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# Lipid Enrichment of Eastern Oyster Broodstock Using Commercially Available Emulsions

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## Introduction

Lipids are a group of nutritional components important in shellfish development. Examples of lipids include fats, waxes, oils and steroids (for example testosterone, cholesterol). In shellfish eggs and larvae, lipids' primary function is the long-term storage of energy; they sustain a fertilized egg until it becomes a feeding larva. Lipids are also important during metamorphosis, when setting larvae cease feeding and depend on lipid reserves until metamorphosis is complete. The most important types of lipid for shellfish are polyunsaturated fatty acids (PUFA) rich in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), both omega-3 fatty acids (Gallagher and Mann, 1986; Wikfors *et al*., 1991; Caers *et al*., 2002).

The settlement rate of competent to set ("eyed") larvae is highly variable, ranging from single digits to over 50%. The total lipid content of the broods of oyster larvae is highly correlated with survival of those broods through the metamorphic process (Gallagher and Mann, 1986). These results strongly suggest that lipids play an important nutritional role during the non-feeding metamorphic process and must be present in certain concentrations if the larvae are to survive metamorphosis. Studies (Gallagher and Mann, 1986; Robinson, 1992; Rivara *et al*., 2005) have shown increased set rates when eggs started out with higher levels of lipids.

Since eastern oysters, *Crassostrea virginica* (like most bivalves) cannot synthesize these essential fatty acids themselves, they must get these lipids from outside sources, primarily phytoplankton (Wikfors *et al.,* 1991; Wikfors *et al.,* 1996). Our NRAC-funded research (Rivara *et al.,* 2005) using a lipid emulsion supplement has concluded that such supplements also offer a way of getting fatty acids to female broodstock eastern oysters and their eggs. This work showed a doubling of set rate with larvae from lipid enriched eggs (18%) versus larvae from eggs from females fed the alga T-ISO alone (9%). Increasing the amount of lipids in eggs through broodstock conditioning can also translate into more competent larvae with a better chance of surviving to and through metamorphosis, especially if those larvae are stressed (Utting and Millican, 1997).

There have been many laboratory-made lipid emulsions used to enrich larval finfish prey such as brine shrimp and rotifers, as well as bivalve broodstock, larvae and post set. However, these emulsions can be time consuming to prepare and often lack shelf life, requiring daily preparation. A few commercial lipid emulsions and powders, such as Selco® (INVE Aquaculture) and SuperHUFA® (Salt Creek), are readily available at a reasonable cost from many aquaculture suppliers and once opened have a shelf life of at least one month if kept refrigerated. (Note that no endorsement for any of these emulsions is implied here.) Droplets formed when the emulsion is mixed with seawater are from 0.5 to 5 microns, a size that makes some of the droplets directly available to filter feeding broodstock. These emulsions probably also adhere to algal cells and other particles in the broodstock tank before they are ingested by the oysters.

## Guidelines for Use

Keep in mind that these preparations are meant to be fed along with algae (and/or algal paste) and do not replace microalgae in broodstock conditioning. While our NRAC-funded research dealt only with eastern oysters, there is quite a body of knowledge in the literature on lipid enrichment of other species (see references). If the hatchery has a high lipid strain of phytoplankton in mass culture, the use of a lipid emulsion may not be warranted. For example, we found similar set rates with oysters fed with *Tetraselmis* (Plat-P) and T-ISO as with oysters fed with T-ISO plus lipid emulsion, but we did not experiment with feeding both species a high lipid strain of algae plus a lipid emulsion.

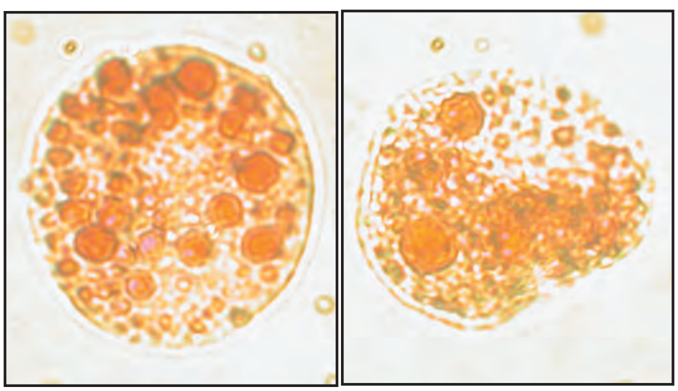

**Figure 1. Oil Red O stained lipid droplets in oyster eggs after conditioning with lipid emulsion and T-ISO (left) and T-ISO alone (right)**

The key to using these lipid emulsions in a hatchery's normal conditioning protocol is balancing lipid feed rate with water quality. The lipids which are desirable for the broodstock can also foul static tanks, especially if overfed, creating a haven for bacteria and protozoa and possible dissolved oxygen issues. Flow-through broodstock systems will have to be shut down or throttled back for hours to allow the shellfish to take up the lipids. Each conditioning system is different and hatchery managers will have to tailor the information given here to fit their particular setup. Note that all oysters being conditioned are fed the supplement, as generally we do not know which are males and which are females before they spawn. Also, lipid enrichment does not shorten the conditioning period.

As with algal rations, determining lipid concentration is less dependent on stocking density than water volume. Lipids must be concentrated in the broodstock tank in order to ensure that each oyster gets a ration each day during the conditioning period. Continuous aeration of the broodstock tank is critical in this regard. While continuous or semi-continuous algal additions to broodstock are preferred (Epifanio, 1981), we generally provide lipid enrichment once per day while conditioning. Alternately, the lipid emulsion can be added to the algae bucket before it is fed to broodstock a number of times each day, prorating the lipid addition (however, never add the emulsion directly to algal cultures).

There are two ways to add lipids to a broodstock tank. The first is to mix up the daily ration from the viscous concentration. Depending on the size of the tank, this may require a sensitive scale or micropipette, since a little goes a long way. A more practical method is to make up a working solution with distilled or spring water that is then added to the tank each day. This is more convenient, saves time and ensures that the correct amount of lipids is fed by anyone on the hatchery staff. Particle size distribution in stock solutions doesn't change appreciably (there is little or no agglomeration or bacterial clumping) over a week or so, although it is a good idea to shake the working solution before each use. In determining how much working solution to make up, figure on using a batch within two weeks.

## Preparation

Based on past research (Samain *et al*., 2000; Caers *et al*., 2003; Nevejan *et al*., 2003), a good place to start is with 2.4 milligrams of emulsion per liter of broodstock tank volume. In this example, we use EZ SELCO® which has 200mg/g dry weight of omega-3 fatty acid. We suggest making a working solution of lipid emulsion since weighing out rations each day would become tedious over the time period required to condition broodstock. To make a working solution, take 24 grams of the emulsion (which is equivalent to a volume of 25 milliliters, for those lacking a sensitive scale) and add this to one liter of distilled or spring water. Using potable rather than filtered seawater will reduce the chance of contamination in the working solution. It will take a number of rinses to get all of the viscous emulsion out of the measuring graduate. Shake the bottle well or use a blender to mix and keep refrigerated.

Once per day during the entire conditioning period, take 10 milliliters of the working solution per 100 liters of broodstock tank volume and disperse the solution throughout the tank. For example, if a broodstock tank holds 300 liters of water, it would take 30 milliliters of working solution once per day. If feeding and enriching three times per day, use 10 milliliters of working solution per feeding. Hand mixing and/or aeration are necessary to distribute the lipids and make them available to the oysters. A slight milky appearance is normal in the broodstock water after lipid enrichment. Aeration should be continuous. Maintain water quality through alternate day water changes or water exchange in flow through systems.

## Conclusions

While enriching oyster broodstock with off-the-shelf lipid emulsions has been shown to increase lipid content of eggs and set rate of eyed larvae in a variety of shellfish species, these compounds are not a replacement for high quality algal cultures during conditioning for spawning. Feeding shellfish broodstock quality microalgae along with supplemental lipids and proper husbandry will help produce high quality eggs, larvae and post set.

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