**Efficacy of calcein as a chemical marker of *Potamocorbula laevis***

**Summary**

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# Supplementary Tables

# Table S1. Chemical labeling conditions for different aquatic organisms.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Size | Fluorescein stain | Concentration | Time | References |
| *Anadara broughtonii* | shell length: 27.24 ± 1.12 mm | calcein | 200, 300 mg/L | 24 h | Zhou et al., 2017 |
| alizarin red | 200, 300 mg/L | 24 h |
| *Danio rerio* | 25 days old | tetracycline hydrochloride | 150, 250 mg/L | 24 h | Xu, 2012 |
| *Apostichopus japonicus* | weight:  3.45 ± 1.34 g | calcein | 200 mg/L | 24 h | Zhao et al., 2011 |

# Table S2. Study on chemical markers of some bivalves. SL: shell length; SH: shell height.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Family | Species | Size | Stain | Concentration | Time | References |
| Ostreidae | *Crassostrea virginica* | (3 – 5) days of age | calcein | 100 mg/L | 48 h | Gancel et al., 2019 |
| *C. virginica* | 2 weeks,  2 months,  3 years of age | calcein | 250 mg/L | 24 h | Spires and North, 2022 |
| *C. virginica* | SL  (15 – 18) mm | calcein | 25 mg/L  50 mg/L | 24, 48 h | Spires et al., 2022 |
| Pteriidae | *Pinctada mazatlanica* | SH  soaking  (14.8 – 42.7) mm  injection  (11.5 – 36.4) mm | calcein  (soaking) | 40 mg/L, | 20.3 h | McCoy and Huato, 2014 |
| calcein  (injection) | 125 mg/L | – |
| *Pteria sterna* | SH  (20.0 ± 1.2) mm | calcein  (injection) | 125 mg/L | – | Cáceres et al., 2011 |
| *P. margaritifera* | SL  (35 ± 0.02) mm | calcein  (Shell soaking) | 150 mg/L | 12, 24 h | Linard et al., 2011 |
| calcein  (Pearl injection) | 200 mg/L | – |
| Pectinidae | *Argopecten irradians* | SL  15 – 30 mm | calcein | 50 mg/L,  125 mg/L | 24 h,  7 h | Hollyman et al., 2013 |
| *A. irradians* | veliger larvae | calcein | 50 mg/L,  100 mg/L | 48 h,  72 h | Moran and Marko, 2005 |
| *Decatopecten radula* | SH  (38.4 – 75.8) mm | calcein | 150 mg/L,  300 mg/L,  600 mg/L | 3,6 h | Thébault et al., 2006 |
| *Pecten novaezelandiae* | SL  (15 – 20) mm | calcein | 100 mg/L | 12 h | Burgess et al., 2015 |
| Arcidae | *Anadara broughtonii* | SL  (27.24 ± 1.12) mm | calcein | 200 mg/L,  300 mg/L | 24 h | Zhou, 2015 |
| Unionidae | *Lampsilis cardium* | 1 day of age | calcein | 125 mg/L,  250 mg/L,  500 mg/ | 6, 12, 24 h | Eads and Layzer, 2002 |
| *Actinonaias pectorosa* | 7 months of age | calcein | 250 mg/L | 12 h |
| Hyriidae | *Westralunio carteri* | SL  (72.8 – 82.8) mm | calcein | 250 mg/L | 24 h | Klunzinger et al., 2014 |
| Veneridae | *Ruditapes philippinarum* | SL  (10.4 – 45) mm | calcein | 50 mg/L,  100 mg/L,  200 mg/L | 0.5 h,  0.5, 1 h,  0.5 h | Mahé et al., 2021 |
| *R. philippinarum* | SL  25 mm | calcein | 300 mg/L,  400 mg/L,  700 mg/L | 17,24 h | Fujikura et al., 2003 |
| Mesodesma-tidae | *Mesodesma donacium* | SL  (14.4 – 88.7) mm | calcein | 100 mg/L | 3,6 h | Riascos et al., 2007 |
| Lucinidae | *Loripes lacteus* | – | calcein | 100, 200, 400, 800 mg/L | 1.3 - 2.6 h | Matthijs et al., 2011 |
| Cardiidae | *Cerastoderma edule* | SL  (1 – 32) mm | calcein | 50 mg/L,  150 mg/L | 3 h,  0.5, 6 h | Mahé et al., 2010 |
| *C. edule* | – | calcein | 250 mg/L | 1.5 h | Andresen et al., 2013 |
| Tellinidae | *Macoma Balthica* | – | calcein | 250 mg/L | 1.5 h | Andresen et al., 2013 |
| Donacidae | *Donax hanleyanus* | SL  (21 – 32 ) mm | calcein | 50 mg/L,  100 mg/L | 3, 6 h | Herrmann et al., 2009 |
| Mytilidae | *Perna perna* | SL  (20 – 30 ) mm,  (60 – 70) mm | calcein  (soaking) | 150 mg/L,  500 mg/L | 4 h | Kaehler and McQuaid, 1999 |
| calcein  (injection) | 125 mg/L,  10 – 640 mg/L | – |
| *P. canaliculus* | 0, 15 and 19 days after fertilization | calcein | 50 mg/L,  100 mg/L,  200 mg/L | 24 h | Fitzpatrick et al., 2011 |

Table S3. Effects of calcein on the fatty acid profiles of *P. laevis* in Experiment 1 (%). The distinct letters labeled for the same line after 2 h or 7 d of labeling indicated the statistically significant difference by Tukey’s honestly significant difference (HSD) test. The values were mean ± SD (*n* = 3).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Fatty acid | 2 h post exposure | | | | 7 d post exposure | | | |
| 0 mg/L+1 h | 20 mg/L+1 h | 20 mg/L+2 h | 50 mg/L+2 h | 0 mg/L+1 h | 20 mg/L+1 h | 20 mg/L+2 h | 50 mg/L+2 h |
| C14:0 | 1.84 ± 0.13 | 2.87 ± 0.60 | 2.03 ± 0.91 | 2.59 ± 0.56 | 2.60 ± 0.60 | 2.81 ± 0.14 | 2.27 ± 0.12 | 1.83 ± 0.63 |
| C15:0 | 1.36 ± 0.15 | 1.70 ± 0.12 | 1.44 ± 0.15 | 1.62 ± 0.07 | 1.62 ± 0.22 | 1.76 ± 0.10 | 1.49 ± 0.07 | 1.39 ± 0.11 |
| C15:1n10 | 0.10 ± 0.01 | 0.11 ± 0.02 | 0.11 ± 0.02 | 0.12 ± 0.01 | 0.13 ± 0.02 | 0.16 ± 0.05 | 0.14 ± 0.01 | 0.12 ± 0.01 |
| C16:0 | 23.51 ± 1.29 | 27.55 ± 1.94 | 24.33 ± 3.60 | 27.29 ± 2.65 | 28.09 ± 3.11 ab | 29.89 ± 1.72 a | 24.73 ± 2.86 ab | 23.13 ± 2.34 b |
| C16:1n9 | 0.64 ± 0.04 | 0.62 ± 0.05 | 0.63 ± 0.04 | 0.66 ± 0.06 | 0.65 ±0.05 | 0.68 ± 0.03 | 0.64 ± 0.03 | 0.60 ± 0.01 |
| C16:1n7 | 5.35 ± 0.09 | 7.34 ± 2.10 | 5.83 ± 1.74 | 7.75 ± 1.12 | 7.12 ± 0.99 | 7.16 ± 0.42 | 6.98 ± 0.53 | 5.57 ± 1.68 |
| C16:1(3t) | 0.26 ± 0.01 a | 0.37 ± 0.02 b | 0.32 ± 0.07 ab | 0.35 ± 0.01 ab | 0.34 ± 0.02 | 0.33 ± 0.02 | 0.32 ± 0.01 | 0.30 ± 0.04 |
| C16:2n6 | 0.42 ± 0.03 | 0.59 ± 0.03 | 0.50 ± 0.10 | 0.54 ± 0.06 | 0.50 ± 0.04 | 0.57 ± 0.06 | 0.49 ± 0.01 | 0.49 ± 0.07 |
| C17:0 | 3.57 ± 0.12 | 3.54 ± 0.47 | 3.47 ± 0.26 | 3.32 ± 0.50 | 3.58 ± 0.09 | 3.62 ± 0.19 | 3.38 ± 0.33 | 3.51 ± 0.33 |
| C16:4n3 | 4.91 ± 0.24 | 4.36 ± 0.21 | 4.71 ± 0.34 | 4.76 ± 0.64 | 4.69 ± 0.17 | 4.95 ± 0.10 | 4.85 ± 0.20 | 4.68 ± 0.07 |
| C18:0 | 3.16 ± 0.06 | 3.24 ± 0.26 | 3.02 ± 0.24 | 3.05 ± 0.35 | 3.55 ± 0.30 | 3.67 ± 0.28 | 3.38 ± 0.31 | 3.21 ± 0.07 |
| C18:1n9 | 2.69 ± 0.08 | 2.98 ± 0.81 | 2.72 ± 0.51 | 3.38 ± 0.42 | 3.19 ± 0.33 | 3.23 ± 0.34 | 3.21 ± 0.36 | 2.83 ± 0.78 |
| C18:1n7 | 2.94 ± 0.03 | 3.41 ± 0.23 | 2.97 ± 0.38 | 3.09 ± 0.10 | 3.37 ± 0.16 | 3.09 ± 0.19 | 3.13 ± 0.13 | 3.05 ± 0.12 |
| C18:2n6 | 0.15 ± 0.02 | 0.17 ± 0.08 | 0.21 ± 0.05 | 0.13 ± 0.08 | 0.14 ± 0.04 | 0.13 ± 0.05 | 0.16 ± 0.05 | 0.20 ± 0.04 |
| C18:3n4 | 0.41 ± 0.03 | 0.48 ± 0.03 | 0.44 ± 0.07 | 0.48 ± 0.03 | 0.46 ± 0.02 | 0.45 ± 0.00 | 0.47 ± 0.07 | 0.43 ± 0.05 |
| C18:3n3 | 0.10 ± 0.04 | 0.16 ± 0.03 | 0.19 ± 0.07 | 0.15 ± 0.09 | 0.11 ± 0.03 | 0.09 ± 0.03 | 0.16 ± 0.09 | 0.15 ± 0.04 |
| C18:4n3 | 0.37 ± 0.01 | 0.43 ± 0.13 | 0.50 ± 0.17 | 0.47 ± 0.25 | 0.35 ± 0.06 | 0.33 ± 0.05 | 0.49 ± 0.19 | 0.40 ± 0.14 |
| C20:0 | 0.21 ± 0.01 a | 0.30 ± 0.02 b | 0.23 ± 0.02 ab | 0.21 ± 0.00 ab | 0.25 ± 0.01 | 0.26 ± 0.02 | 0.27 ± 0.02 | 0.25 ± 0.04 |
| C20:1 | 2.89 ± 0.01 | 3.06 ± 0.08 | 2.79 ± 0.14 | 3.02 ± 0.44 | 3.04 ± 0.12 | 3.16 ± 0.13 | 3.11 ± 0.22 | 2.80 ± 0.15 |
| C20:2n6 | 1.52 ± 0.03 | 1.39 ± 0.04 | 1.53 ± 0.06 | 1.38 ± 0.06 | 1.39 ± 0.16 | 1.35 ± 0.05 | 1.47 ± 0.07 | 1.56 ± 0.05 |
| C20:3n6 | 0.12 ± 0.01 | 0.11 ± 0.00 | 0.11 ± 0.03 | 0.08 ± 0.02 | 0.08 ± 0.04 | 0.06 ± 0.01 | 0.10 ± 0.02 | 0.11 ± 0.02 |
| C20:4n6 | 4.27 ± 0.24 | 3.35 ± 0.13 | 3.91 ± 0.81 | 3.46 ± 0.46 | 3.61 ± 0.35 | 3.71 ± 0.22 | 3.85 ± 0.34 | 4.16 ± 0.43 |
| C20:3n3 | 0.17 ± 0.00 a | 0.20 ± 0.01 b | 0.22 ± 0.07 ab | 0.16 ± 0.08 ab | 0.10 ± 0.06 | 0.13 ± 0.03 | 0.21 ± 0.09 | 0.21 ± 0.03 |
| C20:4n3 | 0.37 ± 0.06 | 0.38 ± 0.05 | 0.41 ± 0.07 | 0.34 ± 0.13 | 0.27 ± 0.08 ab | 0.21 ± 0.04 a | 0.39 ± 0.17 ab | 0.41 ± 0.04 b |
| C20:5n3 | 11.05 ± 0.29 | 9.52 ± 1.04 | 11.34 ±1.75 | 8.88 ± 1.46 | 8.87 ± 1.54 ab | 7.36 ± 0.48 a | 9.93 ± 0.94 ab | 11.68 ± 0.78 b |
| C22:4n6 | 1.63 ± 0.08 | 1.46 ± 0.14 | 1.47 ± 0.19 | 1.52 ± 0.27 | 1.47 ± 0.10 | 1.64 ± 0.16 | 1.59 ± 0.16 | 1.60 ± 0.10 |
| C22:5n3 | 2.33 ± 0.02 | 1.78 ± 0.28 | 2.23 ± 0.27 | 2.16 ± 0.28 | 1.93 ± 0.21 ab | 1.86 ± 0.11 a | 2.29 ± 0.12 ab | 2.35 ± 0.15 b |
| C22:6n3 | 23.66 ± 0.23 | 18.55 ± 1.05 | 22.34 ±4.46 | 19.01 ± 2.14 | 18.53 ± 2.64 ab | 17.35 ± 1.43 a | 20.48 ± 0.91 ab | 22.96 ± 2.20 b |

Table S4. Effects of calcein on the fatty acid profiles of *P. laevis* in Experiment 2 (%). The distinct letters labeled for the same line after 2 h or 7 d of labeling indicated the statistically significant difference by Tukey’s honestly significant difference (HSD) test. The values are mean ± SD (*n* = 3).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Fatty acid | 2 h post exposure | | | | | 7 d post exposure | | | | |
| 0 mg/L+1 h | 20 mg/L+1 h | 20 mg/L+2 h | 50 mg/L+2 h | 50 mg/L+1 h | 0 mg/L+1 h | 20 mg/L+1 h | 20 mg/L+2 h | 50 mg/L+2 h | 50 mg/L+1 h |
| C14:0 | 1.48 ± 0.41 | 1.73 ± 0.46 | 1.77 ± 0.52 | 1.20 ± 0.19 | 1.53 ± 0.53 | 1.52 ± 0.09 | 1.28 ± 0.05 | 1.47 ± 0.12 | 1.47 ± 0.24 | 1.25 ± 0.37 |
| C15:0 | 0.75 ± 0.10 | 0.85 ± 0.15 | 0.80 ± 0.20 | 0.67 ± 0.09 | 0.74 ± 0.08 | 0.77 ± 0.13 | 0.65 ± 0.02 | 0.71 ± 0.04 | 0.76 ± 0.08 | 0.66 ± 0.10 |
| C15:1n10 | 0.16 ± 0.06 | 0.16 ± 0.03 | 0.12 ± 0.01 | 0.12 ± 0.00 | 0.13 ± 0.01 | 0.12 ± 0.03 | 0.11 ± 0.00 | 0.10 ± 0.02 | 0.11 ± 0.01 | 0.13 ± 0.04 |
| C16:0 | 18.38 ± 1.36 | 19.40 ± 1.81 | 19.13 ± 1.33 | 18.56 ± 2.23 | 18.87 ±1.12 | 19.44 ± 1.38 | 18.86 ± 0.84 | 19.37 ± 0.86 | 20.19 ± 2.24 | 17.76 ± 3.12 |
| C16:1n9 | 0.82 ± 0.04 | 0.73 ± 0.02 | 0.75 ± 0.04 | 0.78 ± 0.09 | 0.79 ± 0.06 | 0.77 ± 0.05 | 0.72 ± 0.01 | 0.73 ± 0.02 | 0.80 ± 0.04 | 0.55 ± 0.24 |
| C16:1n7 | 4.66 ± 0.79 | 5.65 ± 0.62 | 5.96 ± 0.93 | 4.67 ± 0.34 | 5.14 ± 1.21 | 5.61 ± 0.24 | 5.20 ± 0.07 | 5.71 ± 0.30 | 5.32 ± 0.65 | 3.93 ± 2.17 |
| C16:1(3t) | 0.08 ± 0.06 | 0.07 ± 0.02 | 0.10 ± 0.06 | 0.12 ± 0.04 | 0.09 ± 0.06 | 0.13 ± 0.06 ab | 0.12 ± 0.05 ab | 0.08 ± 0.01 a | 0.17 ± 0.01 b | 0.09 ± 0.07 ab |
| C16:2n6 | 0.30 ± 0.01 | 0.31 ± 0.04 | 0.39 ± 0.00 | 0.32 ± 0.06 | 0.34 ± 0.06 | 0.44 ± 0.01 | 0.31 ± 0.09 | 0.35 ± 0.10 | 0.33 ± 0.08 | 0.30 ± 0.19 |
| C17:0 | 2.78 ± 0.41 | 2.81 ± 0.09 | 2.75 ± 0.07 | 2.82 ± 0.12 | 2.87 ± 0.28 | 2.79 ± 0.11 | 2.76 ± 0.03 | 2.75 ± 0.06 | 2.90 ± 0.02 | 2.44 ± 0.39 |
| C16:4n3 | 8.08 ± 0.44 | 7.14 ± 0.40 | 7.35 ± 0.03 | 7.87 ± 0.35 | 8.01 ± 0.80 | 6.86 ± 0.82 | 6.82 ± 0.26 | 6.91 ± 0.41 | 7.22 ± 0.45 | 8.02 ± 1.63 |
| C18:0 | 3.78 ± 0.20 | 3.85 ± 0.13 | 3.93 ± 0.14 | 4.06 ± 0.16 | 3.95 ± 0.15 | 4.10 ± 0.16 | 3.97 ± 0.12 | 4.10 ± 0.06 | 4.47 ± 0.34 | 5.58 ± 2.68 |
| C18:1n9 | 3.38 ± 0.29 | 3.75 ± 0.04 | 3.79 ± 0.11 | 3.80 ± 0.18 | 3.78 ± 0.38 | 3.87 ± 0.16 | 4.21 ± 0.02 | 3.91 ± 0.31 | 4.07 ± 0.13 | 4.42 ± 0.86 |
| C18:1n7 | 2.38 ± 0.19 | 2.58 ± 0.07 | 2.64 ± 0.03 | 2.52 ± 0.23 | 2.43 ± 0.37 | 2.77 ± 0.25 | 2.88 ± 0.11 | 2.91 ± 0.02 | 2.85 ± 0.23 | 2.51 ± 0.59 |
| C18:2n6 | 0.65 ± 0.05 | 0.68 ± 0.09 | 0.77 ± 0.16 | 0.72 ± 0.17 | 0.62 ± 0.08 | 0.95 ± 0.05 | 0.92 ± 0.11 | 0.79 ± 0.02 | 0.80 ± 0.02 | 0.93 ± 0.09 |
| C18:3n4 | 0.26 ± 0.04 | 0.27 ± 0.06 | 0.29 ± 0.05 | 0.29 ± 0.05 | 0.25 ± 0.02 | 0.28 ± 0.01 | 0.30 ± 0.02 | 0.25 ± 0.05 | 0.27 ±0.02 | 0.28 ± 0.12 |
| C18:3n3 | 1.34 ± 0.21 | 1.43 ± 0.10 | 1.62 ± 0.10 | 1.45 ± 0.26 | 1.49 ± 0.39 | 1.59 ± 0.28 | 1.80 ± 0.10 | 1.65 ± 0.12 | 1.52 ± 0.03 | 1.10 ± 0.71 |
| C18:4n3 | 1.31 ± 0.14 | 1.50 ± 0.15 | 1.53 ± 0.06 | 1.31 ± 0.11 | 1.36 ± 0.23 | 1.42 ± 0.30 | 1.56 ± 0.06 | 1.47 ± 0.21 | 1.38 ± 0.13 | 1.18 ± 0.26 |
| C20:0 | 0.16 ± 0.07 | 0.22 ± 0.06 | 0.19 ± 0.01 | 0.22 ± 0.03 | 0.19 ± 0.01 | 0.21 ± 0.02 | 0.21 ± 0.00 | 0.20 ± 0.01 | 0.19 ± 0.02 | 0.24 ± 0.02 |
| C20:1 | 1.93 ± 0.46 | 1.63 ± 0.36 | 1.77 ± 0.19 | 2.01 ± 0.16 | 1.84 ± 0.21 | 1.85 ± 0.10 | 1.87 ± 0.04 | 1.80 ± 0.02 | 1.87 ± 0.08 | 2.88 ± 1.85 |
| C20:2n6 | 2.26 ± 0.07 | 2.01 ± 0.13 | 2.01 ± 0.25 | 2.15 ± 0.02 | 2.13 ± 0.19 | 2.09 ± 0.09 | 2.15 ± 0.08 | 2.06 ± 0.05 | 2.17 ± 0.07 | 2.10 ± 0.23 |
| C20:3n6 | 0.14 ± 0.10 | 0.17 ± 0.04 | 0.19 ± 0.05 | 0.18 ± 0.07 | 0.19 ± 0.05 | 0.16 ± 0.08 | 0.21 ± 0.01 | 0.20 ± 0.02 | 0.19 ± 0.01 | 0.32 ± 0.22 |
| C20:4n6 | 5.20 ± 0.33 | 4.63 ±0.41 | 4.82 ± 0.26 | 5.46 ± 0.23 | 5.21 ± 0.60 | 4.83 ± 0.36 | 4.93 ± 0.17 | 4.80 ± 0.46 | 4.77 ± 0.47 | 5.11 ± 0.27 |
| C20:3n3 | 0.38 ± 0.05 | 0.36 ± 0.09 | 0.37 ± 0.10 | 0.37 ± 0.09 | 0.39 ± 0.08 | 0.36 ± 0.13 | 0.45 ± 0.07 | 0.40 ± 0.04 | 0.41 ± 0.02 | 0.36 ± 0.12 |
| C20:4n3 | 0.72 ± 0.05 | 0.81 ± 0.05 | 0.84 ± 0.04 | 0.78 ± 0.12 | 0.65 ± 0.11 | 0.80 ± 0.04 | 0.81 ± 0.08 | 0.74 ± 0.12 | 0.77 ± 0.06 | 0.77 ± 0.15 |
| C20:5n3 | 13.16 ± 0.73 | 13.89 ± 0.99 | 13.59 ± 0.45 | 13.64 ± 0.48 | 13.28 ± 0.58 | 13.49 ± 0.38 | 13.69 ± 0.18 | 13.76 ± 0.29 | 12.78 ± 0.82 | 13.48 ± 1.08 |
| C22:4n6 | 1.90 ± 0.32 | 1.37 ± 0.15 | 1.38 ± 0.32 | 1.59 ± 0.10 | 1.65 ± 0.14 | 1.54 ± 0.07 ab | 1.60 ± 0.06 ab | 1.39 ± 0.07 a | 1.61 ± 0.13 ab | 2.03 ± 0.49 b |
| C22:5n3 | 2.69 ± 0.16 | 2.33 ± 0.23 | 2.03 ± 0.46 | 2.41 ± 0.14 | 2.26 ± 0.41 | 2.30 ± 0.07 | 2.40 ± 0.16 | 2.29 ± 0.11 | 2.34 ± 0.23 | 3.02 ± 0.88 |
| C22:6n3 | 20.88 ± 1.20 | 19.66 ± 1.32 | 19.14 ± 1.32 | 19.90 ± 0.83 | 19.83 ± 1.45 | 18.94 ± 1.29 | 19.23 ± 0.09 | 19.11 ± 0.78 | 18.29 ± 2.06 | 18.55 ± 2.47 |

# Table S5. Recapture data of *P. laevis.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | Survival number | Number of marked individuals | Dead number | Number of marked individuals |
| 50 mg/L -1 | 1 | 1 | 0 | 0 |
| 50 mg/L -2 | 1 | 1 | 0 | 0 |
| 50 mg/L -3 | 2 | 2 | 0 | 0 |
| 75 mg/L -1 | 2 | 2 | 0 | 0 |
| 75 mg/L -2 | 1 | 1 | 1 | 1 |
| 75 mg/L -3 | 1 | 1 | 4 | 4 |

# Table S6. Acute mortality (%) of *P. laevis* at 4 h post exposure.

|  |  |  |  |
| --- | --- | --- | --- |
| Experiment | Treatment  group | Marking scheme | Acute mortality (%) |
| Experiment 1 | A | 0 mg/L + 2 h | 0 |
| B | 20 mg/L + 1 h | 1.11 ± 1.92 |
| C | 20 mg/L + 2 h | 2.22 ± 1.92 |
| D | 50 mg/L + 2 h | 1.11 ± 1.92 |
| Experiment 2 | a | 0 mg/L + 2 h | 0 |
| b | 20 mg/L + 1 h | 0 |
| c | 20 mg/L + 2 h | 0 |
| d | 50 mg/L + 2 h | 0 |
| e | 50 mg/L + 1 h | 0 |
| Supplementary experiment | — | 50 mg/L + 2 h | 0.06 ± 1.58 |
| — | 50 mg/L + 1 h | 1.33 ± 1.15 |

# Table S7. Chemical labeling conditions for three species of shellfish.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Times | Fluorescein stain | Concentration | Time | References |
| *Haliotis rubra* | 6 days after fertilization | calcein | 50, 100 mg/L | 24, 48 h | Chick, 2010 |
| *Perna canaliculus* | 10 days after fertilization | calcein | 50, 100, 200 mg/L | 24 h | Fitzpatrick et al., 2013 |
| *Anadara broughtonii* | shell length: 27.24 ± 1.12 mm | calcein | 200, 300 mg/L | 24 h | Zhou et al., 2017 |
| alizarin red | 200, 300 mg/L | 24 h |

Table S8. Quality evaluation of calcein labeling on *P. laevis* in supplementary experiments. Values in the same column that did not share the same superscripts are significantly different (\**P* < 0.05; one-way ANOVA).

|  |  |  |  |
| --- | --- | --- | --- |
| Temperature (℃) | Marking scheme | Rate of success (%) | Good rate (%) |
| 13.4 ± 0.00 | 50 mg/L + 1 h | 100 | 43.44 ± 3.34 a |
| 50 mg/L + 2 h | 100 | 67.78 ± 5.09 b |
| 24.18 ± 0.04 | 50 mg/L + 1 h | 100 | 42.22 ± 5.09 a |
| 50 mg/L + 2 h | 100 | 70.00 ± 5.77 b |

# Supplementary Methods

**Labeling experiment on *P. laevis* at low temperature with 50 mg/L of calcein for 1 h.**

The experimental time was April 2024, the environmental water temperature was 13.4 °C (Experiment 1: 12.84 ± 0.09 °C), and the experimental material was *P. laevis* with a shell length of 25.7 ± 1.21 mm. A treatment group (calcein 50 mg/L + 1 h) and a control group (calcein 50 mg/L + 2 h) were set up to compare the labeling quality of the two groups under low temperature conditions. Each group had 3 replicates with 30 individuals of *P. laevis* per replicate. The labeling process was carried out in an opaque foam box. The individuals were immersed into 10 L of seawater with 7.95 ± 0.13 mg/L of dissolved oxygen and 33.52 ± 0.17 of salinity during the marking process. The stocking density was 9 ind./L, and the clams were fasting throughout the marking process. Following the immersion, the residual calcein on the surface of the bivalve’s shell was gently rinsed using fresh seawater. Then the clams were transferred to a 0.16 m3 transparent glass tank covered with approximately 5 cm thick of sea sand that was filtered through 30 ~ 50 mesh for 7 d.

Nearly 70% of the cultured seawater was exchanged at regular intervals every day during acclimation. The clams were fed with algal powder in a mixed mass ratio of *Dunaliella salina*, *Chlorella vulgaris* = 1:1, and the feeding proportion was 1% of the soft tissue mass of the clams. The seawater was aerated for 24 h prior to labeling. The temperature, dissolved oxygen and salinity value of seawater were 12.96 ± 0.84 °C, 7.86 ± 0.14 mg/L and 33.54 ± 0.10, during the recovery culture, respectively. During the incubation period, the dead individuals were counted and selected in time. After 7 days of incubation, the Luyor3280-LB fluorescent flashlight (480 ~ 490 nm) was used to count the fluorescence labeling quality of *P. laevis*. The experimental results were shown in Table S7.

# References

Andresen, H., Dorresteijn, I., and Meer, J. (2013). Growth and size-dependent loss of newly settled bivalves in two distant regions of the Wadden Sea. *Mar. Ecol. Prog. Ser.* 472, 141–154. doi: [10.3354/meps10011](https://doi.org/10.3354/meps10011)

Burgess, S., Tuck, I., and Williams, J. (2015). Microstriae identification and growth rates in new zealand scallop (*Pecten novaezelandiae*) juveniles. Conference: 20th International Pecten Workshop 2015. At: Galway City, Ireland. doi: [10.13140/RG.2.1.2909.3842](https://doi.org/10.13140/RG.2.1.2909.3842)

Cáceres, P. J. I., Huato, S. L., Melo, B. F. N., and Saucedo, P. E. (2011). Use of calcein to estimate and validate age in juveniles of the winged pearl oyster Pteria sterna. *Aquat. Living Resour*. 24(3), 329–335. doi: [10.1051/alr/2011139](https://doi.org/10.1051/alr/2011139)

Eads, C. B., and Layzer, J. B. (2002). How to pick your mussels out of a crowd: using fluorescence to mark juvenile freshwater mussels. *J. N. Am. Benthol. Soc*. 21(3), 476–486. doi: 10.2307/1468484

Fitzpatrick, M. P., Jeffs, A. G., and Dunphy, B. J. (2013). Efficacy of calcein as a chemical marker of green‐lipped mussel (*Perna canaliculus*) larvae and its potential use for tracking larval dispersal. *Aquacult. Res*. 44(3), 345–353. doi: 10.1111/j.1365-2109.2011.03034.x

Fujikura, K., Okoshi, K., and Naganuma, T. (2003). Strontium as a marker for estimation of microscopic growth rates in a bivalve. *Mar. Ecol. Prog. Ser*. 257, 295–301. doi: [10.3354/meps257295](https://doi.org/10.3354/meps257295)

Gancel, H. N., Carmichael, R. H., Park, K., Krause, J. W., and Rikard, S. (2019). Field mark-recapture of calcein-stained larval oysters (*Crassostrea virginica*) in a freshwater-dominated estuary. *Estuaries Coasts* 42(6), 1558–1569. doi: [10.1007/s12237-019-00582-6](https://doi.org/10.1007/s12237-019-00582-6)

Herrmann, M., Lepore, M. L., Laudien, J., Arntz, W. E., and Penchaszadeh, P. E. (2009). Growth estimations of the Argentinean wedge clam *Donax hanleyanus*: A comparison between length-frequency distribution and size-increment analysis. *J. Exp. Mar. Biol. Ecol*. 379(1-2), 8–15. doi: [10.1016/j.jembe.2009.07.031](https://doi.org/10.1016/j.jembe.2009.07.031)

Hollyman, P., Luckenbach, M., and Richardson, C. (2013). Nondaily deposition of striae in the bay scallop *Argopecten irradians* (Concentricus) in the laboratory. *J. Shellfish Res*. 32(2), 361–368. doi: [10.2983/035.032.0215](https://doi.org/10.2983/035.032.0215)

Kaehler, S., and McQuaid, C. (1999). Use of the fluorochrome calcein as an *in situ* growth marker in the brown mussel *Perna perna*. Mar. Biol. 133(3), 455–460. doi: [10.1007/s002270050485](https://doi.org/10.1007/s002270050485)

Klunzinger, M. W., Beatty, S. J., Morgan, D. L., Lymbery, A. J., and Haag, W. R. (2014). Age and growth in the Australian freshwater mussel, *Westralunio carteri*, with an evaluation of the fluorochrome calcein for validating the assumption of annulus formation. *Freshwater Sci*. 33(4), 1127–1135. doi: 10.1086/677815

Linard, C., Gueguen, Y., Moriceau, J., Soyez, C., Hui, B., Raoux, A., et al. (2011). Calcein staining of calcified structures in pearl oyster *Pinctada margaritifera* and the effect of food resource level on shell growth. *Aquacult. Int*. 313(1-4), 149–155. doi: [10.1016/j.aquaculture.2011.01.008](https://doi.org/10.1016/j.aquaculture.2011.01.008)

Mahé, K., Bellamy, E., D'Amico, F., and Caill-Milly, N. (2021). In situ fast marking study of manila clams (*Ruditapes philippinarum*). *Int. J. Fish. Aquat. Stud*. 9, 47–51. doi: [10.22271/fish.2021.v9.i1a.2387](https://doi.org/10.22271/fish.2021.v9.i1a.2387)

Mahé, K., Bellamy, E., Lartaud, F., and De-Rafélis, M. (2010). Calcein and manganese experiments for marking the shell of the common cockle (*Cerastoderma edule*): tidal rhythm validation of increments formation. *Aquat. Living Resour*. 23(3), 239–245. doi: [10.1051/alr/2010025](https://doi.org/10.1051/alr/2010025)

Matthijs, V. D. G., Jan, A. V. G., Jaap, V. D. M., Han, O., and Theunis, P. (2011). Suitability of calcein as an *in situ* growth marker in burrowing bivalves. *J. Exp. Mar. Biol. Ecol*. 399(1), 1–7. doi: [10.1016/j.jembe.2011.01.003](https://doi.org/10.1016/j.jembe.2011.01.003)

McCoy, L. P. L., and Huato, S. L. (2014). Efficacy of calcein and Coomassie Blue dyeing of shell growing-edges and micro growth-bands: Ageing juvenile of *Pinctada mazatlanica* (Pterioida: Pteriidae). *Revista de Biología Tropical* 62(3), 969–976. doi: [10.15517/rbt.v62i3.12583](https://doi.org/10.15517/rbt.v62i3.12583)

Moran, A. L., and Marko, P. B. (2005). A simple technique for physical marking of larvae of marine bivalves. *J. Shellfish Res*. 24, 567–571. doi: [10.2983/0730-8000(2005)24[567:ASTFPM]2.0.CO;2](https://doi.org/10.2983/0730-8000(2005)24%5b567:ASTFPM%5d2.0.CO;2)

Riascos, J., Guzman, N., Laudien, J., Heilmayer, O., and Oliva, M. (2007). Suitability of three stains to mark shells of *Concholepas concholepas* (Gastropoda) and *Mesodesma donacium* (Bivalvia). *J. Shellfish Res*. 26(1), 43–49. doi: [10.2983/0730-8000(2007)26[43:SOTSTM]2.0.CO;2](https://doi.org/10.2983/0730-8000(2007)26%5b43:SOTSTM%5d2.0.CO;2)

Spires, J. E., Dungan, C. F., and North, E. W. (2022). Marking the shells of pediveliger eastern oysters *Crassostrea virginica*, with a calcein fluorochrome dye. *J. Shellfish Res*. 40, 479–487. doi: [10.2983/035.040.0304](https://doi.org/10.2983/035.040.0304)

Spires, J. E., and North, E. W. (2022). Marking the shells of juvenile and adult eastern oysters, *Crassostrea virginica*, with the fluorochrome dye calcein and measuring growth and mortality after marking. *J. Molluscan Stud*. 88, eyac004. doi: [10.1093/mollus/eyac004](https://doi.org/10.1093/mollus/eyac004)

Thébault, J., Chauvaud, L., Clavier, J., Fichez, R., and Morize, E. (2006). Evidence of a 2-day periodicity of striae formation in the tropical scallop *Comptopallium radula* using calcein marking. *Mar. Biol*. 149(2), 257–267. doi: [10.1007/s00227-005-0198-8](https://doi.org/10.1007/s00227-005-0198-8)

Zhou, S. (2015). Habitat preference and marking techniques of ark shell *Anadara broughtonii* in stock enhancement. Doctor, Ocean University of China (in Chinese).