

2.7. Heatshock induction in *Parhyale* embryos/hatchlings

- ❑ For heat-shock, fill small Petri-dishes with FASW (for each animal individual dish) and place them covered into an 37°C incubator until the water reached 37°C.
- ❑ Place the animals/embryos into the dishes with prewarmed water and incubate them at 37°C for 20-30min.
- ❑ After the incubation at 37°C the animals should be placed back into room temperature FASW (e.g. place them back into original dishes).
- ❑ First heatshock responses can be expected 3 hours after heatshock (Pavlopoulos et al. 2010). The DsRed expression however can be detected for days after heatshock, therefore it is reasonable to check the DsRed expression 6-12 hours later to make sure that large quantities of the DsRed protein are produced.

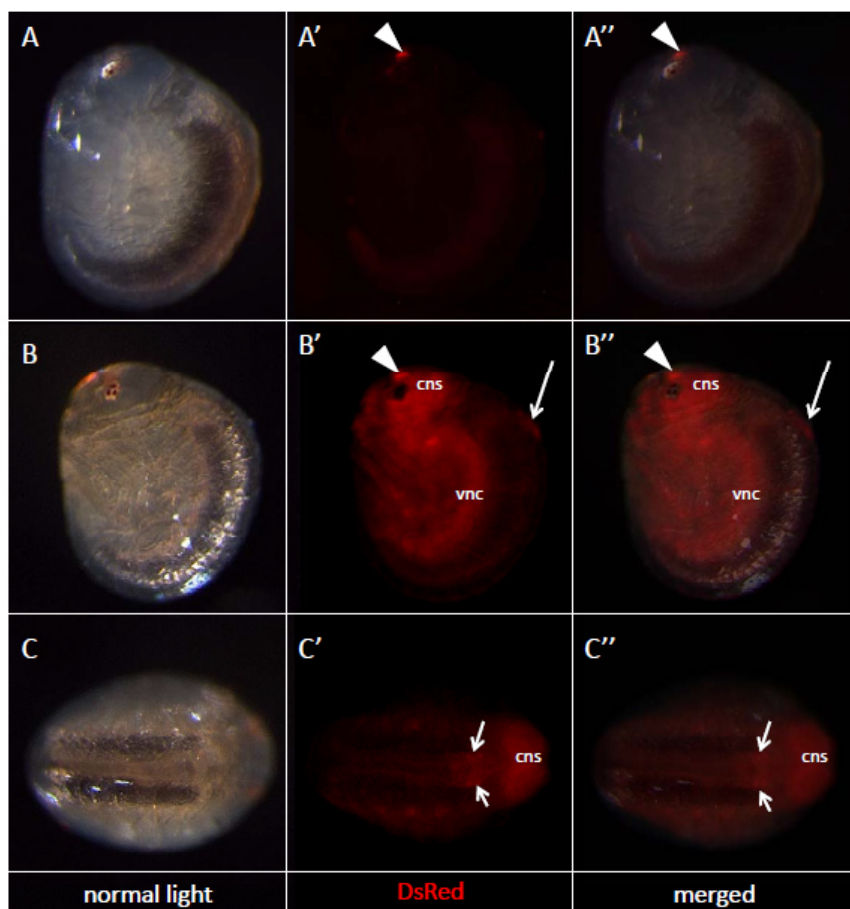


Fig 8 The same embryo before and 1 day after heatshock.

A - A'' Expression before heat shock, lateral view with anterior up: the 3xP3 reporter indicates that this animal is transgenic (arrowhead) . **B - B''** The same embryo after heat shock and documented 24 hours after A - A'' in same view. The heat shock response is visible throughout the embryo, especially in the CNS and VNC. The white arrow points to the putative germ cells. **C - C''** Dorsal view on the same embryo, anterior is to the right. The putative germ cells are indicated by the white arrows.

2.9. References and helpful literature/links

Parhyale embryology:

- Gerberding, M., Browne, W.E., Patel, N.H., 2002. Cell lineage analysis of the amphipod crustacean *Parhyale hawaiiensis* reveals an early restriction of cell fates. *Development* 129, 5789-5801.
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Parhyale transgenesis:

- Pavlopoulos, A., Averof, M., 2005. Establishing genetic transformation for comparative developmental studies in the crustacean *Parhyale hawaiiensis*. *Proc. Natl. Acad. Sci. USA* 102, 7888-7893.
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Parhyale established protocols:

- Rehm, E. J., Hannibal, R.L. , Chaw, R.C. , Vargas-Vila, M.A. , and Patel, N.H., 2009. The Crustacean *Parhyale hawaiiensis*: A New Model for Arthropod Development *Cold Spring Harb Protoc*; doi:10.1101/pdb.emo114
- Rehm, E. J., Hannibal, R.L. , Chaw, R.C. , Vargas-Vila, M.A. , and Patel, N.H., 2009. Protocol Injection of *Parhyale hawaiiensis* Blastomeres with Fluorescently Labeled Tracers *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot5128
- Rehm, E. J., Hannibal, R.L. , Chaw, R.C. , Vargas-Vila, M.A. , and Patel, N.H., 2009. Antibody Staining of *Parhyale hawaiiensis* Embryos. *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot5129
- Rehm, E. J., Hannibal, R.L. , Chaw, R.C. , Vargas-Vila, M.A. , and Patel, N.H., 2009. Fixation and Dissection of *Parhyale hawaiiensis* Embryos. *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot5127
- Rehm, E. J., Hannibal, R.L. , Chaw, R.C. , Vargas-Vila, M.A. , and Patel, N.H., 2009. In Situ Hybridization of Labeled RNA Probes to Fixed *Parhyale hawaiiensis* Embryos. *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot5130

More protocols, images and other information:

- <http://patelweb.berkeley.edu/>
- <http://extavourlab.com/protocols/index.html>

Amphipod development:

- Scholtz, G., Wolff, C., 2002. Cleavage, gastrulation, and germ disc formation of the amphipod *Orchestia cavimana* (Crustacea, Malacostraca, Peracarida). *Contributions to Zoology* 71, 9-28.
- Wolff, C., Scholtz, G., 2002. Cell lineage, axis formation, and the origin of germ layers in the amphipod crustacean *Orchestia cavimana*. *Dev Biol* 250, 44-58