

Harmful Algal Bloom (HAB) Symposium

# *HAB* isolates and cultures from South Australia 2025

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# Clonal cultures of dinoflagellates from HAB

Clonal cultures of the harmful algal bloom-forming dinoflagellates are laboratory populations grown from a single isolated cell.

Clonal cultures are crucial for studying harmful dinoflagellates.

Research Applications:

- Toxin profiling
- Genetic analysis
- Mitigation
- Sensor calibration

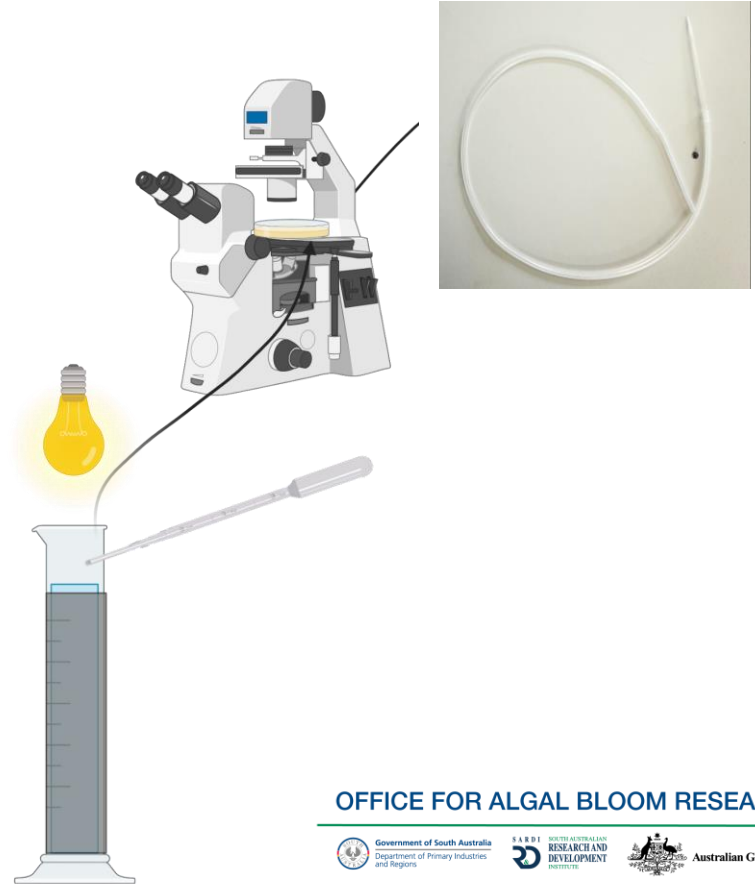
# Procedure for single-cell isolation

Sample collection

Light treatment for cell concentration

Isolation: Using modified cell pickers – glass rod drawn out under flame and suction hose attached, Inverted microscopes

Maintenance and cultivation



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The diagram shows two 96-well plates. The top plate has columns numbered 1 to 6 and rows labeled A to D. The bottom plate has columns numbered 1 to 12 and rows labeled A to H. Arrows indicate the addition of 100 µl of 100 mg/ml protein to the top-left well (A1) and the bottom-right well (H12).

The diagram illustrates two 96-well plates. The top plate is a 4x6 grid with columns labeled 1 to 6 and rows labeled A to D. The bottom plate is an 8x12 grid with columns labeled 1 to 12 and rows labeled A to H. An arrow points from the well at row D, column 5 of the top plate to the well at row A, column 12 of the bottom plate.

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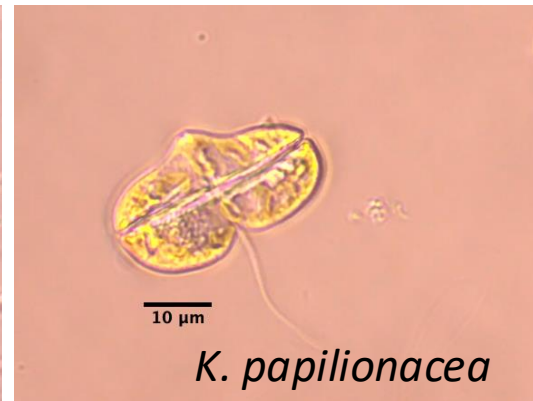
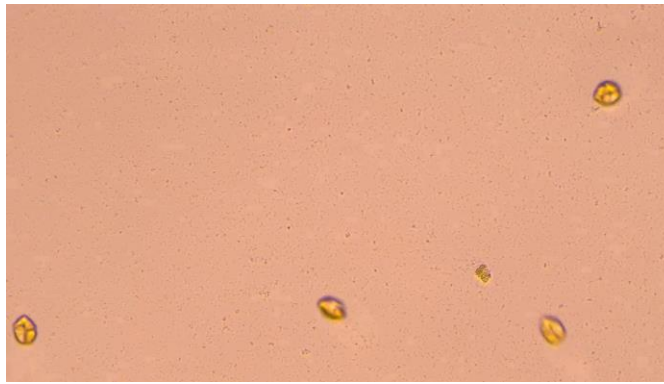
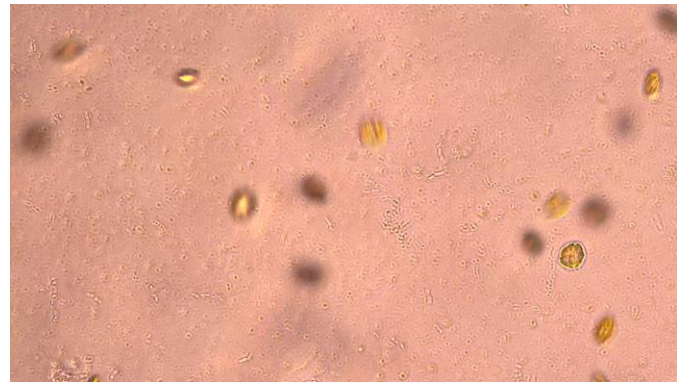
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# Number of single-cell isolates

| Temperature (°C) | Number of independent isolates |             |              |             |          |                 |              |
|------------------|--------------------------------|-------------|--------------|-------------|----------|-----------------|--------------|
|                  | Karenia                        | Karlodinium | Scrippsiella | Gymnodinium | Takayama | Biecheleriopsis | Unknown taxa |
| 15               | 4                              | 26          | 12           | 5           | 3        | 4               | 9            |
| 17-18            | 4                              | 15          | 3            | 5           | 9        | 2               | 5            |
| 20               | 2                              | 1           | 19           | 2           | 9        | 4               | 8            |
| 22               | 0                              | 0           | 0            | 0           | 0        | 0               | 0            |
| Total            | 10                             | 42          | 34           | 12          | 21       | 10              | 22           |

Above 20°C, evaporation caused the culture to crash.

# Cultures of *Karenia* spp. from HAB in SA



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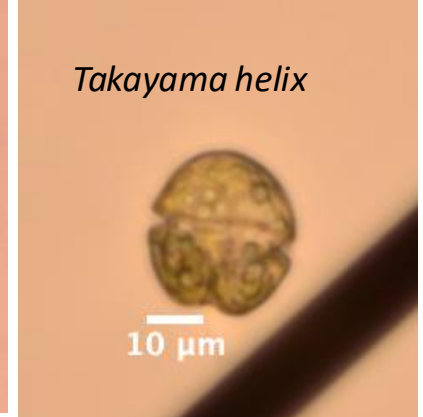
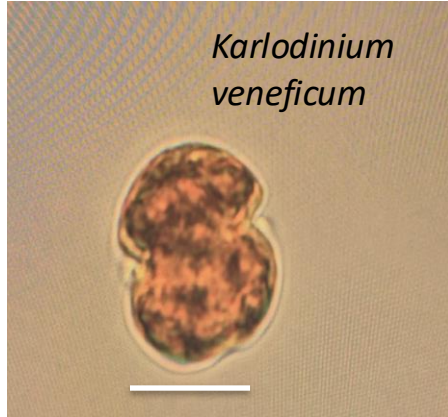


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# Other HAB cultures



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# Large-scale cultures of *Karlodinium veneficum* and *Scrippsiella acuminata*



Used for mitigation trials.

At this time-No successful upscaling of *Karenia* species

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# Culture medium for isolating and cultivating

| Medium                   | Nitrogen source                 |                                         |
|--------------------------|---------------------------------|-----------------------------------------|
| K medium added selenium  | Nitrate and Ammonium            | Many successful <i>Karenia</i> isolates |
| L1 medium added selenium | Nitrate only                    | No successful <i>Karenia</i> isolates   |
| Gse medium               | Nitrate + soil extract organics | Not tested yet                          |

# Highly fragile and sensitive to shear forces, turbulence, and agitation.

Moving *Karenia* cultures across building caused 100% culture crash.

Need to mind when:

- Transporting
- Transferring cells into new medium for upscaling
- Adding medium to culture



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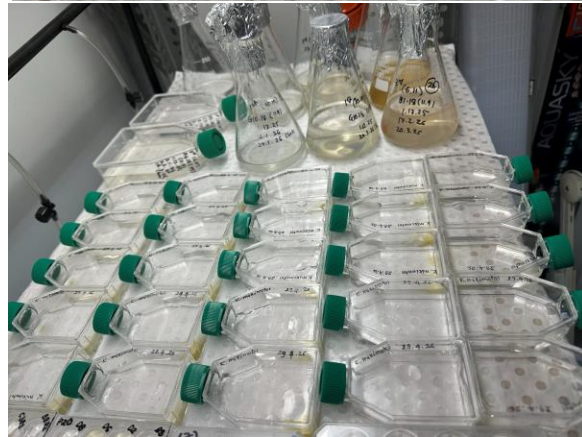
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# Salinity – an important factor to consider

- Original seawater: No success
- Seawater at SARDI (38-39 ppt): Only 2/24 cultures were viable but crashed soon
- Add fresh water to make medium with salinity of 35-37 ppt: 100% culture were viable and healthy.

# Preliminary success in *Karenia* production

| Upscale | <i>Karenia</i> species                                                                                                                                                      |                                                                                                                                                                   |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 mL    | 15 from 4 independent isolates                                                                                                                                              |                                                                                                                                                                   |
| 5 mL    | <ul style="list-style-type: none"> <li>- 10 cultures from single isolate of <i>K. cristata</i></li> <li>- 45 cultures from single isolate of <i>K. mikimotoi</i></li> </ul> | <p>- 90 cultures of <i>K. cristata</i> from two differed isolates have doubled cell number.</p> <p>- 36 cultures of <i>K. mikimotoi</i> were well-established</p> |
| 20 mL   | 50 cultures of <i>K. mikimotoi</i>                                                                                                                                          | Density of <i>K. mikimotoi</i> : $4.6 \times 10^6$ cells L <sup>-1</sup> .                                                                                        |
| 100 mL  | 1 culture of <i>K. mikimotoi</i>                                                                                                                                            | -                                                                                                                                                                 |



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# *Karenia cristata* cultures



A unique strain of *K. cristata*

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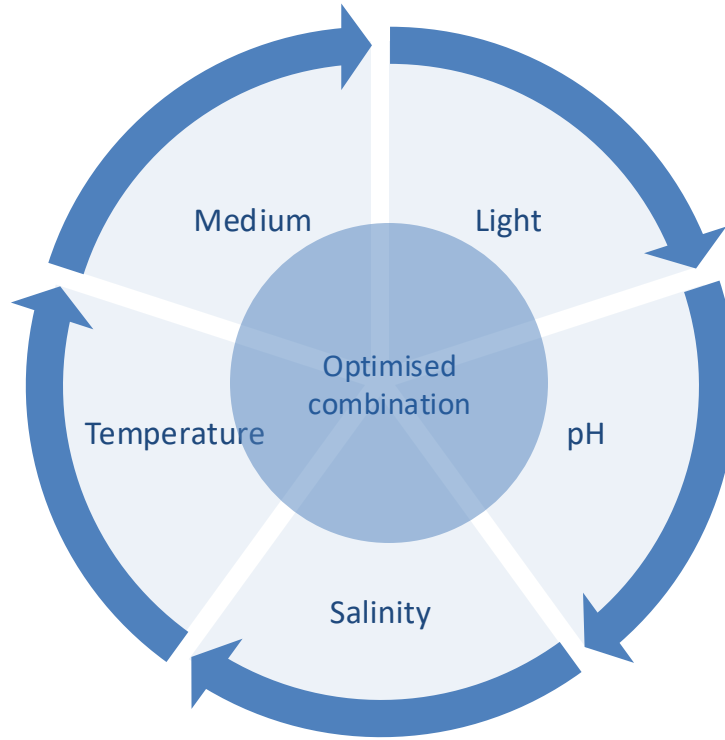


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# Next plan for upscaling



To increase growth rate

To obtain larger-scale  
production of *Karenia  
cristata*

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# Acknowledgement

Algal production team

HAB sampling team

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