Temporal variation in larval release in *Botrylloides violaceus*

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**Abstract**

Despite *Botrylloides violaceus* being a globally invasive species in temperate marine habitats, little is known about its natural dispersal behavior. Previous work (Delton et al, 2011) suggests behavioral inferences based on a related and much more commonly studied species may not be appropriate, potentially due to a large size difference between the non-feeding larvae of these two species. Here, we consider factors that may affect dispersal potential. To study how light affects larval release, we measured timing of larval release in the field and compared larval size and time of release. Colonies were collected from floating docks in Richmond, CA and placed into flow-through acrylic boxes to trap released larvae. Larvae were counted every half hour from morning to mid-day and then brought to the lab for measurement. In contrast to other tunicate species that release closer to sunrise (4), this species on average released more offspring later in the morning. In addition, larval size showed an increasing trend later in the morning. Larger larvae are likely to have more energy reserves that may allow greater dispersal potential and thus a daily difference within colonies in dispersal potential of sibling progeny. This may have implications for population patterns of relatedness, a key feature in the smaller relative that is commonly used as a kin selection model system.

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**Background**

*B. violaceus* larvae are 1-1.5mm in body diameter, non-feeding and fully developed upon release. Larvae may immediately settle or swim actively and sporadically for as long as 12 hours. Ascidian larvae contain a photosensory organ called a photolith, which allows the tadpole larva to respond to light (1). In lab experiments, *Botrylloides schlussleri* and *Polyandrocarpa zonitensis* both release larvae in the morning following a darkened night latency period (4). A field experiment using nonfeeding short-lived larvae from another phylum, the bryozoan *Bugula neritina*, showed a decrease in larval size over the course of a day, while the abundance of larvae increased over the morning, contrary to expectations (2).

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**Field and laboratory methods**

*Sampling occurred on various locations on the floating docks at the Marina Bay Yacht Harbor, Richmond, CA (Image 3)*

*From each location, 5 *B. violaceus* colonies were collected and placed into flow-through acrylic boxes to trap dispersed larvae. A HOBO logger was deployed to record light intensity every 15 minutes (Image 4)*

*Larvae were collected every half hour from morning to mid-day and were preserved in 95% ethanol. (Image 5)*

*Preserved larvae were brought back to the lab and counted. The head length of each larva was measured using a Cannon camera at 40X magnification and ImageJ software. (Image 6)*

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**Results**

**Mid-morning peak in larval numbers and size**

![Image 1: Colony of *B. violaceus*](image1.png)

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![Image 2: Head of tadpole larva of *B. violaceus* at 400X](image2.png)

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![Image 3: Google map of Marina Bay Yacht Harbor. Location of collection sites designated by the yellow stars](image3.png)

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![Image 4: Field set up of collection boxes and HOBO logger](image4.png)

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![Image 5: Larvae from the colonies every half hour were preserved in falcon tubes containing 95% ethanol.](image5.png)

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![Image 6: Lab set up for measuring head length of tunicate larvae.](image6.png)

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**Discussion**

- Larval release numbers and size generally increase with increasing light, producing more larvae later in morning, regardless of weather conditions.
- There is an apparent temporal component to energetic content of dispersing larvae, assuming larger larvae have greater nutrient or caloric content.
- Energetic content is expected to correlate with dispersal potential, substrate selectivity, and juvenile success.
- Thus, there is a predicted daily temporal dispersal polymorphism that may affect population structure of this model taxon for studying kin selection.
- Additional experiments testing the effects of flow on dispersal potential and relatedness are being analyzed.

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**References**


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**Acknowledgements**

**Special thanks to Joseph Spalding, Benson Chee, Chenh Fetkova, The Cohen Lab, STM users, RTC, and the STM Program for making this project possible, and to Ellen Koonin for suggestions.**

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**Figure 1:** Number of larvae released each hour in the field. Data shown are means and std errors for 3 separate days with varying weather-light conditions based on cloudiness and dock exposure.

**Figure 2:** Larval head length measures related to time of day, as described in fig 1 caption.

**Figure 3:** The average amount of larvae released at different light intensities. The average light intensity was averaged from 15 minute interval recordings over each hour that larvae were collected on two separate dates.

**Figure 4:** Average larval size released at different light intensities, measured as above on two separate dates.