Alternative image-based technique for phytoplankton cell counts in shellfish aquaculture.

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Introduction

Shellfish aquaculture presents a sustainable alternative food source to satisfy global demands and conserve species at risk of over-harvest. Standard operations in shellfish aquaculture begin at a hatchery where adults are spawned and larvae are reared (figure 1). Early-life stages have an elevated sensitivity to unfavorable conditions (e.g. diet, seawater chemistry, etc.) and pose a production limitation. A successful hatchery is largely dependent on accurate counts of phytoplankton batch cultures

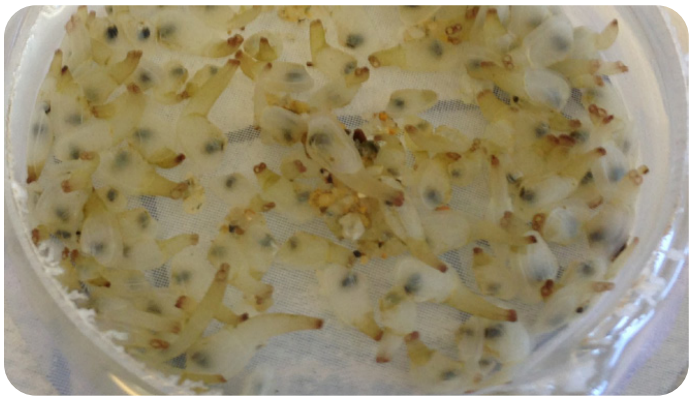

Figure 1: Juvenile Pacific geoduck clams *Panopea generosa* (approximately 5 mm shell length) reared with a mixed-algae diet.

to estimate daily feeding routines, optimize shellfish production, and avoid sub-lethal harm during life-stages susceptible to overfeeding or starvation (figure 2). However, common techniques to quantify phytoplankton cultures place hatcheries at a detriment to labor intensive protocols and instrumentation.

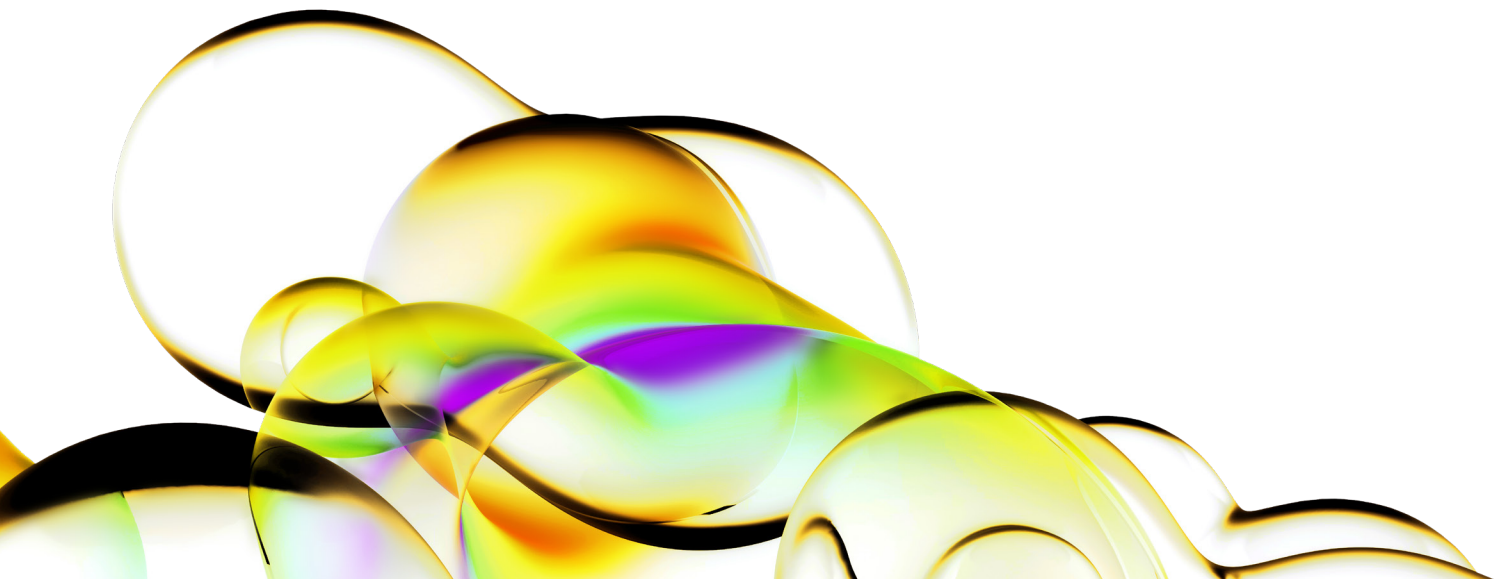

Figure 2: Large batch cultures (approximately 100 L) in translucent polymer fiberglass tanks.

Here, we describe use of the Cellometer® Auto T4 in a commercial hatchery setting as an alternative to conventional methods used to count phytoplankton cells in aquaculture (e.g. hemocytometer and coulter counter). We report on the ability to yield rapid and accurate counts and cell size data with an automated image-based analysis. Results are reported from an experiment when researchers used cell concentrations of phytoplankton cultures to calculate the volume required to feed juvenile shellfish a mixed-species diet of three phytoplankton species.

Method

Samples were taken (2 – 3 mL) from 100 L batch cultures of the genera Isocrysis, Tetraselmis, and Pavlova to count cell density and calculate the volume required to feed juvenile shellfish (5 mm shell length). Test tubes with algae samples were inverted to ensure homogenous mixing before pipetted. Approximately 20 µL of each sample was pipetted into one side of a dual-chamber SD100 Nexcelom slide for image-based analysis of cells < 100 µm in diameter. No reagents or additional solutions were required to image these three phytoplankton.

The Cellometer Auto T4 was used to capture brightfield images of the cells with brighten center and highlighted dark cell perimeter. Species or genera-specific parameters for cell diameter and roundness were adjusted to optimize capture of target cells, avoid unwanted debris, and decluster cell aggregations. Occasional impurities due to sampling were avoided with minor focus adjustments and exposure of the image.

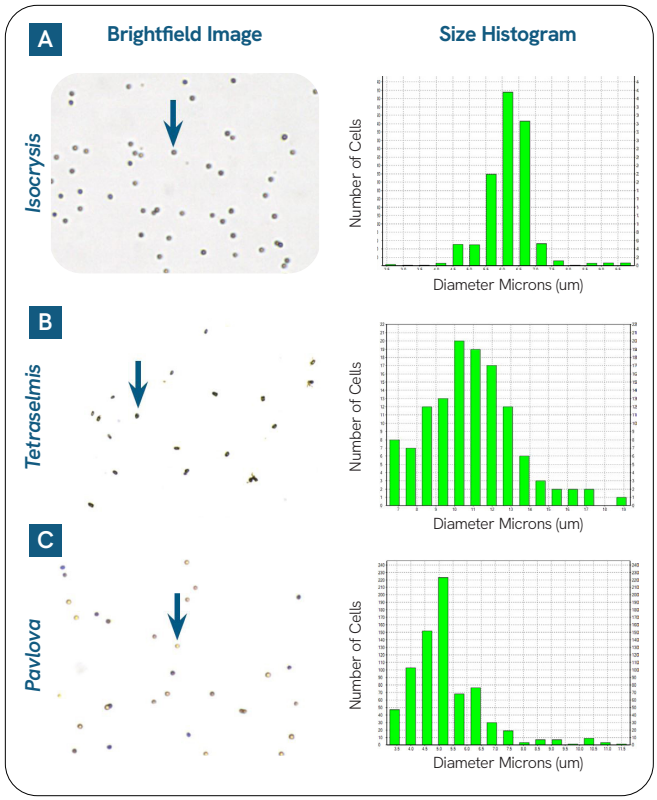

Figure 3: Images and histograms from the Cellometer for samples of *Isocrysis* (A), *Tetraselmis* (B), and *Pavlova* (C). Arrows identify a single cell representative of each culture. Images in B and C were exposed to reduce the impact of background debris and enhance target cell capture.

Table 1: An example of daily batch culture data for a mixed-diet of three phytoplankton. Culture concentration and mean diameter of cells were used to calculate volume of algae to feed clams and monitor status of the cultures.

Genera	Cell count	Mean diameter (µm)	Concentration (cells ml -1)
Isochrysis	1178	6.2	3.18×10^6
Pavlova	749	5.2	2.02×10^6
Tetraselmis	124	10.9	3.39×10^5

Histograms for each culture were observed to adjust for any inaccuracies in overall cell capture and were saved to monitor the status of batch cultures through time. Once verified that the images were accurately counted, cell concentrations reported by the Cellometer were automatically output to a cumulative spreadsheet. Cell counts with the Cellometer were often greater than manual microscopy and were repeated for consistency. An R script, written for the experiments, extracted daily count data to calculate the required volume for shellfish diets. Sample image- analysis and feed calculations were completed in less than 10 minutes.

Discussion and conclusions

Our data demonstrates the reliability of the Cellometer for measuring both small (< 5 µm) and large (> 10 µm) phytoplankton commonly used for shellfish diet in aquaculture industry. Automated image-based counts were consistent and reproducible unlike manual methods that are otherwise labor-intensive and confounded by individual error.

Advantages of the Cellometer are especially evident in analysis of live samples and highly-mobile cells. Phytoplankton such as Tetraselmis present greater potential for miscalculating cell concentrations in manual microscopy (e.g. hemocytometer). Inherent to the function of the Cellometer, image-based counts exclude error associated with mobility and suggest that the Cellometer can also be beneficial for assessing early-life stage shellfish in our study (e.g. D-hinge, trochophore, pediveliger, etc.).

Additionally, cell type settings can be customized with the Cellometer (e.g. size and roundness) for individual targets to count diverse samples in quick succession. Such samples relevant to shellfish aquaculture embody the entire spawning and rearing process (algae, egg, sperm, larvae, etc.).
We conclude that this tool enhances workflow in an aquaculture setting and offers a rapid and reliable method on analysis of phytoplankton and batch culture viability.

Acknowledgements

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