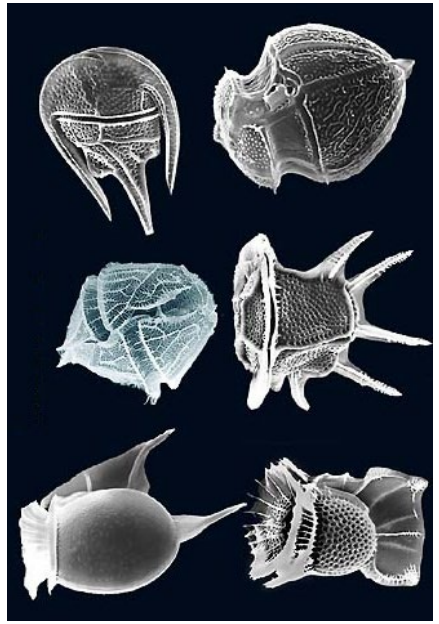
Laboratoire PHYCOTOXINES — Ifremer

VIMS | William & Mary — Virginia Institute of Marine Science

University of Copenhagen

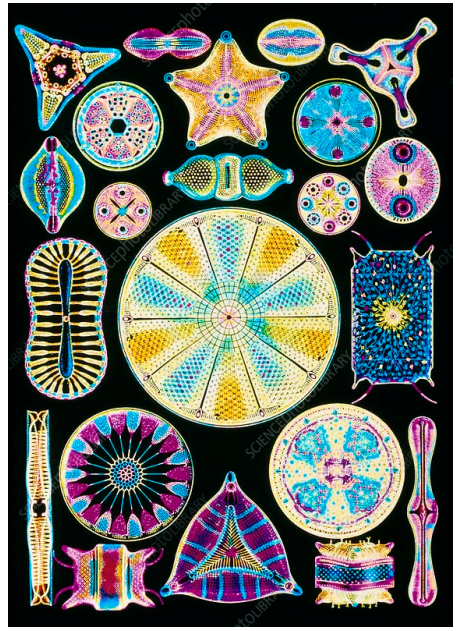
Ecophysiological responses of ***Pseudo-nitzschia*** and ***Dinophysis*** toxic species to environmental variations related to climate change



Dinoflagellates photo
by Fickle and Freckled

Early career scientist
Virginia institute of marine science (VIMS)

Contact: nayache@vims.edu
804.684.7812

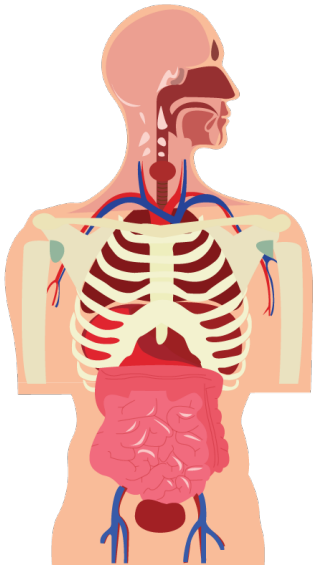


Art of Diatom algae
(from Ernst Haeckel)

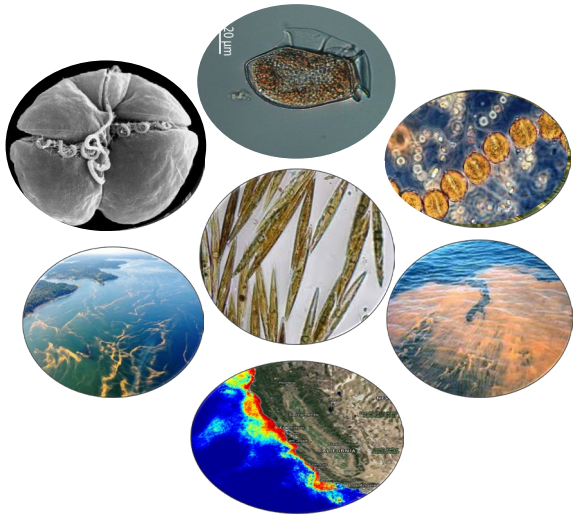
Harmful algal blooms: HAB producers of potent toxins and harmful to human and wildlife health

➢ **Health impact**

Neurological symptoms



Gastro-intestinal symptoms



Transfer to humans

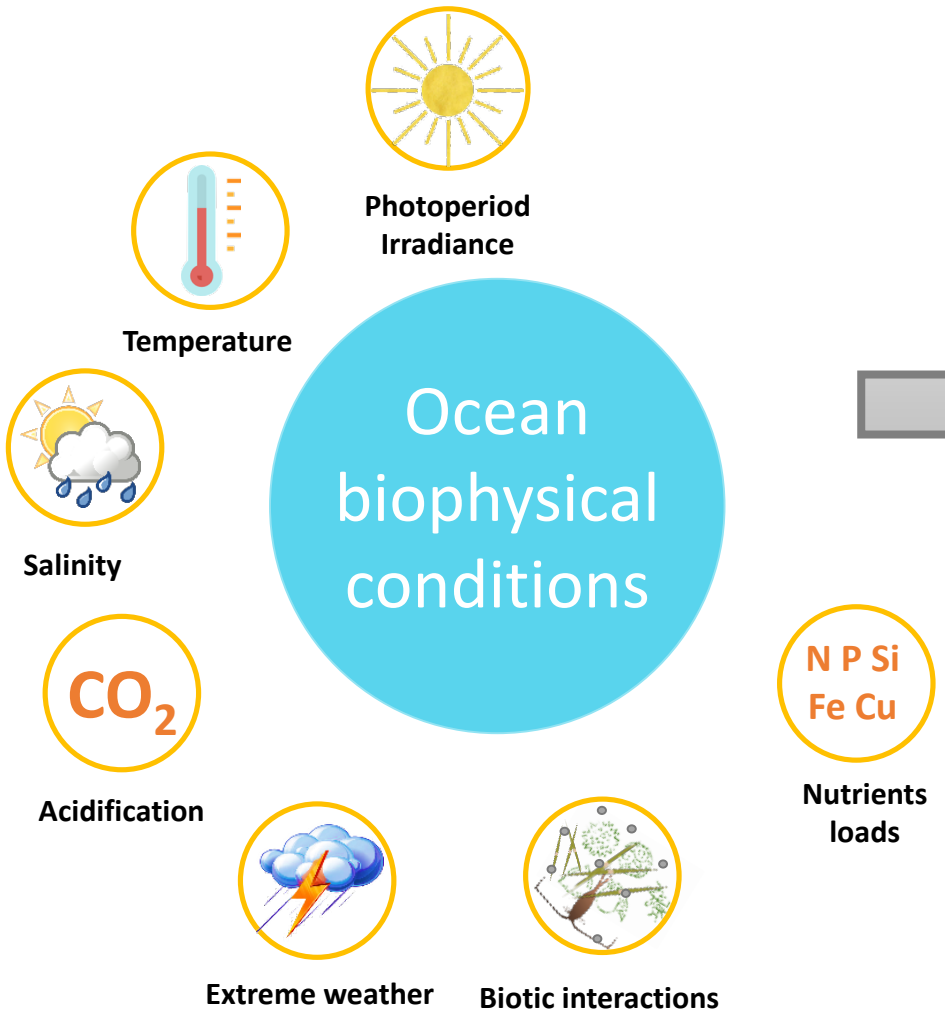
Bioaccumulation and magnification in mollusks and crustaceans

Transfer to fishes

Transfer to marine birds and mammals

Global increase of HABs

Environmental factors that may affect the proliferation and toxicity of HABs

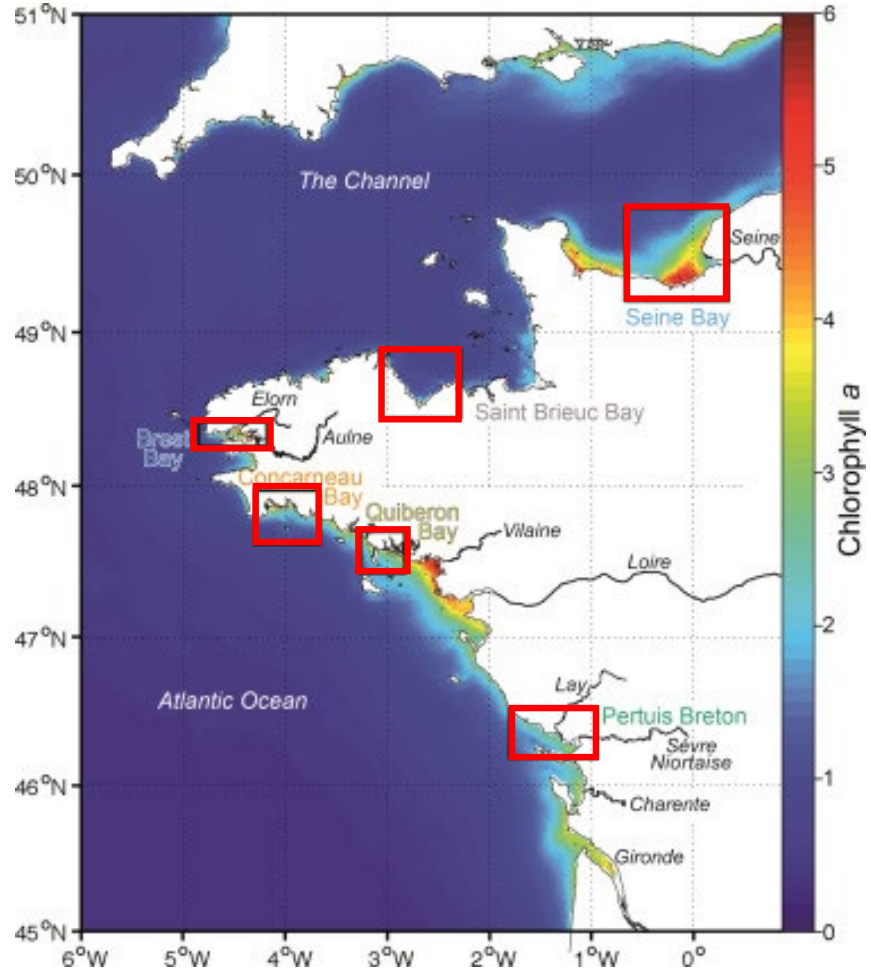


Increase in **frequency**, **intensity** and **distribution** of **HAB blooms** (Wells et al. 2015)

Unusual extreme weather event: Cyclone Xynthia

Highly toxic bloom of ***Pseudo-nitzschia***

Domoic acid exceeded safety thresholds by **150-fold**



Two decades of *Pseudo-nitzschia* spp. blooms and **king scallop contamination** by domoic acid along the French Atlantic and English Channel coasts (Berengere et al. 2016)

Closures of **scallop** fishing activities for **2 years**

(Ifremer-Quadrige^2-REPHY 2014)

Laboratoire PHYCOTOXINES

Ifremer

UNIVERSITY OF COPENHAGEN

Impact of salinity and pH variation on growth and toxin content of several toxic and non-toxic *Pseudo-nitzschia* strains

Ayache et al. 2019, 2020, and 2021

Experimental set-up

pH variation

- **pH 8.07** • **Present pH level**
- **pH 7.77** • **Estimated pH level for 2100 (RCP8.5 scenario)**
- **pH 7.40** • **Estimated pH level for 2300 (RCP8.5 scenario)**

Experimental conditions:

7 strains of ***P. fraudulenta*** **(non toxic)** and ***P. australis*** **(toxic)**

Namibia and **France** (Atlantic, English channel)

Semi-continuous cultures,

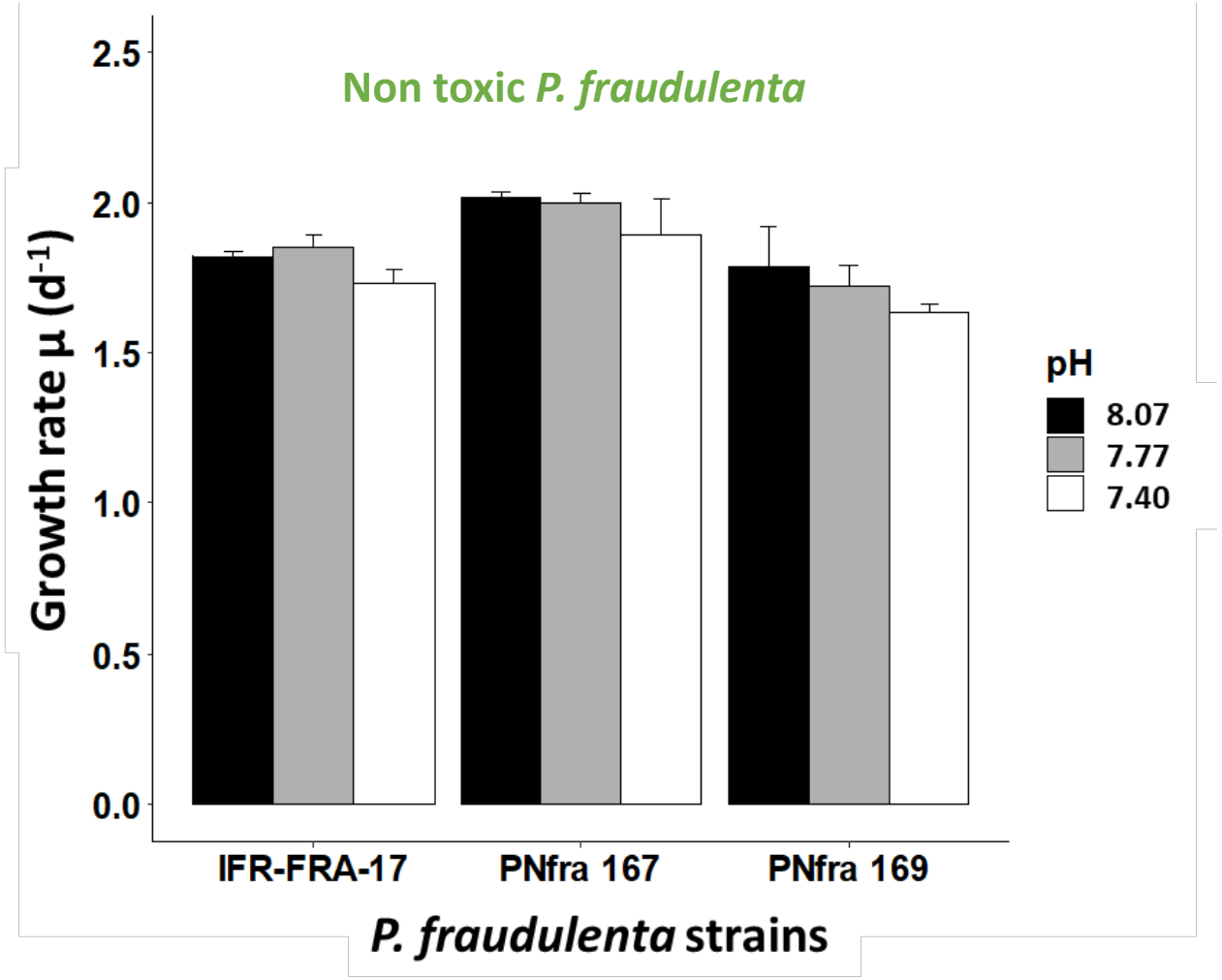
Acclimated > 20 generations

Monitoring

- **Growth rate**
- Total **DA content HPLC-MS/MS**

1. Effect of pH on growth

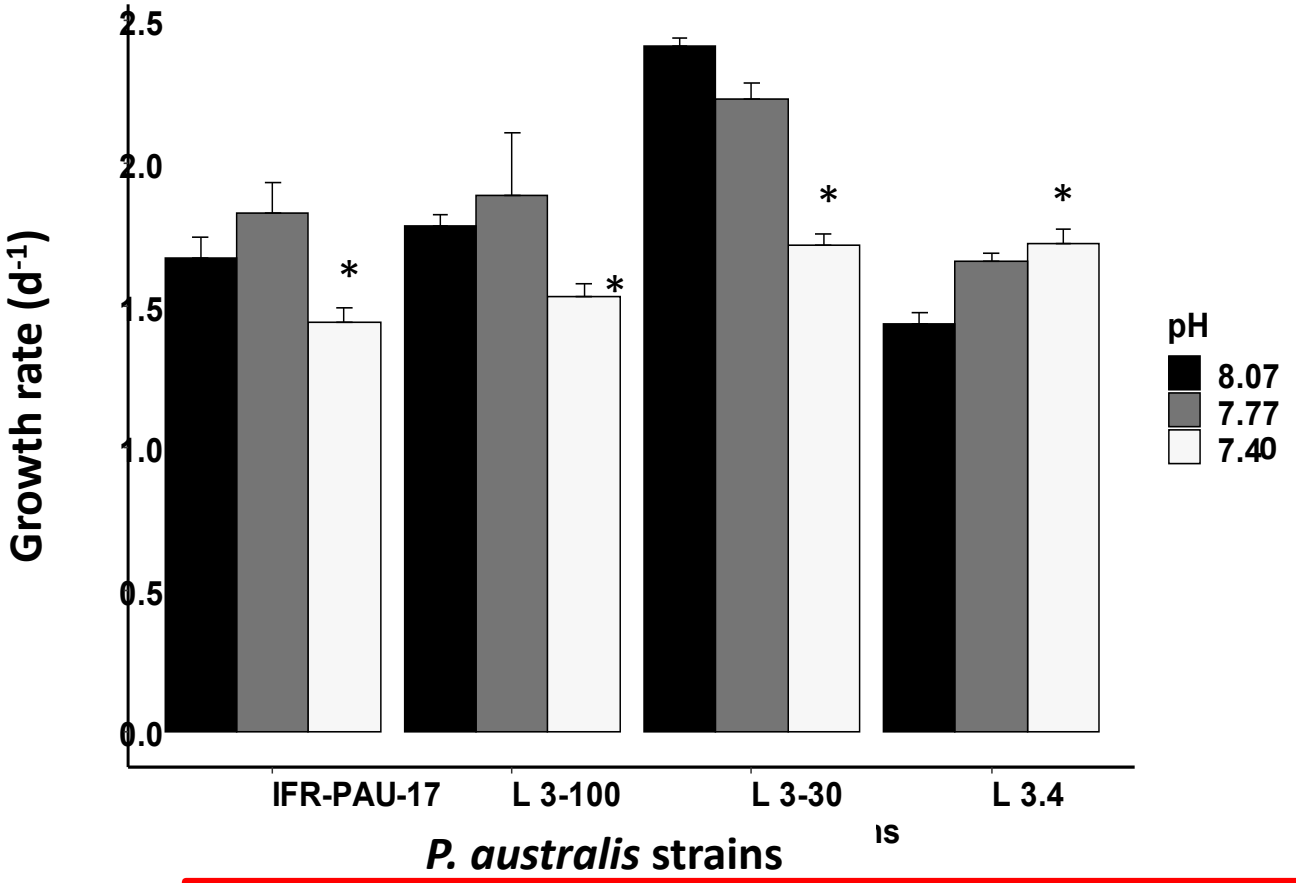
- *Pseudo-nitzschia* rely on **CO_2 and HCO_3^-** uptake for **photosynthesis** (Rost et al. 2003)
- **Theoretically: decrease of pH ➔ increase growth rate**



No effect of pH variation on growth rates

1. Effect of pH on growth

➢ **Toxic *P. australis* strains**



pH 8.07 (present) Vs 7.77 (2100)

➔ **No significant effect on growth**

pH 8.07 (present) Vs 7.40 (2300)

➔ **Slight decrease of growth 15 – 30 %**

pH 8.07 (present) Vs 7.40 (2300)

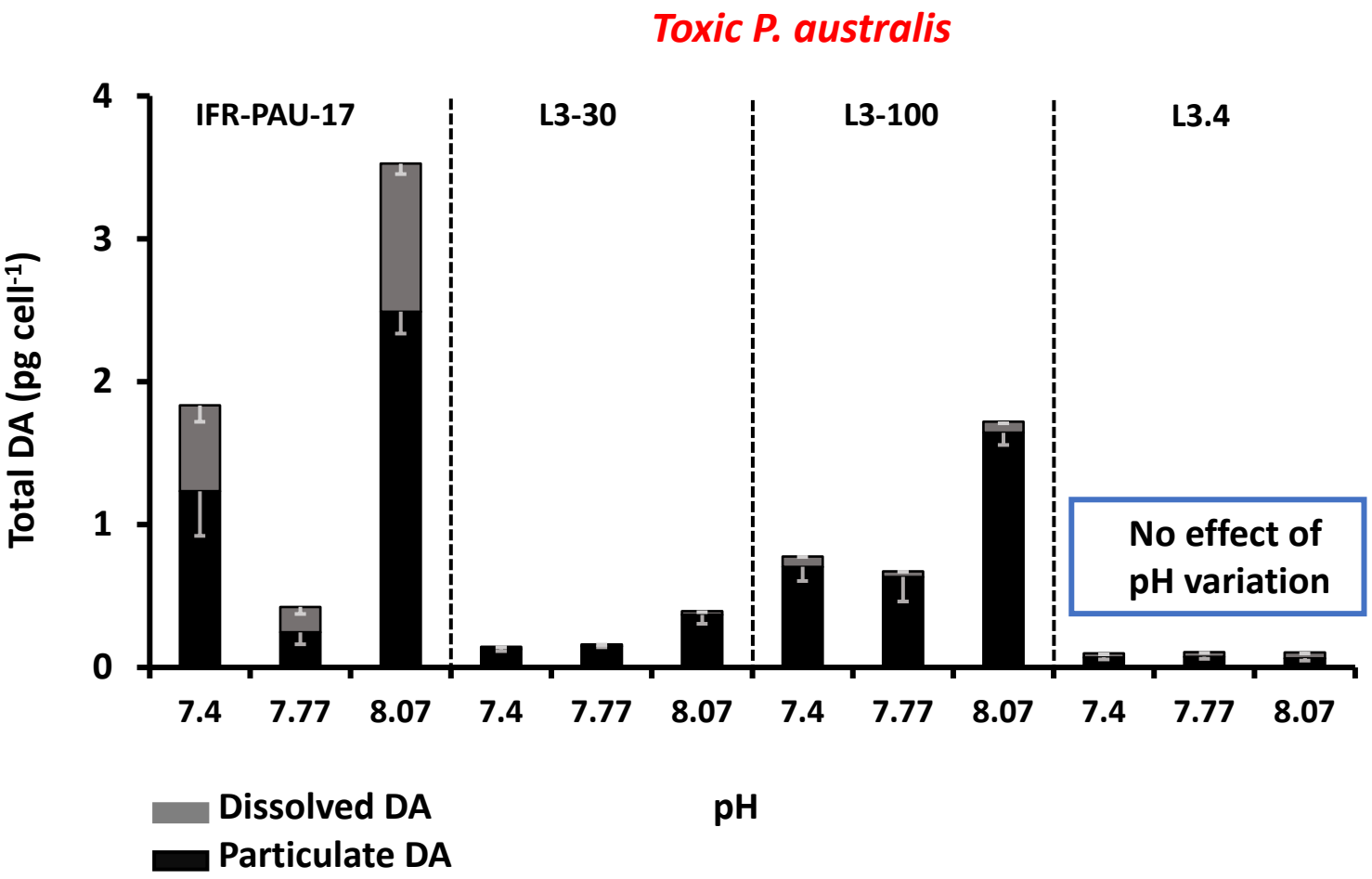
20 % increase *P. australis* **L3.4**

Lower growth rate at pH 7.40 (2300)

➢ High energy required to maintain a steady pH in the cell, necessary for cellular function at the expense of growth

2. Effect of pH on DA content

- **No DA stimulation in any of *P. fraudulenta* strains**



pH 8.07 (present) Vs 7.77 (2100)

➔ **Higher total DA content at current pH for most strains**

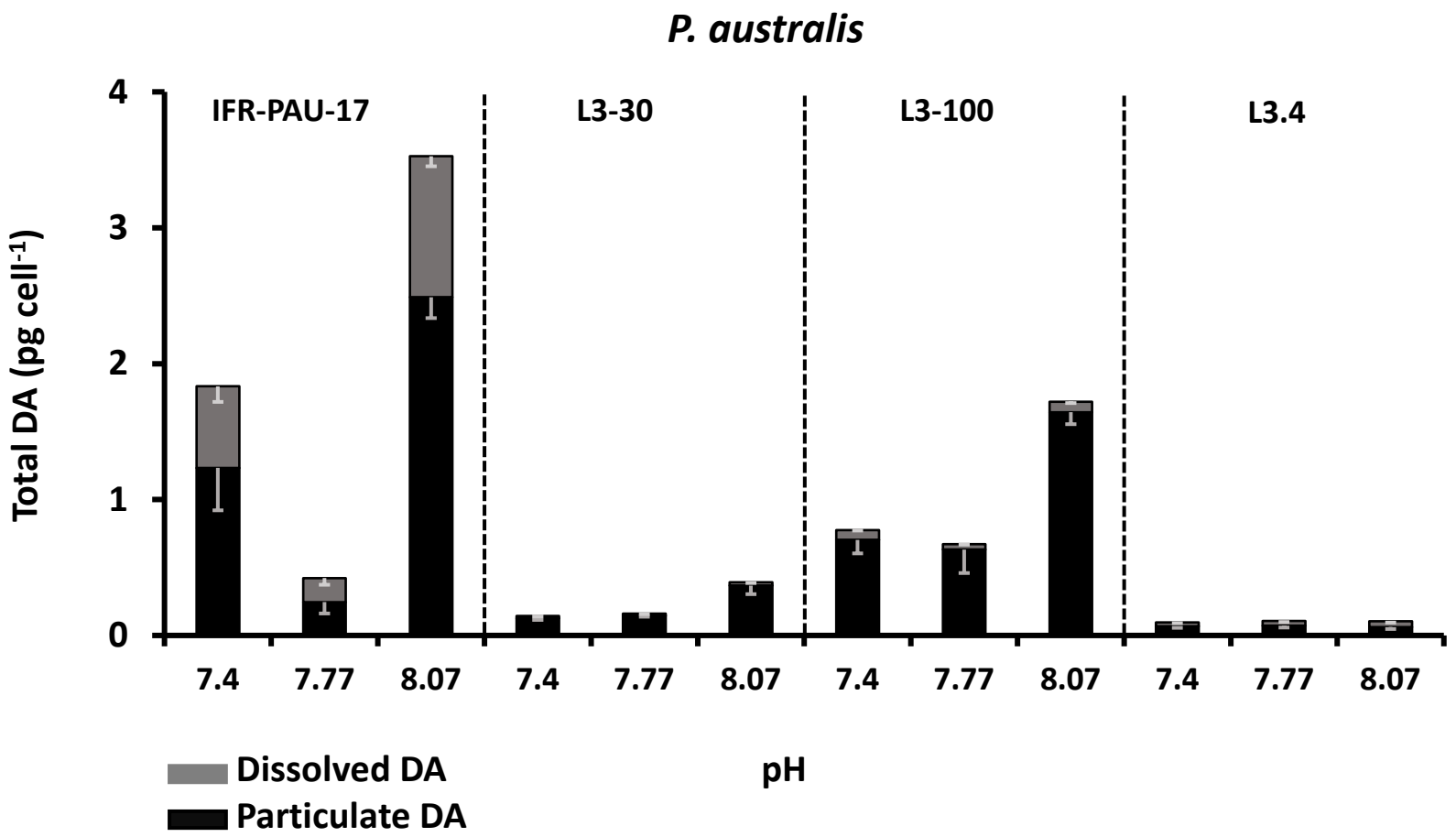
➔ **At the lowest pH levels: amount of pDA was 50 % to 90 % lower**

2. Effect of pH on DA content

Shift from optimum intracellular pH

➔ **Affected physiological processes: enzyme activity, protein function and nutrient uptake**

➔ **High energy spent on maintaining high growth at the expense of DA production**



Summary

- **No effect of pH variation on growth**

➔ *P. fraudulenta* and *P. australis* strains **acclimated** and maintained **high growth**

- **Significant lower cellular DA content at lower pH levels (up to 10-fold)**

- **Significant variability**: species (***P. fraudulenta* vs *P. autralis*)**, strain, geographical origin

Future perspectives

- **DA biosynthesis pathway** (Brunson et al. 2018)

➔ Gene expression (+/-) under conditions of decrease pH

- **Interactive effects of decrease pH + irradiance and T in response to climate change**

2- Ecophysiological responses of *Dinophysis* toxic species to environmental variations related to climate change

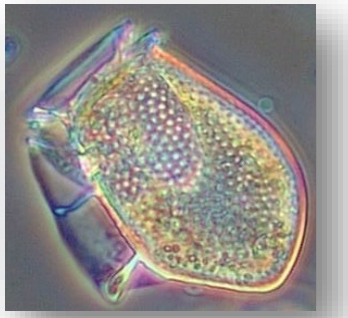
Why *Dinophysis*?

Since 2008: "*Dinophysis* emerging threat to Human health and fisheries in USA"

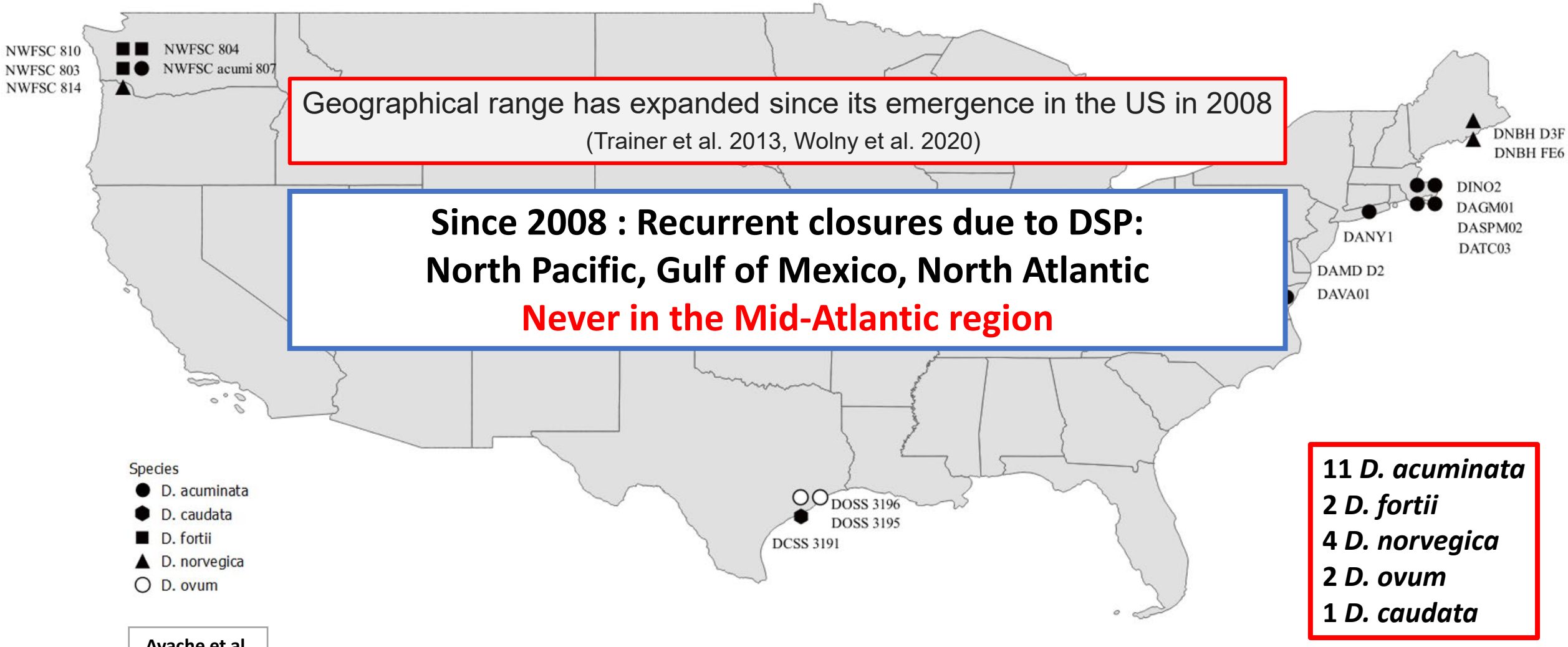
Toxin shuts down Sequim Bay shellfish harvests

Originally published August 11, 2011 at 6:42 pm | *Updated August 12, 2011 at 10:48 am*

A new biotoxin found on the Olympic Peninsula has caused a shellfish closure after an adult and two children were sickened when they harvested and ate mussels from Sequim Bay.



Characterization of 20 toxin-producing *Dinophysis* strains isolated from US coastal waters



Geographical range has expanded since its emergence in the US in 2008

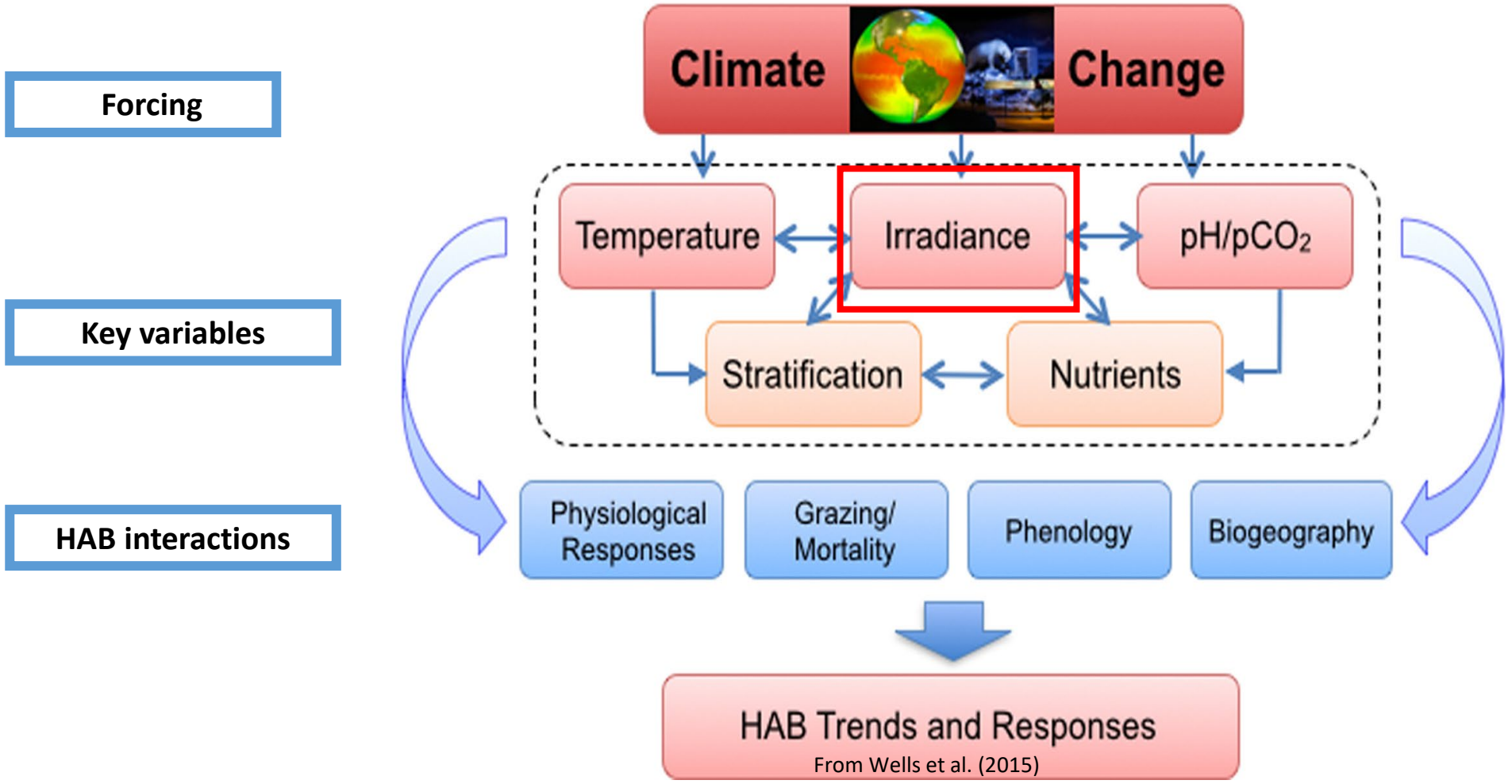
(Trainer et al. 2013, Wolny et al. 2020)

Since 2008 : Recurrent closures due to DSP:
North Pacific, Gulf of Mexico, North Atlantic
Never in the Mid-Atlantic region

11 *D. acuminata*
2 *D. fortii*
4 *D. norvegica*
2 *D. ovum*
1 *D. caudata*

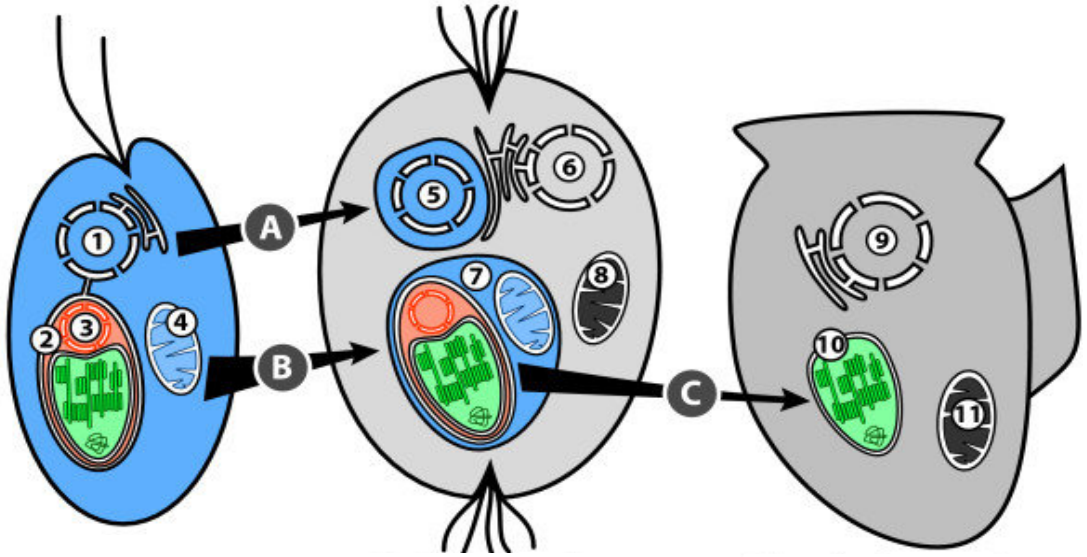
Ayache et al. submitted

Investigate environmental and biological drivers of *Dinophysis* blooms and toxicity *in situ* within and across regions



From Wells et al. (2015)

Dinophysis trophic mode: obligate mixotroph



Cryptophyte ***Teleaulax amphioxeia***

Ciliate ***Mesodinium*** **rubrum**

Dinoflagellate ***Dinophysis***

First successful cultures of *Mesodinium* (Yih et al. **2004**) and *Dinophysis* (Park et al. **2006**)

➢ ***Mesodinium*** **and** ***Dinophysis*****:**

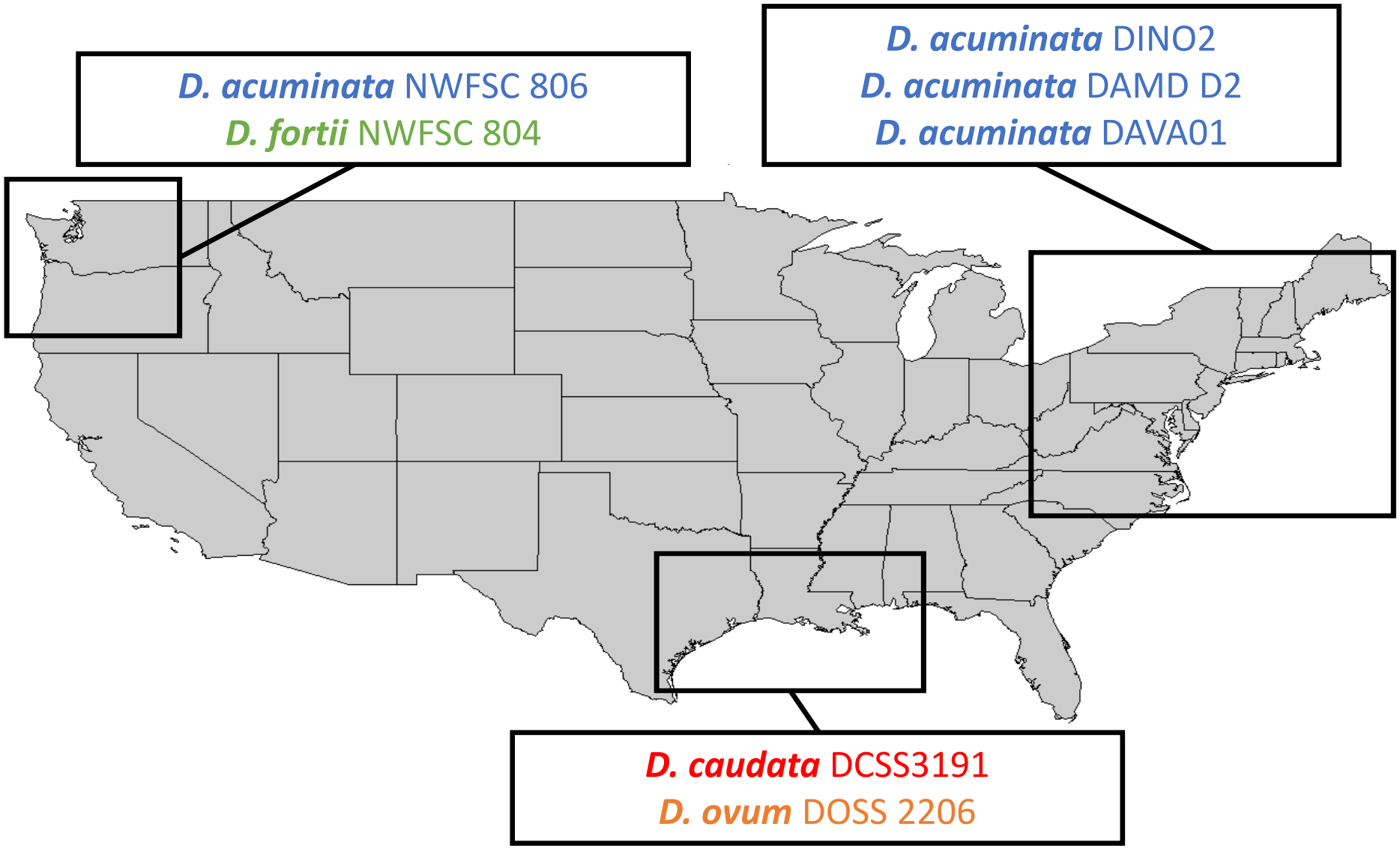
Combining both phototrophy and heterotrophy - **Kleptoplast** (Hansen et al., 2019)

➢ *Dinophysis* rely on chloroplast uptake from preys for **photosynthesis**

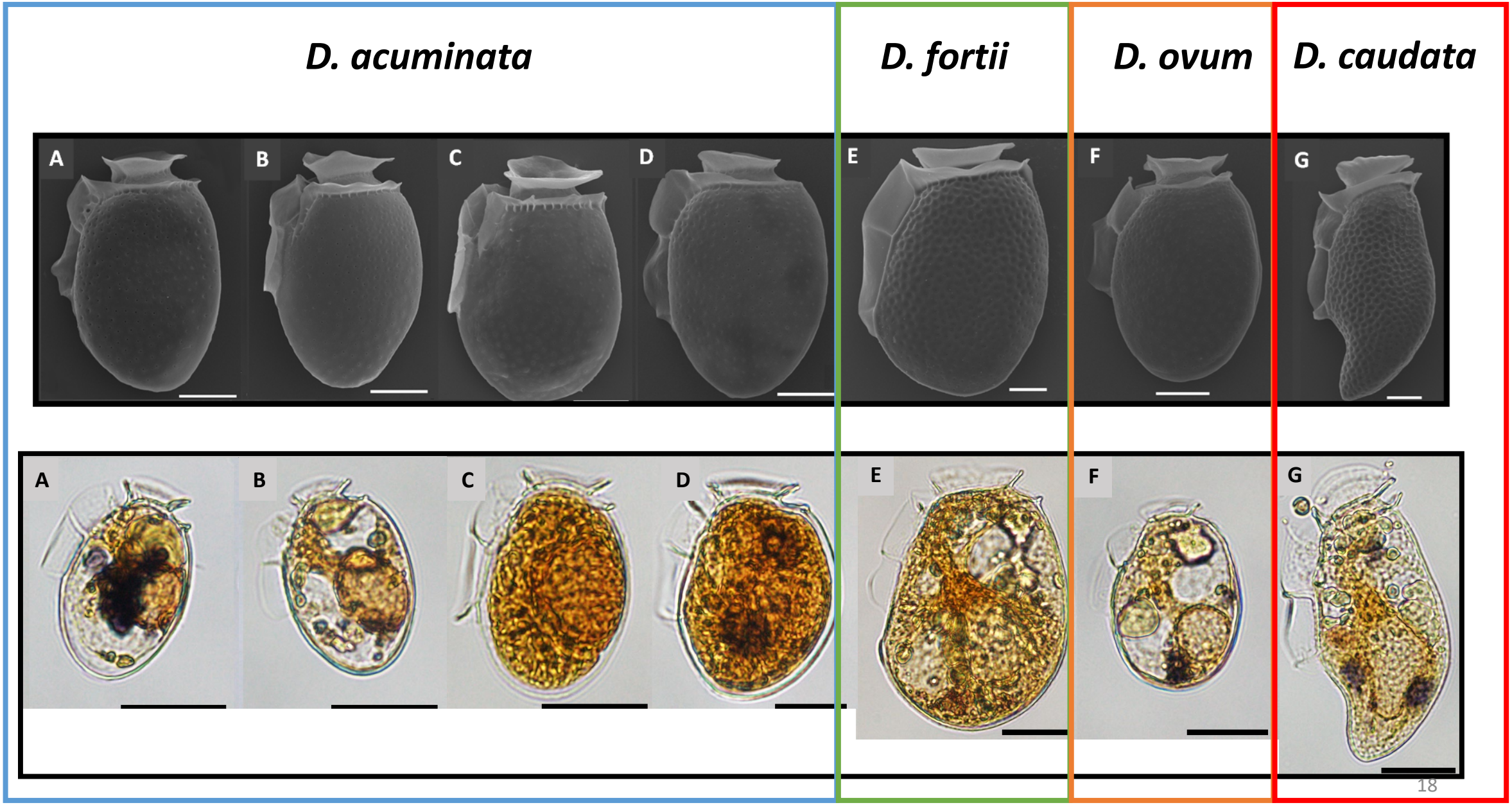
➔ **growth, metabolite synthesis**

Effect of irradiance

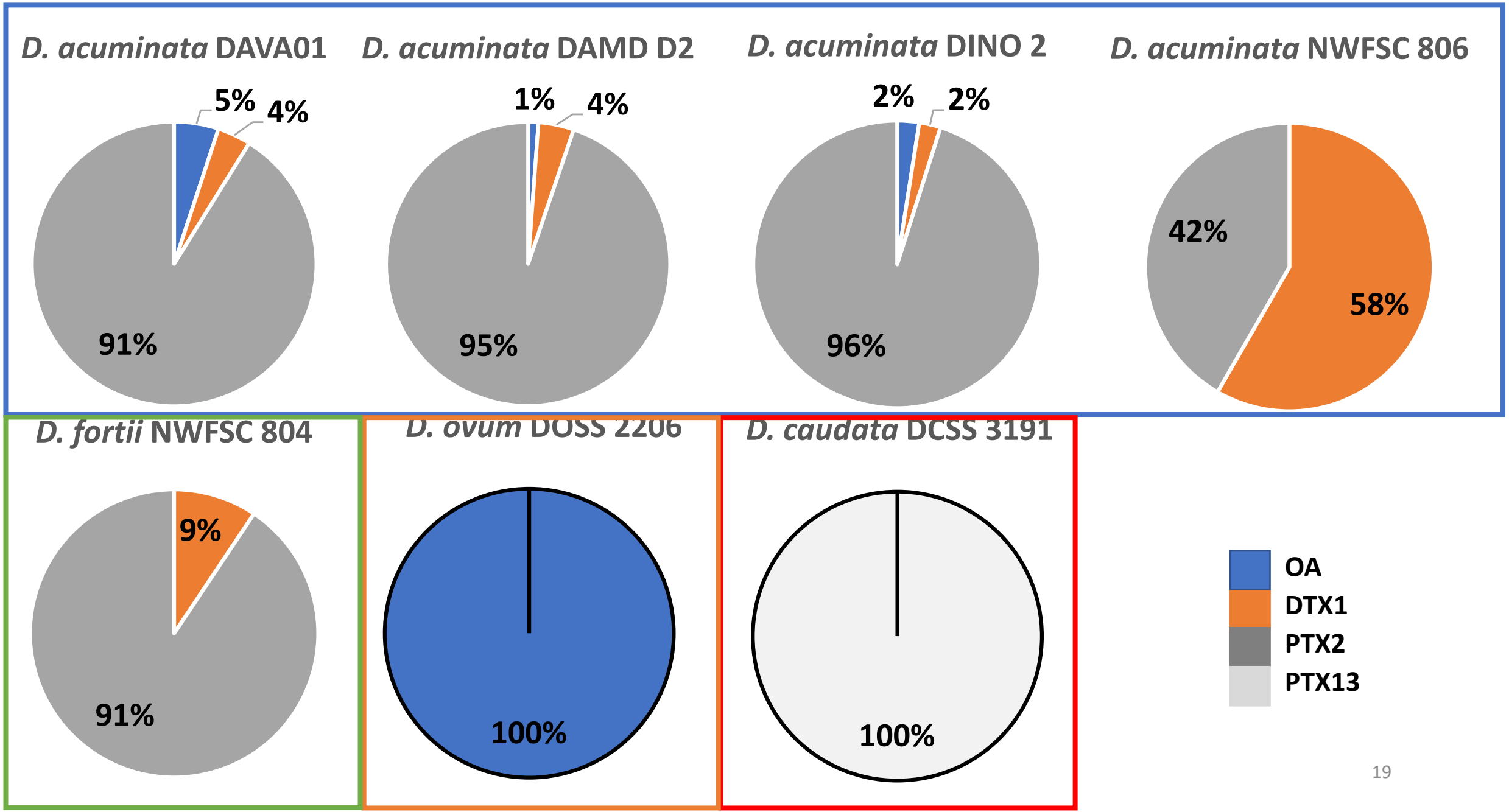
7 different isolates from 3 estuarine and coastal regions



7 different isolates from 3 estuarine and coastal regions



Toxin profiles: species- and origin-specific



Experimental set-up

Irradiance

- **L 10** • Low light: pycnocline
- **L 100** • Control
- **L 400** • High light: photosaturation

Monitoring:

Acclimated 4 months > 10-20 generations

Maintained in exponential growth phase

- **Growth rate (counting)**
- **Photosynthetic efficiency F_v/F_m (Phyto-PAM)**
- **Toxin content and production rates** (LC-MS/MS)

1. Effect on growth

Strong variability

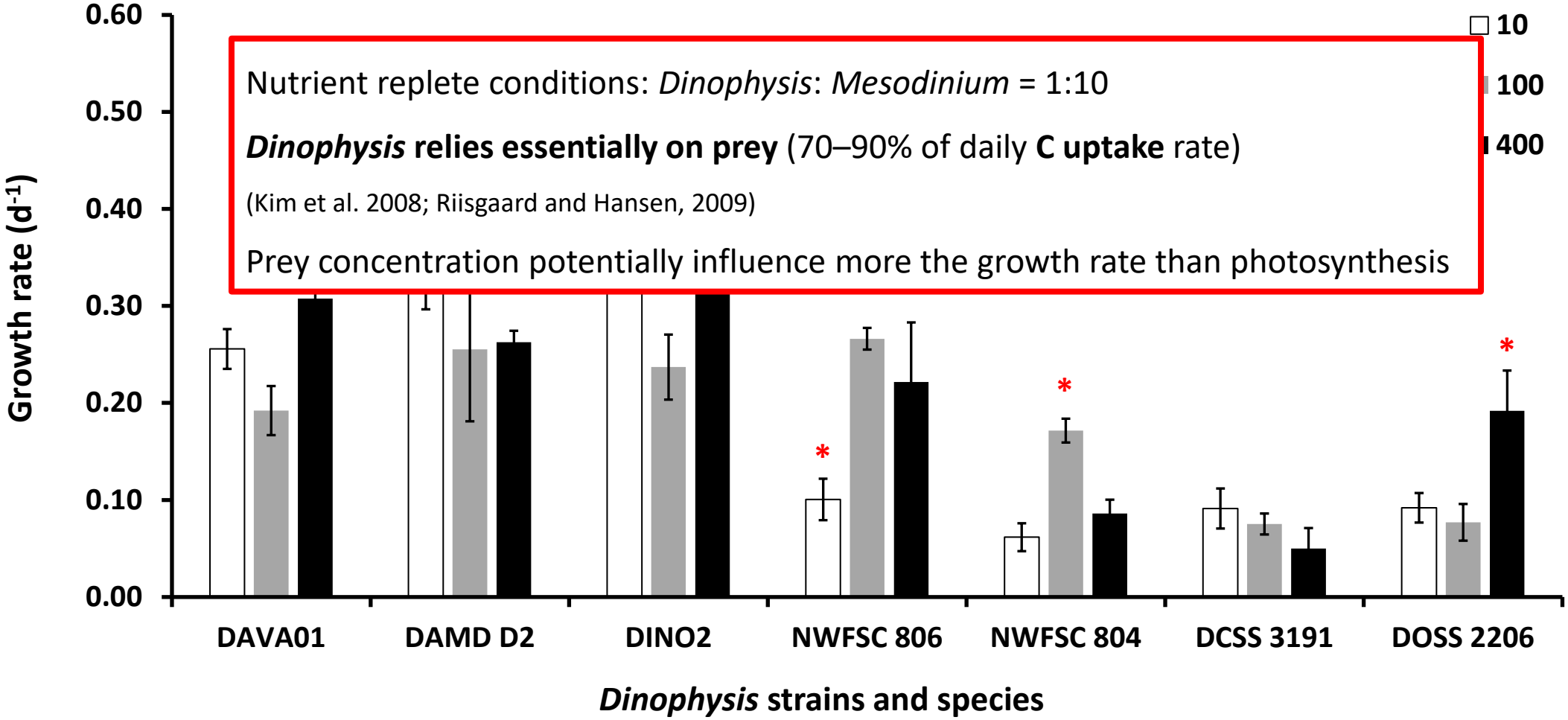
Independent of the intra and interspecific variation, geographical origin

Nutrient replete conditions: *Dinophysis*: *Mesodinium* = 1:10

***Dinophysis* relies essentially on prey** (70–90% of daily **C uptake** rate)

(Kim et al. 2008; Riisgaard and Hansen, 2009)

Prey concentration potentially influence more the growth rate than photosynthesis



2. Effect on photosynthetic efficiency F_v/F_m

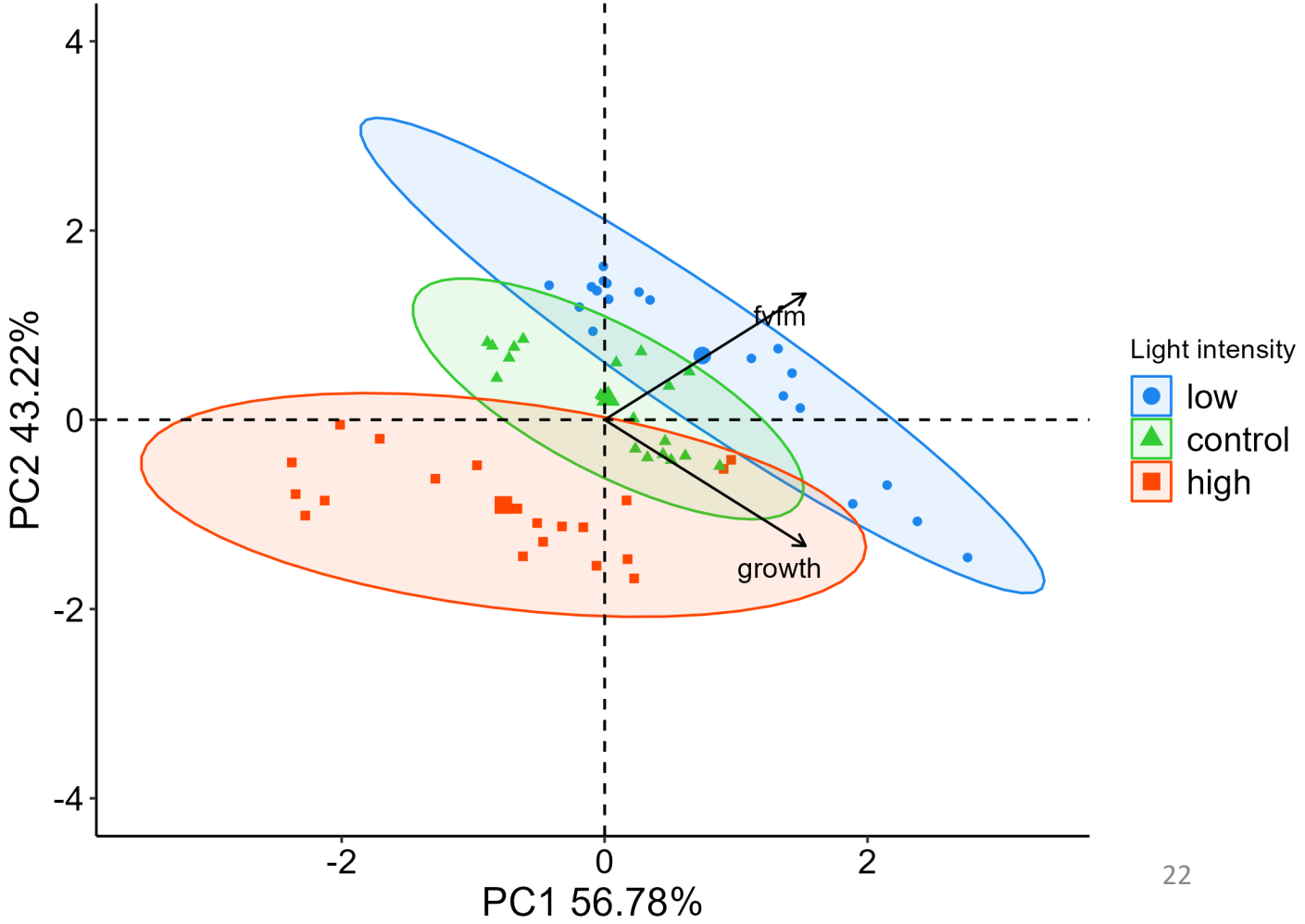
➢ **F_v/F_m varied as a function of irradiance ➔ Higher F_v/F_m at lower irradiance**

At low irradiance

Higher pigment content:

Chl a, phycoerythrin

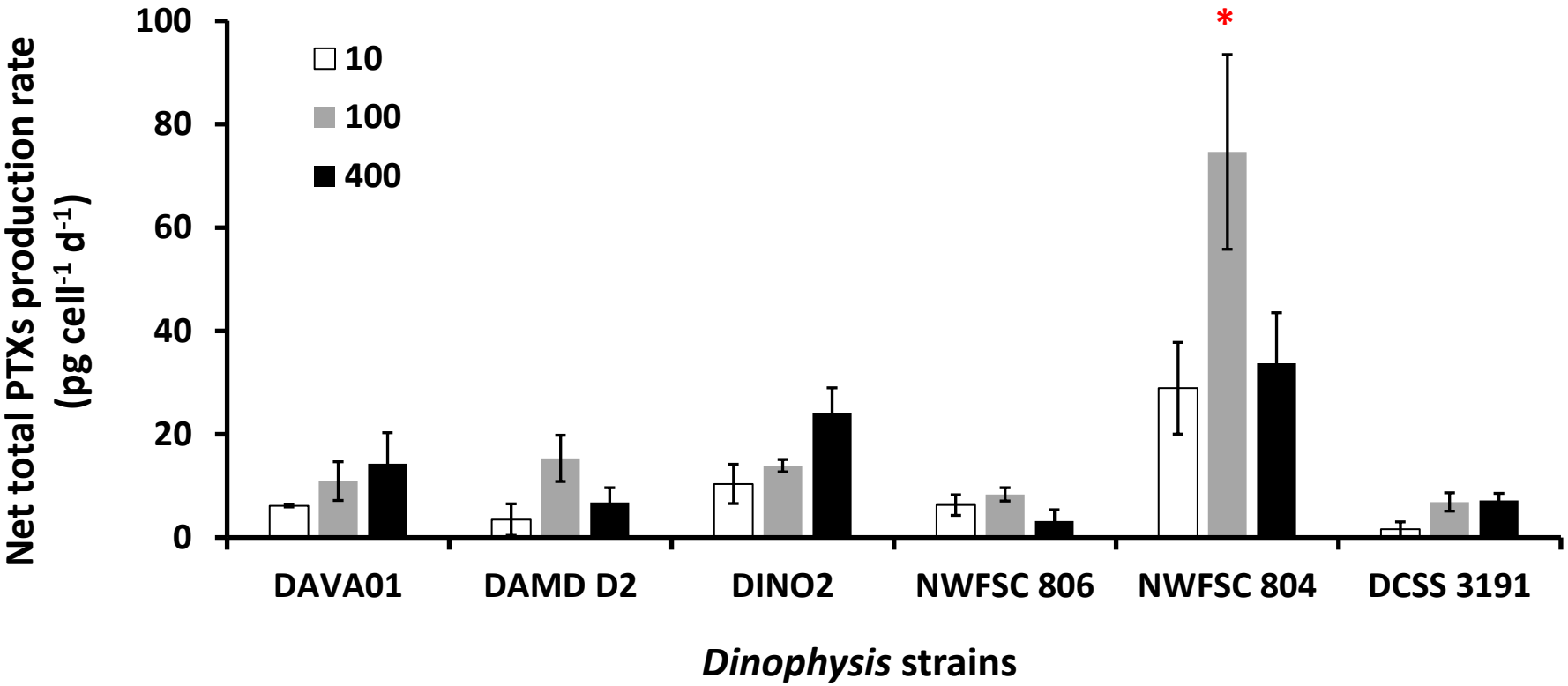
Photosystems more efficient/active to capture the max available light



3. Effect on toxin production

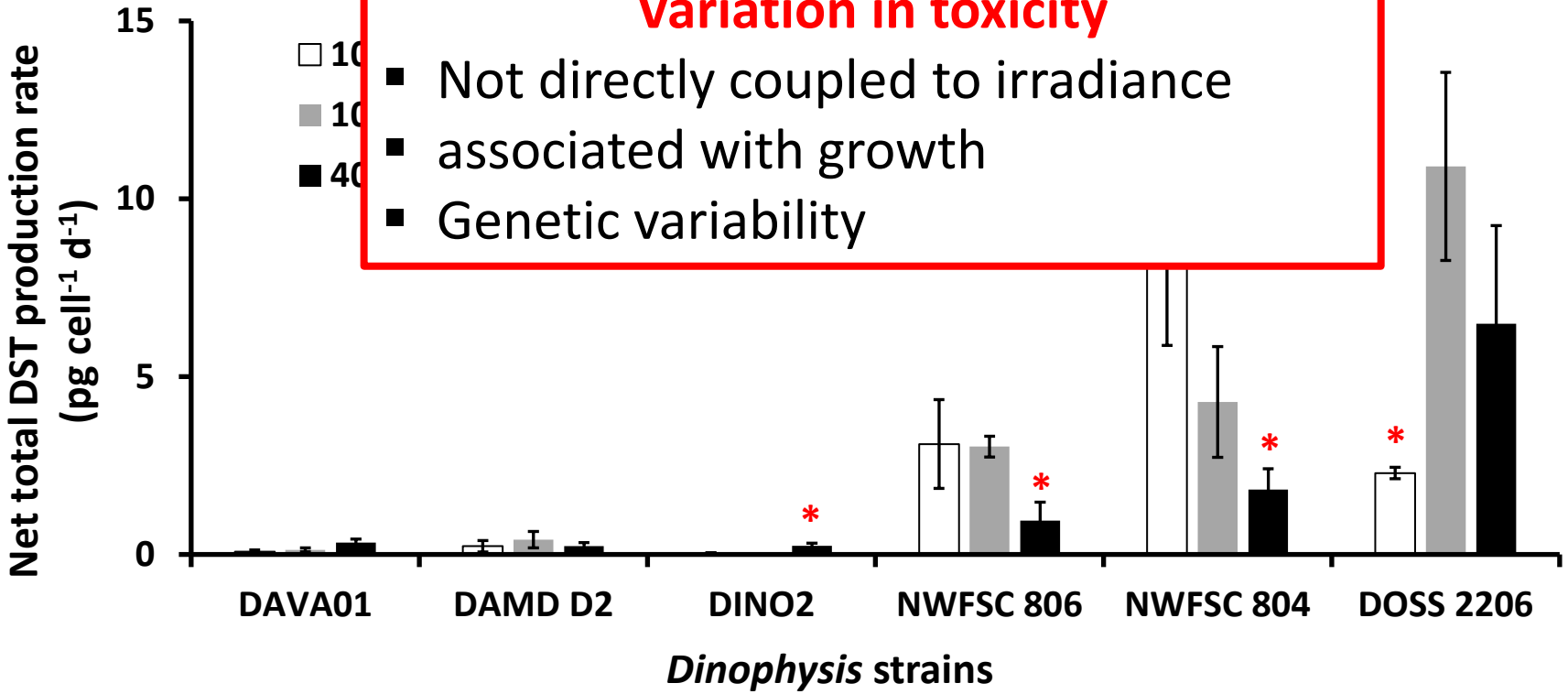
➢ **Total PTXs: PTX2, PTX13 (pg cell^{-1} d^{-1})**

Overall
No effect of irradiance on PTXs production rates
Exception
D. fortii **➔Highest PTXs** production rates at **100**



3. Effect on toxin production

➢ **Total DST: OA + DTX1 (pg cell^{-1} d^{-1})**



- Mid-Atlantic isolates: **no effect**
- **West coast** isolates: **Highest DST** production rates at **10 and 100**
- **Gulf of Mexico: Highest DST** production rates at **100 and 400**
- **North East Atlantic strain: Highest DST at 400**

Summary

- **All strains able to photo-acclimate and tolerate a large range of irradiance (10 to 400)**
- **But no direct effect of irradiance on growth and toxicity**

➔ **Strong intraspecific variations** are most likely controlled by genetic differences between *Dinophysis* species

Perspectives

- **Other collaborators investigating: salinity, turbulence, pH, T ° for the same isolates**
- **Interactive effects of irradiance + T° in response to climate change scenarios** (Wells et al. 2021, Boyd et al. 2018) **including transcriptomics**

➔ **Modelling effect of these changes ➔ predict bloom formation and toxicity triggers**

Ayache et al.
In prep