

STOMATOPOD LARVAL EYE DEVELOPMENT: A MORPHOLOGICAL COMPARISON OF TWO SYMPATRIC SPECIES OF PULLOSQUILLA

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Abstract. Stomatopod adult and larval retinas develop within the same eyestalk before the final metamorphosis, the post-larval molt, into stomatopodid. This post-larval molt leads to an astounding ocular adjustment with the larval retina reducing in size and the adult retina growing. The two organisms studied here are *Pullosquilla litoralis* and *Pullosquilla thomassini*, two sympatric species within the same genus. Thirty-nine individuals were captured in their terminal larval stage and photographed for a period of four days, during which they went through their post-larval molt. Both species had similar rates of eye reduction and growth, as a result of their similar sizes and bathometric overlap. However, there was one difference with regards to eye development. *Pullosquilla litoralis* began adult eye growth after the post-larval molt while *Pullosquilla thomassini* began adult eye growth before the post-larval molt.

Key words: *stomatopods; Lysiosquilloidea; Pullosquilla; Moorea, French Polynesia; vision; sympatric*

INTRODUCTION

There are many different levels of complexity with regards to structure and function of eyes across life. From photosensitive proteins in bacteria to the sharp focus of the lens in a Peregrine falcon (*Falco peregrinus*), eyes provide a selective advantage in most environments. Vision plays multiple roles including prey capture, predator avoidance, and mate selection. For example, a study on the kangaroo rat (*Dipodomys merriami*) revealed that vision had a significant effect on predator avoidance. When the hearing of the rodent was reduced but there was some light available, the kangaroo rat was able to avoid the strike of a sidewinder rattlesnake (*Crotalus cerastes*), emphasizing importance of vision to survival (Webster and Webster 1971). However, eyes are energetically expensive and in the case of cave dwelling species such as the Mexican blind cavefish (*Astyanax mexicanus*), they can be lost if they provide no benefit (Jeffery 2005).

One group of organisms that has been a model for the study of vision are the stomatopods. Adult stomatopod vision differs

greatly from their larvae's vision which only uses one photoreceptor (Jutte et al. 1998). Even though the larval eye only contains one photoreceptor it is still a complex sensory organ that is very effective for the larva's pelagic life (Schram and Charmantier-Daures 2013). The stomatopod larvae is transparent, except for their eyes which contain an opaque core that is produced by screening pigments. The function of these screening pigments is to keep light from interfering with separate ommatidia, if stray light was allowed to freely move throughout the eye then it would disrupt information and ruin spatial resolution. Three separate mechanisms have been evolved in order to combat the reduction in transparency caused by the requirement of screening pigments. One of these mechanisms is a reduction in number of screening pigments, dramatically reducing the size of the retina and producing transparent apposition eyes (Nilsson 1997). Another mechanism is the presence of photostable pigments. These pigments absorb only a certain wavelength of light and allows all others to pass through which offers a degree of transparency (Jutte et al. 1998). The final mechanism is the presence

of iridescent pigments that create an eyeshine that reduces contrast of the eye within the certain environment that the larvae is found in (Cronin and Jinks 2001, Feller and Cronin 2014).

Like all crustaceans, the larvae of stomatopods must go through a metamorphosis from larvae to post-larvae. This metamorphic change from larvae to stomatopodid involves a drastic ocular reorganization as the adult eye begins developing within the same stalk as the larval eye (Williams et al. 1985, Jutte et al. 1998).

Stomatopod eyes are complex, some possessing up to 16 photoreceptors that can decipher wavelengths between infra-red and ultra violet (Marshall et al. 2007). The eye is one of the most notable features of a stomatopod. They are placed anteriorly on independently moving stalks driven by well-developed muscles (Jones 1994). On close inspection, the eye is made up of hundreds of individually isolated ommatidia, which are the functional unit that contains the photoreceptive cells (Land 1997). Stomatopods within the families Gonodactyloidea and Lysiosquilloidea possess the highest number of photoreceptive classes within Stomatopoda. The eye is divided into three distinct regions, the dorsal and ventral hemispheres and the midband (Marshall et al. 2007). Mantis shrimps within these families have well-developed midbands made up of six rows of ommatidia and containing 14 of the 16 known photoreceptors. This allows a wide range of color vision and the ability to see into the ultra-violet and differentiate polarized light (Marshall et al. 2007). Ommatidia located in the hemispheres contain two types of photoreceptive cells which provide ample detection of motion and space (Cronin and Marshall 2004).

The overall goal of this study was to test if there was a difference between two sympatric species of *Pullosquilla* larvae: *Pullosquilla litoralis* and *Pullosquilla thomassini*, which are a part of the superfamily Lysiosquilloidea and the family Nannosquillidea (Manning 1980). They are both spearing monogamous stomatopods that live in small U-shaped burrows (Jutte 1997). Previous research has indicated that the two species had very different depth ranges with some overlap, *P.*

litoralis with a range of 0-2 meters of depth and *P. thomassini* with a range of 1-37 meters (Marshall et al. 2007). Moreover, Cheroske et al. (2003) showed that *P. litoralis* has a breadth of change in one of its photoreceptor classes, being able to have a more blue-shifted photoreceptor in midband row 3, the midband being where majority of color photoreceptors are located. This would indicate that *P. litoralis* can live in deeper environments, the reason for this is the result of light attenuation. These differences in environmental conditions set up the potential for differences in eye development between the two species which leads to my hypothesis that *Pullosquilla litoralis* and *Pullosquilla thomassini* will have very different eye development as a result of their habitat preferences.

METHODS

Study site and field collections

Surveys for *P. litoralis* and *P. thomassini*, were conducted nightly for a full moon cycle, new moon to new moon, from October 12th to November 11, 2015th. Collections were made adjacent to the dock at the Richard B. Gump Pacific Research Station in Moorea, French Polynesia (-17.490437, -149.826153, Fig. 1).

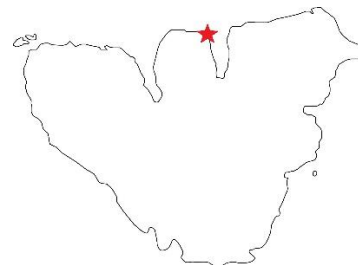


FIG. 1. Map of Moorea, French Polynesia. The red star indicates the location of the Gump Station at the entrance of Cook's Bay.

The majority of the *Pullosquilla* that were measured were collected from Nov 2nd to Nov 7th. Specimens were acquired through the use of a dive light and a plankton net from between 8:30-11:30 p.m. Once collected, the stomatopods were placed in a large container and kept in the dark to prevent the animals from molting. Individuals that molted before

being photographed were returned back to the bay the night they were collected.

Identification

Individuals were identified after the post-larval molt. *Pullosquilla litoralis* has a ventrally smooth telson and *P. thomassini* has a ventrally spiny telson (Ahyong 2001, Fig. 2).

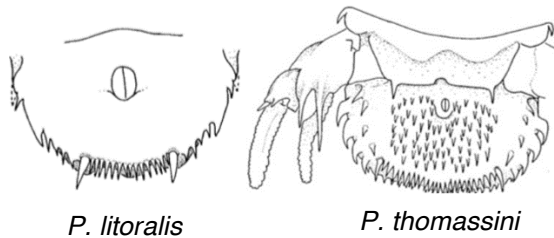


FIG. 2. Ventral surface of the telson of *P. litoralis* and *P. thomassini* (Ahyong 2001).

Care of specimens

Stomatopods were each placed in their own cup after initially being caught. Each animal was only kept for a four-day period and during that time they were each fed two copepods every other day. Each day their water was changed with new salt water from the nearby bay. After four days the stomatopods were released where they were collected.

Photography

Organisms were photographed with a Nikon D90 camera mounted on a Leica dissecting microscope. All photos were captured at 1.6 magnification in order to have uniformity throughout measurements of the height and width of both the larval and adult eyes. The stomatopod was placed on its side for capturing three measurements; the height and width of the larval eye and the height of the adult eye. In order to get the width of the adult eye the stomatopod was positioned on its dorsal side. Two photos were then taken, one where the eyes were flat and one where the animal had turned its eyes upwards, revealing the entire adult eye. Individuals were photographed five separate times. First after the initial capture, then four hours later to measure adult eye growth before the post-

larval molt. The following three other pictures were taken over the course of three days.

Analyzing the photos

Height and width of both the eyes were measured using ImageJ (Schneider et al. 2012, Figs. 3 and 4). In order to keep measurements concise all photos were analyzed using the same scale bar, a 7mm ruler at 1.6 magnification. A photo was saved as the scale bar and applied to every photo used in order to standardize all measurements.

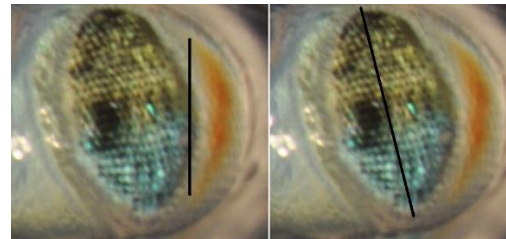


FIG. 3. Measurements of the height of the larval eye (to the right) and adult eye (to the left)

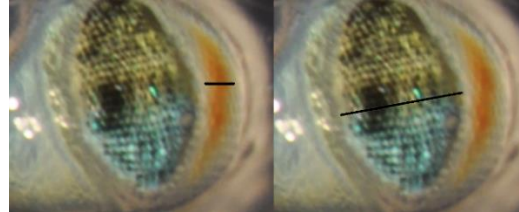


FIG. 4. Measurements of the width of the larval eye (to the right) and adult eye (to the left).

Statistical analysis

A linear regression was used to analyze the data. Height and width of larval and adult eyes were examined separately. Difference in size was correlated to the change in time from zero to 63 hours after initial capture.

RESULTS

The terminal larval eye of *P. litoralis* is black on the dorsal half and blue ventrally, while the terminal larval eye of *P. thomassini* is green on the dorsal half and blue ventrally (Fig. 5). The

only exception was the occasional *P. litoralis* that had a completely black larval eye (Fig. 6). Verification of species identity following post-larval molt, where there is a reliable published character for species assignment, Ah Yong (2001), suggest that larval eye color is 100% effective in species identification.

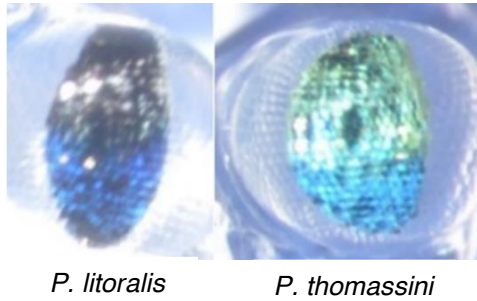


FIG. 5. Larval eyes of both species prior to the post-larval molt.

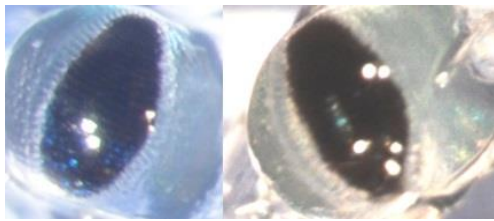


FIG. 6. Variation in *Pullosquilla litoralis* eyes, note the varying amount of blue from a small amount to none.

The larval height of *P. litoralis* was 15.4% larger than *P. thomassini*. The larval width of *P. litoralis* was 30.3% larger than *P. thomassini*. The adult height of *P. litoralis* was 3.9% larger than *P. thomassini*. The adult width of *P. litoralis* was 2.4% larger than *P. thomassini* (Fig. 7).

The rate of larval eye size decrease was 11.8% faster in *P. thomassini* than for *P. litoralis*. The rate of decrease for width was $-0.072 \text{ mm hr}^{-1}$ and for height was $-0.047 \text{ mm hr}^{-1}$ for *P. thomassini*. The rate of decrease for width was $-0.062 \text{ mm hr}^{-1}$ and for height was $-0.043 \text{ mm hr}^{-1}$ for *P. litoralis* (Fig. 8A and 8B).

The rate of adult eye size growth was 14.9% faster in *P. litoralis* than for *P. thomassini*. The rate of growth for width was 0.141 mm hr^{-1} and for height was 0.168 mm hr^{-1} for *P. litoralis*. The rate of growth for width was 0.131 mm hr^{-1} and for height was 0.132 mm hr^{-1} for *P. thomassini* (Fig. 8C and 8D).

Adult eye growth began before the post-larval molt for *P. thomassini*. No adult eye growth occurred in *P. litoralis* before the post-larval molt. Notice in both Fig. 8C and 8D, there were no detected adult eyes in *P. litoralis* before 18 hours. During this time all stomatopods measured were in their terminal larval stage and were going to molt into their post-larval form by the third photo of 18 hours after initial capture.

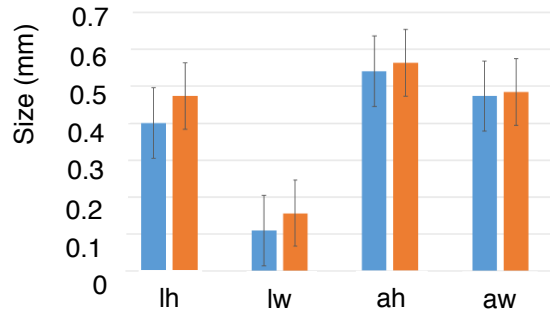


FIG. 7. Final average sizes of larval and adult eyes. Blue is *P. thomassini* and orange is *P. litoralis*. Measurements include: lh= larval height, lw= larval width ah= adult height, and aw= adult width. Error bars represent standard error.

DISCUSSION

The rate of larval eye reduction and adult eye growth were fairly similar in *P. litoralis* and *P. thomassini*. The slight difference in the rates and the final sizes can be explained by the size difference between the species. Both organisms are pygmy stomatopods, however, *P. litoralis* is slightly bigger with the largest recorded being 19 mm while the largest *P. thomassini* recorded was 17 mm (Ah Yong 2001). The final eye sizes of the two species were similar with *P. thomassini* having both smaller larval and adult eyes. This similarity in eye development could also be a factor of a greater overlap of habitat. *Pullosquilla litoralis* has been observed at depths of 30 meters where *P. thomassini* depth range reaches (Caldwell pers. obs.).

These two species of stomatopods had very different larval eye colorations which suggests a difference in their pelagic lifestyles. It has been shown that *P. thomassini* has a half green half blue larval eye and that the eyeshine of the larval stomatopod eye is a unique mechanism

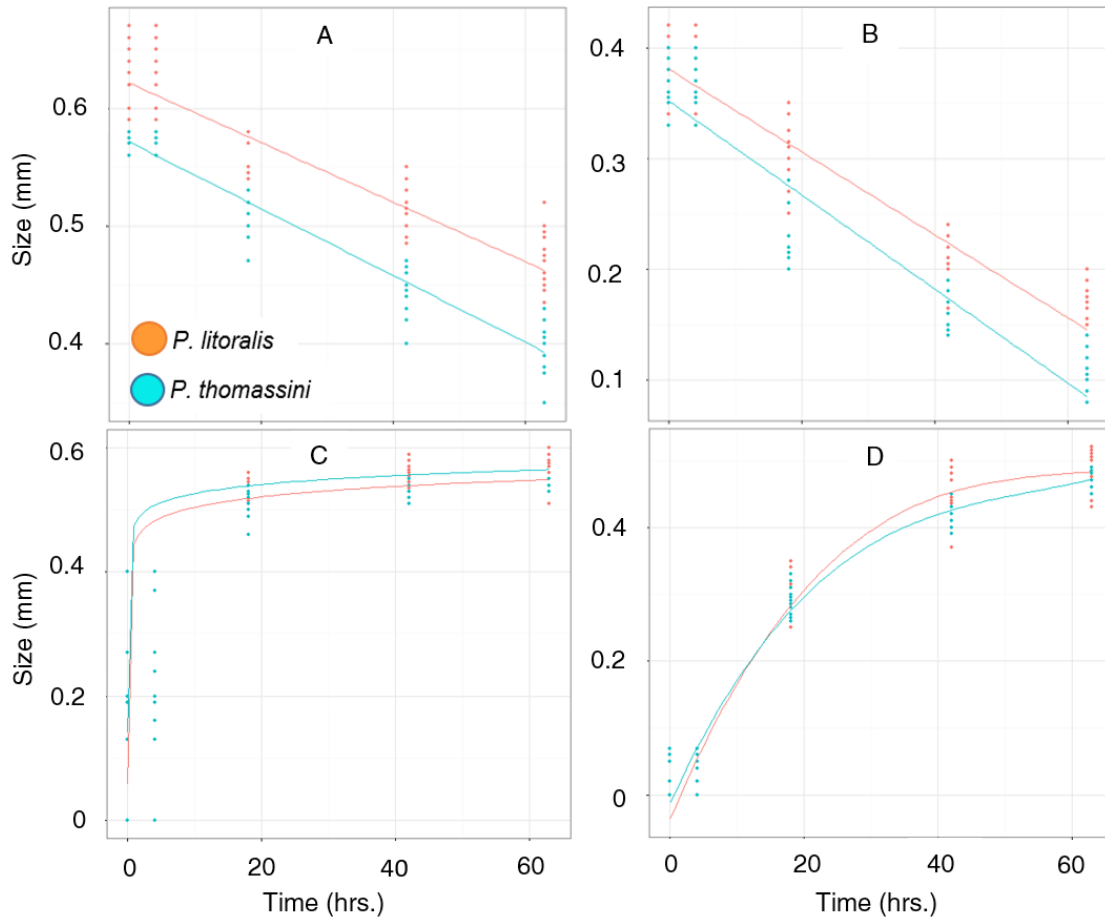


FIG. 8 A-D) Decrease in height of larval eye, the line is a line of best fit. B) Decrease in width of larval eye, the line is a line of best fit. C) Growth in height of adult eye, the line is a logarithmic growth curve. D) Growth in width of the adult eye, the line is a polynomial curve.

to increase transparency (Feller and Cronin 2014). This could suggest that these two stomatopods have different photic environments when in their pelagic larval form. *Pullosquilla litoralis* possess a half blue half black eye while *P. thomassini* possess a half blue half green eye. With the opaque eye being the only feature of the larval stomatopod that reduces its transparency, the coloration of the eye offers a breadth of contrast with the surrounding environment. This difference in coloration between these two *Pullosquilla* species could be explained by the phase of moon in which they are in their free-swimming form.

The most notable difference between the two stomatopods was the timing of the adult eye growth. *Pullosquilla thomassini* began adult

eye development before the post-larval molt while *P. litoralis* began adult eye development after the post-larval molt. This difference cannot be tied to the preferential habitat of each organism since it has been observed that they have large bathometric overlap. It is believed that stomatopods begin adult eye growth before the post-larval molt (Schram et al. 2013), however, *P. litoralis* defied this expectation. This difference can indicate that the two species are in fact more distantly related than previously thought. This outcome raises a question for future research: what are the consequences of having different timings of adult eye development in relation to the surrounding environment?

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LITERATURE CITED

- Ahyong, S. T. 2001. Revision of the Australian stomatopod crustacea. Australian Museum Sydney.
- Cheroske, A. G., T. W. Cronin, and R. L. Caldwell. 2003. Adaptive color vision in *Pullosquilla litoralis* (Stomatopoda, Lysiosquilloidea) associated with spectral and intensity changes in light environment. *The Journal of Experimental Biology* **206**(Pt 2):373-379.
- Cronin, T. W., and R. N. Jinks. 2001. Ontogeny of vision in marine crustaceans. *American Zoologist* **41**(5):1098-1107.
- Cronin, T. W., and J. Marshall. 2004. The unique visual world of mantis shrimps. *Complex Worlds from Simpler Nervous Systems* 239.
- Cronin, T. W., N. J. Marshall, R. L. Caldwell, and D. Pales. 1995. Compound eyes and ocular pigments of crustacean larvae (Stomatopoda and Decapoda, Brachyura). *Marine and Freshwater Behaviour and Phy* **26**(2-4):219-231.
- Feller, K. D., and T. W. Cronin. 2014. Hiding opaque eyes in transparent organisms: A potential role for larval eyeshine in stomatopod crustaceans. *The Journal of Experimental Biology* **217**(Pt 18):3263-3273.
- Jeffery, W. R. 2005. Adaptive evolution of eye degeneration in the Mexican blind cavefish. *The Journal of Heredity* **96**(3):185-196.
- Jones, J. 1994. Architecture and composition of the muscles that drive stomatopod eye movements. *The Journal of Experimental Biology* **188**(1):317-331.
- Jutte, P. A. 1997. The ecology, behavior, and visual systems of *Pullosquilla litoralis* and *P. thomassini*, two monogamous species of stomatopod crustacean. University of California, Berkeley.
- Jutte, P. A., T. W. Cronin, and R. L. Caldwell. 1998. Photoreception in the planktonic larvae of two species of *Pullosquilla*, a lysiosquilloid stomatopod crustacean. *The Journal of Experimental Biology* **201** (Pt 17):2481-2487.
- Land, M. F. 1997. Visual acuity in insects. *Annual Review of Entomology* **42**(1):147-177.
- Manning, R. B. 1980. The superfamilies, families, and genera of recent stomatopod crustacea, with diagnoses of six new families. *Proceedings of the Biological Society of Washington* **93**:362-372.
- Marshall, J., T. W. Cronin, and S. Kleinlogel. 2007. Stomatopod eye structure and function: A review. *Arthropod Structure and Development* **36**(4):420-448.
- Nilsson, D. 1997. Eye design, vision and invisibility in planktonic invertebrates. *Zooplankton: Sensory Ecology and Physiology* 149-162.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**(7):671-675.
- Schram, F., and M. Charmantier-Daures. 2013. *Treatise on zoology-anatomy, taxonomy, biology. the crustacea*, volume 4 Brill.
- Webster, D., and M. Webster. 1971. Adaptive value of hearing and vision in kangaroo rat predator avoidance. *Brain, Behavior and Evolution* **4**(4):310-322.
- Williams, B., J. Greenwood, and J. Jillett. 1985. Seasonality and duration of the developmental stages of heterosquilla tricarinata (Claus, 1871) (Crustacea: Stomatopoda) and the replacement of the larval eye at metamorphosis. *Bulletin of Marine Science* **36**(1):104-114.