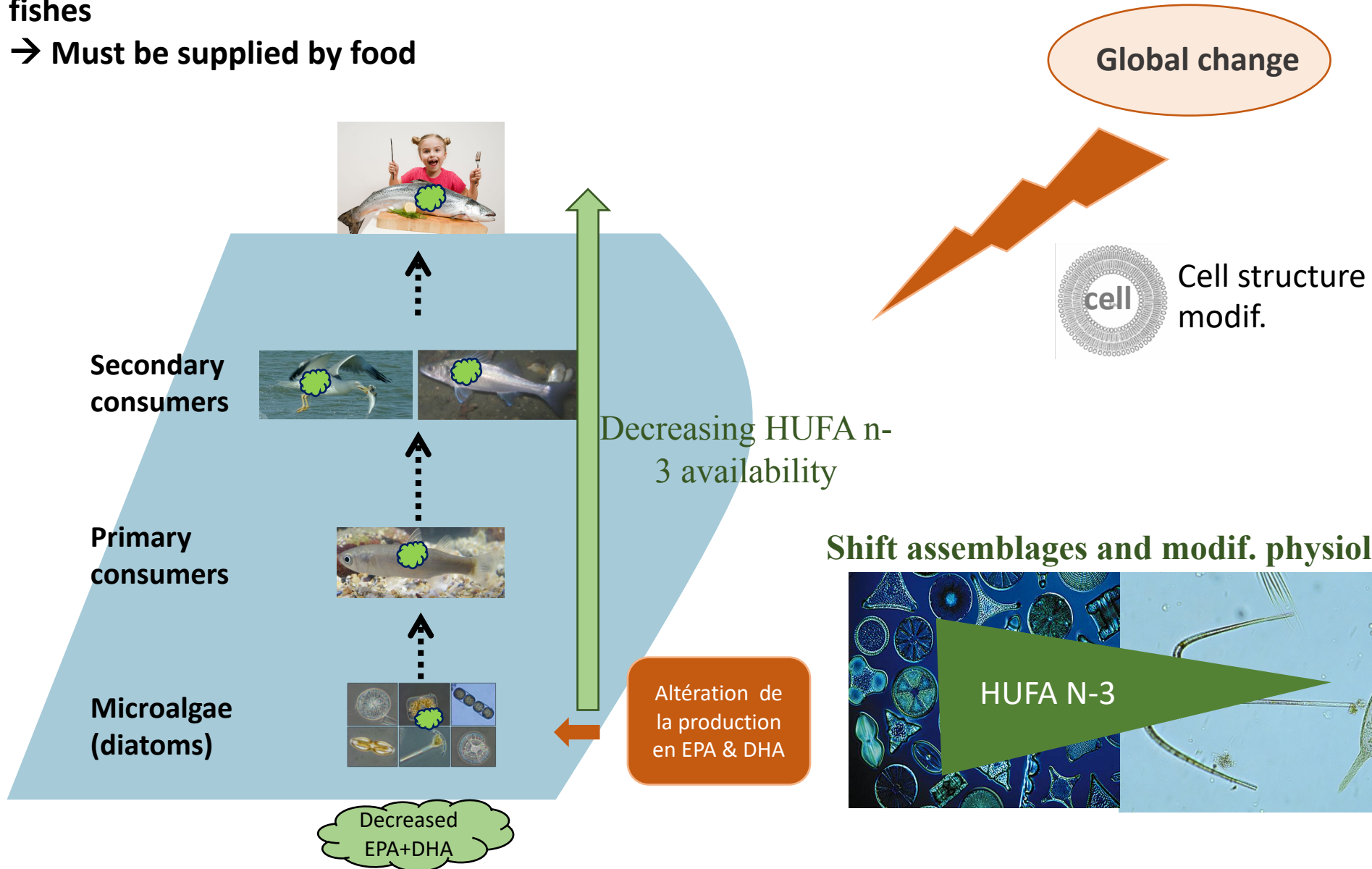
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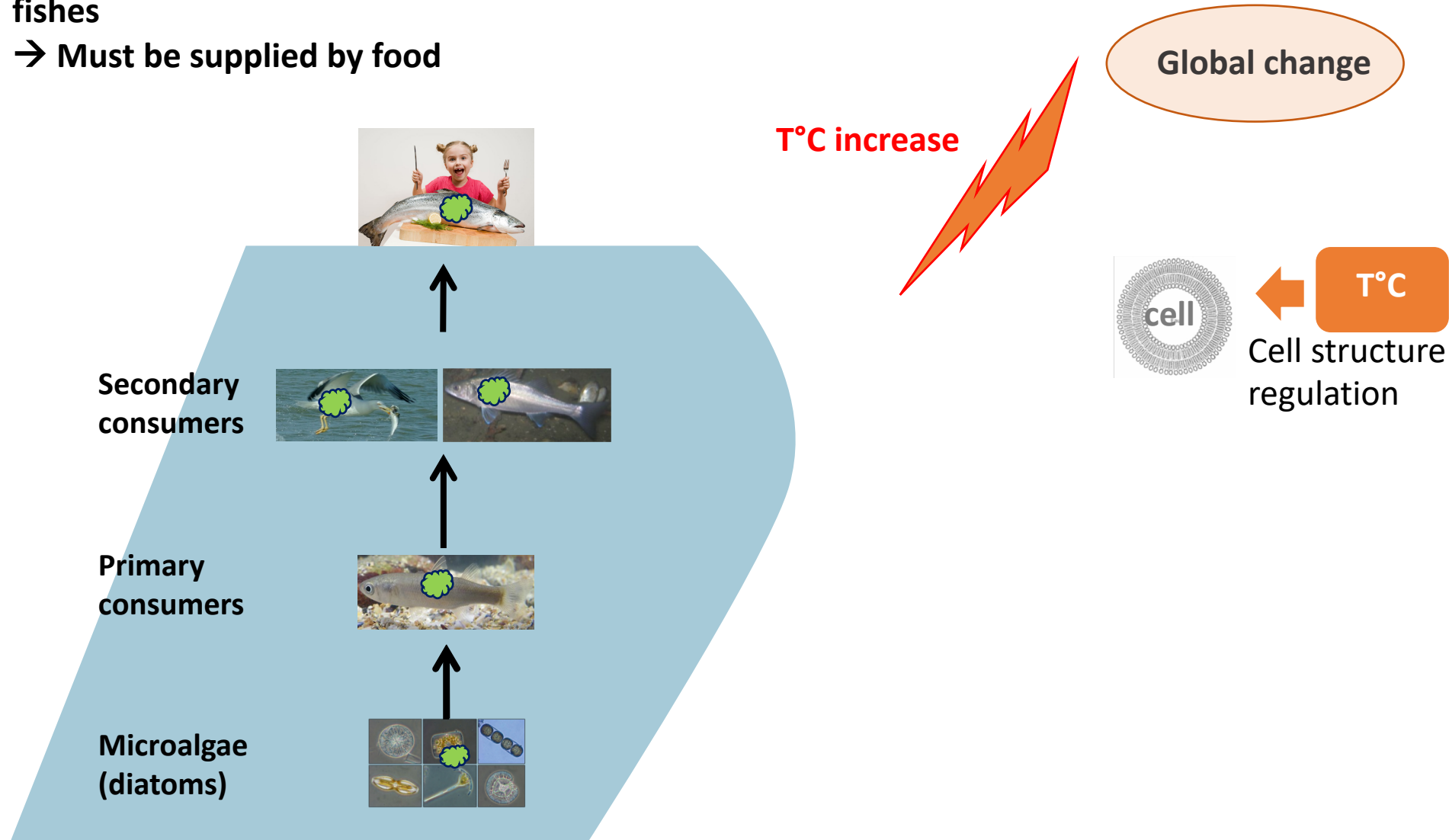
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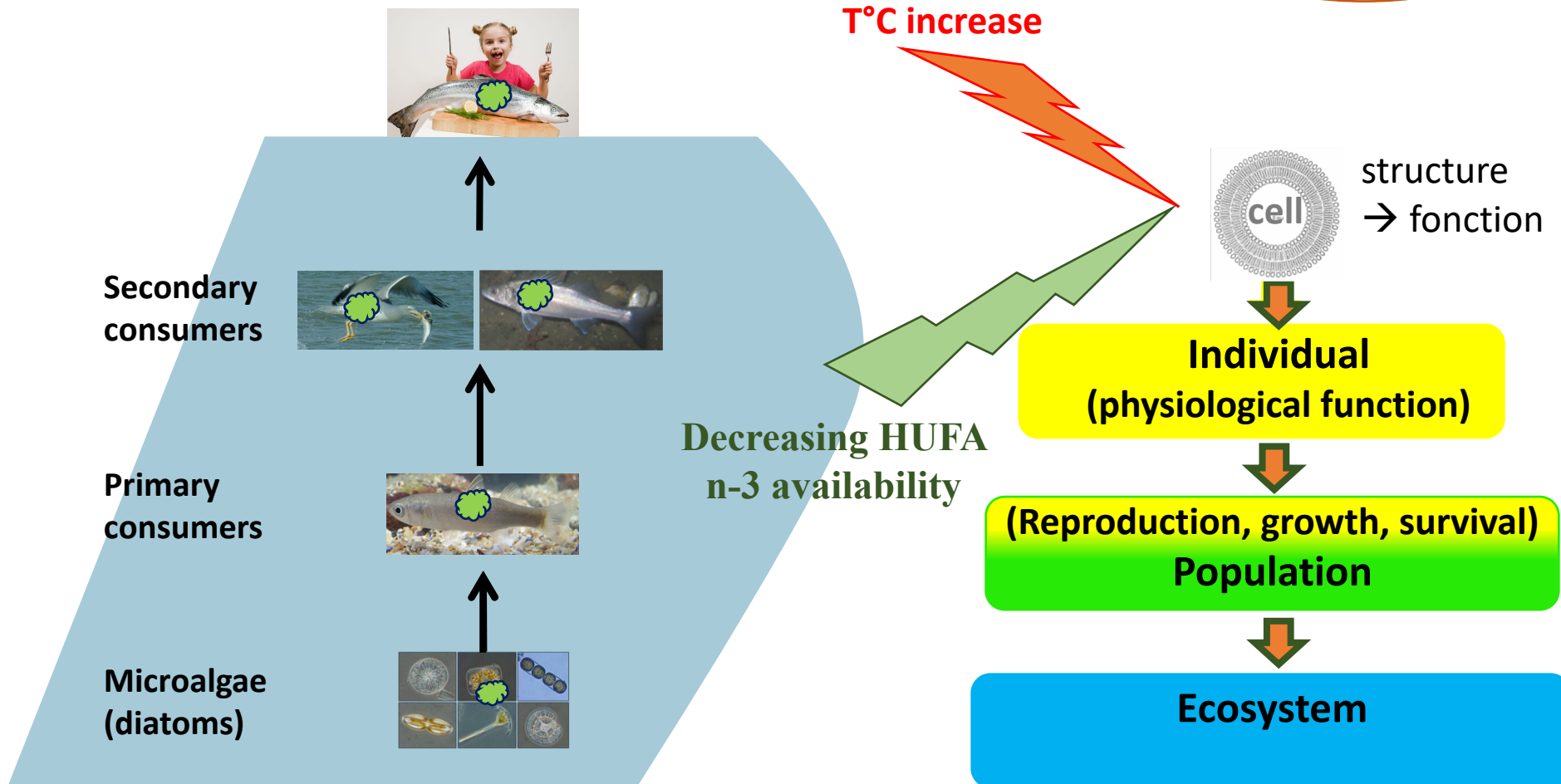
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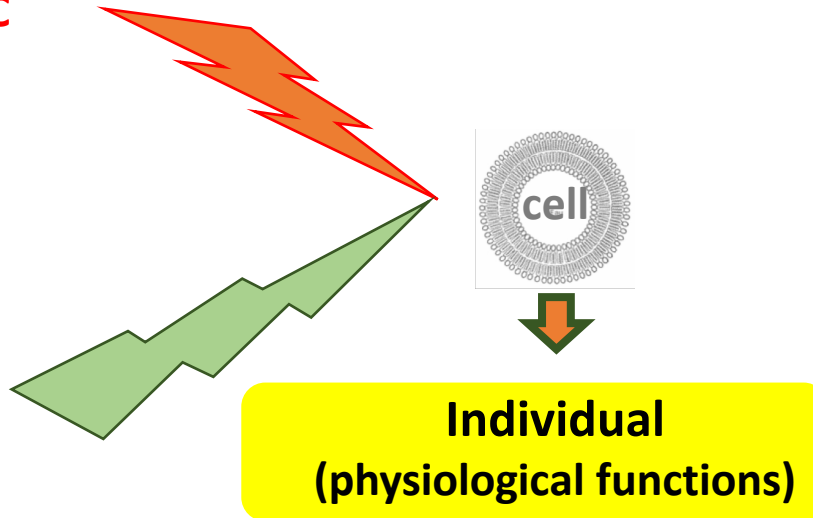
Global change





Increasing T°C

HUFA n-3
deficiency



RESEARCH ARTICLE

Depletion of Essential Fatty Acids in the Food Source Affects Aerobic Capacities of the Golden Grey Mullet *Liza aurata* in a Warming Seawater Context

Marie Vagner^{1✉*}, Thomas Lacoue-Labarthe^{1✉}, José-Luis Zambonino Infante², David Mazurais², Emmanuel Dubillot¹, Hervé Le Delliou², Patrick Quazugue², Christel Lefrançois¹

1 UMR 7266 Littoral Environnement Sociétés, La Rochelle, France, **2** Ifremer, UMR 6539 Laboratoire des sciences de l'Environnement Marin, Plouzané, France

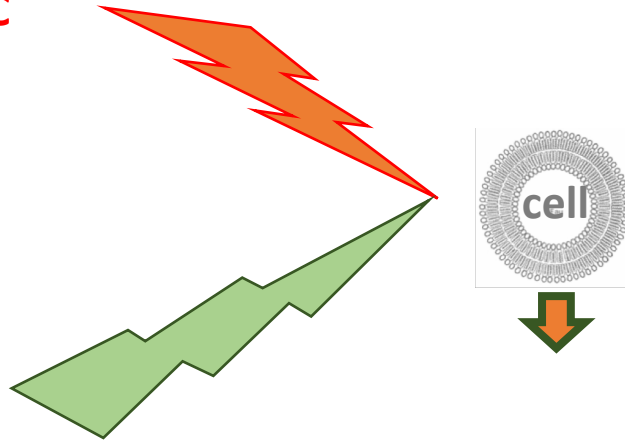
✉ These authors contributed equally to this work.



Greater energy expenditure to achieve a good swimming speed

Increasing T°C

HUFA n-3
deficiency



HYPOTHESIS:

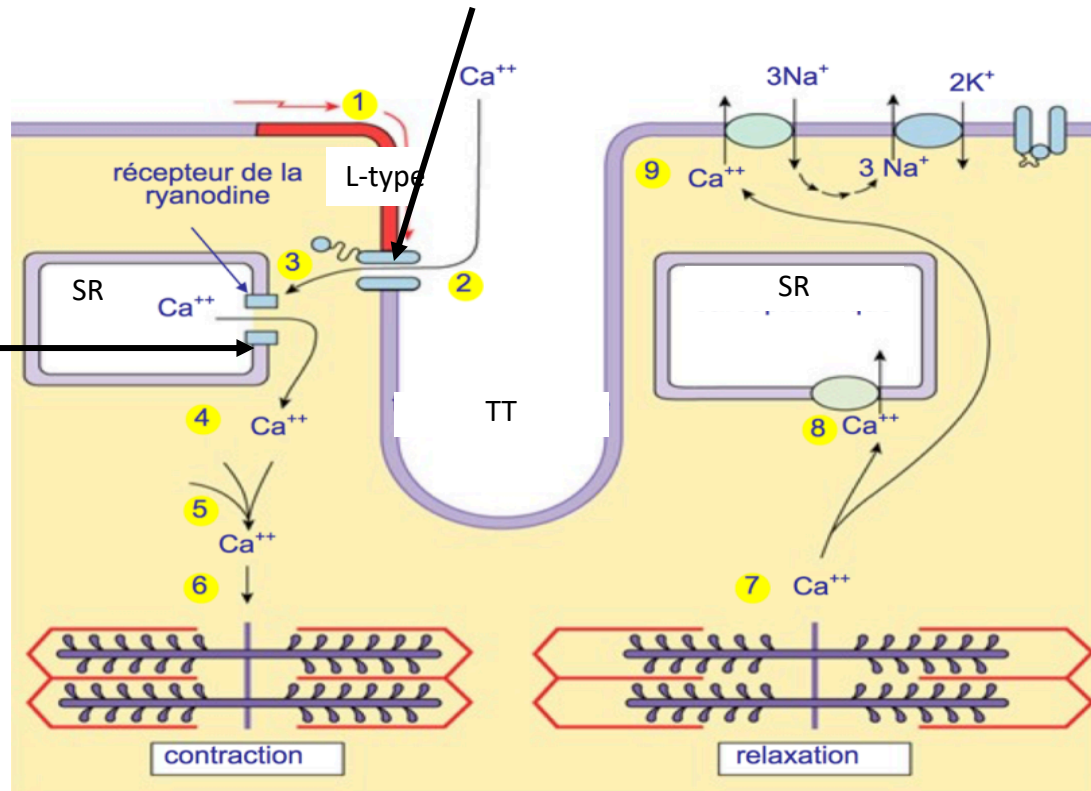
Cardiac function, which is essential for supplying the required amount of O₂ and nutrients to the tissues, is affected

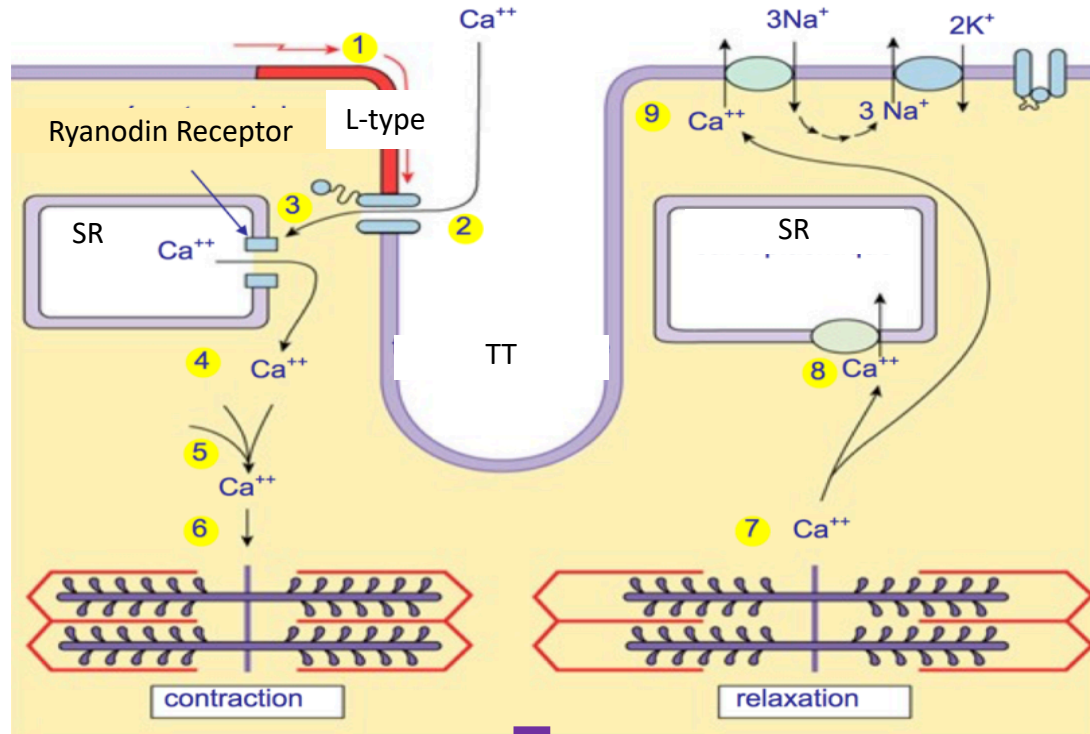


Greater energy expenditure to achieve a good swimming speed

Cardiac function in fish

Sarcolem channels



T°C increase**HUFA n-3
deficiency****Conformation of ionic pumps and embedded channels in cell membranes****Capacity of cardiomyocytes contraction****Greater energy expenditure to achieve a good swimming speed**

Objective

T°C increase

HUFA n-3
deficiency

Individual
(physiological function)

Integrated Cardiac response

INDIVIDUAL

CELLULAR

MOLECULAR



TGCATTATGCCTGGTCACGTGCAA

CTGTGATTGGAAGACTGCATAGTAC

4 Experimental conditions

- 2 isolipidic & isoproteic diets with \neq n-3 HUFA content:
 - **LH: 0.2%** EPA+DHA
 - **HH: 4.8%** EPA+DHA
- 2 T°C :
 - **12°C**: winter-like
 - **20°C**: summer-like in the studied area, i.e. Pertuis Charentais)

Low n-3 x 20°C
(LH20)

High n-3 x 20°C
(HH20)

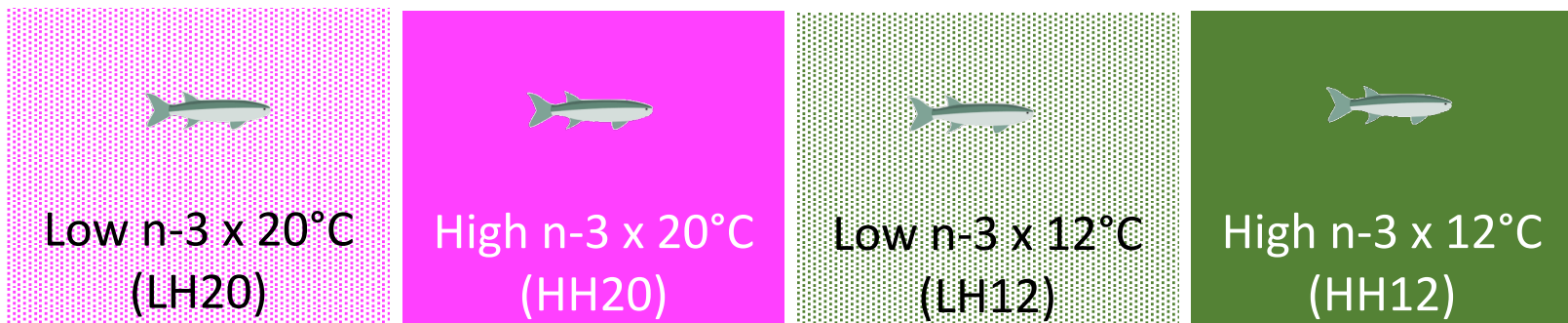
Low n-3 x 12°C
(LH12)

High n-3 x 12°C
(HH12)

for 4 months

4 Experimental conditions

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for 4 months



INTEGRATIVE RESPONSE

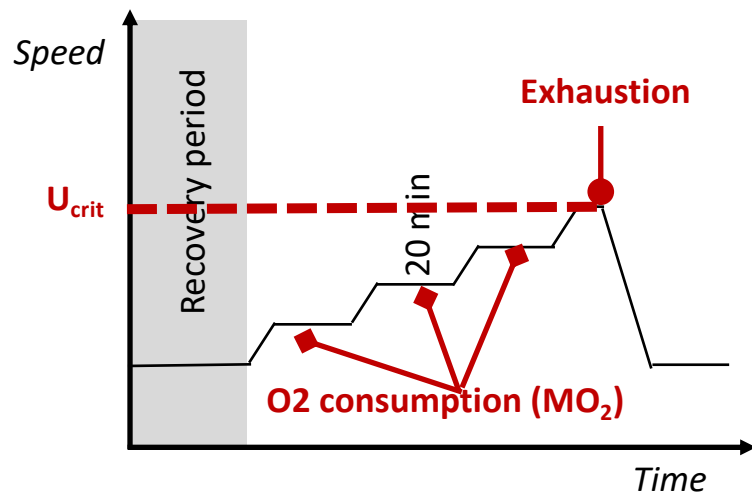
INDIVIDUAL	CELLULAR	MOLECULAR
Swimming test	<i>In vivo</i> heart performance	RNA seq

INDIVIDUAL

Swimming test



Step-protocol (Brett, 1964)

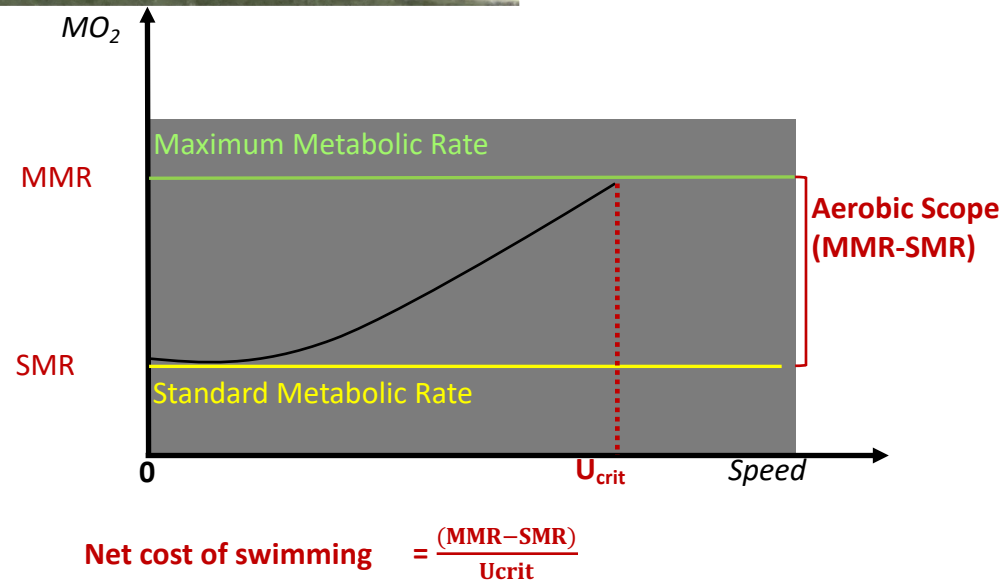
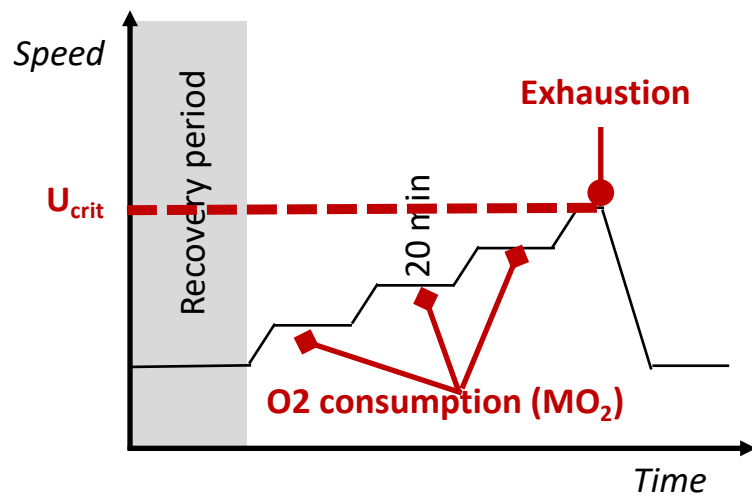


INDIVIDUAL

Swimming test



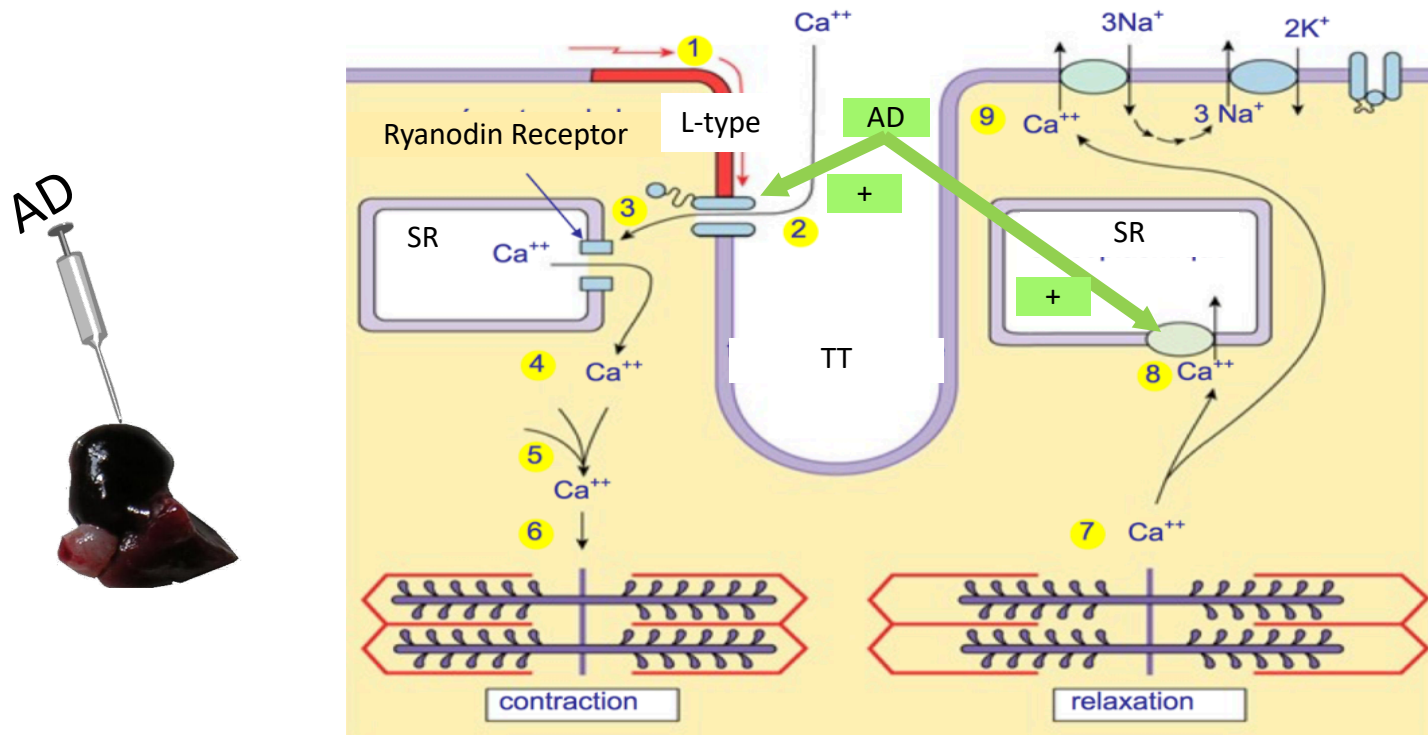
Step-protocol (Brett, 1964)



CELLULAR

In vivo heart performance

Measurement of contraction capacity in response to different stimulation frequencies

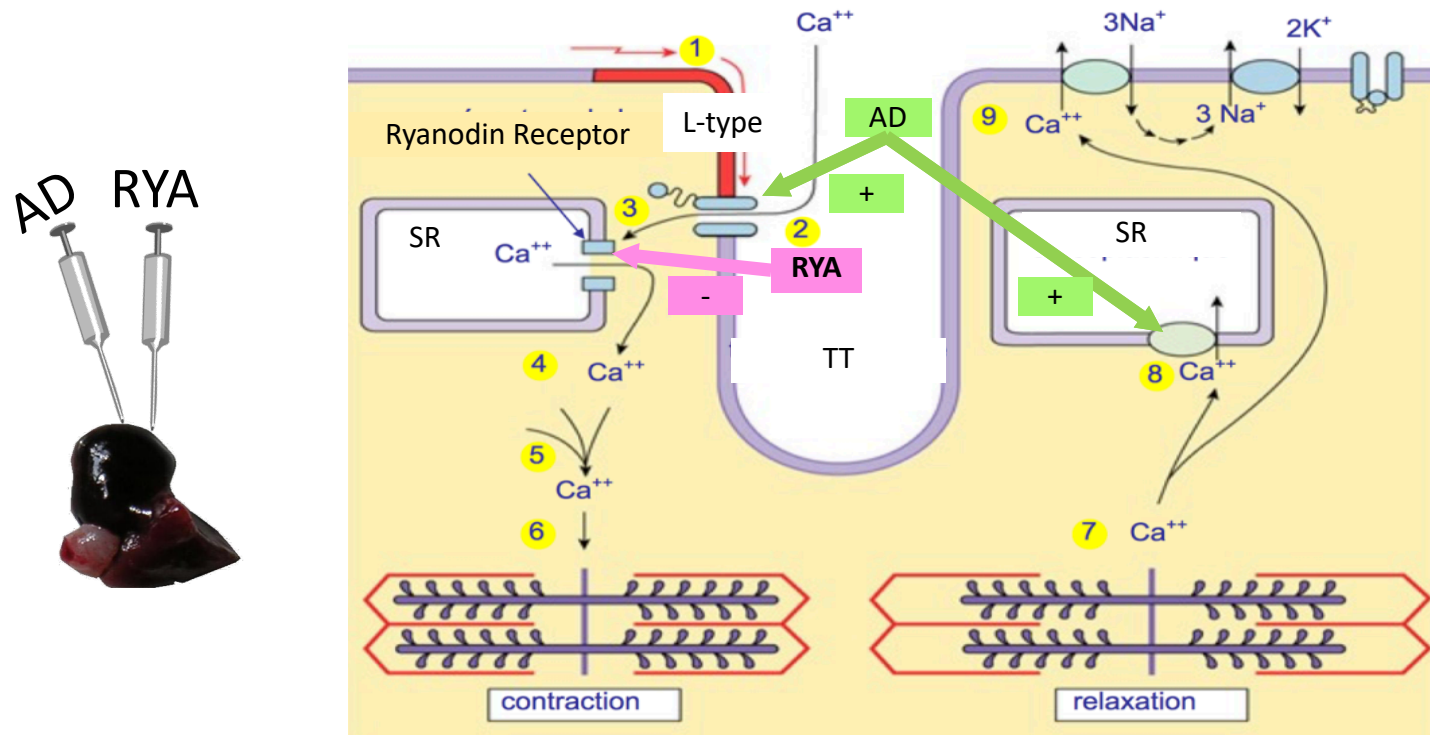


- Evaluation of the involvement of sarcolemmal channels: injection of AD dose 10 nmol (mimicking rest) & 10 μmol (mimicking exercise)

CELLULAR

In vivo heart performance

Measurement of contraction capacity in response to different stimulation frequencies



- Evaluation of the involvement of sarcolemmal channels: injection of AD dose 10 nmol (mimicking rest) & 10 μ mol (mimicking exercise)
- Evaluation of SR involvement: injection of RYA to block ryanodine receptors, after injection of basal & stress AD

MOLECULAR

RNA seq

1. RNA extraction



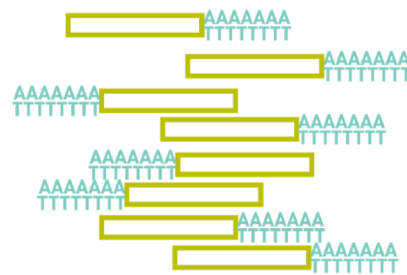
2. cDNA libraries



3. Sequencing



4. Analyses



```

aacgtccaaaggagt
gttacctacggctaa
aacgtccaaaggagt
ttcgagcatacgact
cacgtcgaatgagt
attacgtacgggtaa
tacgtgcttacgagt
tacgtgcttacgagt
atcgaaggctagctat
atcgaaggctagctat

```



MOLECULAR

RNA seq

1. RNA extraction



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aacgtccaaaggagt
gttacctacggctaa
aacgtccaaaggagt
ttcgagcatatcgact
cacgtcgaatgagt
attacgtacgggtaa
tacgtgcttacgagt
tacgtgcttacgagt
atcgaaggctagctat
atcgaaggctagctat

```



5. Analyses

- **Differential expression:** a transcript can be under or over expressed



Over-expression



Under-expression

MOLECULAR

RNA seq

1. RNA extraction



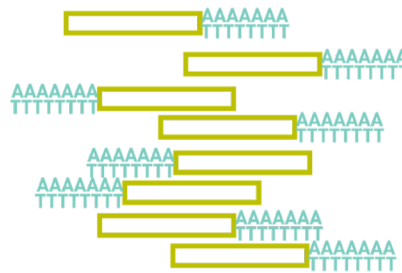
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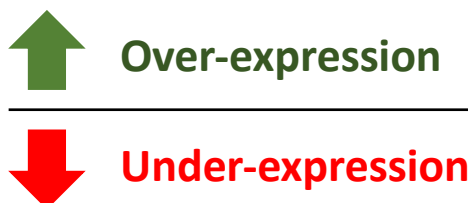
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tacgtgcttacgagt
tacgtgcttacgagt
atcgaaggctagctat
atcgaaggctagctat

```



5. Analyses

- **Differential expression:** a transcript can be under or over expressed
- **Functional enrichment** => gene ontology (GO) terms



Over-expression

Under-expression

Is there over-representation of a functional process (biol. process/cell. component/mol. function) ?

INDIVIDUAL

Swimming test

Group	U_{crit} (BL s ⁻¹)	SMR (mg O ₂ kg ⁻¹ h ⁻¹)
HH20	8.8±0.4 (n=14)	20.1±2.2 ^a (n=16)
LH20	9.1±0.3 (n=13)	23.6±3.0 ^a (n=12)
HH12	9.6±0.3 (n=14)	37.4±4.1 ^b (n=12)
LH12	9.1±0.3 (n=12)	27.9±3.9 ^b (n=12)
Statistics	NS	Temp**

INDIVIDUAL

Swimming test

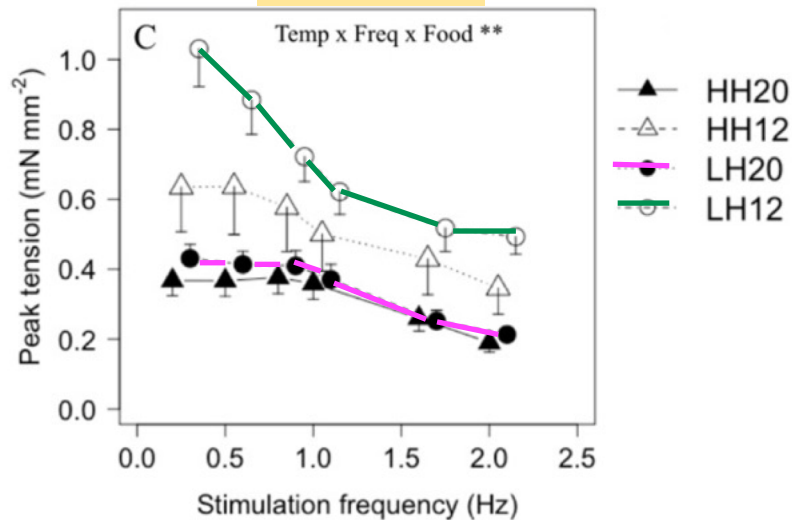
Group	U_{crit} (BL s ⁻¹)	SMR (mg O ₂ kg ⁻¹ h ⁻¹)	MMR (mg O ₂ kg ⁻¹ h ⁻¹)	AS (mg O ₂ kg ⁻¹ h ⁻¹)	NCOT (mg O ₂ kg ⁻¹ m ⁻¹)
HH20	8.8±0.4 (n=14)	20.1±2.2 ^a (n=16)	211.6±10.0 ^b (n=14)	191.2±10.4 ^b (n=14)	22.4±1.4 ^b (n=13)
LH20	9.1±0.3 (n=13)	23.6±3.0 ^a (n=12)	234.9±7.2 ^c (n=13)	213.8±6.3 ^c (n=12)	23.9±1.0 ^b (n=13)
HH12	9.6±0.3 (n=14)	37.4±4.1 ^b (n=12)	57.8±13.1 ^a (n=10)	128.6±13.8 ^a (n=11)	12.9±1.5 ^a (n=11)
LH12	9.1±0.3 (n=12)	27.9±3.9 ^b (n=12)	135.5±10.7 ^a (n=11)	100.5±9.5 ^a (n=10)	12.0±1.5 ^a (n=10)
Statistics	NS	Temp**	Food×Temp*	Food×Temp*	Temp***

MMR and AS highest in LH20, and lowest in LH12

CELLULAR

In vivo heart performance

AD repos

**LH12**

At rest → Higher pumping capacity than in other groups

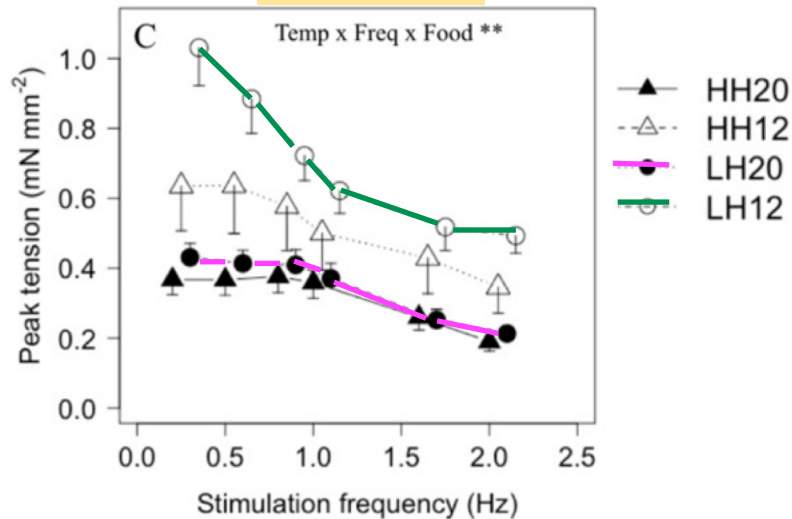
LH20

At rest → lower pumping capacity

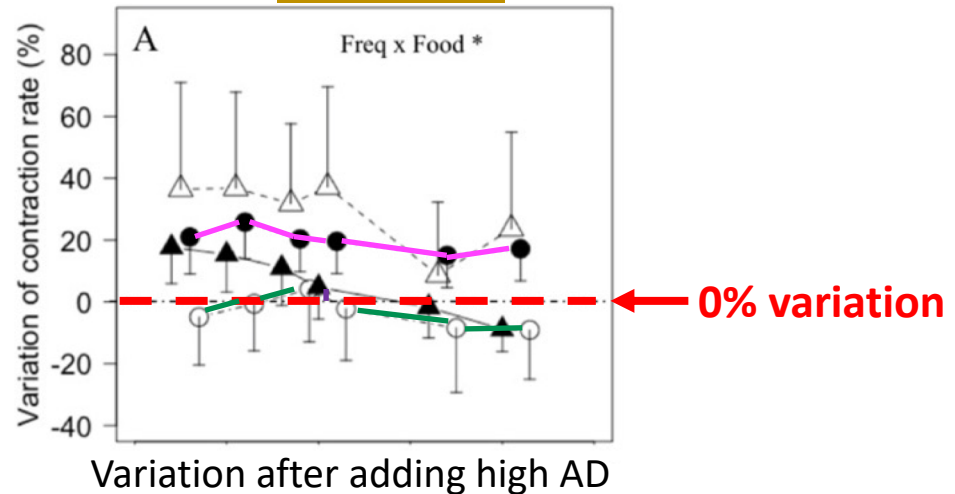
CELLULAR

In vivo heart performance

AD repos



AD Effort



LH12

At rest → Higher pumping capacity than in other groups

Under exercise/stress → do not change (already at its maximum when at rest)?

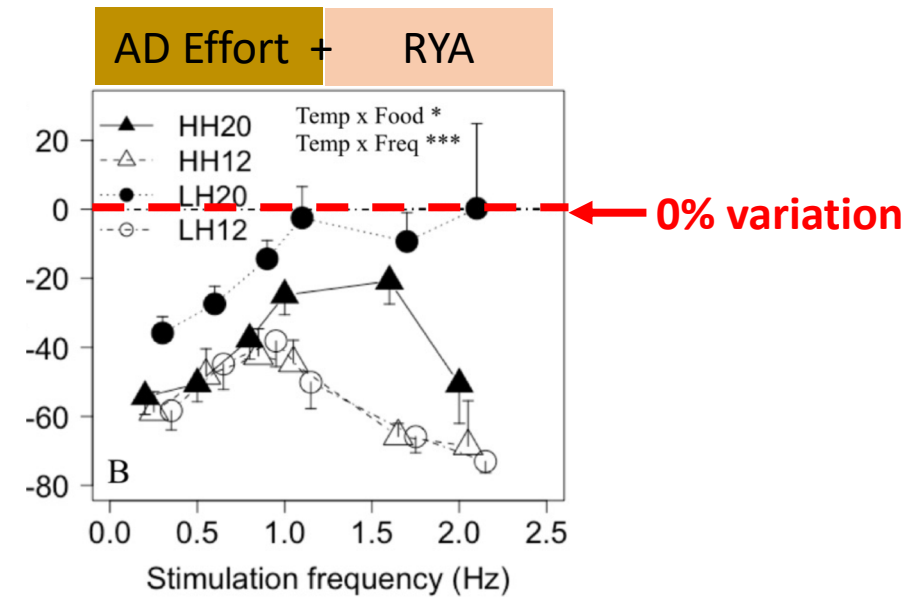
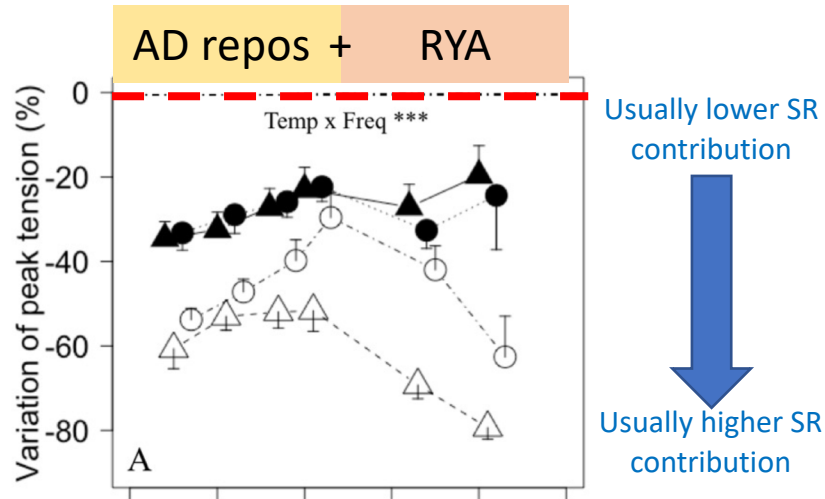
LH20

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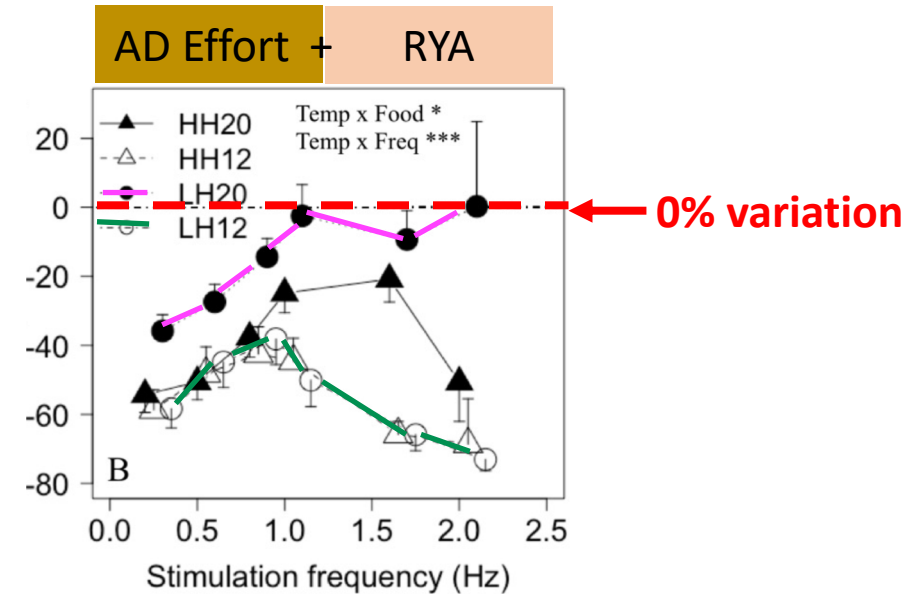
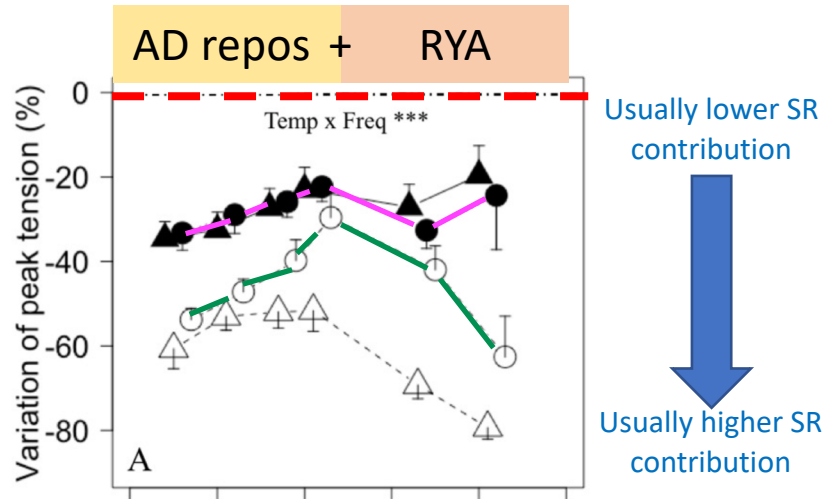
Under exercise/stress → increased => higher adrenergic sensitivity than LH12?

Higher AD sensitivity in LH20 than in LH12

CELLULAR

In vivo heart performance

CELLULAR

In vivo heart performance

⇒ **LH12**: Higher SR contribution reinforced under stressing conditions

⇒ **LH20**: Lower SR involvement than in other groups?

LH20

LH12

CELLULAR

In vivo heart performance

- Greater contraction under effort
 - **Higher** adrenergic sensitivity
 - **Lower** SR contribution
- Contraction at effort = contraction at rest
 - **Lower** adrenergic sensitivity
 - **Higher** SR contribution increasing under stressful conditions

LH20

LH12

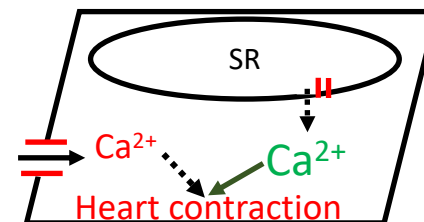
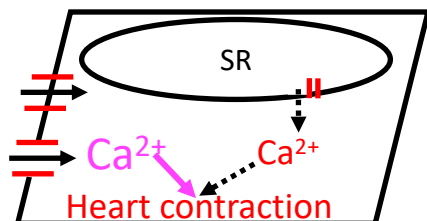
CELLULAR

In vivo heart performance

- Greater contraction under effort
 - **Higher** adrenergic sensitivity
 - **Lower** SR contribution
- Contraction at effort = contraction at rest
 - **Lower** adrenergic sensitivity
 - **Higher** SR contribution increasing under stressful conditions



+ adrenergic receptors?
+ Ca²⁺ entry through sarcolemmal
channels?



LH20

LH12

MOLECULAR

RNA seq

- Differential expression of genes involved in membrane Ca^{2+} transport via Ca^{2+} channels (L-type)
- Greater number of transcripts encoding L-type channels in **LH20**
- 11 GO terms 'biological processes' related to the regulation of ion transmembrane transport over-represented in **LH20** vs **LH12** & HH12

LH20

LH12

MOLECULAR

RNA seq

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-
- Higher number of transcripts encoding **RYA receptors**
 - GO terms involving SR only over-represented in **LH20** (e.g. GO "regulation of Ca^{2+} release from the cytosol by SR")

Global change

LH20

INTEGRATIVE RESPONSE

MOLECULAR

CELLULAR

INDIVIDUAL

RNA seq

In vivo heart performance

Swimming test

High T°C

- Proliferation of SL & SR Ca²⁺ channels
- Under stressful/exercise conditions (10 μmol l⁻¹ AD), stronger contraction force probably due to strong increased extracellular Ca²⁺ influx through sarcolemmal channels and SR Ca₂₊ stores

- Higher MMR, AS and relative NCOT

HUFA n-3
depletionAssumption of a costly Ca²⁺ mobilisation strategy

Global change

LH20

INTEGRATIVE RESPONSE

MOLECULAR

CELLULAR

INDIVIDUAL

RNA seq

In vivo heart performance

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HUFA n-3 depletion

Assumption of a costly Ca²⁺ mobilisation strategy

Quelles conséquences écologiques?

Hypothesis

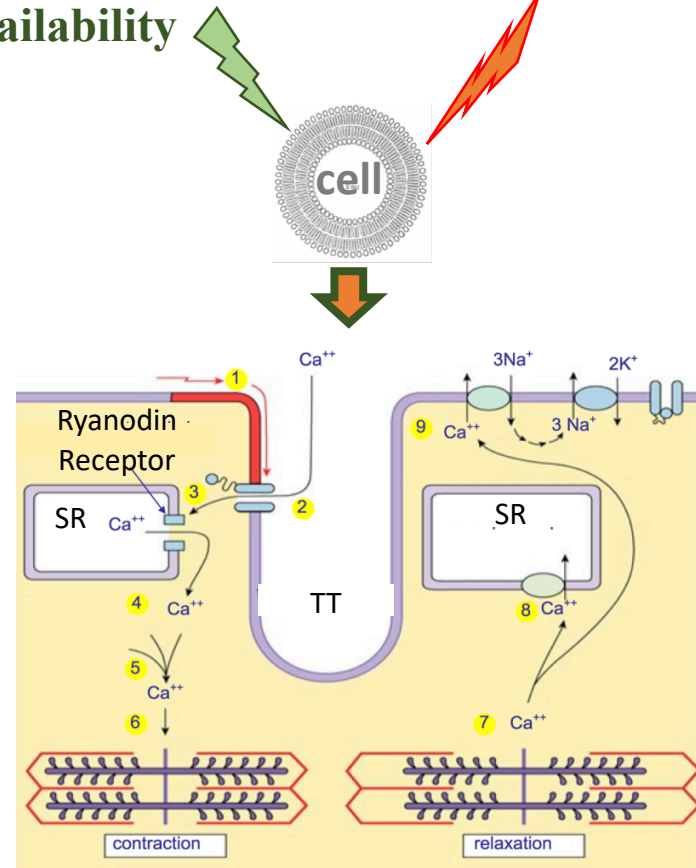
Impacted cell structure:
EPA and DHA content

Impacted cell function:
Impacted functionality of the
embedded channels and receptors

**Individual
(physiological function)**

Decreasing HUFA
n-3 availability

T°C increase



Hypothesis

LH20

Muscle DHA level

Muscle EPA level

=

<

LH12

Muscle DHA level

Muscle EPA level

- EPA could be an efficient driver in the functioning of cardiomyocyte membrane sub-types
- EPA deficiency in fish cardiomyocytes would result in a higher energetic cost in order to maintain an efficient cardiomyocyte functionality



MERCI

Mickael Le Gac & Julien Quéré for help with the preparation of DNA libraries

M. Durollet for help with fish rearing

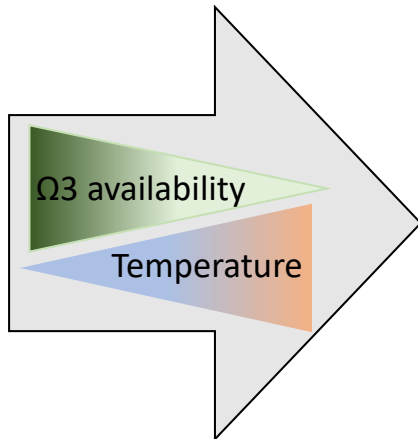
GenoToul platform for help and computing resources

Pour en savoir plus

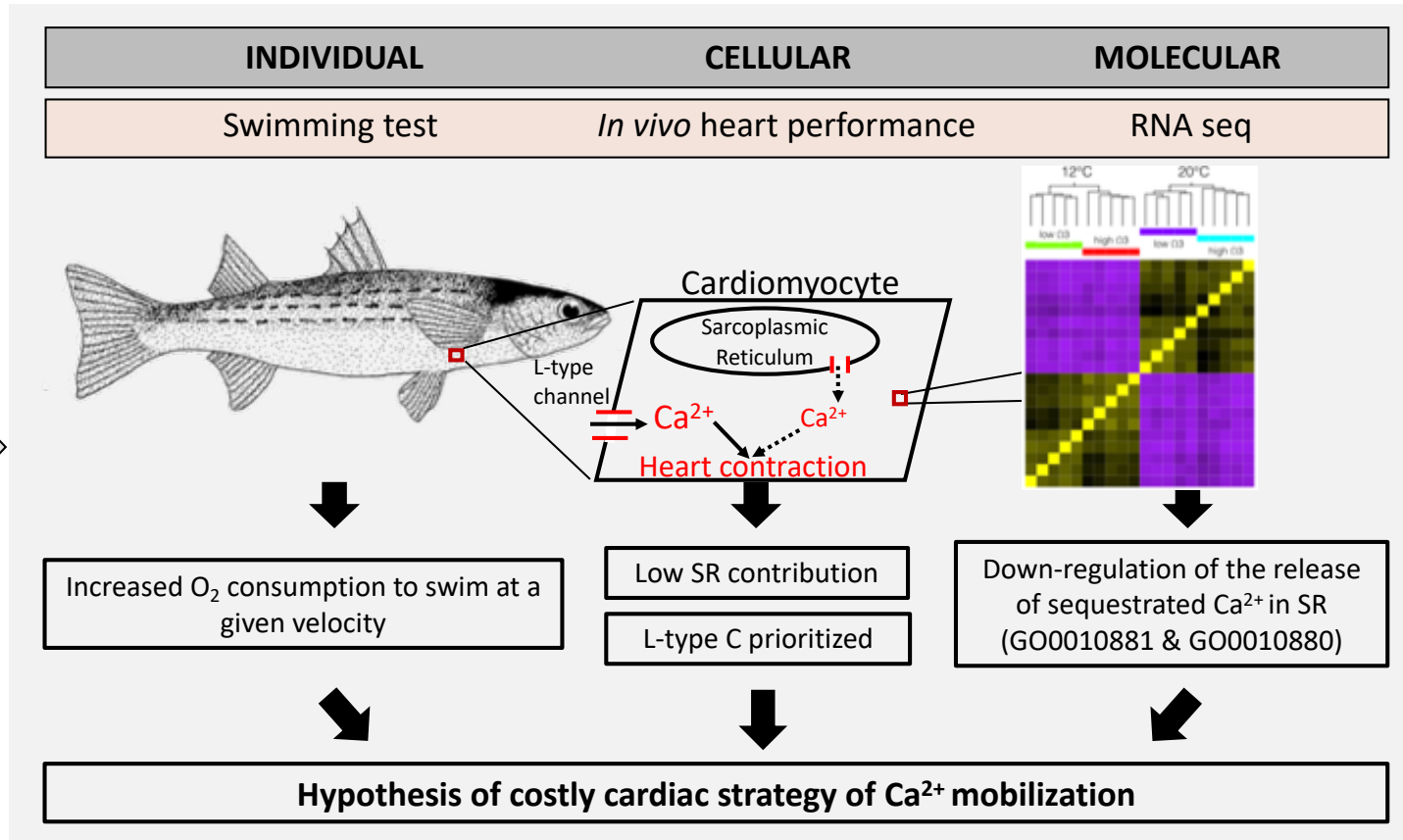
Vagner M, Pante E, Viricel A, Lacoue-Labarthe T, Zambonino-Infante J-L, Quazuguel P, Dubillot E, Huet V, Le Delliou H, Lefrançois C, Imbert-Auvray N (2019) Ocean warming combined with lower omega-3 nutritional availability impairs the cardio-respiratory function of a marine fish. The Journal of Experimental Biology jeb.187179. doi:

[10.1242/jeb.187179](https://doi.org/10.1242/jeb.187179)

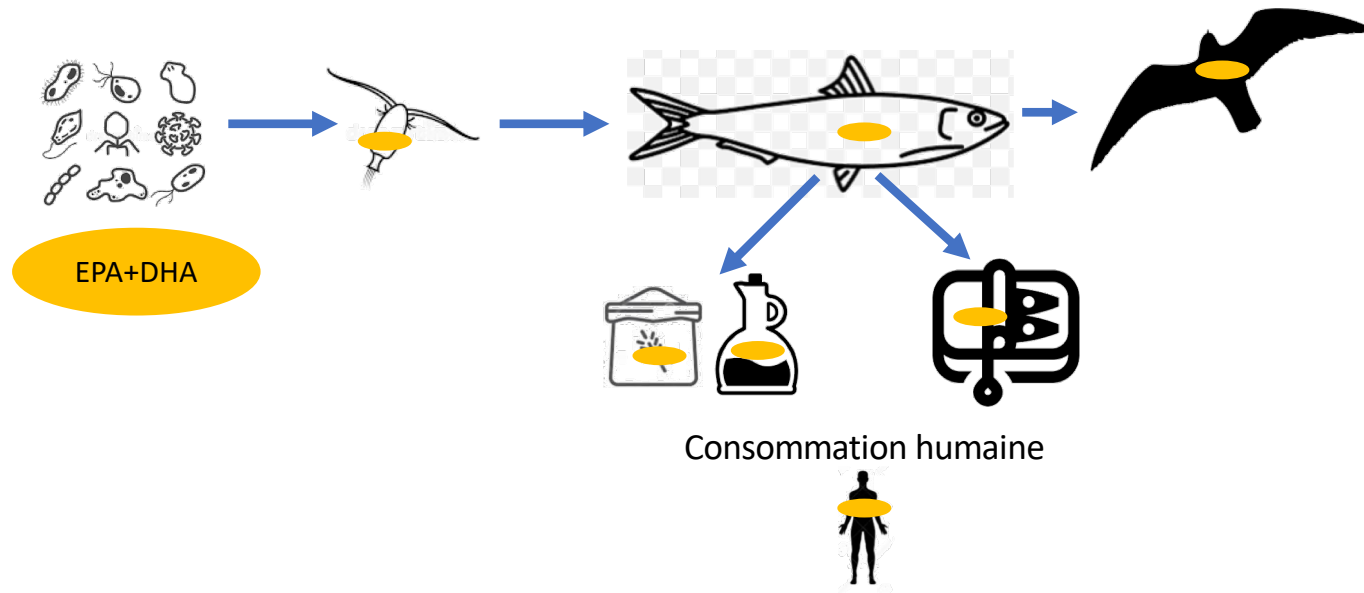
PREDICTION UNDER CLIMATE CHANGE

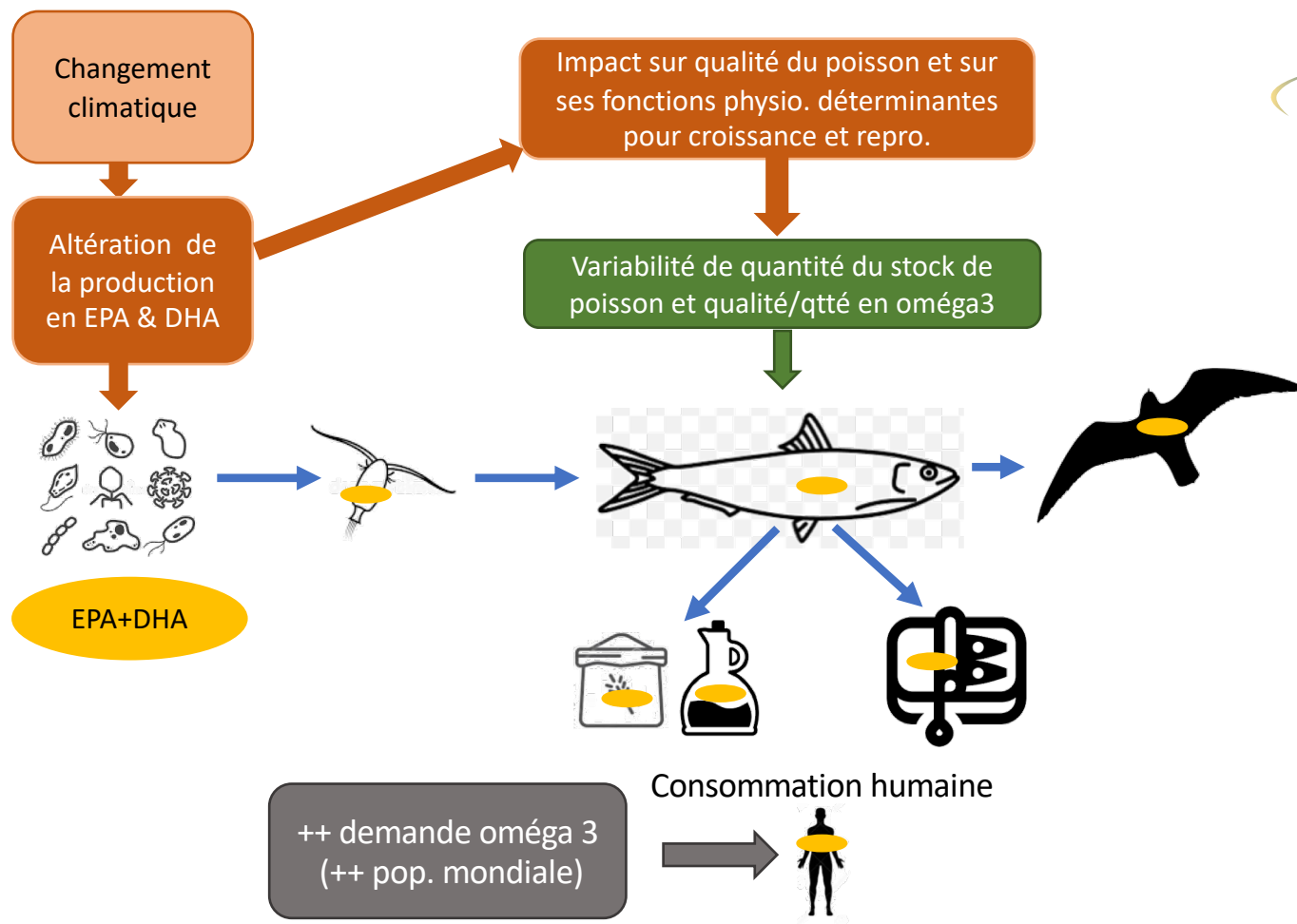


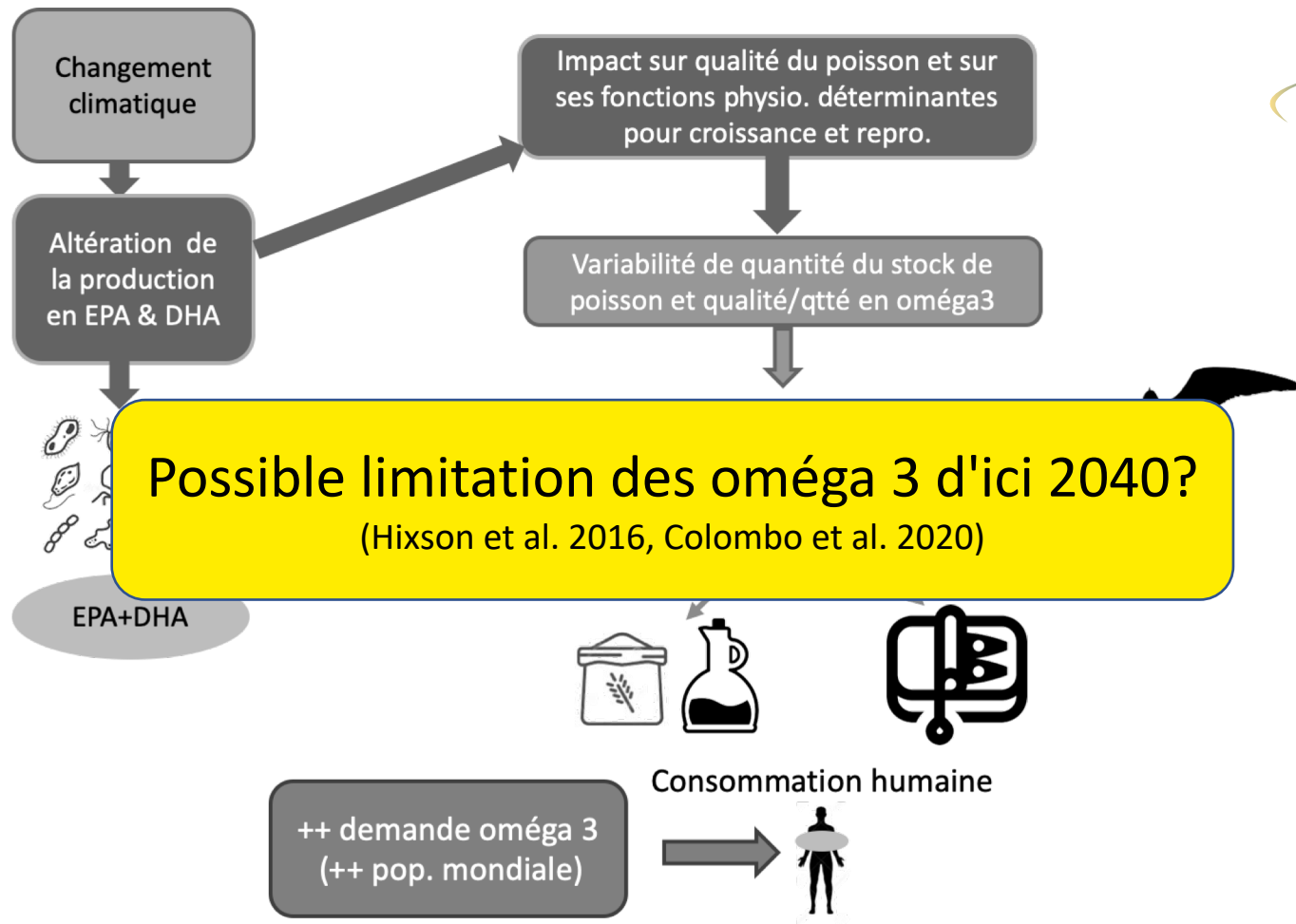
INTEGRATIVE RESPONSE



- ❖ Oméga-3 d'origine marine (**EPA et DHA**) nécessaires au développement et aux différentes fonctions vitales des organismes vivants, dont l'être humain.
- ❖ Oméga-3 d'origine terrestre (huiles végétales, **précurseurs de EPA et DHA**) ne sont pas en mesure de remplacer les oméga-3 d'origine marine pour la santé humaine, car faible bioconversion en EPA et DHA







While necessary to maintain vital functions, HUFA n-3 weakly synthesized *de novo* by marine fishes

→ **Must be supplied by food**

Low enzymatic activities in marine fishes and Humans



FOOD

18:3 n-3

Delta 6 désaturase

18:4 n-3

C18-C20 élongase

20:4 n-3

Delta 5 désaturase

20:5 n-3

EPA

C20-C22 élongase

22:5 n-3

C22-C24 élongase

24:5 n-3

Delta 6 désaturase

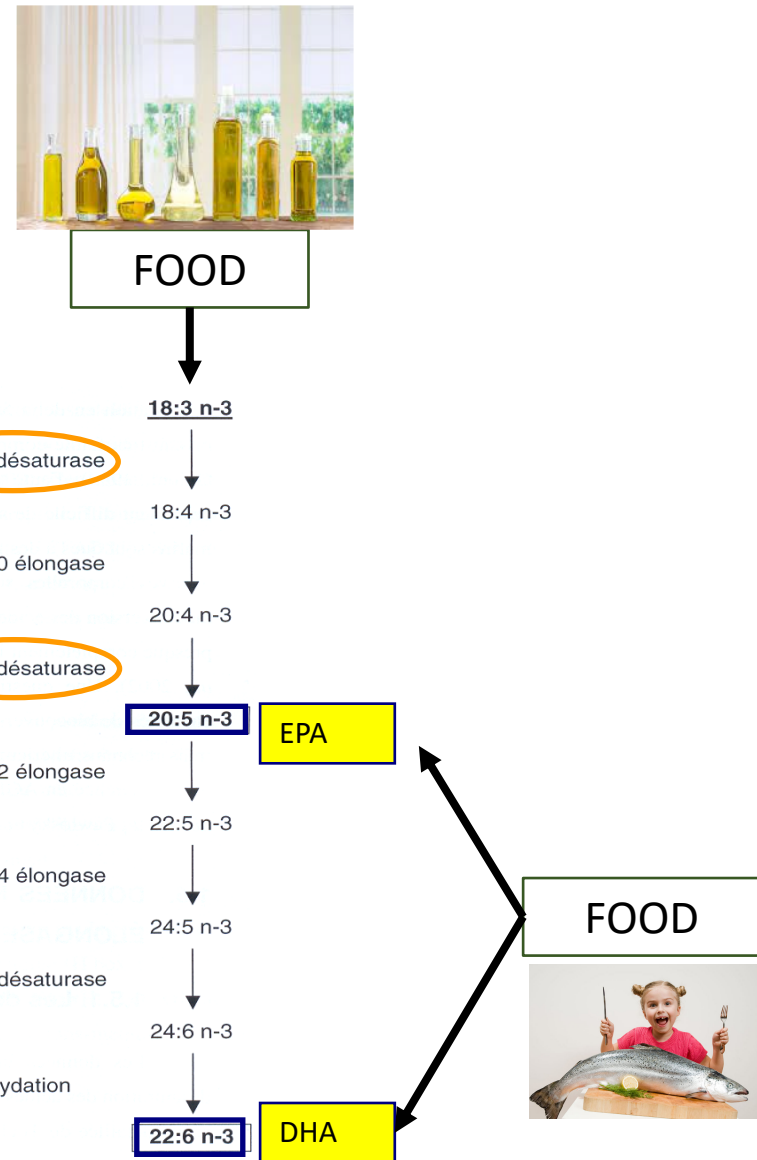
24:6 n-3

β -oxydation

22:6 n-3

DHA

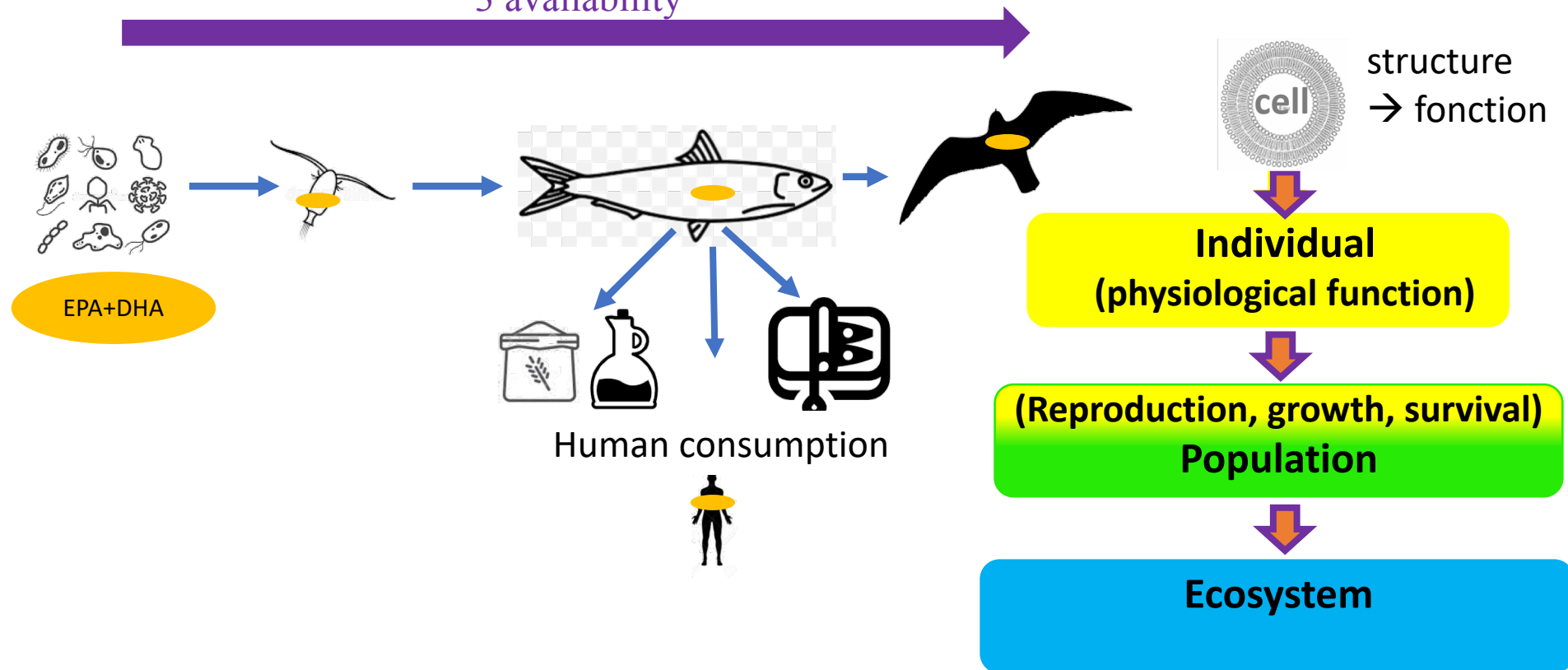
FOOD



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→ Must be supplied by food

Decreasing HUFA n-3 availability



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