The Effects of Including *Nannochloropsis oculata* in a Mixed Algal Diet on Pacific oyster *Crassostrea gigas* Larvae

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PACIFIC OYSTERS IN AQUACULTURE

The Pacific oyster, *Crassostrea gigas*, is the most commonly reared shellfish on the Pacific Coast and a large part of United States aquaculture, making up 20.3% of total mollusk production value in the U.S. in 2018[1]. An oyster's larval stages are vital to its growth and production, so effective rearing is paramount to oyster aquaculture success[2]. Typical larval rearing techniques use mixtures of microalgae that vary between producers, but the effects of including microalgae species *Nannochloropsis oculata* as a larval food source is currently not well understood, though studies show high potential in larval growth and survival[2,3,4,5]. **Quantifying the effects of *N. oculata* inclusion in larval diets may have a huge impact on global oyster aquaculture, as it may present a beneficial alternative to current larval rearing techniques.**

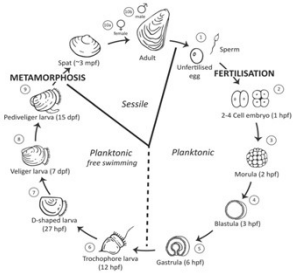

Fig 1. Life stages of the Pacific oyster. Hours post-fertilization (hpf) and days post-fertilization (dpf) are denoted. From: Vogeler, S., Bean, T. P., Lyons, B. P., & Galloway, T. S. (2016). Dynamics of nuclear receptor gene expression during Pacific oyster development. *BMC Developmental Biology*, *16*(1). https://doi.org/10.1186/s12861-016-0129-6

HYPOTHESIS

The project aims to quantify effects of introducing *Nannochloropsis oculata* into microalgal mixtures containing commonly used microalgae. In addition to this, the project compares the hatchery feeding method of incremental algal rations ("non-grazing") with a quantified algal ration method ("grazing"). **It is hypothesized that introduction of *Nannochloropsis oculata* will result in an increase in larval growth and survival,** due to prior studies showing increased growth and metamorphosis rates in larval models[2] and high total fatty acid content which is imperative in larval growth and development[4].

SPAWNING AND LARVAL REARING METHODS

Spawning Methods

- 3 females crossed with 5 males
  - 4/5 sires and 3/3 dams represented in crosses; chosen by highest number of D-larvae
- Each cross contributed equal number of larvae to experimental unit

Rearing Methods

- Larvae reared in static tanks for 24h, then standardized to density of 40 larvae/mL and stocked at ~100 larvae per sample into Hatfield Ultra Dense Larval System (HUDLS) at D-stage. First exposed to food when placed into HUDLS tanks.

Hatfield Ultra Dense Larval System (HUDLS): *A flow-through seawater system that produces more stable environmental conditions between tanks*

Fig. 2 Picture depicts Hatfield Ultra Dense Larval System at Hatfield Marine Science Center.

EXPERIMENTAL DESIGN

Microalgal Diet Treatments

Treatment 1: Grazing;
Diet includes *N. oculata, T. lutea, and C. muelleri*

Treatment 2: Non-grazing;
Diet includes *N. oculata, T. lutea, and C. muelleri*

Treatment 3: Grazing;
Diet includes *T. lutea and C. muelleri*

"Grazing" vs. "Non-grazing"

Grazing Method:
Larval feeding quantified and calculated feed to 10,000 algal cells in outflow

Non-grazing Method:
Incremental feeding method analogous to standard hatchery protocols (Helm, 2004 hatchery guide: strategy 1)

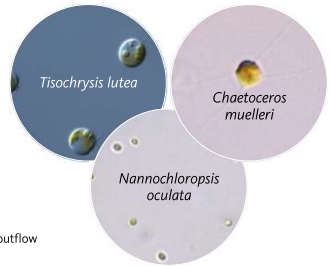

Fig. 3 Microscopic images of the microalgae used in experimental treatments. Not to scale. From: Bigelow National Center for Marine Algae and Microbiota

DATA COLLECTION

Data Collection

Each treatment performed in quadruplicate, **samples collected 24 hours, six days, and 12 days post-fertilization** and immobilized in formalin for analysis. Larvae pictured using Leica DM IL LED Inverted Laboratory Microscope with K3C Microscope Camera attachment and corresponding software. Larvae counted then measured using ImageJ, an open-source image analysis software.

Fig. 4 Microscopic images used in data collection showing larvae at the three timepoints data collection took place. Images taken by Zachary Kowash

DATA ANALYSIS

Data analysis on shell length completed via 3-way repeated measures ANOVA in R. *Table 1* displays R output table, showing statistically significant difference due to treatment. Due to the insignificance of grazing on length, one can attribute the statistically significant difference to inclusion of *N. oculata* in treatments. No statistical analysis completed on survival data due to initial data visualization showing no difference.

Significance p <0.05	Factor	Df	Sum of Squares	Mean of Squares	F-value	P-Value
	Grazing	1	31420	31420	2.584	0.151983
	Treatment	2	555160	555160	45.657	**0.000263*** **
	Date	2	101186	50593	4.161	0.064459
	Residuals	7	85116	12159	*NA*	*NA*

Table 1 3-way repeated measures ANOVA output table. Statistical significance displayed between lengths due to treatments. Sum of squares and mean of squares inform on variance in samples from sample mean, i.e. larger sum/mean of squares equals more variance across samples. Residuals show how much different sample values are from sample mean i.e. positive residual means more values greater than sample mean

RESULTS

Shell Length

Treatments fed with *N. oculata* (Treatments 1 and 2) showed statistically significant higher growth in terms of shell length (p = 0.000263). Best seen at 12 days post-fertilization. No significant difference observed between algal feeding methods (grazing vs. nongrazing) (p = 0.151983). Variability in treatments also observed.

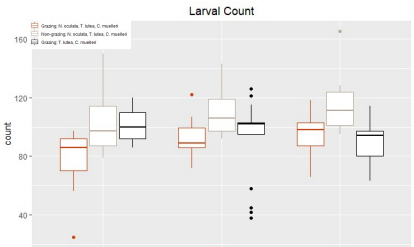

Fig 6. Larval counts at 24 hours, six days, and 12 days post-fertilization. Samples taken at a target volume of ~100 larvae per sample. No discernible difference between treatments.

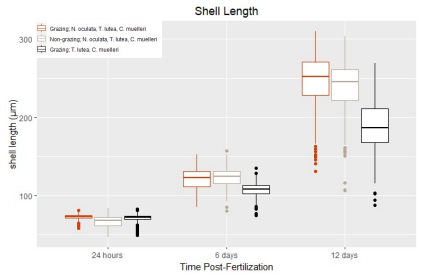

Fig 5. Larval counts at 24 hours, six days, and 12 days post-fertilization. Samples taken at a target volume of ~100 larvae per sample. No discernible difference between treatments.

Survival

Larval count was used as measure of survival, as samples were collected with a target larval volume of approximately 100. No statistical difference in larvae survival observed between treatments, either due to algal feeding method or addition of *N. oculata*.

CONCLUSIONS

Data show a statistically significant benefit on larval growth and no observed effect on larval survival with inclusion of *N. oculata*. No strong effect observed from feeding method.

Future Steps with *Nannochloropsis oculata:*

- ***Quantification*** of exact effects on larval growth and development.
- ***More research across various husbandry environments*** due to differences in aquaculture environments.
- ***Evaluation*** of its effects in different microalgal mixtures.
- ***Cost-benefit analysis*** of *N. oculata* inclusion in current hatchery protocols.

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