

Swimming velocities and patterns of *Bugula californica* larvae through the early stages of development

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Abstract

The larval swimming patterns prior to settlement play an important role in geographic dispersal and genetic connection for sessile marine organisms. This study examined how swimming patterns and size change with age for larvae of the bryozoan, *Bugula c.f. californica*. We observed three different swimming patterns, including upward swimming, downward swimming, and sinking which were observed every hour post hatch for four hours until the onset of settlement each day. We used analysis of digital photographs to measure the body length and width of larvae within the same cohort. Almost all early stage larvae swam up to the top in the first hour, but in later stages larvae changed their swimming pattern to swim down or sink. Upward velocity averaged at about 2mm per second and decreased over time, and in the fourth hour the larvae started to sink at about 2mm per second. In addition, there is no significant difference among the values of the lengths or widths of the larvae over time, but these phenotypic characteristics did vary within the age cohorts.

1. Introduction

Larvae of many marine invertebrates have three distinct phases through early ontogeny: a swimming phase, a settlement phase, and a metamorphosis phase (Wendt, 2000). The swimming phase results in larval dispersal which has a

profound impact on the location and types of settlement habitat that larvae encounter (Wendt, 2000). Larval behaviors during this phase play important roles in spreading cohorts, and in the likelihood of encountering favorable habitats. Larval behavior is likely to be affected by important factors such as larval age, size, and swimming velocities (Burgess *et al.*, 2009). For sessile organisms such as bryozoans, planktonic larval dispersal is critical to determine their geographical range and genetic linkage among populations (Kosman and Pernet, 2009).

Bryozoan species in the genus *Bugula* are ideal candidates to study the planktonic swimming patterns and speed of larvae, since their larvae have short (hrs) planktonic periods before the onset of settlement and because the adult colonies release multiple larvae around the same time (Wendt, 2000). *B. c.f. californica*, an arborescent bryozoan, characterized by light brown branches arranged in whorls releases free swimming, lecithotrophic larvae that spend only hours in the plankton before settling down permanently on suitable habitat, such as hard substrates (Lamb and Hanby, 2005).

Many studies have looked at larval sizes, energy cost, and swimming behaviors (Wendt, 2000; MacDonald, 2004; Kosman and Pernet, 2009), but few have examined larval velocities for multiple stages of development. Due to an apparent lack of information about how swimming characteristics such as speed and direction vary through time during the planktonic larval phase, we analyzed changes in speed and swimming patterns of the planktonic larvae

of *Bugula c.f. californica*, from when they hatched to the onset of settlement. Additionally, we compared the body size (length and width) of the larvae of different ages to see if size is related to changes in swimming speed and patterns, and related to the settlement of this species.

2. Method and materials

2.1 Sampling procedure

Sexually mature colonies of *Bugula c.f. californica* that could be recognized by the orange coloration due to brooded larval stages were collected from the docks located at Friday Harbor Laboratories, Friday Harbor, WA, between July 31 and August 2 of 2014. They were kept overnight in plastic containers fitted with micro-mesh screens immersed in about 12°C circulating seawater inside a large opaque tank with a black lid. In the morning and after 12-16 hours in the dark, the colonies were transferred into clean glass jars that were partially filled with filtered sea water (FSW). These jars with colonies were placed in an outdoor tank with circulating seawater that ranged from about 12°C to 13°C and exposed to natural sun light for 1 to 2 hours to induce the release of the larvae. After one hour, we checked jars for released larvae every 15 minutes until a large number of larvae were present, which was approximately between 1 and 2 hours after sunlight exposure. We harvested the larvae by pipetting them into crystalizing dishes filled with FSW to contain them until they were transferred into the water column tanks. Only

the larvae collected within 30 minutes of the first observation of larval release were used for the experiment in order to ensure the analysis of the same cohort.

2.2 Speed and pattern of swimming measurements

Individual larvae were gently pipetted one at a time into the water column measuring 1.5cm x 1.5cm x 17 cm and filled with FSW (Fig. 1B). The water in the column was kept at about 12°C by an outer cooling tank hooked up to a small chiller running continuously with reverse osmosis water. (Fig. 1A). Each larva was placed approximately 2.5 cm from the bottom (except in the third and fourth hours, where the larvae were placed in the approximate middle) of the water column (Fig. 1B). Velocities and patterns of swimming were measured for 5-7 larvae that could be measured within a 30 minute period every hour from the time of release to the onset of settlement.

The column has division marks every 2.0 cm, and these marks were used to calculate the velocity by measuring the time that it took the larvae to cross the distance between two division marks, and the distance was divided by the time. The velocity vector was only considered when the movement was vertical. The time was recorded by stopwatch. *B. c.f. californica* larvae are phototactic (Wendt, 1996), thus in order to induce swimming light was present at the top of the column. Each larva was observed for about 2 to 3 minutes within the column. The hour of development when each larva was observed was also recorded along with the swimming patterns exhibited by the larvae.

Swimming patterns were evaluated and described as follows:

- *Swimming up* (SU): larvae moved to the top of column.
- *Swimming down* (SD): larvae moved in a spiral pattern to the bottom of the column.
- *Sink* (S): larvae moved in a straight line to the bottom.
- *Up/Down & Up* (UDU): larvae swim up and down and finish on the top of the column.
- *Up/Down & Down* (UDD): larvae swim up and down and finished on the bottom of the column.

2.3 Measurement of larval size

When measuring the larvae, five of each age cohort were delicately transferred to depression slides and covered with glass cover slips. Larvae from the same age cohort of those used to analyze the swimming features were observed every hour for 4 hours under a compound microscope (Nikon ECLIPSE E600) connected to a camera (Infinity 2), and digital photographs were taken at 10X magnification. These 5 photos were analyzed later to measure the body length and width at the longest and widest part of each larva (Fig. 2) with Infinity Capture and Image J software version 1.49b. The Image J software was calibrated to a stage scale bar under the same 10X magnification.

2.4 Statistical analyses

Data were analyzed with the statistical software SPSS v.20.0. The assumption of normality of the data was tested using the Kolmogorov-Smirnov test; when data did not meet this assumption, differences among groups were assessed using the corresponding non-parametric test. An Analysis of Variance (ANOVA) or a Kruskal-Wallis test (according to the normality of the data) was applied to analyze differences in the speed of a particular swimming pattern (up, down, sink) between ages, and a Kruskal-Wallis test was used to compare speed between swimming patterns. To analyze changes through age in the proportion of individuals performing a particular swimming pattern, a Chi Square test was applied. For analysis of changes in body size the corresponding parametric or non-parametric test was applied (ANOVA, Student t-test, or Kruskal-Wallis test).

3. Results

Speed measurements and observations of swimming behavior were realized in a total of 75 (1h: 17, 2h: 17, 3h: 19, and 4h: 22) larvae of *Bugula c.f. californica* during a period of three days. Due to significant differences (Tukey's test, $p < 0.05$) between the first day of the experiment with the rest of the days of the experiment; the first day's data was not included in the statistical analyses (Table 1). The upward swimming speed did not differ significantly through ages, although a decreasing tendency can be observed (Kruskal-Wallis H-Test, $H = 7.3$, $p = 0.06$). The downward swimming

behavior was not observed during the first hour, and the average speed did not differ through the rest of the larval development (ANOVA, $F = 0.7$, $F = 0.1$, $p > 0.05$), and individuals started to sink in the fourth hour after hatching.

Upward swimming, downward swimming, and sinking patterns were observed in the fourth hour on the second and third day of the experiment. The swimming down speed is noticeably slower compared with the swimming up and sinking speed (Kruskall-Wallis H-Test_{4h}, $H = 5.4$, $p < 0.05$). Larvae swimming tendencies changed through time (Fig. 3). A higher proportion of individuals swam to the top of the water column in the earlier ages, and in the later stages there is an increasing tendency to swim to the bottom of the column or sink ($X^2 = 41.7$; $df = 3$; $p < 0.001$).

Neither length nor width differed significantly across ages (Kruskall-Wallis H-Test, $H_{\text{length}} = 1.9$, $H_{\text{width}} = 2.1$, $p > 0.05$). However, means of both of these phenotypic characteristics increased through time, see (Table 2).

4. Discussion

In this study we found that the upward swimming velocity of the larvae of the *B. c.f. californica* did not differ significantly across the four hours but the number of larvae that exhibited this upward swimming behavior decreased over time as the larvae aged and entered the settlement phase within several hours. This decrease seems to be caused by a change in the swimming patterns or behaviors of the larvae. During the first hour the larvae were actively

swimming up and as the hours passed a higher proportion of the larvae started to spiral downward in a controlled descent to the bottom of the water column. By the fourth hour post-hatch a fraction of the larvae started to sink to the bottom of the column while the rest descended to the bottom.

As lecithotrophic larvae, the *B. c.f. californica* offspring are endowed with enough energy content to sustain them through their short planktonic durations, but this short amount of time spent in the plankton can reduce their dispersal radius (Levitan, 2000; Kosman and Pernet, 2009). Towards the end of the planktonic period the larvae are most likely reallocating their energy reserves to prepare for the settlement phase and metamorphosis phases, so they adjust their swimming behavior to conserve their remaining energy content (Wendt, 2000; Kosman and Pernet, 2009). The larvae do not necessarily reduce their swimming behavior during the 3rd and 4th hours, but are more likely undergoing metamorphosis so they are gradually losing their cilia and their mode for swimming. Since these larvae have a shorter planktonic period they will not be transferred too far away from favorable settling habitat, and they will avoid most causes of mortality such as predation (Burgess *et al.*, 2009). However, in addition to benthic predation, with a smaller dispersal radius they will have to face spatial competition and patch resources within pre-established adult sessile colonies (Kosman and Pernet, 2009).

Although larval size shows a slight tendency to increase over time, there is not a significant difference through hourly ages. Larval size varies within the same age, which could be the result of a parental strategy to produce offspring with different dispersal capabilities (Burgess *et al.* 2009). Within the same species larger larvae tend to swim for longer periods than the smaller ones and consequently disperse and colonize habitats farther away, while the smaller larvae are most likely to settle near the maternal colony (Burgess *et al.* 2009; Kosman and Pernet 2009). Therefore, our observations of larger larvae throughout the experiment may be due to a procedure bias, since the smaller larvae may have already settled by the time we sampled from the crystal dish to perform the experiment each hour, especially during the third and fourth hours. We advise to keep the sample pool of larvae in conditions that might discourage settlement when conducting an experiment that analyzes larval velocities and size changes over time (Wendt, 2000). This study has shown the swimming velocities and changes in swimming behavior of *Bugula c.f. californica* larvae as they transitioned from the swimming to the settlement phase. Since cilia are the main mode of swimming for most bryozoan larvae, in future studies it would be beneficial to compare the cilia lengths and the surface area of the body that has cilia for this species of bryozoan larvae over the same transitional timeline. More cilia might explain these fast larvae, or the ciliary beat speed could be much faster than expected.

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Table 1. *Bugula c.f. californica* larvae speed across four hours according to swimming pattern.

Experiment Day	Swimming direction	Age							
		I		II		III		IV	
		Mean speed (cm/s)	N	Mean speed (cm/s)	N	Mean speed (cm/s)	N	Mean speed (cm/s)	N
1	Up	0.20 ± 0.02	5	0.17 ± 0.01	5	0.15 ± 0.07	2	-	0
	Down	-	0	0.08 ± 0.03	4	0.08 ± 0.01	3	0.06 ± 0.03	2
	Sink	-	0	-	0	-	0	-	0
2+3	Up	0.26 ± 0.04	12	0.23 ± 0.05	12	0.23 ± 0.03	6	0.17 ± 0.06	3
	Down	-	0	0.09 ± 0.01	3	0.09 ± 0.01	4	0.09 ± 0.03	11
	Sink	-	0	-	0	-	0	0.20 ± 0.09	6

Table 2. The Body length and width (μm) of *Bugula c.f. californica* larvae

across four hours.

Experiment day		Age (hr)							
		1h		2h		3h		4h	
		Mean	N	Mean	N	Mean	N	Mean	N
1	Length	207.5 \pm 19.9	6	221.0 \pm 24.5	3	-	0	-	0
	Width	177.3 \pm 14.61		187.8 \pm 24.3		-		-	
2+3	Length	185.6 \pm 18.4	12	193.1 \pm 15.6	7	210.1 \pm 40.9	9	217.1 \pm 44.7	7
	Width	170.1 \pm 19.7		186.3 \pm 21.3		196.2 \pm 38.4		207.5 \pm 54.7	

Figures

Figure 1. Water column tank. **A.)** Water column tank with cooling bath and chiller system, and a thermometer. **B.)** The scale of the water column (dimensions: 1.5cm x 1.5cm x 17 cm) and the placement of the larvae at the start of the 1st and 2nd hours and the 3rd and 4th hours for velocity measurements according to swimming patterns.

Figure 2. The Placement of length and width measurements on the longest and widest sections of the *Bugula c.f. californica* larvae that were analyzed in Image J.

Figure 3. Swimming patterns for larvae of *Bugula c.f. californica* as a function of age.

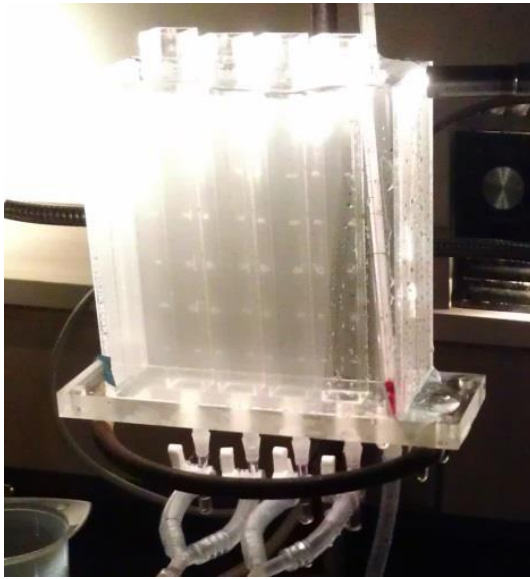


Figure 1A

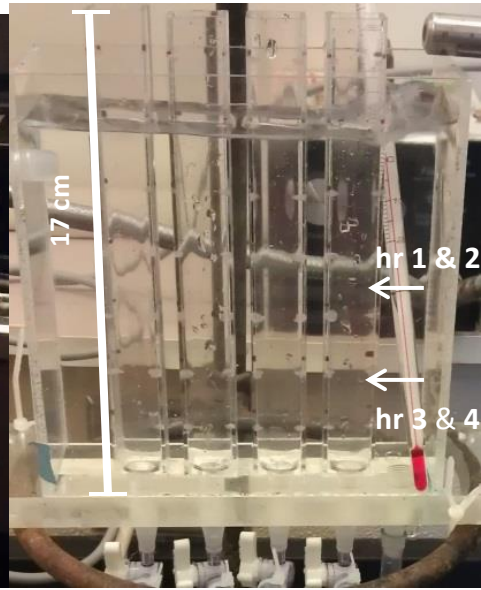


Figure 1B

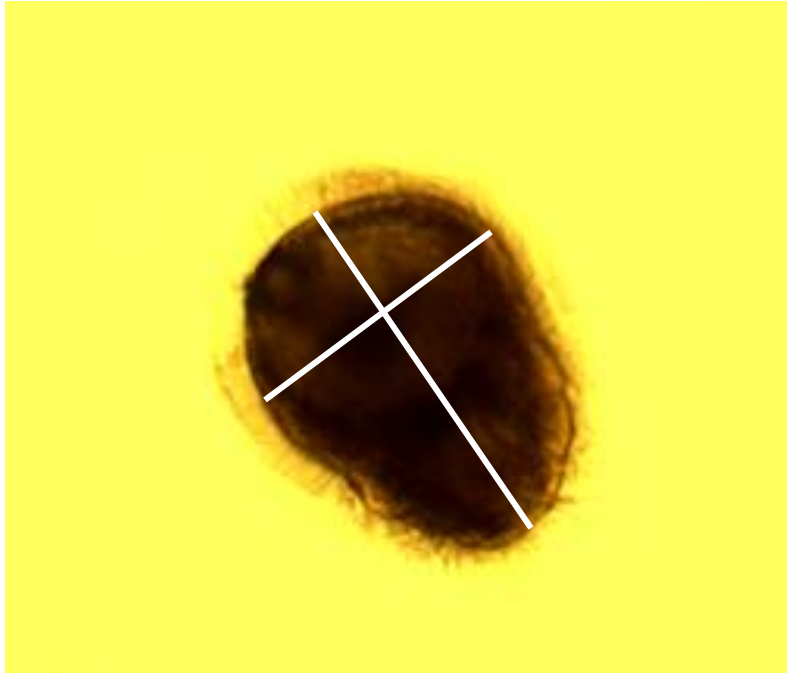


Figure 2

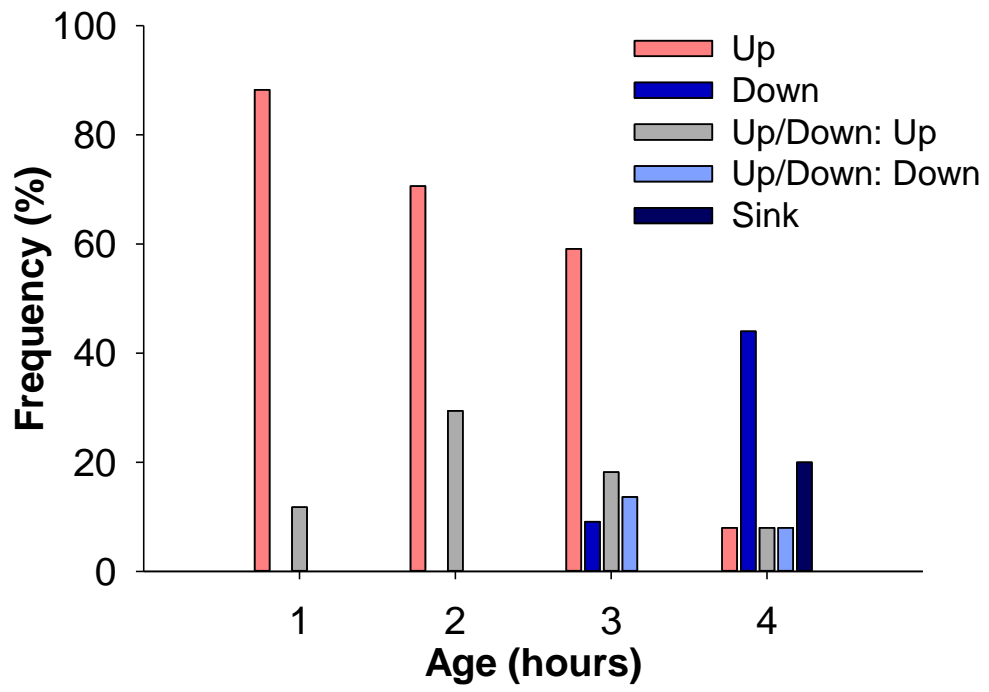


Figure 3