

8-1-2015

# The Effects Of Triiodothyronine, Glucose, Alanine, And Iodide As Nutrients On The Survival And Successful Metamorphosis Of *Aeolidiella* *Stephanieae* Veligers

Bryan Tate  
*University of New England*

Follow this and additional works at: <http://dune.une.edu/theses>

 Part of the [Biology Commons](#), [Developmental Biology Commons](#), and the [Marine Biology Commons](#)

© 2015 Bryan Tate

---

## Preferred Citation

Tate, Bryan, "The Effects Of Triiodothyronine, Glucose, Alanine, And Iodide As Nutrients On The Survival And Successful Metamorphosis Of *Aeolidiella Stephanieae* Veligers" (2015). *All Theses And Dissertations*. 40.  
<http://dune.une.edu/theses/40>

This Thesis is brought to you for free and open access by the Theses and Dissertations at DUNE: DigitalUNE. It has been accepted for inclusion in All Theses And Dissertations by an authorized administrator of DUNE: DigitalUNE. For more information, please contact [bkenyon@une.edu](mailto:bkenyon@une.edu).

THE EFFECTS OF TRIIODOTHYRONINE, GLUCOSE, ALANINE, AND IODIDE  
AS NUTRIENTS ON THE SURVIVAL AND SUCCESSFUL METAMORPHOSIS OF  
*AEOLIDIELLA STEPHANIEAE* VELIGERS

BY

Bryan Tate  
B.S. Eckerd College, 2011

THESIS

Submitted to the University of New England  
in Partial Fulfillment of the  
Requirement for the Degree of

Master of Science

in

Biological Sciences

August, 2015

This thesis has been examined and approved.

*Jeri L Fox*

---

Thesis Director, Dr. Jeri Fox  
Associate Professor of Biology  
and Marine Science

*Stine Brown*

---

Dr. Stine Brown  
Chair and Professor of Biology

*Stephan Zeeman*

---

Dr. Stephan Zeeman  
Professor of Marine Sciences

*Anna Bass*

---

Dr. Anna Bass  
Associate Researcher and  
Visiting Assistant Lecturer

*AUGUST 25, 2015*

---

Date

© 2015 Bryan Tate  
All Rights Reserved

## ACKNOWLEDGEMENTS

I would like to take the time to thank my advisor, Dr. Jeri Fox, for her constant support these last couple years; her wisdom, patience, and insight into aquaculture and animal nutrition. I'm very grateful for the time she took to help me reach this point; even when she was pressed for time, she still made sure I had what I needed. I'm glad I had her as my primary professor at UNE, and I wish her the best in her future work with students in aquarium sciences.

I would also like to thank the other members of my thesis committee for their dedication, for taking the time to ensure my thesis work would be successful. Dr. Stine Brown for taking the time to brainstorm with me and help me explore new methods and angles for my final thesis. Dr. Anna Bass for her enthusiasm into the subjects of my thesis work and for her valuable advice and insight as I plotted my course through many thesis ideas. Dr. Stephan Zeeman for his help navigating statistics and data analysis, helping to ensure my analyses were sound.

Thanks are due to Rick Oellers at New England Aquarium Services, for providing space and resources for me to conduct my research. I also want to thank Rema Lee Weston for her unflinching aid in providing much-needed thesis supplies and technical advice. I'd like to thank Mike Esty for his time and technical help early on in my thesis project, as well as my parents for their encouragement from afar in Connecticut.

Many thanks to the University of New England and the UNE Biology/Marine Sciences Graduate Program for providing crucial funding as well as the teaching assistantship which allowed me to conduct my research and complete my thesis work.

And finally, many thanks to my wife and best friend, Kateri. She was a huge blessing and encouragement to me as I did troubleshooting and progressed through the long hours of animal culture and data collection. I'm all the richer for having her unconditional love and support.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
ABSTRACT.....	viii
<b>CHAPTER</b>	<b>PAGE</b>
INTRODUCTION.....	1
MATERIALS AND METHODS.....	6
Stock Animal Culture.....	6
Experimental Procedure.....	10
Chemical Treatments.....	11
RESULTS.....	13
DISCUSSION.....	25
Triiodothyronine.....	25
Glucose.....	26
Iodide.....	27
Alanine.....	28
Alanine: Survival.....	30
Alanine: Juveniles.....	31
Alanine: Veligers.....	31
Conclusions.....	32
LITERATURE CITED.....	34

## LIST OF TABLES

TABLE	PAGE
Table 1. Comparisons for larval <i>Aeolidiella stephanieae</i> survival after four days exposed to varying alanine concentrations.....	15
Table 2. Comparisons for successful survival and metamorphosis of <i>Aeolidiella stephanieae</i> larvae to juveniles after four days exposed to varying alanine concentrations.....	16
Table 3. Comparisons for survival of <i>Aeolidiella stephanieae</i> larvae without successful metamorphosis after four days exposed to varying alanine concentrations.....	17

## LIST OF FIGURES

FIGURE	PAGE
Figure 1. Sexually-mature <i>Aeolidiella stephanieae</i> (Valdés 2005) with egg mass, cultured in captivity 2015.....	8
Figure 2. Heated water system for culture of <i>Aeolidiella stephanieae</i> stock animals and incubation of eggs and egg masses for experiments procedures.....	9
Figure 3. Survival of <i>Aeolidiella stephanieae</i> larvae at three alanine concentrations after four days of exposure.....	18
Figure 4. Survival of <i>Aeolidiella stephanieae</i> larvae at three T <sub>3</sub> concentrations after four days of exposure.....	19
Figure 5. Survival of <i>Aeolidiella stephanieae</i> larvae at three glucose concentrations after four days of exposure.....	20
Figure 6. Survival of <i>Aeolidiella stephanieae</i> larvae at three iodide concentrations after four days of exposure.....	21
Figure 7. Survival and successful metamorphosis of <i>Aeolidiella stephanieae</i> larvae at three alanine concentrations after four days of exposure.....	22
Figure 8. Survival and unsuccessful/incomplete metamorphosis of <i>Aeolidiella stephanieae</i> larvae at three alanine concentrations after four days of exposure.....	23
Figure 9. Dose-response relationship for survival of <i>Aeolidiella stephanieae</i> larvae at three alanine concentrations after four days of exposure.....	24

## ABSTRACT

### THE USE OF TRIIODOTHYRONINE, GLUCOSE, ALANINE, AND IODIDE AS NUTRIENTS ON THE SURVIVAL AND SUCCESSFUL METAMORPHOSIS OF *AEOLIDIELLA STEPHANIEAE* VELIGERS

by

Bryan Tate

University of New England, August, 2015

During early life stages, nutrients are crucial to the proper development of larval marine invertebrates. Many such larvae are lecithotrophic and therefore do not actively feed; however, a large body of research has shown that lecithotrophic larvae take in dissolved organic material (DOM) including amino acids and sugars, contributing heavily to metabolic requirements. Another dissolved nutrient, iodine, is useful to marine invertebrates for the production of organic compounds. Some of these compounds (thyroid hormones, THs) are historically thought to be used almost exclusively by vertebrates, though studies have shown that THs are also useful to some marine invertebrates for developmental and homeostatic processes. *Aeolidiella stephanieae* is an aeolid nudibranch found exclusively in the Florida Keys. The purpose of this study was to determine whether dissolved nutrients are beneficial to *A. stephanieae* veligers during development. The effects of a thyroid hormone (triiodothyronine), a sugar (glucose), an amino acid (alanine), and a form of iodine (iodide) added to rearing water prior to metamorphosis were investigated. Survival and successful metamorphosis of *A. stephanieae* individuals at 15 days after oviposition were observed for each treatment. *A. stephanieae* veliger survival and successful metamorphosis were not negatively impacted by the addition of triiodothyronine, glucose, or iodide. Survival and successful

metamorphosis, as well as the presence of veligers at the 15-day mark were all negatively affected by the presence of alanine. Alanine concentrations had the most marked effect on survival, showing declines in survival proportionate to alanine concentration. Significant declines in survival were present even at the lowest alanine concentration. The rapid reproduction of heterotrophic bacteria in response to the abundance of free amino acids is the most likely culprit for mortality seen with alanine supplementation. It is unlikely that triiodothyronine, glucose, alanine, or iodide alone are beneficial to *A. stephanieae* veligers; however, it is still possible that these nutrients or a combination thereof at optimal concentrations may positively affect body mass and soft tissue growth. Future research involving lecithotrophic larvae paired with these and other nutrients would benefit from using species with larger eggs and larval stages for the purpose of protein and body mass analysis. In turn, this research could foster a better understanding of the importance of dissolved nutrients to lecithotrophic invertebrate larvae.

## INTRODUCTION

Aquaculture is a rapidly growing field in biology and finding new and improved ways to culture aquatic organisms is crucial for the success of both commercial and ornamental fisheries. The ability to maintain animals at egg and embryo stages is especially important due to high rates of mortality for larval vertebrates and invertebrates alike. The availability of nutrients plays a key role in survival and successful development. For many decades it was assumed that most non-mammalian, externally developing animals relied completely on maternally-invested energy reserves (Shilling and Bosch 1994). Numerous studies, however, have discovered that dissolved organic material plays a significant role in embryonic survival, development, and growth for numerous invertebrates (Manahan and Crisp 1982; Jaeckle and Manahan 1989; Shilling and Bosch 1994; Shilling et al. 1996).

Dissolved organic material (DOM) in relation to animal development usually refers to amino acids, fatty acids, and monosaccharides dissolved in the water column (Shilling and Bosch 1994). Numerous invertebrate species have the ability at the larval stages to acquire these nutrients from the surrounding water to help meet energetic and nutrient-bound requirements of development. Furthermore, many of these larvae are lecithotrophic (non-feeding), but do take up local dissolved nutrients. Lecithotrophic larvae of the gastropod *Haliotis rufescens* take up amino acids from seawater, providing significant energy for metabolism (Jaeckle and Manahan 1989). It is also likely that DOM provides energy equivalent to maternal oocyte investment up to the early juvenile

stage for this species (Shilling et al. 1996). Lecithotrophic larvae of two echinoderm species (*Odontaster validus* and *Acodontaster hodgsoni*) were shown to have the potential to meet metabolic requirements and increase in biomass prior to active feeding (Shilling and Bosch 1994). These biomass increases were related to phytoplankton blooms, which were responsible for increasing DOM (including free amino acids) (Shilling and Bosch 1994). Sugars in DOM are also considered to be significant sources of energy for larvae. Veligers of *Crassostrea gigas* (a bivalve) and *H. rufescens* have shown an affinity for taking up sugars, largely glucose and maltose, from seawater (Welborn and Manahan 1990). Similarly, soft tissue growth (in the form of proteins, measured as amino acids) of adult and juvenile manila clams (*Ruditapes philippinarum*) is promoted by the addition of sugar (glucose) to the seawater, indicating that amino acid production is encouraged by an abundance of available glucose (Uchida et al. 2010). It is likely that other invertebrate species may respond similarly to the addition of amino acids and sugars at larval stages.

Triiodothyronine ( $T_3$ ) is a thyroid hormone, the active form of thyroxine ( $T_4$ ). In humans and other vertebrates,  $T_3$  and  $T_4$  boost physiological processes; For example,  $T_3$  affects pituitary function by repressing production of thyroid stimulating hormones (TSH) and stimulating expression of growth hormones (GH), which affect tissue growth rates (Jameson and De Groot 2010; Melmed et al. 2011). Thyroid hormones (hereafter abbreviated as THs) affect marine species of fish and invertebrates in various ways. In particular, studies have focused on the function of THs in echinoderms, marine and freshwater fish, ascidians, and *Aplysia californica*. THs have been shown to cause simultaneous promotion of juvenile structures and early degeneration of larval structures

in numerous vertebrate and invertebrate species (Heyland et al. 2004; Heyland and Moroz 2006). For example, THs affect marine ascidian species at pre- and post-settlement stages of life by accelerating differentiation of body structures (Heyland and Moroz 2006; Heyland et al. 2006). The sea urchin, *Lytechinus variegatus*, synthesizes its own THs when exposed to iodine and treating these urchins with THs causes accelerated development leading to metamorphosis (Heyland et al. 2006). In one freshwater fish, *Brycon amazonicus*, application of exogenous THs resulted in longer, heavier individuals, with swim bladders becoming functional significantly sooner (Urbinati et al. 2008; Leonardo et al. 2013). In another freshwater fish, *Danio rerio*, inhibition of the thyroid hormone – thyroid receptor (TH-TR) axis yielded fatal deformities in fish larvae, including late development of the swim bladder, gastrointestinal tract, and cartilaginous structures (Liu and Chan 2002). Research concerning THs has been conducted on an opisthobranch gastropod, *Aplysia californica*. *A. californica* synthesizes the thyroid hormone triiodothyronine ( $T_3$ ) in the presence of iodine (Heyland et al. 2006). This  $T_3$  is used for partial sulfate and phosphate homeostasis, via protein synthesis tied to sulfate-sodium and sodium-phosphate symporters (Gerencser et al. 2002; Gerencser et al. 2003). It is likely that other invertebrates also use THs for various physiological functions.

THs have multiple iodine atoms in their structures (three in  $T_3$ , four in  $T_4$ ), and certain forms of iodine are known to be useful to marine algae and animals. Common iodine species found in seawater are as follows: Iodine ( $I_2$ ), Iodide ( $I^-$ ) and Iodate ( $IO_3^-$ ). Marine algae will often reduce iodate to iodide and typically use iodide as an antioxidant against reactive oxygen species (Wong et al. 2002; Kupper et al. 2008). Some marine invertebrates use iodine for manufacturing THs (as previously mentioned). In addition,

iodine is sequestered for production of other organoiodine compounds by other invertebrate species, such as gorgonians, antipatharians, and sponges (Goldberg et al. 1994; Kingsley et al. 2001; Dembitsky 2002). It is possible that iodide provided to other invertebrates may boost survival, even during the larval stages.

*Aeolidiella stephanieae* (Valdés 2005), previously known as *Berghia verrucicornis*, is an aeolid nudibranch found only in the Florida Keys, USA, and common in the ornamental aquarium trade (See Figure 1). *A. stephanieae* is well-known as a “pest-control” animal in marine aquaria because it is an obligate feeder on a hardy and prolific anemone, *Aiptasia pallida*. Life history and neuromuscular development are well documented for *A. stephanieae*, and laboratory culture has been highly successful (Carroll and Kempf 1990; Kristof and Klussmann-Kolb 2010). *A. stephanieae* individuals ingest nematocysts and zooxanthellae from *A. pallida* and store them in cerata; the zooxanthellae incorporation is similar to the chloroplast-sequestration by some sacoglossan sea slugs and may represent a similar, but short-lived “symbiosis” of about six days (Kempf 1991; Obermann et al. 2012). Recently it was confirmed that nematocysts incorporated into *A. stephanieae* cnidosacs for defense against predators are immature upon entering cnidosacs and are made functional by a rise in pH at those locations (Obermann et al. 2012).

Primary egg capsules are deposited in spirals, enclosed in a gelatinous secondary membrane. The permeability of the primary and secondary membranes to various nutrients has not been heavily investigated. Fertilized eggs of *A. stephanieae* develop into lecithotrophic (non-feeding) larvae; hatching from primary egg capsules as free-swimming veligers approximately 11-12 days after oviposition (DAO). After 1-3 days the

veligers settle and metamorphose (Carroll and Kempf 1990). The recently metamorphosed juveniles begin feeding on *A. pallida* about 15 DAO, or approximately two days following metamorphosis (approximately 16 to 17 DAO) (Carroll and Kempf 1990). It is possible that these animals can take in dissolved nutrients at non-feeding stages much like other marine invertebrates species with lecithotrophic larval stages.

In this study, the effects of T<sub>3</sub>, glucose, alanine and iodide on the successful survival and metamorphosis of *A. stephanieae* larvae were investigated using closed-system larval incubation. It was hypothesized that at optimal concentrations, especially as concentration levels for each treatment increased from low to high, survival and successful metamorphosis percentages would increase accordingly. T<sub>3</sub>, glucose, and alanine were chosen for their positive effects on invertebrate larval development in past studies, while iodide was chosen for its potentially antioxidizing properties and/or role in producing THs and organoiodine compounds.

## MATERIALS AND METHODS

An adaptation of a closed-system larvae incubation assay was developed to assess survival in *Aeolidiella stephanieae* under the aforementioned chemical treatments (T<sub>3</sub>, glucose, alanine, and iodide) using a range of concentrations (Figure 2). Experiments were conducted during the winter and spring of 2015 using eggs collected from F<sub>2</sub> generation stock animals.

### Stock animal culture

Six *Aeolidiella stephanieae* individuals were purchased from Inland Aquatics (inlandaquatics.com) and maintained in bowls of artificial seawater at 22-24°C within a heated water system (see Figure 2). Artificial seawater at 30ppt salinity was prepared by mixing Instant Ocean sea salt with deionized (DI) water. Artificial seawater for *A. stephanieae* culture was stored in plastic carboys and allowed to age at least 24 hours prior to use in animal upkeep. Bowls contained approximately 600mL of seawater and were partially covered with plastic wrap to reduce evaporation. Fifty percent water changes were performed every two days. Sexually-mature F<sub>1</sub> individuals were obtained about 65 days following acquisition of parent slugs, and sexually-mature F<sub>2</sub> individuals were obtained 55-60 days after that. Experimental replicates were accomplished using eggs produced by F<sub>2</sub> individuals.

Adult *A. stephanieae* were fed every two days using clipped oral disks from *Aiptasia pallida* stock animals obtained from local marine aquaria. *A. pallida* were maintained using artificial seawater and fed with flake fish food every two days to

promote overall growth and recovery following oral disk harvesting. *A. pallida* were maintained under 12 hour day/night light cycles.

The sterile filtered seawater (SFS) used for experiments was filtered with Steritech 1.3 $\mu$ m pore glass filters and autoclaved. Final salinity was corrected using sterile DI water.



Figure 1. Sexually-mature *Aeolidiella stephanieae* (Valdés 2005) with egg mass, cultured in captivity 2015.

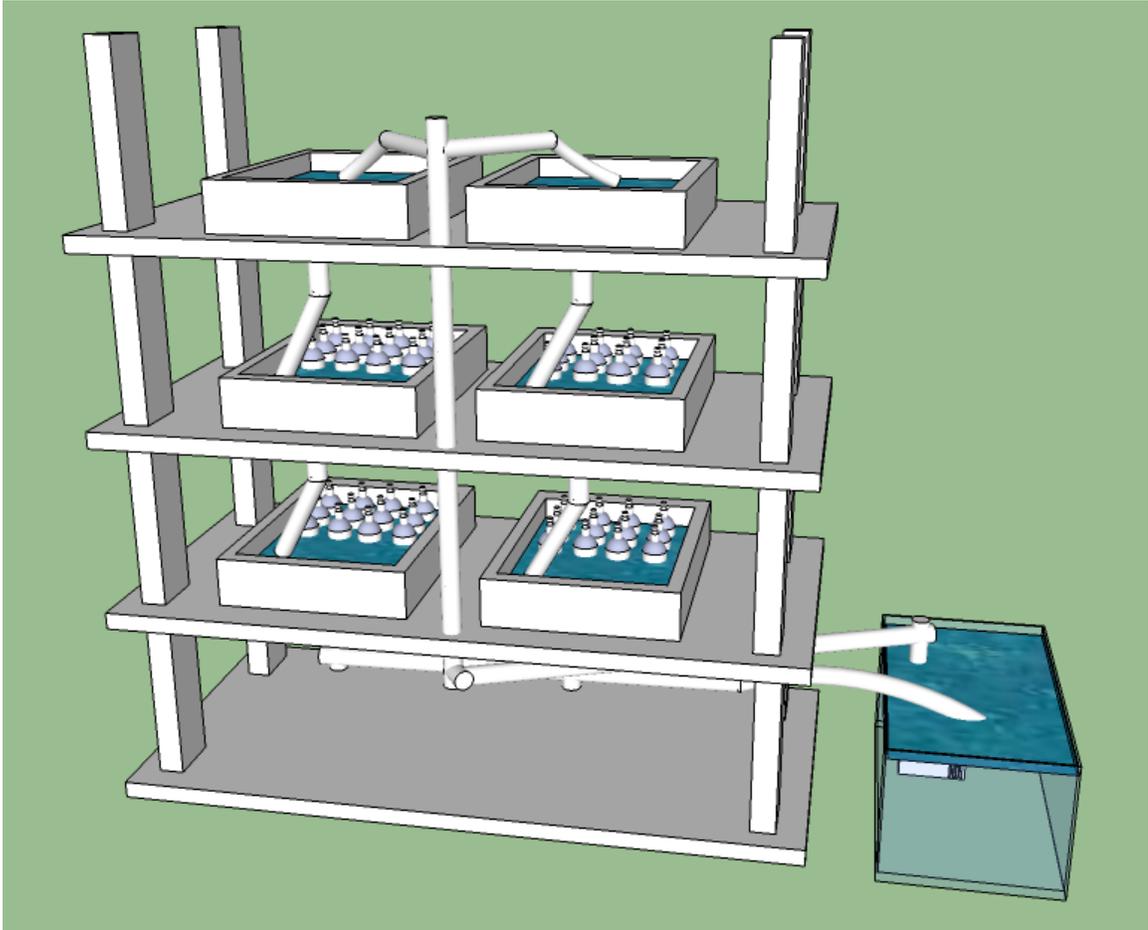


Figure 2. Heated recirculating water system for culture of *Aeolidiella stephanieae* stock animals and incubation of eggs and egg masses for experimental procedures. The heater for this system is located in the sump (bottom-right).

## Experimental procedure

Bowls containing approximately ten adult F<sub>2</sub> *Aeolidiella stephanieae* and 600mL of artificial seawater were checked every 8 hours for new egg masses; only egg masses containing >100 fertilized eggs at the one to four-cell stages were retained for subsequent culture. Satisfactory egg masses were then placed into sterile glass 300mL BOD (Biochemical Oxygen Demand) bottles containing 150mL of SFS (see stock animal culture for details). Fifty percent water changes were performed daily with sterile filtered seawater, and bottles were maintained at 22-24°C in the heated water system, separate from stock animals.

The maintained egg masses were examined at eleven DAO. If viable, the egg masses were opened using a sterile ceramic blade and divided using a sterile pipette into four sets of 25 veligers. Each set of 25 veligers was placed into a sterile BOD bottle containing 125mL of the chosen treatment concentration (control, low, middle, and high). Water was not changed in the experimental treatment bottles, however, the tops were opened daily for a brief time to equalize air pressure in the bottles. The number of individuals that had survived and the number that had successfully metamorphosed were determined at 15 DAO. Each bottle was shaken for 15 seconds and then rinsed into a beaker. This was repeated for each bottle two additional times. The contents of the beaker were then allowed to settle for approximately 30 seconds, and surviving larvae were counted with the aid of a dissecting scope. The number of successfully metamorphosed juveniles versus the number of veligers or larvae in transition to juveniles were determined and recorded. 16 egg masses were processed in this way for each treatment,

yielding 16 replicates for each treatment concentration and controls. A total of 64 egg masses were successfully processed over the course of this experiment.

The 11-day timepoint was chosen as the time for dividing larvae into the different concentration levels due to larvae having consistently reached a mobile veliger stage but not yet progressing beyond that point (Carroll and Kempf, 1990; pers. obs.). The 15-day timepoint was chosen because larvae begin to feed on *A. pallida* approximately 3-5 days after metamorphosis (Carroll and Kempf 1990). This ensured that juveniles would survive for counting without requiring food because the addition of *A. pallida* to treatment bottles could introduce confounding factors and possibly increase mortality.

#### Chemical treatments

Four different chemical treatments were used in this study. They were as follows: T<sub>3</sub>, glucose, alanine, and iodide. Each chemical treatment had high, middle, and low concentrations.

The sodium salt of 3,3',5-Triiodo-L-thyronine (Sigma T6397) was dissolved in 1N NaOH at a ratio of 1mg T<sub>3</sub> to 1mL NaOH solution was mixed with sterile filtered seawater (SFS, see stock animal culture) to make an active stock solution of 1mg/L T<sub>3</sub>. Aliquots from this stock were used to make two additional concentrations: 0.1mg/L and 0.01mg/L T<sub>3</sub>. Solutions were stored at 4-6°C and kept no longer than 48 hours.

D-(+)-Glucose (Sigma G8270) was dissolved in SFS to make a stock solution at a ratio of 100mg glucose per liter. Aliquots from this stock were used to make two additional concentrations: 10mg/L and 1mg/L. Solutions were stored at 4-6°C and kept no longer than 24 hours.

L-Alanine (Sigma 05130) was dissolved in SFS to make a stock solution of 10mg alanine per liter. Aliquots from this stock were used to make two additional concentrations: 5mg/L and 2.5mg/L. Solutions were stored at 4-6°C and kept no longer than 24 hours.

Sodium iodide (Sigma 409286) was dissolved in SFS to make a stock solution of 10mg iodide per liter. Aliquots were taken from this stock to make two additional concentrations: 1mg/L and 0.5mg/L. Factoring in the ambient iodide concentration of 0.206mg/L in the SFS at 30ppt, the true iodide concentrations were 0.206, 0.706, 1.206, and 10.206mg/L. Solutions were stored at 4-6°C and kept no longer than 24 hours.

One-way ANOVAs were performed on the data for each type of treatment (T<sub>3</sub>, glucose, alanine, or iodide) for juveniles, veligers, and total surviving larvae. A Tukey's HSD was also performed on the data for each ANOVA to check for significance.

## RESULTS

*Aeolidiella stephanieae* larval survival and successful development to metamorphosis were not positively affected by the experimental conditions. Overall survival of *A. stephanieae* larvae was not significantly affected compared to controls for T<sub>3</sub>, glucose, or iodide treatments (One-way ANOVAs, P = 0.464, 0.366, and 0.389 respectively, see Figures 4, 5, and 6). Successful survival and metamorphosis to juveniles was not significantly affected compared to controls for T<sub>3</sub>, glucose, or iodide treatments (One-way ANOVAs, P = 0.238, 0.45, and 0.785 respectively). Similar results were seen for surviving larvae still in the veliger stage after the four days (15-day timepoint) for T<sub>3</sub>, glucose, and iodide treatments (One-way ANOVAs, P = 0.196, 0.871, and 0.927 respectively). Tukey's HSD analyses confirmed that there were no significant differences between any means for these treatments and concentrations.

Survival of *A. stephanieae* larvae in alanine treatments showed significantly reduced results for total survival, surviving juveniles, and veligers present after 15 days (P = < 0.001 for each). Delving further, Tukey's HSD analyses revealed significant interactions between means for all three independent variables.

Total survival of *A. stephanieae* larvae in alanine treatments was significantly different for nearly every possible comparison of means (Table 1, Figure 3). There were significant differences between the control and each alanine concentration; only the comparison between the 5 and 10mg/L alanine concentrations was not significant (P = 0.551, see Table 1). A figure further illustrating the consistency of the dose-response

relationship between alanine concentration and average larval survival was constructed from the mean data (Figure 9, Linear Regression  $R^2 = 0.948$ ).

Successful survival and metamorphosis to juveniles in alanine treatments was significantly reduced with increasing alanine concentration. Survival was significantly different between the control and the two higher alanine concentrations (5 and 10mg/L,  $P = <0.001$  and  $<0.001$ , respectively), as well between 2.5 and 10mg/L alanine concentrations ( $P = <0.001$ ) (See Table 2 and Figure 7). The other three comparisons were not significantly different (Table 2).

Survival but incomplete development to the mobile juvenile stage (development not surpassing the veliger stage) was significantly different between the control and each alanine concentration spanning 2.5, 5, and 10mg/L ( $P = 0.00289$ ,  $<0.001$ , and  $<0.001$  respectively) (See Table 3 and Figure 8). Comparisons between non-control concentrations did not yield any significant differences (Table 3).

Table 1. Comparisons for larval *Aeolidiella stephanieae* survival after four days exposed to varying alanine concentrations. A total of 16 replicates were collected for each alanine concentration, with counts represented out of 25 initial veligers. Concentrations include control (0mg/L), 2.5, 5, and 10mg/L. P-values (Tukey's HSD) that varied significantly for individual comparisons have been italicized and bolded.

	0 mg/L	2.5mg/L	5mg/L	10mg/L
0mg/L	-	<b><i>0.0115</i></b>	<b><i>&lt;0.001</i></b>	<b><i>&lt;0.001</i></b>
2.5mg/L		-	<b><i>0.0247</i></b>	<b><i>&lt;0.001</i></b>
5mg/L			-	0.0551
10mg/L				-

Table 2. Comparisons for successful survival and metamorphosis of *Aeolidiella stephanieae* larvae to juveniles after four days exposed to varying alanine concentrations. A total of 16 replicates were collected for each alanine concentration, with counts represented out of 25 initial veligers. Concentrations include control (0mg/L), 2.5, 5, and 10mg/L. P-values (Tukey's HSD) that varied significantly for individual comparisons have been italicized and bolded.

	0 mg/L	2.5mg/L	5mg/L	10mg/L
0mg/L	-	0.275	<b>&lt;0.001</b>	<b>&lt;0.001</b>
2.5mg/L		-	0.0734	<b>&lt;0.001</b>
5mg/L			-	0.058
10mg/L				-

Table 3. Comparisons for survival of *Aeolidiella stephanieae* larvae without successful metamorphosis after four days exposed to varying alanine concentrations. 16 replicates were collected for each alanine concentration, with counts represented out of 25 initial veligers. Concentrations include control (0mg/L), 2.5, 5, and 10mg/L. P-values (Tukey's HSD) that varied significantly for individual comparisons have been italicized and bolded.

	0 mg/L	2.5mg/L	5mg/L	10mg/L
0mg/L	-	<b><i>0.00289</i></b>	<b><i>&lt;0.001</i></b>	<b><i>&lt;0.001</i></b>
2.5mg/L		-	0.849	0.977
5mg/L			-	0.977
10mg/L				-

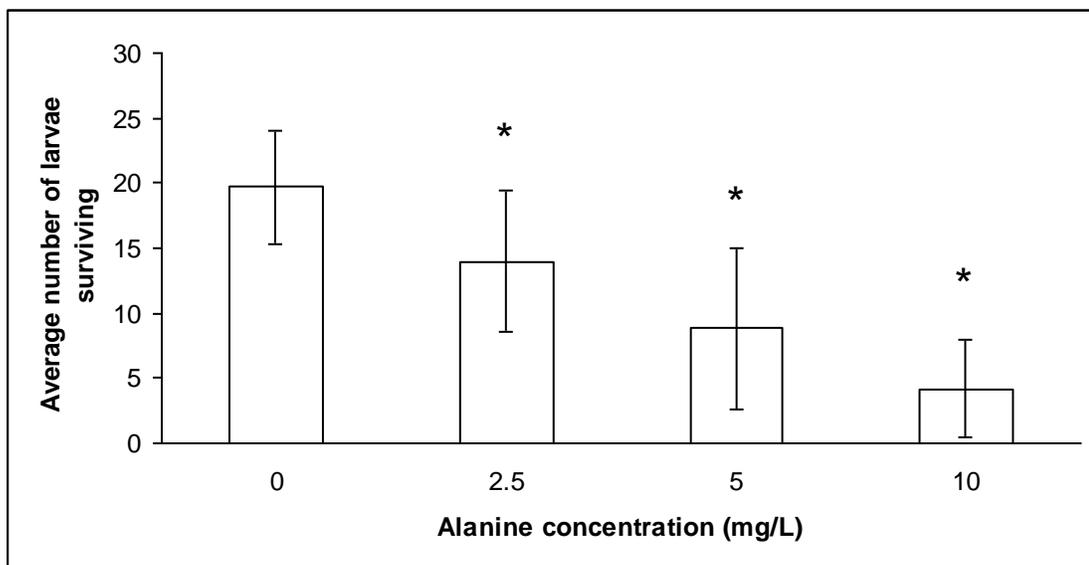


Figure 3. Survival of *Aeolidiella stephanieae* larvae at three alanine concentrations after four days of exposure. The x-axis indicates the concentrations of alanine used, with the control (0) for reference. The y-axis shows the average number of *Aeolidiella stephanieae* individuals surviving after four days. Alanine concentrations were 0, 2.5, 5, and 10mg/L. Twenty-five veligers were used for each replicate, and surviving individuals were counted after the four days. A total of 16 replicates were collected for each concentration. The error bars represent the standard deviation for each column. Mean survival significantly different from the control is marked with an asterisk.

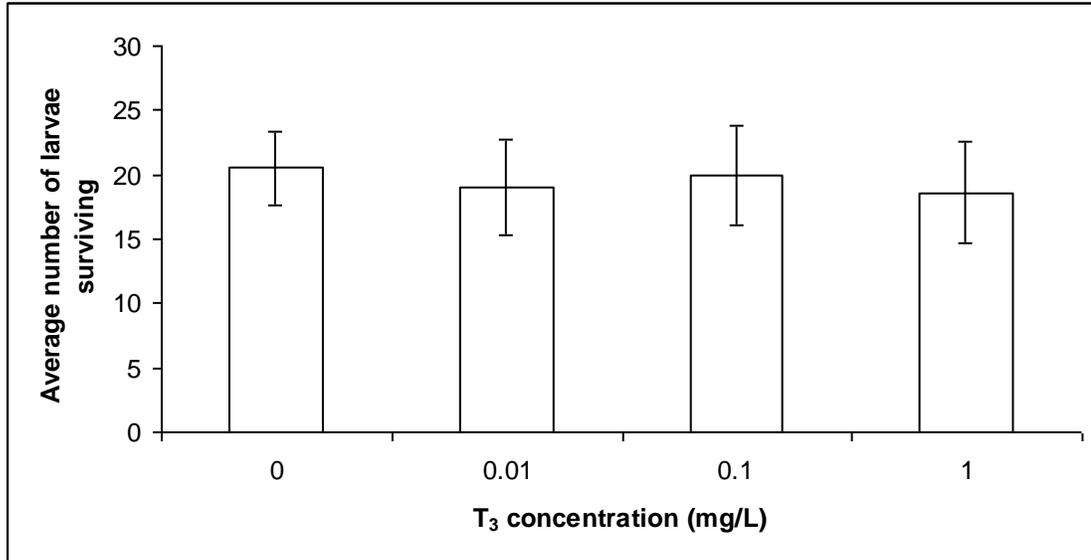


Figure 4. Survival of *Aeolidiella stephanieae* larvae at three T<sub>3</sub> concentrations after four days of exposure. The x-axis indicates the concentrations of T<sub>3</sub> used, with the control (0) for reference. The y-axis shows the average number of *Aeolidiella stephanieae* individuals surviving after four days. T<sub>3</sub> concentrations were 0, 0.01, 0.1, and 1mg/L. Twenty-five veligers were used for each replicate, and surviving individuals were counted after the four days. A total of 16 replicates were collected for each concentration. The error bars represent the standard deviation for each column.

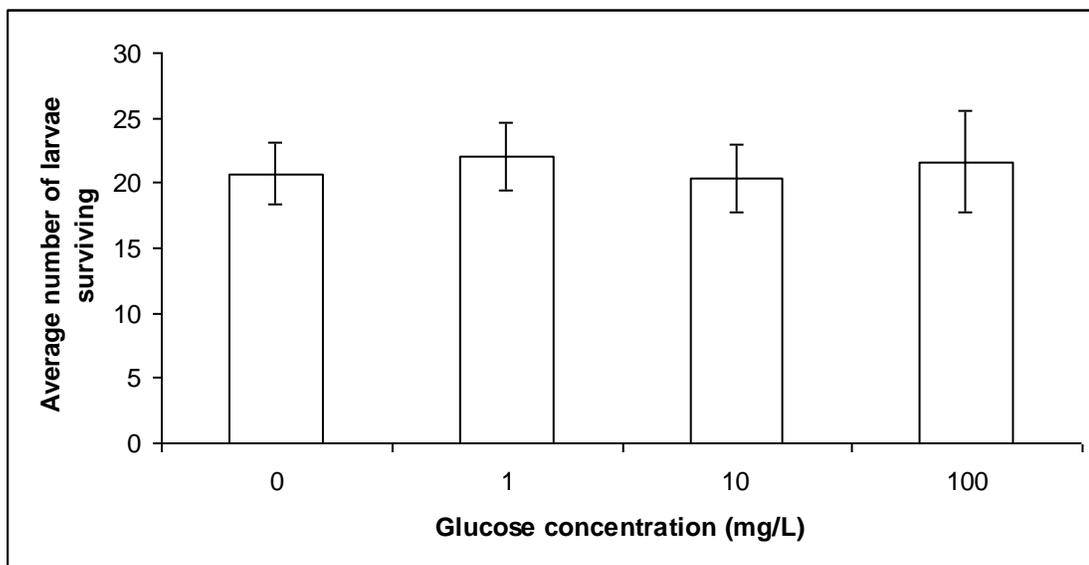


Figure 5. Survival of *Aeolidiella stephanieae* larvae at three glucose concentrations after four days of exposure. The x-axis indicates the concentrations of glucose used, with the control (0) for reference. The y-axis shows the average number of *Aeolidiella stephanieae* individuals surviving after four days. Glucose concentrations were 0, 1, 10, and 100mg/L. Twenty-five veligers were used for each replicate, and surviving individuals were counted after the four days. A total of 16 replicates were collected for each concentration. The error bars represent the standard deviation for each column.

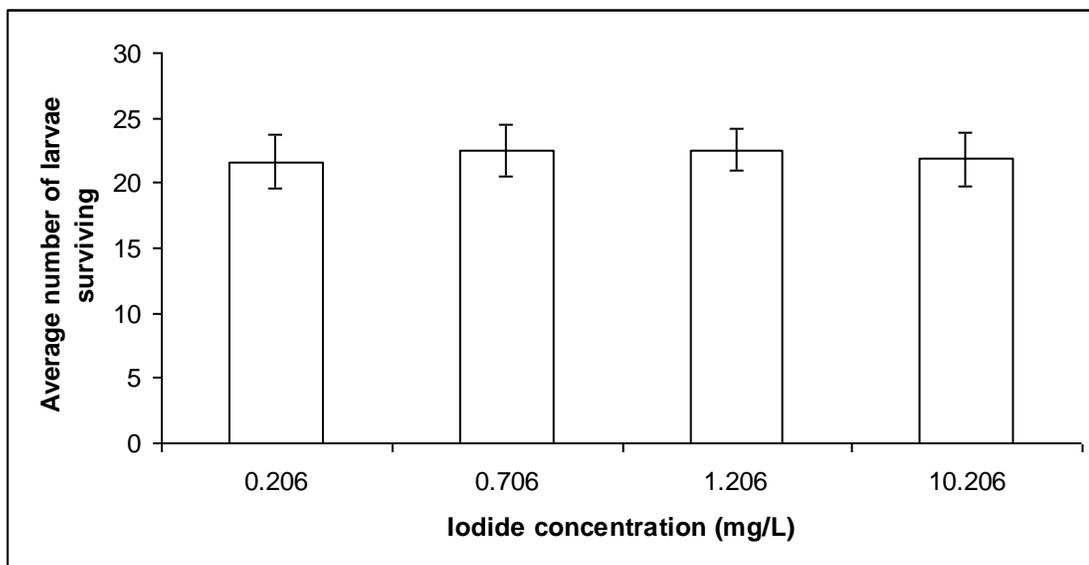


Figure 6. Survival of *Aeolidiella stephanieae* larvae at three iodide concentrations after four days of exposure. The x-axis indicates the concentrations of iodide used, with the control (0.206mg/L) for reference. The y-axis shows the average number of *A. stephanieae* individuals surviving after four days. Iodide concentrations were 0.206, 0.706, 1.206, and 10.206mg/L. Twenty-five veligers were used for each replicate, and surviving individuals were counted after the four days. A total of 16 replicates were collected for each concentration. The error bars represent the standard deviation for each column.

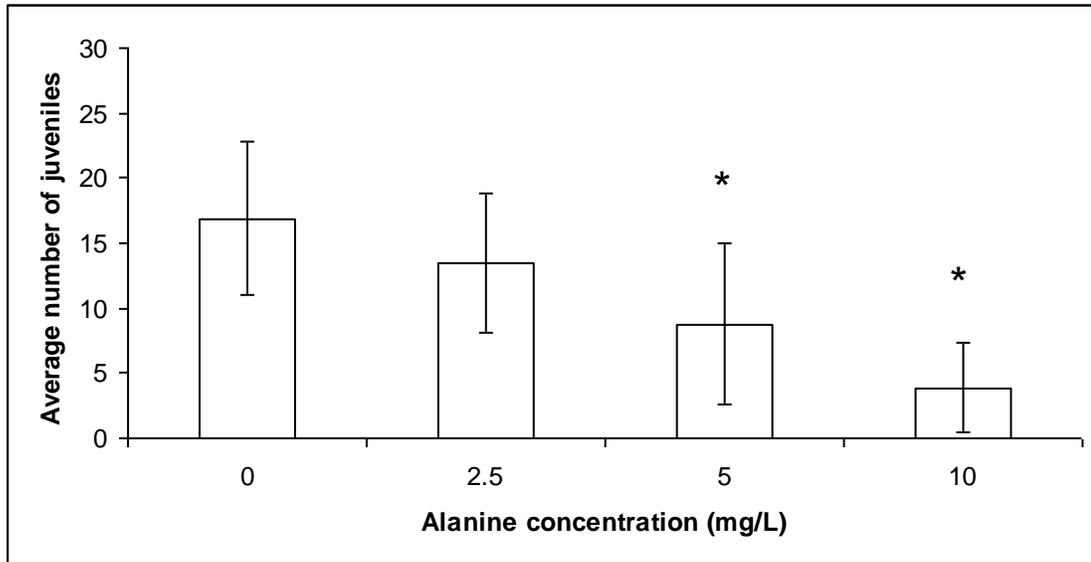


Figure 7. Survival and successful metamorphosis of *Aeolidiella stephanieae* larvae at three alanine concentrations after four days of exposure. The x-axis indicates the concentrations of alanine used, with the control (0) for reference. The y-axis shows the average number of *Aeolidiella stephanieae* individuals that survived and metamorphosed into juveniles after four days. Alanine concentrations were 0, 2.5, 5, and 10mg/L. 25 veligers were used for each replicate, and surviving juveniles were counted after the four days. A total of 16 replicates were collected for each concentration. The error bars represent the standard deviation for each column. Mean survival significantly different from the control is marked with an asterisk.

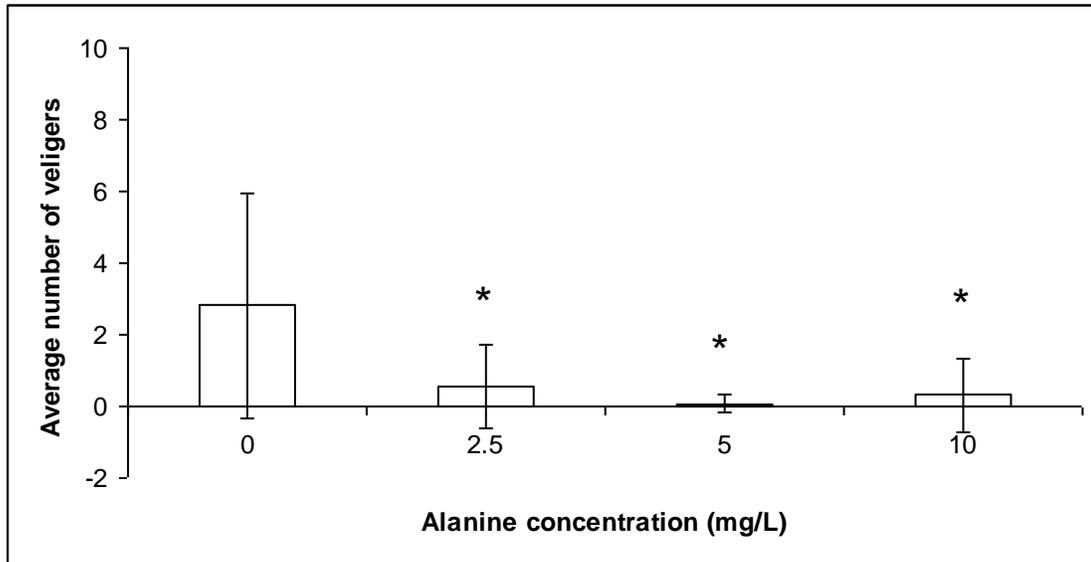


Figure 8. Survival and unsuccessful/incomplete metamorphosis of *Aeolidiella stephanieae* larvae at three alanine concentrations after four days of exposure. The x-axis indicates the concentrations of alanine used, with the control (0) for reference. The y-axis shows the average number of *Aeolidiella stephanieae* individuals that survived and remained veligers and/or had not yet completed metamorphosis after four days. Alanine concentrations were 0, 2.5, 5, and 10mg/L. 25 veligers were used for each replicate, and surviving veligers/larvae in transition were counted after the four days. A total of 16 replicates were collected for each concentration. The error bars represent the standard deviation for each column. Mean survival significantly different from the control is marked with an asterisk.

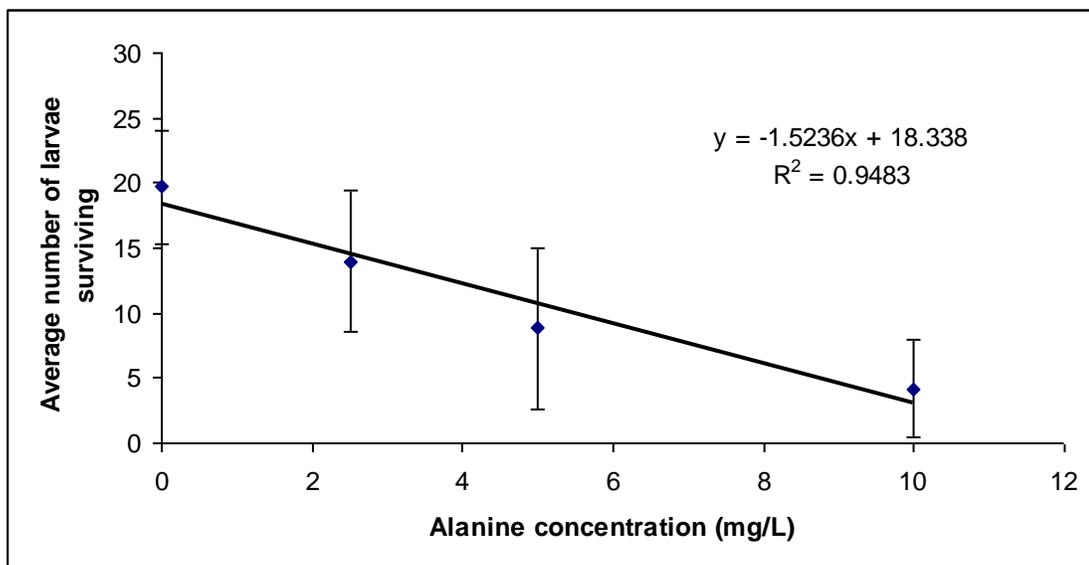


Figure 9. Dose-response relationship for survival of *Aeolidiella stephanieae* larvae at three alanine concentrations after four days of exposure. The x-axis indicates the concentrations of alanine used, with the control (0) for reference. The y-axis shows the average number of *Aeolidiella stephanieae* individuals surviving after four days. Alanine concentrations were 0, 2.5, 5, and 10mg/L. 25 veligers were used for each replicate, and surviving individuals were counted after the four days. A total of 16 replicates were collected for each concentration. A linear regression was fitted to the plot ( $R^2 = 0.9483$ ) to illustrate the negative relationship between alanine concentration and larval survival for *Aeolidiella stephanieae*. The error bars represent the standard deviation for each column.

## DISCUSSION

It was hypothesized that *Aeolidiella stephanieae* larvae would respond to various potentially relevant nutrients with increased survival and a greater proportion of successful metamorphosis. Our results do not support a positive response to the nutrient supplementation used here for *A. stephanieae*. Indeed, for the alanine treatments, a significant decrease in survival was seen at all concentrations compared with controls (Figures 3 and 9). A significant rise in total larval survival, successful metamorphosis to juveniles, or a decrease in veliger presence after the four days was not seen in this study.

### Triiodothyronine

T<sub>3</sub> is an important hormone, primarily for vertebrates, but certain invertebrates can also make use of T<sub>3</sub> and other thyroid hormones. Previous research has demonstrated that an opisthobranch gastropod, *Aplysia californica*, synthesizes T<sub>3</sub> in the presence of iodine and uses it in sulfate and phosphate homeostasis (Gerencser et al. 2002; Gerencser et al. 2003; Heyland et al. 2006). T<sub>3</sub> was chosen as a nutrient treatment to investigate the potentially positive effects of T<sub>3</sub> on another opisthobranch gastropod, *Aeolidiella stephanieae*, using survival and successful metamorphosis as the response variables. The chosen concentrations (0.01, 0.1, and 1mg/L) were based upon previous literature examining the effects with a fish species, *Brycon amazonicus* (Urbinati et al. 2008; Leonardo et al. 2013). The highest concentration was chosen to determine whether an overabundance of T<sub>3</sub> might also be beneficial or detrimental to the larvae.

Survival of *A. stephanieae* larvae did not differ from the control for any T<sub>3</sub> concentrations. The presence of T<sub>3</sub> did not significantly affect survival of larvae at any level, indicating T<sub>3</sub> does not promote survival of *A. stephanieae* larvae. It must be stated that the effects of T<sub>3</sub> may not be easily detected when looking solely at survival. Protein assays may be an alternative approach, but the extremely low mass of larvae makes this type of assay difficult. The presence of T<sub>3</sub> also did not significantly alter the presence of veligers or juveniles after the four-day time period. The presence of T<sub>3</sub> was predicted to promote larval health and positively affect development and successful metamorphosis such that fewer veligers and more juveniles would be present after the four days. This effect was not observed, as the numbers of veligers and juveniles did not vary significantly from controls; therefore, T<sub>3</sub> did not positively or negatively influence development or metamorphosis. We can conclude that T<sub>3</sub> is not an effective nutrient for *A. stephanieae* larval survival or metamorphosis.

### Glucose

Glucose and other simple sugars have been examined as potent nutrients for invertebrate larvae and adult sessile invertebrates alike. Simple sugars have been shown to provide energy to veliger larvae of *Crassostrea gigas* and *Haliotis rufescens*, and to increase soft tissue growth for *Ruditapes philippinarum* juveniles and adults (Welborn and Manahan 1990; Uchida et al. 2010). The concentrations (1, 10, and 100mg/L) were chosen based upon previous literature with *R. philippinarum*, where these same concentrations were used to investigate subsequent soft tissue growth, which increased at 10mg/L and 100mg/L (Uchida et al. 2010).

The addition of sugars to the rearing water of adult and juvenile marine invertebrates can present a challenge to survival by increasing microbial growth, thus increasing water turbidity (Uchida et al. 2010). We sought to eliminate this factor by using sterile artificial seawater, and overall survival was not significantly reduced by the presence of sugars during the 4-day period. In addition, overall survival was not impacted significantly by the addition of glucose. The presence of *Aeolidiella stephanieae* veligers or juveniles at the terminal timepoint was also not significantly affected. We predicted that glucose would provide extra energy to promote efficient development and successful metamorphosis. Using sterile seawater prevented significant larval mortality, however an overabundance of glucose did not significantly affect the process or overall speed of larval development and metamorphosis. Using sterile artificial seawater in future nutrient studies may still reduce or prevent larval mortality and enable the detection of positive nutrient effects on marine larvae.

### Iodide

Iodine is useful to all marine organisms because vertebrates and invertebrates can use iodine for synthesizing various organic compounds (including THs), while marine algae can use one form, iodide, as an antioxidizing agent (Goldberg et al. 1994; Kingsley et al. 2001; Dembitsky 2002; Kupper et al. 2008). It is unknown whether an abundance of iodide may also provide benefits to *Aeolidiella stephanieae* larvae in the form of increased survival and/or successful metamorphosis. The true iodide concentrations utilized in this study were 0.206, 0.706, 1.206, and 10.206mg/L, based on a variety of factors. First, natural seawater should contain about  $7.62 \times 10^{-3}$ mg/L of iodide, while the salt mix used for the SFS (Instant Ocean) produces saltwater with iodide concentrations

of ~0.24mg/L at 35ppt (or ~0.206mg/L at 30ppt) (Tian and Nicolas 1995; United Pet Group, personal communication March 2015). Due to the presence of an already high concentration of iodide in the SFS used for this study, we determined adding iodide above Instant Ocean levels would be optimal for gauging the effects of what would be considered an abundance of iodide.

Survival of *A. stephanieae* larvae was not significantly affected by any of the iodide levels used in this study. It is possible that the levels of iodide already present in the sterile filtered seawater are more than sufficient to boost survival and mask the effects of adding excess iodide, however this can not be determined based on our study design. Survival was not negatively impacted by such high iodide concentrations (0.706, 1.206, and 10.206mg/L). The presence of veligers and/or juveniles at the terminal timepoint was also not significantly affected by the addition of iodide. This finding may be due to the level of iodide already present, and indeed 2.4, 4.8, and 48.5-fold additions of iodide above Instant Ocean artificial seawater concentrations cannot be considered anything but complete saturation. It is possible that the control seawater contained sufficient iodide for synthesizing organoiodine compounds and/or for use in essential biological processes, and the added abundance of iodide was unnecessary. The exact effects of an abundance of iodide on *A. stephanieae* larvae are still unknown, but no negative impact was observed at these levels.

#### Alanine

Alanine is an amino acid commonly found in natural seawater, as a component of DOM. Lecithotrophic larvae of *Haliotus rufescens* are known to take up alanine and other amino acids from the surrounding water to provide energy for metabolism; up to 70% and

39% of metabolic rates may be accounted for by amino acid uptake for 2 and 3 day-old veliger larvae respectively (Jaeckle and Manahan 1989). Furthermore, research with *H. rufescens* revealed that the capacity for larvae to uptake dissolved alanine from seawater increased 3-fold during metamorphosis; this increase in transport capacity of amino acids in relation to body size increase and/or metamorphosis is a phenomenon seen in many other marine invertebrate species (Shilling et al. 1996). The effects of alanine concentrations on lecithotrophic *Aeolidiella stephanieae* larvae were examined in regard to survival and the presence of veligers and/or juveniles at the terminal timepoint.

Dissolved amino acid levels can have a wide range in natural seawater, from 0.2 $\mu$ M to 17 $\mu$ M depending on location; 0.2  $\mu$ M is low for offshore waters, while 17  $\mu$ M is high for interstitial waters from coastal sediments (Manahan and Crisp 1982). For comparison, the alanine concentrations chosen for these tests (2.5, 5, and 10mg/L) were based upon work done in 1979 with a sea star, *Asterias rubens*, where 90 $\mu$ M (~8mg/L) amino acid levels showed increased body mass and high mortality while concentrations below 30 $\mu$ M (~2.67mg/L) did not show an increase of body mass for adult sea stars (Siebers 1979). These concentrations were also chosen in order to determine whether similar results regarding survival and mortality held true for *A. stephanieae* larvae during the time period overlapping metamorphosis, using similar high and low concentrations, with a complementary middle concentration. The concentrations listed above are extremely high compared to ambient levels, in order to account for effects manifested with an abundance of amino acids (Uchida et al. 2010).

## Alanine: Survival

Overall survival of *Aeolidiella stephanieae* larvae in alanine treatments declined significantly compared to controls as concentration increased (Figure 3, Table 1). Additionally, nearly every concentration level varied significantly with every other concentration level (Table 1). The greatest mortality was seen at the highest concentration (10mg/L), similar to mortality seen at a comparable concentration with *Asterias rubens* (Siebers 1979). Although mortality was lowest at the 2.5mg/L alanine concentration, it was also significantly more than control conditions, indicating that this concentration was high enough to affect survival, but not so high as to increase larval mortality (as was seen at 10mg/L). It is likely that the optimal concentration for survival of larvae lies below 2.5mg/L of alanine, and mortality may be due to the growth of heterotrophic bacteria within the time frame.

Heterotrophic bacteria will preferentially sequester amino acids over other forms of dissolved organic nitrogen, promoting rapid reproduction and consumption of larval tissues from weakened or dying larvae (Middelburg and Nieuwenhuize 2000). It is also worth noting that although glucose concentrations were extremely high, mortality due to the presence of abundant heterotrophic bacteria was not an issue, indicating that sugar is not as limiting for bacterial growth as available nitrogen, especially amino acids. Reduction of water quality via heterotrophic bacterial growth therefore seems to be the most likely culprit for larval mortality concerning alanine supplementation, and may be due to the addition of approximately 2mL of rearing water to each experimental bottle accompanying the initial veligers. To illustrate the relative predictability of larval survival, a simple linear regression was applied to the data spanning from 0 to 10mg/L

(Figure 9). For future studies involving invertebrate larvae and amino acids, amino acids concentrations below 2.5mg/L would be recommended. Survival should not vary significantly with controls at certain levels below this concentration, proving useful for studies examining other factors such as body weight and energy requirements.

#### Alanine: Juveniles

The presence of juveniles at the terminal timepoint was significantly reduced for the two highest alanine concentrations when compared to controls (5 and 10mg/L) (Figure 7, Table 2). The presence of juveniles is most-directly related to overall survival, as veliger presence after the terminal timepoint was minor compared to juvenile presence (Figure 7 and 8). Presence of juveniles (i.e. survival and successful metamorphosis to juveniles) gradually decreased as alanine concentration increased, similar to observations on overall survival. The two highest concentrations (5 and 10mg/L) were significantly different from controls, indicating significant mortality at these levels (Figure 7). Even though not all alanine concentrations yielded results that differed significantly from controls, the effects of the added alanine on water quality are still seen at the two higher concentrations.

#### Alanine: Veligers

The presence of veligers was significantly reduced at all alanine concentrations compared to controls (Table 3, Figure 8). Mortality of veliger stages increased likely due to an increase in heterotrophic bacteria, facilitated by the presence of free alanine. Consequently, it was anticipated that the presence of veligers after the terminal timepoint would correspond to the water quality, as veliger presence was much higher in controls

than for any alanine concentration (Figure 8). Survival of veligers may not be significantly affected at lower alanine levels (<2.5mg/L), but this bears further investigation. It is very likely that veliger-stage larvae are more susceptible to reduced water quality in general, as illustrated by significant mortality estimates at all alanine levels (Figure 8).

### Conclusions

The purpose of this study was to determine whether a variety of nutrients could positively affect survival of *Aeolidiella stephanieae* larvae and successful metamorphosis, with the hope of extrapolating these results to the culture of other marine larvae. Ultimately, we hypothesize that heterotrophic bacterial growth may pose a major problem to nutrient studies when certain limiting nutrients (amino acids for example) are provided in relatively large quantities. Even though SFS was used for all experiments, the addition of ~2mL of rearing water containing veligers to each experimental bottle is the most likely source of bacterial contamination. Though bacterial presence was not quantified in this study, it is possible that higher concentrations of alanine were better for promoting bacterial growth. The optimal concentration for alanine and/or general amino acid addition may lie below 2.5mg/L. T<sub>3</sub>, glucose, and iodide did not affect survival of *A. stephanieae* larvae in any way, and it is possible that these nutrients are not subsumed from the surrounding seawater during lecithotrophic larval development. This scenario is unlikely as the lecithotrophic larvae of many species are known to acquire nutrients from surrounding seawater that contribute significantly to metabolism and growth (Jaeckle and Manahan 1989; Shilling and Bosch 1994; Shilling et al. 1996). Bacterial growth does not appear to be a noteworthy factor when these nutrients are involved, however, experiments

using non-sterile media would be necessary to determine whether this is true in normal marine culture conditions. The fairly small size of *A. stephanieae* larvae prevented protein assays from being a useful indicator of nutrient uptake and discouraged nutrient application via microinjector. For future work involving nutrient uptake by *A. stephanieae* larvae, there are a few approaches to consider. Radiolabeling of nutrients such as amino acids and sugars should provide insight into whether these nutrients are uptaken by the veligers. It is also possible that a combination of nutrients may yield significant results where single nutrient treatments may not, combining for example a sugar and amino acid, or a sugar with a thyroid hormone.

Future studies into lecithotrophic larval nutrition may benefit from using protein analysis for marine invertebrates with larger eggs and larvae. Regarding whether DOM is beneficial for early development, eliminating DOM from natural seawater used in testing may demonstrate the degree to which DOM is important to lecithotrophic larvae. Natural seawater is also low in iodine, and may prove useful for determining whether an abundance of iodine is useful or even crucial at early stages. Additionally, exploring the effects of parental investment in egg masses related to stock animal size and/or diet may be helpful. Parental investment was largely eliminated as a factor in this study due to the use of paired replicates across treatment concentrations; however, taking into account stock animal and live food nutrition may also be significant in regard to offspring survival and body mass prior to feeding. Overall it is still possible that these nutrients may positively affect non-feeding larvae, and future studies utilizing animals with lecithotrophic larvae, especially larger larvae, may yield more definitive results and shed light on efficient and effective nutrient supplementation techniques.

## LITERATURE CITED

- Carroll DJ, Kempf SC. 1990. Laboratory culture of the aeolid nudibranch *Berghia verrucicornis* (Mollusca, Opisthobranchia): Some aspects of its development and life history. *Biol Bull.* 179:243-253.
- Dembitsky VM. 2002. Bromo- and iodo-containing alkaloids from marine microorganisms and sponges. *Russ J Bioorg Chem.* 28(3):170-182.
- Gerencser GA, Cornette KM, Zhang J. 2002. Thyroid-hormone-induced phosphate absorption in *Aplysia californica* gut is mediated through protein synthesis. *Can J Physiol Pharmacol.* 80(12):1195-1198.
- Gerencser GA, Loo SY, Zhang J. 2003. Thyroid hormone-induced sulfate absorption in *Aplysia californica* gut is mediated by protein synthesis. *Can J Physiol Pharmacol.* 81(4):405-408.
- Goldberg WM, Hopkins TL, Holl SM, Schaefer J, Kramer KJ, Morgan TD, Kim K. 1994. Chemical composition of the sclerotized black coral skeleton (Coelenterata: Antipatharia): a comparison of two species. *Comp Biochem Physiol, Part B: Biochem Mol Biol.* 107(4):633-643.
- Heyland A, Reitzel AM, Hodin J. 2004. Thyroid hormones determine developmental mode in sand dollars (Echinodermata: Echinoidea). *Evol & Dev.* 6(6):382-392.
- Heyland A, Moroz LL. 2006. Signaling mechanisms underlying metamorphic transitions in animals. *Integr Comp Biol.* 46(6):743-759.
- Heyland A, Price DA, Bodnarova-Buganova M, Moroz LL. 2006. Thyroid hormone metabolism and peroxidase function in two non-chordate animals. *J Exp Zool Part B: Mol Dev Evol.* 306(6):551-566.
- Jaeckle WB, Manahan DT. 1989. Feeding by a “non-feeding” larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. *Mar Biol.* 103(1):87-94.

- Jameson JL, De Groot, LJ, editors. Endocrinology, Adult and Pediatric. 6<sup>th</sup> ed. Vol. II. Philadelphia: Saunders; 2010. 2834 p.
- Kempf SC. 1991. A 'primitive' symbiosis between the aeolid nudibranch *Berghia verrucicornis* (A. Costa, 1867) and zooxanthellae. J. Mollus Stud. 57(Supplement Part 4):75-85.
- Kingsley RJ, Corcoran ML, Krider KL, Kriechbaum KL. 2001. Thyroxine and vitamin D in the gorgonian *Leptogorgia virgulata*. Comp Biochem Physiol, Part A: Mol Integr Physiol. 129(4):897-907.
- Kristof A, Klussmann-Kolb A. 2010. Neuromuscular development of *Aeolidiella stephaniaeae* Valdéz, 2005 (Mollusca, Gastropoda, Nudibranchia). Front Zool. 7:5.
- Kupper FC, Carpenter LJ, McFiggans GB, Palmer CJ, Waite TJ, Boneberg E-M, Woitsch S, Weiller M, Abela R, Grolimund D, et al. 2008. Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. PNAS 105(19):6954-6958.
- Leonardo AFG, Hoshiba MA, Urbinati EC, Senhorini JA. 2013. Improvement of matrinxã, *Brycon amazonicus*, larviculture by exposing eggs to triiodothyronine. J World Aquacult Soc. 44(1):141-147.
- Liu YW, Chan WK. 2002. Thyroid hormones are important for embryonic to larval transitory phase in zebrafish. Differentiation. 70(1):36-45.
- Manahan DT, Crisp DJ. 1982. The role of dissolved organic material in the nutrition of pelagic larvae: amino acid uptake by bivalve veligers. Amer Zool. 22(3):635-646.
- Melmed S, Polonsky KS, Larsen PR, Kronenberg HM. Williams Textbook of Endocrinology. 12<sup>th</sup> ed. Philadelphia: Saunders; 2011. 1897 p.
- Middelburg JJ, Nieuwenhuize J. 2000. Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. Mar Ecol Prog Ser. 203:13-21.

- Obermann D, Bickmeyer U, Wägele H. 2012. Incorporated nematocysts in *Aeolidiella stephanieae* (Gastropoda, Opisthobranchia, Aeolidoidea) mature by acidification shown by the pH sensitive fluorescing alkaloid Ageladine A. *Toxicon*. 60(6):1108-1116.
- Shilling FM, Bosch I. 1994. 'Pre-feeding' embryos of Antarctic and temperate echinoderms use dissolved organic material for growth and metabolic needs. *Mar Ecol Prog Ser*. 109:173-181.
- Shilling FM, Hoegh-Guldberg O, Manahan DT. 1996. Sources of energy for increased metabolic demand during metamorphosis of the abalone *Haliotis rufescens* (Mollusca). *Biol Bull*. 191(3):402-412.
- Siebers D. 1979. Transintegumentary uptake of dissolved amino acids in the sea star *Asterias rubens*. A reassessment of its nutritional role with special reference to the significance of heterotrophic bacteria. *Mar Ecol Prog Ser*. 1:169-177.
- Tian RC, Nicolas E. 1995. Iodine speciation in the northwest Mediterranean Sea: method and vertical profile. *Mar Chem*. 48:151-156.
- Uchida M, Kanematsu M, Miyoshi T. 2010. Growth production of the juvenile clam, *Ruditapes philippinarum*, on sugars supplemented to the rearing water. *Aquaculture*. 302:243-247.
- Urbinati EC, Vasques LH, Senhorini JA, Souza VL, Goncalves FD. 2008. Larval performance of matrinxã, *Brycon amazonicus* (Spix and Agassiz 1829), after maternal triiodothyronine injection or egg immersion. *Aquacult Res*. 39:1355-1359.
- Valdés A. 2005. A new species of *Aeolidiella* Bergh, 1867 (Mollusca: Nudibranchia: Aeolidiidae) from the Florida Keys, USA. *Veliger*. 47(3):218-223.
- Welborn JR, Manahan DT. 1990. Direct measurements of sugar uptake from seawater into molluscan larvae. *Mar Ecol Prog Ser*. 65:233-239.
- Wong GTF, Piumsonboon AU, Dunstan WM. 2002. The transformation of iodate to iodide in marine phytoplankton cultures. *Mar Ecol Prog Ser*. 237:27-39.