The Microalga Pavlova contains an Analog for the Hormone Ecdysone that Promotes Metamorphosis of Larval Bay Scallops (Argopecten irradians irradians)

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Abstract:
Nutritional requirements of bivalve mollusks (oysters, clams, mussels, scallops), especially young stages, must be known for effective hatchery production of “seed” shellfish for subsequent grow-out to market. Species in the Genus Pavlova, a prymnesiophyte alga, have been used for many years as dietary components that are mass-cultured and fed to larval shellfish because they are known to contain essential lipids. In this project, we have determined that a Pavlova strain also contains an analog for the hormone Ecdysone. When added to the diet of Bay Scallops (Argopecten irradians irradians) Pavlova induces metamorphosis earlier than in those scallops fed a diet with no Pavlova. A sterol unique to the Genus Pavlova, named ethyl-Pavlovol, with a structure very similar to Ecdysone appears to be the bioactive compound in Pavlova cells. Addition of synthetic ecdysone also induced early metamorphosis in scallop larvae, and metamorphosis induction by both Pavlova and ecdysone was inhibited by the ecdysone-blocking insecticide Azasol. These findings provide strong evidence that pavlovols have hormonal effects upon mollusk larvae.

Introduction:
Nutritional requirements of bivalve mollusks, in terms of which microalgal species promote growth and development, have been investigated experimentally since the beginning of modern shellfish aquaculture (Ukeles and Rose, 1976). Subsequently, the importance in bivalve nutrition of long-chain, polyunsaturated fatty acids (Langdon and Waldok, 1988, Patterson et al. 1996) have been recognized. Among the most-effective microalgal strains for feeding larval and post-set bivalves are diatoms, prasinophytes, and several prymnesiophyte taxa (Brown et al., 1989).

The prymnesiophyte Class of microalgae includes the Genus Pavlova. Strains of Pavlova have been used for many years as components of larval feeds for molluscan shellfish (Ukeles, 1971). Pavlova strains appear to complement the nutritional profile of the T-ISO strain of Isochrysis sp., which lacks sterols that mollusks can convert to cholesterol (Patterson G. W, 1994, Wikfors G. H. 2005). While analyzing the sterol composition of Pavlova strains, a previously-unknown class of sterols that were named “pavlovols”, were discovered (Patterson et al. 1993). The biochemical structures of pavlovols are remarkably similar to the hormone ecdysone, which is known to regulate life-history transitions (e.g., molting) in insects and other arthropods (e.g., crustaceans) (King and Siddal, 1969). The genetic sequence for the hormone ecdysone has been found in bivalve mollusks (refs), but a role for this hormone in life-history transitions in bivalves has not been demonstrated. Nevertheless, this structural similarity between Pavlovols and ecdysone led researchers to suspect that roles of Pavlova in the diets of shellfish larvae may include both nutritional and hormonal activities. Previous experiments demonstrated that inclusion of Pavlova in T-ISO diets fed to larval bay scallops, varying both the percentage and timing of Pavlova supplementation, caused changes in metamorphosis in addition to growth (Ghosh, et al, 1997). The most striking response was early metamorphosis of larvae at an unusually small size two days after high-percentage Pavlova supplementation.

Materials and Methods:
Hydroxyecdysone (H5142), a hormone shown to cause life stage transitions in arthropods, was tested for the ability to stimulate setting of Northern bay scallop larvae (Argopecten irradians irradians) and will be referred to as Ecdysone from this point on. Azasol Insecticide (Arborjet), a neem-based product with the primary ingredient Azadirachtin, is a chemical that has been shown to act as blocker of Ecdysone in insects and will be referred to as AZA from this point on.

As no data was available on the possible toxicity of ecdysone or Azasol to larval bay scallops, a 48-hour LC50 assay was performed using 2-day old Bay Scallop larvae and 7 concentrations each of Ecdysone and AZA to determine if these concentrations were lethal to the larvae. The stocking
density for this bioassay was 15 scallop larvae per ml in 4 ml of 1.0-µm-filtered seawater (FSW) per well of a 12-well, polystyrene plate. The Ecdysone 1x solution had a concentration of 2.46 mg/l (LC50 data Sigma-Aldrich), dissolved in Isopropanol, while the AZA 1x concentration was 47 ug/ml (Peng, 2000). The 12-well plates each contained three concentrations, 4 replicate wells each: Plate 1 contained 10x, 5x, and 1.5x Ecdysone concentrations. Plate 2 contained 1x, .5x, and .25x Ecdysone concentrations. Plate 3 contained the Larvae-only control, 25x Ecdysone concentration, and Isopropanol control. The Isopropanol control contained 4ml FSW, larvae, and 4 ul of Isopropanol. Plate 4 contained 25x, 10x, and 5x AZA concentrations. Plate 5 contained the 1.5x, 1x, and .5x AZA concentrations. Finally, plate 6 contained a larvae-only and FSW control and the .25x AZA concentration. Results indicated that concentrations up to 25x Ecdysone and 10x AZA were not lethal to the larvae, thus the experiment could be conducted without concern for toxicity.

A factorial design was chosen for the main experiment (Fig. 1). Two grids of 16 one-liter beakers each were set-up and given different experimental treatments with increasing amounts of Ecdysone and AZA. Into each beaker bay scallop larvae were added, using a stocking density of 10 larvae/800 ml of filtered seawater (FSW). The upper left beaker served as a control for the grid, having no Ecdysone or AZA added. Across the top of the grid, the following treatments were added to each column: 0 Ecdysone, 1x Ecdysone, 10x Ecdysone, 100x Ecdysone. The 1X ecdysone treatment was calculated to equal the pavlovol content of the Pavlova culture fed each day, e.g. 2 mg/g Pavlova dry weight (Patterson, 1991). On the left side of the grid, the following treatments were added to the rows: 0 AZA, .1 ug/ml AZA, 1.0 ug/ml AZA, and 10 ug/ml AZA. The first grid (beakers 1-16) was fed a diet of 75% T-ISO and 25% Pavlova (CCMP 459) and the second grid (beakers 17-32) was fed a diet of 100% T-ISO. Each beaker was aerated using Pasteur pipettes (Fig. 2). The chemical additions were given to both grids, dosed every day, just after feeding. (Patterson G. W., 1998) The sub-samples of larvae were taken on days 5, 7, 9, 12, and 14. Larvae were preserved using Formalin and Lugol’s Iodine. A live count was done on the controls of each grid to assure that scallops were alive and growing. The preserved specimens were counted with a dissecting, light microscope at a later date. Counts of live, dead, and metamorphosed scallops were analyzed using Statgraphics™ software. A Multifactor Analysis of Variance model was applied to cumulative counts of metamorphosed (set) scallops from days 9, 12, and 14 with diet, Azasol concentration, and ecdysone concentration as independent variables, and 2-way interactions only included in the model (df=3 for each interaction term). The same statistical analysis was used to explore possible effects of experimental treatments upon survival.
Results and Discussion:

The primary purpose of this experiment was to test the hypothesis that Pavlova contains a chemical component, likely pavlovol, that acts as an analog for the hormone Ecdysone in promoting metamorphosis in Northern bay scallop larvae. A secondary purpose was to test the hypothesis that ecdysone promoted early metamorphosis of scallop larvae. This experiment also tested whether the insecticide Azasol, known to block ecdysone promotion of pupation in lepidopteran insects (Martinez, 2001) blocks larval scallop metamorphosis stimulated by both ecdysone and Pavlova.

MANOVA results indicate that Pavlova and Ecdysone both promote metamorphosis of larval bay scallops, and this activity is blocked by Azasol (Table 1). Evidence of this is shown in Figure 3; by day 12, Pavlova induced significantly higher metamorphosis than the T-ISO diet (Figure 5). The lowest AZA concentration tested, 0.1 µg/ml, was as effective as the higher concentrations at blocking metamorphosis induced by Pavlova; therefore, the threshold of effect is <0.1 µg/ml (Fig. 6).

<table>
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<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F-Ratio</th>
<th>P-Value</th>
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<td>A: AZA</td>
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<td>3</td>
<td>48.0417</td>
<td>4.03</td>
<td>0.0451</td>
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<td>Total (Corrected)</td>
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All F-Ratios are based on the residual mean square error.
This study has also shown that AZA inhibits setting of Bay Scallop larvae at day 12. This data, shown in Figure 6, corroborates the finding of Peng (Peng, 2000). Finally, this experiment has shown that Ecdysone promotes setting in 12 day-old bay scallop larvae. This is shown in Figure 5, and helps to corroborate the work of Karlson. (Karlson, 1956)

Conclusions:
This experiment has demonstrated that Pavlova and ecdysone promote metamorphosis of 12 day-old bay scallop larvae. A common mechanism for the hormone ecdysone and a component of Pavlova also is supported by the finding that Azadiractin blocks the effects of both Pavlova and Ecdysone in larvae (Figure 7). The effective concentration range of hormonal effects for both Ecdysone and AZA has also been determined and can be used in future research projects. The major practical implication of these findings is confirmation that addition of cultured Pavlova to the diet of larval bay scallops, and presumably other bivalve species, can be used to promote setting, thereby improving hatchery production of shellfish seed for aquaculture and restoration.

Bibliography:


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Acknowledgements:

Thanks to the University of New Haven and the SURF program for making the research possible and to Mr. and Mrs. Carrubba for their support of the SURF program. Thank you to the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center Milford Laboratory for the use of their facilities. Also thanks to Jen Alix, Mark Dixon, and Dr. Shannon Meseck for all their assistance with various aspects of this project. Finally, special thanks to Dr. Gary Wikfors for mentoring me and assisting me with this project.

About the Author:

Derrick Chelikowsky is currently a senior at UNH majoring in Marine Biology. He hopes to continue his research and education in graduate school, working towards a Ph.D. in Marine Biology or Aquaculture. This was Derrick’s first experience with research and it has confirmed for him that research is what he wishes to do with his life. Derrick is the Sargent at Arms of the Marine Biology Club as well as President of Science Fiction and Fantasy Club at UNH.