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

Derrick J. Chelikowsky
Marine Biology
Advisors: Carmela Cuomo, University of New Haven
Diane Kapareiko, Dorothy Jeffress, and Gary H. Wikfors, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, North East Fisheries Science Center, Milford, CT 06460

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 LC_{50} 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 (LC_{50} 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[tm] 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.

Figure 1 – Factorial Design

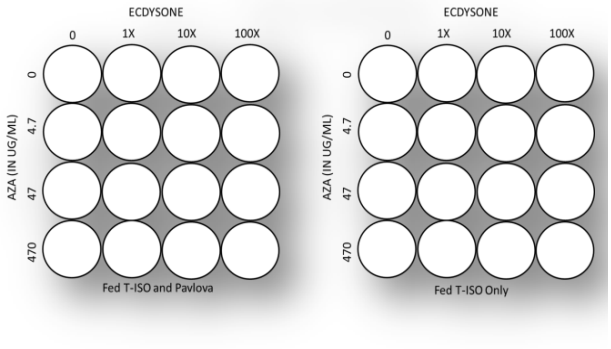


Figure 2: Beakers showing aeration using Pasteur pipets

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 1: MANOVA Results

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main Effects					
A: AZA	144.125	3	48.0417	4.03	0.0451
B: ECDY	106.375	3	35.4583	2.98	0.0892
C: PAV	288	1	24.17	24.17	0.0008
Interactions					
AB	262.375	9	29.1528	2.45	0.0994
AC	38.75	3	12.9167	1.08	0.4042
BC	61	3	20.3333	1.71	0.2348
Residual	107.25	9	11.9167		
Total (Corrected)	1007.88	31			
All F-Ratios are based on the residual mean square error					

Figure 3: Effects of AZA on larval setting

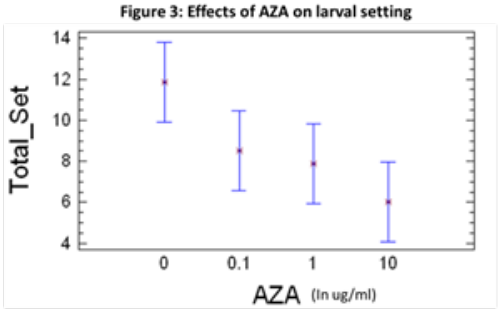


Figure 4: Effect of Ecdysone on larval setting

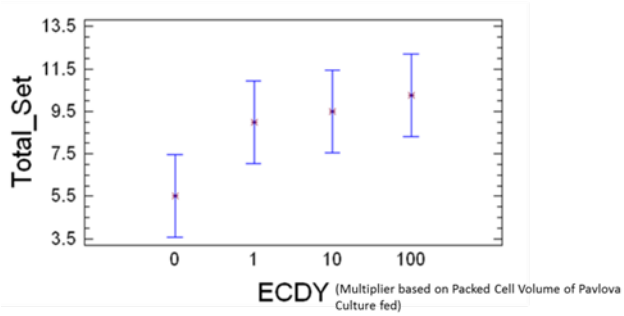


Figure 5: Effects of Pavlova on larval setting

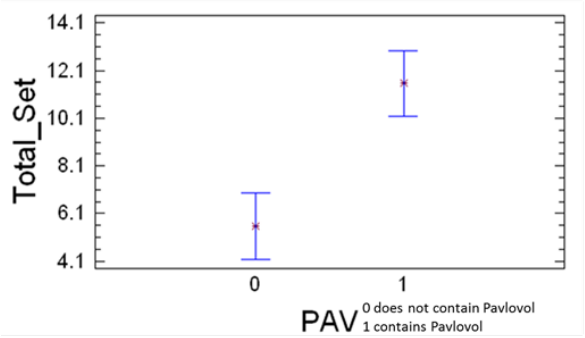


Figure 6: Effect of Pavlova and AZA on larval setting

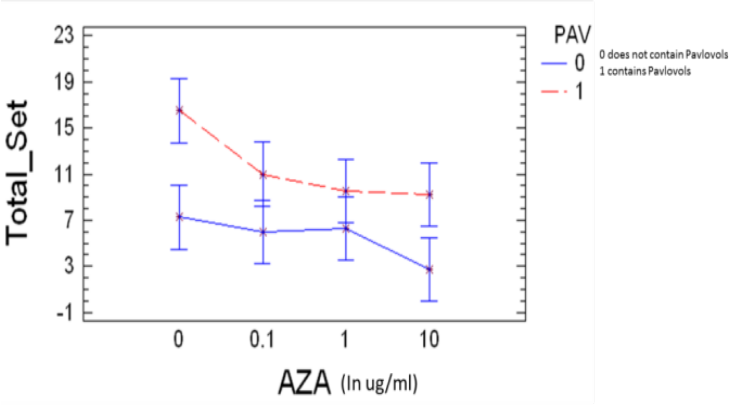
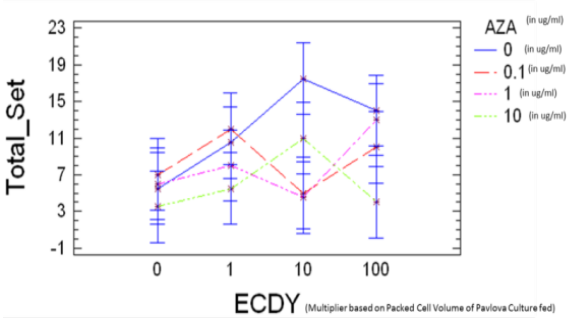


Figure 7: Effects of AZA and Ecdysone on larval setting



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 (Figure7). 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.

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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.