UNDERSTANDING THE TRANSITION FROM BENTHIC EGG TO DISPERSIVE LARVAE: OBSERVATIONS ON THE INTRA-CAPSULAR GROWTH AND DEVELOPMENT OF A MARINE SNAIL (*KELLETIA KELLETII)*

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by

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Abstract

Understanding the transition from benthic egg to dispersive larvae: observations on the intra-capsular growth and development of a marine snail (*Kelletia kelletii*)

Megan Wilson

It has long been understood that the larval life stage is responsible for the dispersion of many marine organisms across their biogeographic range. Such organisms have a bipartite life cycle, existing in the water column and subject to oceanographic processes as planktonic larvae before settling to suitable habitat along the benthos where they grow and mature. Previous studies have demonstrated that larval growth rate and behavior in the water column can alter larval position in relation to ocean currents and affects their dispersal pathway. However, there is a paucity of information regarding the growth rate of the earliest larval stage for organisms whose larvae first exist in protective, benthic capsules. In this study, I observed the reproductive process, oviposition, and intra-capsular larval development and growth of an ecologically and economically important marine snail, the Kellet's whelk (*Kelletia kelletii*). I observed an abnormally long incubation period for the egg capsules that challenges previous studies, and I found that the Gompertz and Gaussian models of growth best fit the larval whelks' growth. My results can be used to refine dispersion models guiding the management of the Kellet's whelk fishery, and provide a window of insight into the biological mechanisms that facilitate marine population connectivity.

Keywords: Kellet's whelk, population connectivity, dispersal, larval biology, growth rate, model

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I. Introduction

Mounting evidence from across marine taxa have shown that larval behavior is a key mechanism structuring dispersal pathways. The notion that larvae act as passive particles in the water column, subject to ocean large-scale oceanographic processes, has been largely replaced by the understanding that larvae actively adjust their position in the water column, thereby controlling their exposure to currents along a depth gradient (Sponaugle et al., 2002 and Morgan and Fisher, 2010). As such, an increasing number of modelling studies now include generalized larval behavior in population connectivity and dispersal models (i.e. Drake et al., 2013). However, due to a paucity of data concerning species-specific larval behaviors, durations, and growth rates, few models have been able to incorporate these variables, which are particularly critical in species-specific conservation or management objectives (Miller and Shanks, 2004). Furthermore, little attention has been given to the larval development, growth rate, incubation time, or behavior of larvae that exist in a protective capsule during their earliest stages.

The Kellet's whelk, *Kelletia kelletii*, is an organism of interest due to the recent expansion of its biogeographical range and its status as an emerging fishery species (California Department of Fish and Wildlife, 2006). Historically, the biogeographical range of this species extended from Isla Asuncion, Baja California, Mexico (McLean, 1978), to Point Conception, California, USA. In the 1980's, this species experienced a range expansion northward to Monterey, California, USA (Herrlinger, 1981), which may have been correlated with a major El Niño event (Zacherl et al., 2003) (Fig. 1). *K. kelletii* is a benthic marine gastropod that relies upon its pelagic larval stage to disperse; thus, understanding the paths and mechanisms by which these larvae travel provides a window of insight into the stratification and maintenance of marine populations.

K. kelletii undergo mixed development; in the case of this species, they develop from an embryo to a trochophore larvae, and finally to a veliger larva within a protective egg capsule and emerge as swimming veliger larvae. Previous studies have shown that the larvae are released from protective egg capsules after a period of 30-35 days (Rosenthal, 1970), and remain pelagic, planktotrophic larvae in the water column for at least 5.5 - 9 weeks (Romero and Zacherl, unpublished data). Near the end of their planktonic phase, larvae develop into pediveligers and hence become competent to settle.

A laboratory study by Romero et al. (2012) of larval migration in *K. kelletii* showed that larvae exhibited a nocturnal diel vertical migration behavior throughout the larvae's pelagic phase. During the fifth week of planktonic development, 60% of larvae (100 sibling larvae, n=5 replicates) were demersal at week 5 regardless of time of day. One possible interpretation of this result is that upon reaching competency, larvae become demersal to explore the benthos in search of suitable substrate upon which to settle. After finding suitable substrate, larvae shed their velum, marking the irreversible transition from the out of the water column to the benthos. The swimming ability of the larvae affects their ability to vertically migrate, and thus their ability to feed, orient in relation to currents, and ultimately, settle on suitable habitat. The growth that the larvae undergo within their capsule and their size at hatching may be highly predictive of larval condition, swimming ability, and settlement success.

My study uses *K. kelletii* as a model organism to: 1) observe the incubation stages and time line of development of intra-capsular larvae and 2) measure and model the growth rate of the dispersive stage of the larvae. Coupling the total incubation period with the individual growth rate and hatching size of the larvae within can provide information about larval condition as they enter the water column. Adding this data to a species-specific model of *K. kelletii* population

connectivity may strengthen the model's power to predict recruitment success and thereby inform the management of its fishery.

II. Materials and Methods

Study Organism: Kelletia kelletii

K. kelletii is a large predatory marine gastropod belonging to the family Buccinnidae (Forbes, 1850). They are kelp forest inhabitants and are commonly found on benthic hard substrate or cobble-sand interfaces at depths of 2 to 70 meters (Rosenthal, 1970). *K. kelletii* have separate sexes and reproduce annually via internal fertilization. Mating generally occurs between March and May and oviposition between April and May.

Collection and Aquaria

K. kelletii individuals were collected on SCUBA on May 4th, 2016 from Santa Rosa Reef (Fig. 1), located outside of Avila Bay in San Luis Obispo, California at depths ranging from 45 to 60 feet by Cal Poly scientific divers. The individuals were transported to the Cal Poly Pier in Avila Bay and maintained by Cal Poly Pier staff. They were kept at ambient temperatures (14-15C) via a flow through system of filtered seawater and fed a mixture of frozen anchovies and market squid weekly. The whelks shared a tank with kelp, cobble, juvenile rockfish, and other local gastropods.

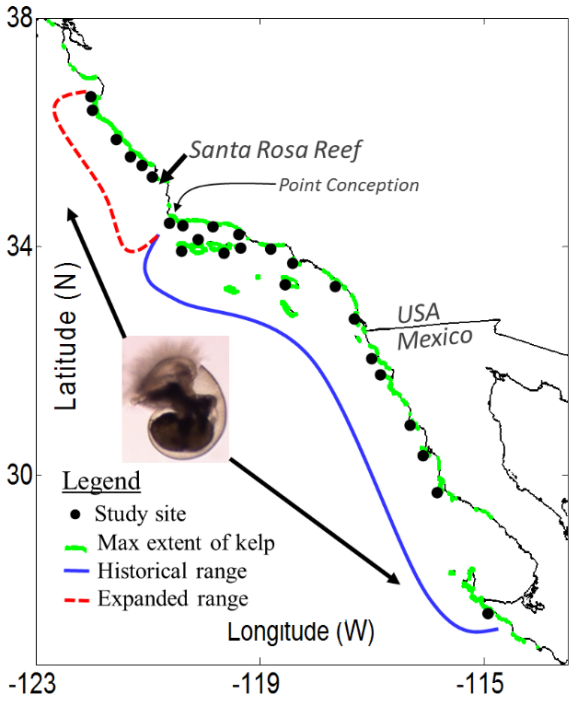


Figure 1: The historic (blue) and extended (red) biogeographic range of *K. kelletii*. The veliger larvae image represents the dispersive ability of this life stage. The collection site (Santa Rosa Reef) as well as a major oceanographic barrier (Point Conception) are marked. Graphic credit: Dr. Crow White.

Egg Capsule Collection and Maintenance

Oviposition began on May 19th, 2016 in a tank on the Cal Poly Pier and continued into the month of June. Capsules were laid on May 31st, 2016, and collected the same day and brought to a laboratory on the Cal Poly main campus. Capsules were maintained in a 15C incubator on a 12:12 L:D schedule, and kept in 800ml beakers containing 650ml of aerated FSW that was changed every other day until hatching. Beakers and aeration stones and tubing were rinsed and scrubbed thoroughly in first de-ionized and then distilled water.

Larval Rearing

Within 24 hours of hatching, 400 larvae were transferred to 4000ml beakers filled with 3000ml of FSW at 15C and 50ml of *I. galbana.* The cultures were maintained at 15C on a 12:12 L:D light cycle. The cultures were not aerated via air stones but rather were continuously stirred by a swinging paddle contraption as described by Richard Strathmann (2014). The water was changed in the cultures twice a week. The cultures were gently decanted over a 210 micron sieve. The larvae were hand-pipetted into a clean 4000ml beaker containing clean FSW and returned to the incubator. Cultures were fed 50ml *I. galbana* the day following a water change.

Observations on Intracapsular Development

Each week post-oviposition, a single egg capsule was sacrificed. A subset of the capsule, 10 individuals, was observed. Swimming behavior, development stage, length and width were recorded (Table 1). In order to slow the larvae enough to measure them the larvae (Fig. 2), the individuals were treated with ethanol. The length was defined as the longest distance on the larval shell, parallel to the operculum. The width was measured perpendicular to the length and the operculum (Fig. 2). Egg capsules were taken from the same brood each week to control for potential differences in maternal provision and/or embryo quality.

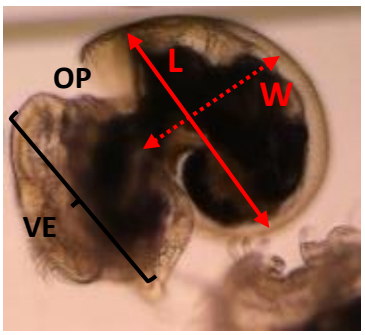


Figure 2: An example of the measurements taken on each individual larvae. "L" and "W" represent length and width, respectively. The operculum (OP) and velum (VE) are structures used for orientation.

Modeling and Statistics

The program JMP 11.1 was used to run an ANOVA and Tukey post-hoc test to identify differences in larval size during their *in-vitro* rearing.

Larval shell growth was fit to the Von Bertlanffy growth function expressed by the Von Bertlanffy (1938), Gompertz (1825), Richards (1959), Logistic (1938), Tanaka (1959), logistic growth (Verhulst, 1938), and Gaussian (Rogers, 1983) growth functions (Table 2) using Matlab R2013b.

III. Results

Oviposition occurred on May 31st, 2016. (Fig. 3). First hatching from the capsule from which the intra-capsular measurements were taken occurred July 15th, 2016; thus the total incubation period was observed to be 49 days. Intra-capsular measurements were taken each week from week one until hatching at seven weeks. Weeks one through three are excluded from growth modeling analyses because they represent the embryonic and trochophore larval stages,

whose shapes are incomparable to the veliger larval stage (Table 1). All of the larvae died by August 6, 2016; the total PLD achieved was 4.5 weeks. This falls short of the total PLD time recorded by Romero and Zacherl (unpublished data).

There were significant differences in length between weeks four, five, and six (p-value < 0.0001), however, weeks six and seven were not significantly different (Fig. 5).

Few egg capsules from the same brood remained unhatched by nine weeks post oviposition. These larvae were forcibly removed from their capsule and measured as well. Their size was most similar to week five larvae, though were not significantly different from week five, six, or seven larvae that hatched naturally (Fig. 5).

The Gaussian and Gompertz function were both accepted based on the lowest Akaike Information Criterion (AIC) score (Fig. 6). Both were accepted because the AIC cannot differentiate within two points.

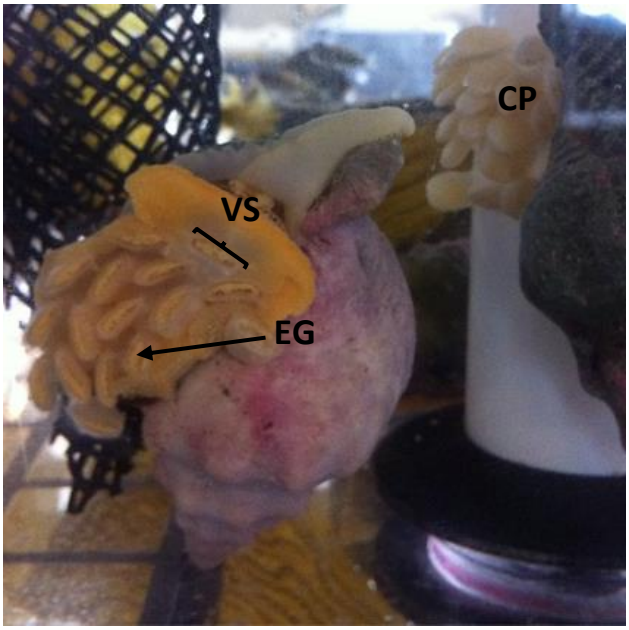


Figure 3: A *K. kelletia* female undergoing oviposition on the side of an aquarium tank. Capsules (CP) are behind the female, and under the female's orange foot. Additionally, the female is in the process of forming a capsule at the ventral slit (VS). Many eggs (EG) are visible in each capsule.

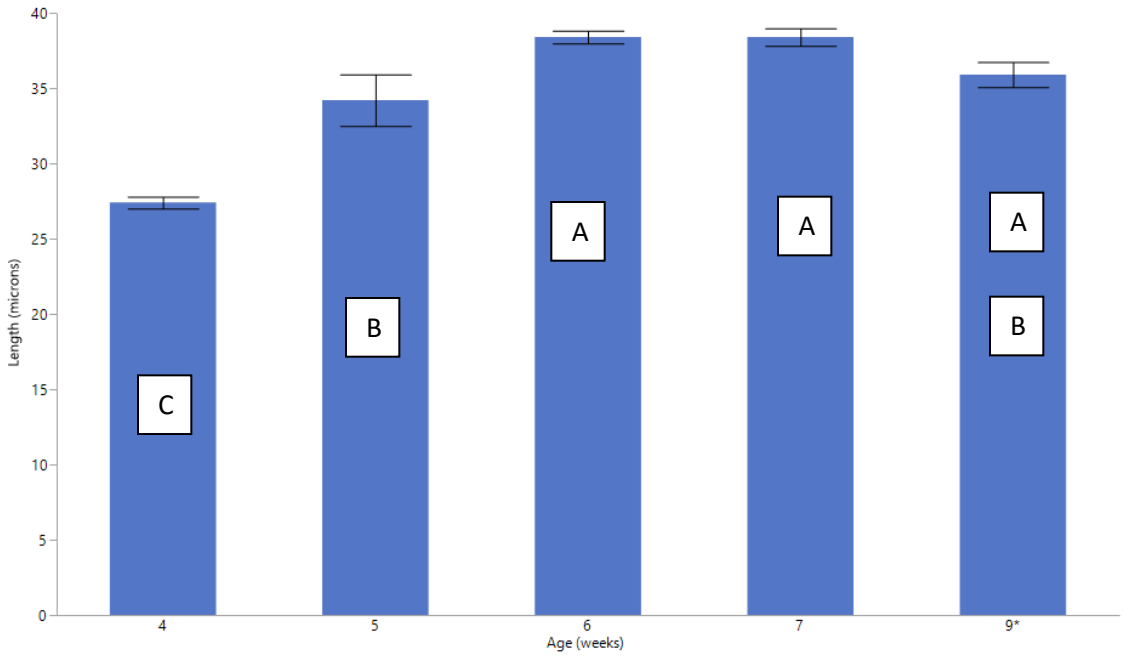


Figure 4: Mean shell length of intra-capsular veliger larvae from four to seven weeks of age, and nine week old larvae that were forcibly hatched (9*). Error bars represent the standard error of the mean, and bars connected by letters are not significantly different.

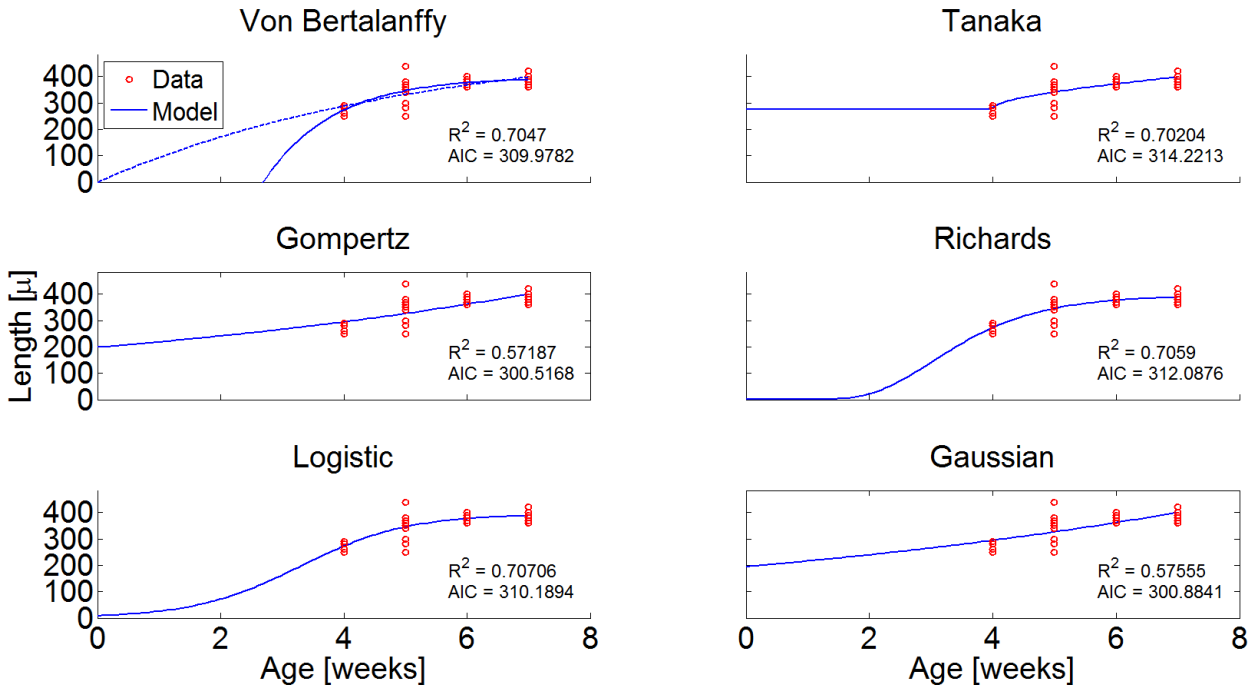


Figure 5: Intra-capsular veliger stage larval shell length as a function of age for six different growth models. Two best models, as determined by AIC score, are boxed.

IV. Discussion

My study quantifies both the total development time and the larval growth rate for the *K. kelletii* during their earliest life history stage: from the time the eggs are laid to the time the larvae escape their protective capsule and enter the water column. I observed that this process takes 49 days, a departure from the average 30-34 day development period observed by Rosenthal (1970) and Romero et al. (2012). I maintained the capsules in the same culture conditions as described in Romero et al. I reared our capsules at 15.5C, while Rosenthal reared his capsules at 14.5-17.5C. The only outstanding difference between my study and previous studies is the collection location and time of the adults who laid the capsules. Adults were collected from Santa Rosa Reef, in San Luis Obispo County, CA in 2016. Romero et al. collected

capsules from Monterey, California in 2005. Rosenthal observed adults from reefs in San Diego County, California in 1968-1969.

Seasonal and temporal differences in adult environment may affect the condition of the eggs laid, and thereby alter the intra-capsular development period and growth rate. One recent study suggests that external cues, such as environmental conditions or physical disturbance, may result in hatching plasticity in some marine invertebrates (Oyarzun and Strathmann, 2010). Variability in time to hatching changes the oceanographic conditions larvae encounter; variability in development stage and size at hatching changes the ability of the larvae to cope with challenges in the water column. One notable difference in my study specimen and those utilized in Rosenthal and Romero et al. is that my specimen were subject to anomalous ocean conditions. The specimen were collected during the major El Niño event of 2014-2016, which also coincided with the presence of the abnormally warm water mass termed “The Blob”.

It is possible that stress, due to elevated temperature, limited food availability, or other factors could have resulted in poor quality eggs or mothers, thus lengthening the development period. This was observed in a study of tropical damselfish by McCormick (2003), which found that female fish with increased access to food had fuller guts and a higher condition factor and their larvae had larger yolk sacs and oil globules, which conferred survival benefits to the larvae, than mothers with less access to this food source; thus, environmental condition was shown to directly impact the condition of the next generation. Though this line of questioning is outside the scope of this study, I did notice that the capsules of poor condition, which did not hatch naturally but rather were forcibly hatched, contained on average smaller larvae. This pattern may corroborate the idea that poor quality capsules result in a longer development period. However, a

much more robust study would be required to establish a correlation between ocean condition and *K. kelletii* intra-capsular development period.

Because I did not have wild control egg capsules, I was unable to test for the effect of the laboratory setting on my results. The egg capsules were laid by adults maintained in flow through aquaria at the Cal Poly Pier. This environment closely resembles the natural environment in oxygenation, water temperature, water chemistry, and thus I do not believe differences in adult condition caused the observed lengthened intra-capsular development period. Though the exact feeding schedule of the whelks was not recorded, I assumed that their access to food was equal or better than wild conditions as there were few whelks sharing a high energy food item regularly. However, when capsules were moved to a laboratory incubator, water oxygenation, water chemistry, and photoperiod were variables that may have differed from natural conditions and therefore may have caused the extended development period. Because the larvae were wholly encompassed in capsules, I do not believe laboratory differences in water chemistry significantly affected intra-capsular growth rates or the development period. In order to control for these differences, future studies could incorporate weekly egg capsule collections, as long as it could be ensured that capsules from the same brood were being collected each week (perhaps caging the egg capsules). Alternatively, egg capsules could be kept in flow through aquaria as long as it could be ensured that the hatching event was not missed.

Furthermore, my study was limited in that I only assessed the development period and growth rate of individuals within capsules from one brood. *K. kelletii* females mate multiple times, often laying eggs following or simultaneously during copulation (Rosenthal, 1970). Thus, one brood may encompass genetic diversity because of the multiple paternities. However, maternal condition may impact the provisioning a female provides within the egg capsules. Only

including samples from one female severely biases my results. Future studies should increase the number broods sampled, ensuring that each brood is laid by a different female.

In my analysis of the growth rate of intra-capsular *K. kelletii* veliger larvae, I found that the Gompertz and Gaussian models best fit my data. The elucidation of the individuals' growth rate and pattern, coupled with their average incubation period, results in knowledge of individuals size and growth pattern upon their release into the water column. An interesting future study would assess the predictability of settling or juvenile condition given newly hatched larval condition, which may be predicted from incubation period, growth rate and/or hatch size. Moreover, hatch size and/or condition, incubation period, and intra-capsular growth rates for are much easier metrics to assess than, for example, larval density in the water column. Benthic egg capsules are more easily found, collected, and maintained due to their robustness compared to larvae in the open ocean. Altogether, the ability to predict juvenile success from these data is a very powerful tool in management.

Much remains to be discovered concerning the larval biology of *K. kelletii*. Rearing the larvae *in vitro* for their entire PLD and quantifying the growth rate over their entire larval period, as well as observing ontogenic shifts in behavior will establish foundational knowledge of this species larval biology. Establishing this baseline data will allow for future manipulation studies such as quantifying the change in growth rate and/or behavioral to environmental conditions such as changes in current patterns, chemical cues, acidity, temperature, or food availability.

Lastly, large-scale changes in ocean condition such as those due to global climate change immediately necessitate the establishment of baseline data because it is only with such data that we will be able to discern directional change. Elucidating species-specific variables such as larval growth and behavior across all development stages will undoubtedly require innovative

methodology and meticulous study. However, it will provide the baseline observations and quantifications of life history traits that govern the life history stage paramount to the maintenance of marine populations. Moreover, it will allow us to observe climate change driven changes in larval populations that will predict recruitment success and adult population dynamics. Indeed, this line of study may further shift existing paradigms in marine population dynamics that will clarify our current understanding of marine systems.

	$L_t = L_\infty \cdot \left(1 - e^{-\kappa(t-t_0)}\right)$
	$S_t = \frac{1}{\sqrt{f}} \cdot \ln\left(2f(t-c) + 2\sqrt{f^2(t-c)^2 + fa}\right) + d$
	$y = K\left(1 + e^{(d-abt)}\right)^{(-1/b)}$
	$y(t) = ae^{-be^{-ct}}$
	$L_t = 1 + be^{-kt}$
	$y = ae^{(-(x-c)/b)^2}$

Von Bertlanaffy (1938)	$L_t = L_\infty \cdot \left(1 - e^{-\kappa(t-t_0)}\right)$
Tanaka (1982)	$S_t = \frac{1}{\sqrt{f}} \cdot \ln\left(2f(t-c) + 2\sqrt{f^2(t-c)^2 + fa}\right) + d$
Richards (1959)	$y = K\left(1 + e^{(d-abt)}\right)^{(-1/b)}$
Gompertz (1825)	$y(t) = ae^{-be^{-ct}}$
Logistic Growth* (1838)	$L_t = 1 + be^{-kt}$ *solution form
Gaussian (1983)	$y = ae^{(-(x-c)/b)^2}$

Table 1: List of functions modeled and a key to variables and parameters

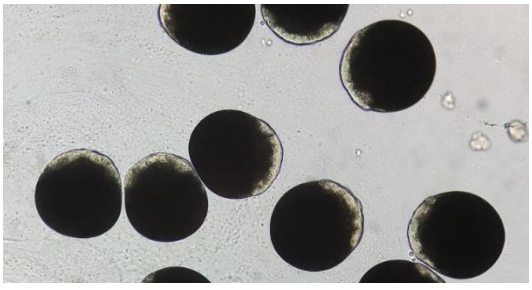
Functions

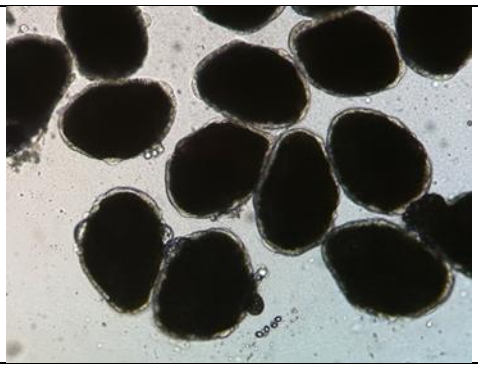
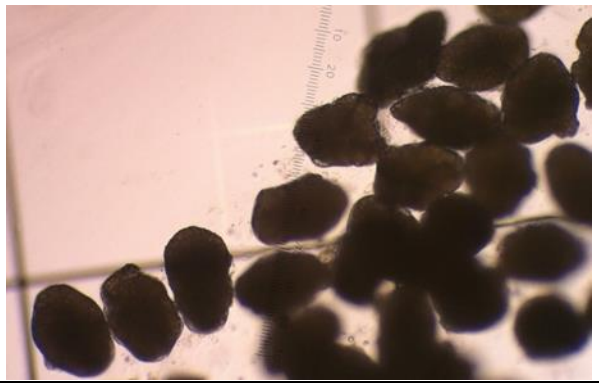
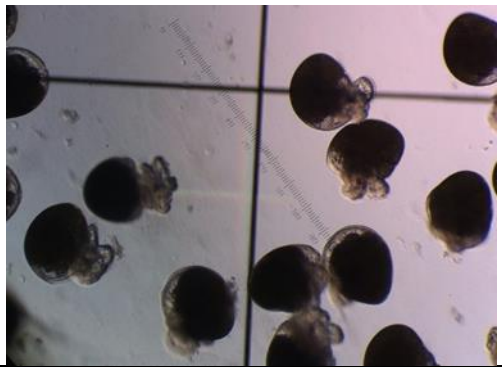
Parameter	Description
t_0	time zero (curve fitting parameter)
κ	Constant that controls decrease in growth rate as the animal matures
σ	Standard deviation of the distribution of max growth vs. size
a, b, c, d, f	Curve fitting parameters
K	Upper asymptote of y*
b	Parameter to define asymmetric curves*
d	Parameter allowing for the time at which y=K/2 to be varied*
a	Maximum intrinsic rate of increase of y*

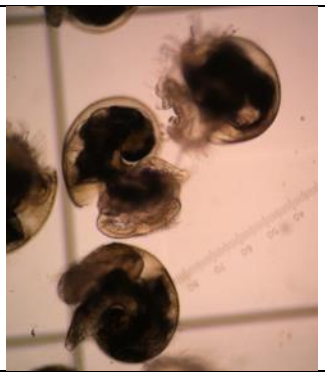
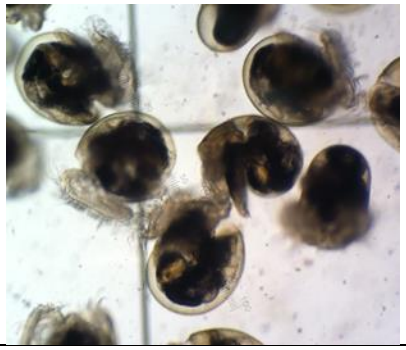
Variable	Description
L_t	Length at time t
L_∞	Maximum size
y	Variable also representing length at time t
t/x	time (age)

*In relation to the Richards function

Table 2: Weekly observations on larval development stage

Weeks post-oviposition	Date	Developmental Stage	Picture
1	6/7/2016	Embryo	
2	6/14/2016	Trochophore	

			
3	6/22/2016	Trochophore	
4	6/28/2016	Veliger	
5	7/8/2016	Veliger	
6	7/15/2016	Veliger	

			
7	7/19/2016	Veliger	

REFERENCES

California Department of Fish and Wildlife. 2006. Review of some California fisheries for 2005: coastal pelagic finfish, market squid, Dungeness crab, sea urchin, abalone, Kellet's whelk, groundfish, highly migratory species, ocean salmon, nearshore live-fish, Pacific herring, and white seabass.

Gompertz, B. 1825. On the Nature of the Function Expressive of the Law of Human Mortality, and on a New Mode of Determining the Value of Life Contingencies. *Philosophical Transactions of the Royal Society of London* 115: 513-583.

Drake, P.T., Edwards, C.A., Morgan, S.G., Dever, E. P. 2013. Influence of larval behavior on transport and population connectivity in a realistic simulation of the California Current System. *Journal of Marine Research* 71 (4): 317-350.

Herrlinger, T. J. 1981. Range extension of Kelletia kelletii. *Veliger* 24 (1): 78-78.

McCormick, M.I. 2003. Consumption of coral propagules after mass spawning enhances larval quality of damselfish through maternal effects. *Oecologia.* 136 (1): 37-45.

McLean, J.H. Marine Shells of Southern California, vol. 24 of Science Series, Natural History Museum of Los Angeles County, Los Angeles, Calif, USA, 1978.

Miller, J.A., and Shanks, A. L. 2004. Evidence for limited larval dispersal in black rockfish (*Sebastes melanops*): implications for population structure and marine-reserve design. *Canadian Journal of Fisheries and Aquatic Sciences.* 61 (9): 1723-1735.

Morgan, S.G., and Fisher, J.L. 2010. Larval behavior regulates nearshore retention and offshore migration in an upwelling shadow and along the open coast. *Marine Ecology Progress Series*. 404: 109-126.

Oyarzun, F.X., and Strathmann, R.R. 2011. Plasticity of hatching and the duration of planktonic development in marine invertebrates. *Integrative and Comparative Biology*. 51 (1): 81-90.

Sponaugle, S., Cowen, R.K., Shanks, A., Morgan, S.G., Leis, J.M., Pineda, J., Boehlert, G.W., Kingsford, M.J., Lindeman, K.C., Grimes, G., Munro, J.L. 2002. Predicting self-recruitment in marine populations: Bio physical correlates and mechanisms. *Bulletin of Marine Science*. 20: 341-375.

Richards, F.J. 1959. A flexible growth function for empirical use. *Journal of experimental Botany*, *10*(2): 290-301.

Rogers, D. W. 1983. BASIC microcomputing and biostatistics, 274 p. The Humana Press Inc., Clifton, NJ.

Romero, M. R., Walker, K.M., Cortez, C.J., Sanchez, Y., Nelson, K.J., Ortega, D.C., Smick, S.L., Hoese, W.J., Zacherl, D.C. 2012. Larval Diel Vertical Migration of the Marine Gastropod *Kelletia kelletii* (Forbes, 1852). *Journal of Marine Biology.* Vol. 2012.

Rosenthal, R. J. "Observations on the reproductive biology of the Kellet's whelk, Kelletia kelletii." *Veliger* 12.3 (1970): 319-324.

Tanaka, M. 1982. A new growth curve which expresses infinite increase. *Amakusa Marine Biology Laboratory* 6: 167-177.

Verhulst, P.F. 1838. Notice sur la loi que la population suit dans son accroissement. correspondance mathématique et physique publiée par a. *Quetelet* 10: 113-121.

Von Bertlanffy, L.A. 1983. A quantitative theory of organic growth (inquiries on growth laws. II. *Human Biology* 10.2: 181-213.

Zacherl, D., Gaines, S.D., Lonhart, S.I. 2003. The limits to biogeographical distributions: insights from the northward range extension of the marine snail, *Kelletia kelletii* (Forbes, 1852). *Journal of Biogeography* 30 (6): 913-924.