

*The following supplement accompanies the article*

# **Eyestalk ablation affects larval morphogenesis in the swimming crab *Portunus trituberculatus* during metamorphosis into megalopae**

**Shigeki Dan<sup>1</sup>, Takaaki Kaneko<sup>2</sup>, Satoshi Takeshima<sup>3</sup>, Masakazu Ashidate<sup>1</sup>, Katsuyuki Hamasaki<sup>2,\*</sup>**

<sup>1</sup>Tamano Laboratory, National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Tamano, Okayama 706-0002, Japan

<sup>2</sup>Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan, Minato, Tokyo 108-8477, Japan

<sup>3</sup>Hakatajima Laboratory, National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Imabari, Ehime 794-2305, Japan

\*Corresponding author: hamak@kaiyodai.ac.jp

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**Supplement.** Morphological measurements of last stage (fourth stage) zoeae (Tables S1 & S2) and fifth-instar larvae (Tables S3, S4 & S5) of the swimming crab *Portunus trituberculatus*

Table S1. Morphological measurements (CL, CHL, PL, DSL and FL) of fourth stage zoeae of *Portunus trituberculatus* treated during the postmoult and premoult of the second (Z2) or third (Z3) zoeal stages. Values are mean  $\pm$  SD. See Table 1 and Fig. 1 in the main text for definitions of measurement abbreviations and further details

Developmental stage at treatment	Treatment	Morphological measurement				
		CL ( $\mu\text{m}$ )	CHL ( $\mu\text{m}$ )	PL ( $\mu\text{m}$ )	DSL ( $\mu\text{m}$ )	FL ( $\mu\text{m}$ )
Z2-postmoult	Control	1488 $\pm$ 8	541 $\pm$ 16	345 $\pm$ 7	1182 $\pm$ 58	971 $\pm$ 36
	Darkness	1399 $\pm$ 36	500 $\pm$ 5	318 $\pm$ 8	1116 $\pm$ 46	992 $\pm$ 7
	Dorsal spine ablation	1510 $\pm$ 22	551 $\pm$ 15	345 $\pm$ 7	587 $\pm$ 133	968 $\pm$ 8
	Eyestalk ablation	1388 $\pm$ 15	300 $\pm$ 18	148 $\pm$ 13	1318 $\pm$ 74	993 $\pm$ 37
Z2-premoult	Eyestalk ablation	1385 $\pm$ 24	354 $\pm$ 40	217 $\pm$ 16	1269 $\pm$ 71	947 $\pm$ 58
Z3-postmoult	Control	1469 $\pm$ 38	524 $\pm$ 14	338 $\pm$ 9	1253 $\pm$ 2	1031 $\pm$ 26
	Darkness	1443 $\pm$ 30	515 $\pm$ 21	333 $\pm$ 12	1243 $\pm$ 55	1017 $\pm$ 48
	Dorsal spine ablation	1494 $\pm$ 23	538 $\pm$ 17	348 $\pm$ 10	386 $\pm$ 48	998 $\pm$ 40
	Eyestalk ablation	1473 $\pm$ 24	458 $\pm$ 14	293 $\pm$ 14	1457 $\pm$ 33	1000 $\pm$ 34
Z3-premoult	Eyestalk ablation	1471 $\pm$ 10	505 $\pm$ 9	322 $\pm$ 9	1320 $\pm$ 42	1014 $\pm$ 21

Table S2. Morphological measurements (CHLr, PLr, DSLr and FLr) of fourth stage zoeae of *Portunus trituberculatus* treated during the postmoult and premoult of the second (Z2) or third (Z3) zoeal stages. Values are mean  $\pm$  SD. See Table 1 and Fig. 1 in the main text for definitions of measurement abbreviations and further details

Developmental stage at treatment	Treatment	Morphological measurement			
		CHLr (%)	PLr (%)	DSLr (%)	FLr (%)
Z2-postmoult	Control	36.4 $\pm$ 0.9	23.2 $\pm$ 0.4	88.2 $\pm$ 4.0	72.4 $\pm$ 1.3
	Darkness	35.8 $\pm$ 1.3	22.7 $\pm$ 0.8	83.1 $\pm$ 3.9	73.8 $\pm$ 1.3
	Dorsal spine ablation	36.5 $\pm$ 0.7	22.8 $\pm$ 0.4	41.9 $\pm$ 9.6	69.2 $\pm$ 2.0
	Eyestalk ablation	21.6 $\pm$ 1.2	10.7 $\pm$ 0.9	99.5 $\pm$ 2.5	75.0 $\pm$ 3.1
Z2-premoult	Eyestalk ablation	25.5 $\pm$ 2.5	15.6 $\pm$ 1.0	96.6 $\pm$ 3.3	72.3 $\pm$ 3.6
Z3-postmoult	Control	35.7 $\pm$ 1.0	23.0 $\pm$ 0.3	86.1 $\pm$ 2.8	70.9 $\pm$ 2.4
	Darkness	35.7 $\pm$ 0.9	23.0 $\pm$ 0.5	87.3 $\pm$ 3.9	71.5 $\pm$ 2.8
	Dorsal spine ablation	36.0 $\pm$ 1.2	23.2 $\pm$ 0.8	26.4 $\pm$ 2.9	68.6 $\pm$ 4.1
	Eyestalk ablation	31.1 $\pm$ 0.7	19.9 $\pm$ 0.7	98.3 $\pm$ 11.7	70.8 $\pm$ 1.5
Z3-premoult	Eyestalk ablation	34.3 $\pm$ 0.6	21.9 $\pm$ 0.6	91.9 $\pm$ 3.6	70.6 $\pm$ 2.0

Table S3. Morphological measurements (CL, CHL, DSL and FL) of fifth-instar larvae of *Portunus trituberculatus* treated during the postmolt of the second (Z2), third (Z3) or fourth (Z4) zoeal stages, premolt of Z2 or Z3, or intermolt or premolt of Z4. Z5 = supernumerary fifth zoeal stage occurring after eyestalk ablation in Z2 or Z3; MG = megalopae. Values are mean  $\pm$  SD. See Table 1 and Fig. 1 in the main text for definitions of measurement abbreviations and further details

Developmental stage at treatment	Treatment	Morphological measurement				
		CL-Z5 ( $\mu\text{m}$ )	CL-MG ( $\mu\text{m}$ )	CHL ( $\mu\text{m}$ )	DSL ( $\mu\text{m}$ )	FL ( $\mu\text{m}$ )
Z2-postmolt	Control	–	2550 $\pm$ 54	1401 $\pm$ 30	0 $\pm$ 0.0	25 $\pm$ 14.7
	Darkness	–	2416 $\pm$ 27	1353 $\pm$ 8	1 $\pm$ 2.0	49 $\pm$ 35.7
	Dorsal spine ablation	–	2535 $\pm$ 53	1394 $\pm$ 28	4 $\pm$ 3.0	30 $\pm$ 23.9
	Eyestalk ablation	1594 $\pm$ 14	–	480 $\pm$ 13	1425 $\pm$ 77.7	1044 $\pm$ 81.3
Z2-premolt	Eyestalk ablation	1670 $\pm$ 33	–	596 $\pm$ 18	1368 $\pm$ 74.1	1026 $\pm$ 149.1
Z3-postmolt	Control	–	2468 $\pm$ 33	1377 $\pm$ 15	17 $\pm$ 3.1	20 $\pm$ 5.8
	Darkness	–	2424 $\pm$ 36	1370 $\pm$ 11	2 $\pm$ 2.6	36 $\pm$ 10.7
	Dorsal spine ablation	–	2464 $\pm$ 28	1359 $\pm$ 19	15 $\pm$ 9.4	24 $\pm$ 12.1
	Eyestalk ablation	1756 $\pm$ 57	–	655 $\pm$ 20	1595 $\pm$ 57.1	867 $\pm$ 205.2
Z3-premolt	Eyestalk ablation	1873 $\pm$ 52	2440 $\pm$ 132	1048 $\pm$ 86	569 $\pm$ 233.5	757 $\pm$ 103.7
Z4-postmolt	Control	–	2484 $\pm$ 12	1412 $\pm$ 21	15 $\pm$ 6.2	2 $\pm$ 2.5
	Darkness	–	2451 $\pm$ 28	1376 $\pm$ 17	2 $\pm$ 1.1	0 $\pm$ 0.0
	Dorsal spine ablation	–	2528 $\pm$ 30	1403 $\pm$ 12	21 $\pm$ 9.6	3 $\pm$ 4.8
	Eyestalk ablation	–	2501 $\pm$ 48	1364 $\pm$ 16	3 $\pm$ 0.7	21 $\pm$ 22.0
Z4-intermolt	Eyestalk ablation	–	2637 $\pm$ 68	1396 $\pm$ 25	4 $\pm$ 6.3	1 $\pm$ 2.2
Z4-premolt	Eyestalk ablation	–	2649 $\pm$ 43	1394 $\pm$ 11	69 $\pm$ 45.5	39 $\pm$ 26.5

Table S4. Morphological measurements (CHLr, DSLr and FLr) of fifth-instar larvae of *Portunus trituberculatus* treated during the postmoult of the second (Z2), third (Z3) or fourth (Z4) zoeal stages, premoult of Z2 or Z3, or intermoult or premoult of Z4. Z5 = supernumerary fifth zoeal stage occurring after eyestalk ablation in Z2 or Z3; MG = megalopae. Values are mean  $\pm$  SD. See Table 1 and Fig. 1 in the main text for definitions of measurement abbreviations and further details

Developmental stage at treatment	Treatment	Morphological measurement					
		CHLr-Z5 (%)	CHLr-MG (%)	DSLr-Z5 (%)	DSLr-MG (%)	FLr-Z5 (%)	FLr-MG (%)
Z2-postmoult	Control	–	56.0 $\pm$ 1.5	–	0.0 $\pm$ 0.0	–	1.0 $\pm$ 0.6
	Darkness	–	56.1 $\pm$ 0.7	–	0.0 $\pm$ 0.1	–	2.0 $\pm$ 1.5
	Dorsal spine ablation	–	56.1 $\pm$ 1.1	–	0.2 $\pm$ 0.1	–	1.2 $\pm$ 0.9
	Eyestalk ablation	29.4 $\pm$ 0.8	–	89.9 $\pm$ 5.1	–	64.3 $\pm$ 3.8	–
Z2-premoult	Eyestalk ablation	35.4 $\pm$ 0.8	–	81.9 $\pm$ 2.6	–	61.3 $\pm$ 8.7	–
Z3-postmoult	Control	–	55.8 $\pm$ 0.9	–	0.7 $\pm$ 0.1	–	0.8 $\pm$ 0.2
	Darkness	–	56.3 $\pm$ 0.8	–	0.1 $\pm$ 0.1	–	1.5 $\pm$ 0.5
	Dorsal spine ablation	–	55.0 $\pm$ 0.9	–	0.6 $\pm$ 0.4	–	1.0 $\pm$ 0.5
	Eyestalk ablation	38.4 $\pm$ 0.8	–	91.3 $\pm$ 5.9	–	48.5 $\pm$ 12.8	–
Z3-premoult	Eyestalk ablation	37.1 $\pm$ 3.5	46.8 $\pm$ 2.4	86.6 $\pm$ 4.8	17.6 $\pm$ 13.5	61.9 $\pm$ 8.2	29.0 $\pm$ 6.1
Z4-postmoult	Control	–	56.8 $\pm$ 0.8	–	0.6 $\pm$ 0.2	–	0.1 $\pm$ 0.1
	Darkness	–	57.1 $\pm$ 0.8	–	0.1 $\pm$ 0.0	–	0.0 $\pm$ 0.0
	Dorsal spine ablation	–	56.7 $\pm$ 0.8	–	0.8 $\pm$ 0.4	–	0.1 $\pm$ 0.2
	Eyestalk ablation	–	54.4 $\pm$ 0.8	–	0.1 $\pm$ 0.0	–	0.8 $\pm$ 0.9
Z4-intermoult	Eyestalk ablation	–	54.2 $\pm$ 2.0	–	0.2 $\pm$ 0.2	–	0.0 $\pm$ 0.1
Z4-premoult	Eyestalk ablation	–	54.6 $\pm$ 1.1	–	2.6 $\pm$ 1.7	–	1.5 $\pm$ 1.0

Table S5. Morphological measurements (Z5f, DSf and TELf) of fifth-instar larvae of *Portunus trituberculatus* treated during the postmoult of the second (Z2), third (Z3) or fourth (Z4) zoeal stages, premoult of Z2 or Z3, or intermoult or premoult of Z4. Values are mean  $\pm$  SD. See Table 1 and Fig. 1 in the main text for definitions of measurement abbreviations and further details

Developmental stage at treatment	Treatment	Morphological measurement		
		Z5f (%)	DSf (%)	TELf (%)
Z2-postmoult	Control	0	0.0 $\pm$ 0.0	19.6 $\pm$ 6.8
	Darkness	0	2.5 $\pm$ 5.0	24.1 $\pm$ 11.0
	Dorsal spine ablation	0	7.1 $\pm$ 4.8	23.3 $\pm$ 20.5
	Eyestalk ablation	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0
Z2-premoult	Eyestalk ablation	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0
Z3-postmoult	Control	0	21.6 $\pm$ 6.7	26.9 $\pm$ 10.1
	Darkness	0	2.8 $\pm$ 3.2	22.1 $\pm$ 12.6
	Dorsal spine ablation	0	18.9 $\pm$ 5.7	27.5 $\pm$ 12.1
	Eyestalk ablation	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0
Z3-premoult	Eyestalk ablation	14.1 $\pm$ 4.6	77.6 $\pm$ 10.1	95 $\pm$ 3.6
Z4-postmoult	Control	0	26.3 $\pm$ 3.9	2.1 $\pm$ 2.4
	Darkness	0	3.8 $\pm$ 2.6	0.0 $\pm$ 0.0
	Dorsal spine ablation	0	35.9 $\pm$ 17.0	3.6 $\pm$ 4.8
	Eyestalk ablation	0	6.5 $\pm$ 1.8	12.3 $\pm$ 11.3
Z4-intermoult	Eyestalk ablation	0	6.4 $\pm$ 8.1	2.8 $\pm$ 5.6
Z4-premoult	Eyestalk ablation	0	57.3 $\pm$ 27.0	21.0 $\pm$ 10.0