Origin, characterization and mitigation of oil films in tanks used for intensive culture of larval walleye

by

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GENERAL INTRODUCTION

Justification

Noninflation of the gas bladder (NGB) is considered the dominant obstacle to the mass culture of many larval fishes (Colesante et al. 1986; Barrows et al. 1988; Kindschi and MacConnell 1989; Summerfelt 1991). Species experiencing this culture problem include walleye (Stizostedion vitreum)(Summerfelt 1991), striped bass (Morone saxatilis)(Doroshev and Cornacchia 1979), red sea bream (Pagrus major)(Kitajima et al.1981), and gilt-headed bream (Sparus auratus)(Chatain and Ounais-Guschemann 1990).

The etiology of the NGB problem is not completely understood, but environmental, physiological, developmental, and genetic factors have been explored (Battaglene and Talbot 1990; Bristow 1993; Bulak and Heidinger 1980). The most widely accepted explanation for noninflation of the gas bladder in an intensive culture environment is that an oily microfilm at the air-water interface of culture tanks prevents the larvae from penetrating the surface to gulp air. Walleye are physostomous as larvae and, although experimental observations are lacking, contact with the air-water interface may be necessary to initiate inflation of the gas bladder.

The origin of the oily surface film commonly seen in larviculture tanks is unknown, but the oil globule of the fish themselves and oily feed introduced to the tanks are considered possible sources. Colesante et al. (1986) suggested that the oil film originated from the formulated feed introduced to the culture system. Chapman et al. (1988) suggested that the oil may have come from food and feces or released from the oil globules of dead fish.

Various techniques have been devised to alleviate the oil film problem. Barrows et al. (1988), operating under the assumption that the oil originated from feed introduced to the tanks, used a plastic ring to contain the spread of the feed (feeding ring) and oil film. Kindschi and MacConnell (1989), however, did not find the feeding ring to be effective. Oil absorbent cloth was tested to evaluate the possibility that it could reduce oil films in culture systems (Friedmann and Bates 1988). This strategy was ineffective in improving gas bladder inflation of larval striped bass (Van Olst et al. (1990) and walleye (Summerfelt 1991).

The method that has shown the most promise in improving gas bladder inflation in an intensive culture environment has been the use of a surface water spray to homogenize the oil film in the water column or to push the oil film to one side of the tank and down a surface drain. Summerfelt (1991) reported that a water-spray and surface-drain system improved gas bladder inflation in larval walleye to above 90%. Barrows et al. (1993) reported that inflation rates of larval walleye were significantly greater when reared with a water spray (98.4%) than without a spray (51.7%).

The objective of this research was to determine the origin of the oily material responsible for the formation of the oil surface microlayer by monitoring the development of the oil layer in tanks used for the intensive culture of larval walleye and by characterizing oil samples from tank surfaces and from larval walleye using gas chromatography technology. Two methods for reducing the oil surface microlayer (surface-water-sprays and an air-blower and trap apparatus) were evaluated for their ability to improve gas bladder inflation. Eight different screen types and two mesh sizes commonly used in larviculture systems were also evaluated for their ability to allow an oil surface microlayer to pass out of larval fish culture tanks.

Explanation of Thesis Format

This thesis consists of a general introduction, five papers, and a general summary. References cited in the general introduction are listed following the general summary. Each of the five separate papers will be submitted for publication in scientific journals, they are formatted in the style of the appropriate journal. The first paper will be submitted to *Progressive Fish-Culturist*. The second paper will be submitted to *Aquaculture*. The third paper will be submitted to *Progressive Fish-Culturist*. The fourth paper will be submitted to *Applied Aquaculture* and the fifth paper will be submitted to *Progressive Fish-Culturist*.

The research reported was conducted by Charles T. Boggs under the supervision of Dr. Robert C. Summerfelt. Mr. Boggs is the primary author of the papers and was principally involved in the collection, analysis and interpretation of the data as well as the writing of these papers.

PAPER I.

A METHOD FOR SAMPLING AND QUANTIFYING OIL SURFACE MICROLAYERS IN LARVICULTURE TANKS

ABSTRACT

Oily surface films are of common occurrence in intensive larviculture of many fish species; these films are regarded as the cause for noninflation of the gas bladder. A method for sampling and quantifying oil surface microlayers in larval fish culture tanks is described. A glass plate was used to sample oil from the surface of tanks and the solvent 1,1,1 trichloroethane (TCE) used to separate the oil from water. A spectrophotometric procedure was used to quantify the concentrations of oil in TCE by comparison with standard curves prepared with a food-grade cod oil.

PROCEDURE

The formation of an oil (lipid) film on the surface of larval fish culture tanks has been regarded as the cause for noninflation of the gas bladder of larval fish. Species experiencing this larval culture problem include striped bass (Morone saxatilis)(Doroshev and Cornacchia 1979), red sea bream (Pagrus major), gilt-headed bream (Sparus auratus)(Kitajima et al. 1981; Chatain and Ounais-Guschemann 1990) and walleye (Stizostedion vitreum)(Colesante et al. 1986; Summerfelt 1991). Larvae without inflated gas bladders exhibit decreased growth rates and lordotic skeletal malformities and are more susceptible to stress (Chapman et al. 1988; Chatain and Ounais-Guschemann 1990; Kindschi and MacConnell 1989).

The origin of the oily surface film commonly seen in larviculture tanks is unknown, but the oil globule of the fish themselves and oily feed introduced to the tanks are considered possible sources (Colesante et al. 1986; Chapman et al. 1988). Oils that are fluid under ambient temperatures spread horizontally and form a film that overlays the air/water interface (Larsson et al. 1974). Oil in water will continue to spread until it reaches a monomolecular thickness, approximately 0.1 µm (Butt et al. 1986). Although generally recognized as a cause or a contribution to the noninflation of the gas bladder problem, no one has heretofore measured the thickness of the oily surface film. The purpose of the present study was to describe a method to quantify oil thickness on the surface of tanks used for the mass culture of larval walleye on formulated feed.

A glass plate has been used to sample lipid films from water surfaces. Harvey and Burzell (1972) used a glass plate for the collection of microfilms from the ocean's surface, and Muir et al. (1992) used the same method to collect insecticide

samples from the surface of prairie ponds. We used the glass plate method and an adaptation of a colorimetric procedure (HACH Company 1989: 427-429) to sample and quantify an oil surface microlayer from larval walleye culture tanks.

We compared the absorption spectra of several fish oils to determine whether there are species specific differences in spectrophotometric absorption of oils from different fish. Samples of cod (Gadus morhua), menhadden (Brevoortia tyrannus), and herring (Clupea harengus) oil were dissolved in 1,1,1 Trichloroethane (TCE), and the absorption spectra of these solution were determined. The absorptivities of each of these oils were measured from 0 to 800 nm in 50-nm increments. A reagent blank containing pure TCE was used to null the spectrophotometer (HACH DR3000). A sample cell (2.54-cm pathway) containing a 30-ppm concentration of oil and TCE was placed in the spectrophotometer, and the absorption recorded at 50-nm increments. When the area of maximum absorbance was located, the increments were decreased to 5 nm and then 1 nm until the wavelength with maximum absorption was identified. All oils had a single peak at 328 nm; no secondary peaks were found throughout the rest of the spectrum. Although the wavelength of maximum absorption was the same for all fish oils, the peak for cod oil was greater in magnitude at the same wavelength than the herring and menhaden oils (Figure 1). For this reason, cod oil was used to prepare all standard solutions.

Brown (Iowa State University, Ames, personal communication) used procedures as described by Gunstone (1991) for high-resolution nuclear magnetic resonance (NMR) spectroscopy to measure ^{13}C spectra of fish oils from cod, menhaden, herring, and walleye. Brown's findings indicate that these oils had signals in the ^{13}C-NMR spectra similar to those of the n-3 polyene acids and DHA (docosahexanenoic acid) and EPA (eicosapentaenoic acid). The similarity in absorption spectra with both light

and NMR methods was used as confirmation for the selection of cod oil for preparing standard solutions for estimating oil thicknesses in tanks used for the culture of larval walleye.

Data for a standard concentration curve were collected three times with standards of 10, 20, 30, and 100 ppm. The absorbance and concentration values were plotted and described with a second-degree polynomial curve (Figure 2).

Oil thickness on tank surfaces was determined by comparison with a standard curve based on calculations of oil microlayer thicknesses developed in aquaria. Clean glass aquaria (25.5 cm x 50.0 cm x 30.0 cm) were wiped down with TCE and then filled with 20 L of tap water. The surface area of the aquaria were calculated (1.275×10^{11} μm^2), and enough oil was introduced to form a microlayer of a known thickness according to the equation:

$$\text{Oil thickness } (\mu m) = \frac{\text{volume of oil } (\mu m^3)}{\text{surface area } (\mu m^2)}$$

A volume of 0.01 cm^2 (1.0×10^{10} μm^3) of cod oil had a calculated thickness of 0.078 μm in these aquaria.

Oil sampling was done using a glass plate (200 mm x 100 mm x 3 mm). The plate was held in hand perpendicular to the water surface, then submerged in the water and slowly removed, carrying with it oil from the water's surface. Both sides of the plate were rinsed with a 30-ml aliquot of TCE from an autopipet into a 500-ml separatory funnel (Figure 3). A cotton plug was inserted into the delivery tube of the separatory funnel to remove any suspended water and solids. The lower TCE layer was then drained into a glass spectrophotometer cell with a 2.54-cm path length, and its absorbance determined by using the spectrophotometer. Any water adhering to the glass plate remained in the separatory funnel or the cotton plug; water did not contaminate the oil-TCE mixture. Aquaria with oil layers 0.3, 0.5, 0.7 and 1.0 μm

thick were sampled with the glass plate three times each to develop a curve to relate absorption to an oil microlayer thickness (Figure 4). This relationship was used to measure the thickness of oil layers on larval culture tanks.

This spectrophotometric methodology can be used to measure either oil concentration in water or oil thickness on the surface of culture tanks. The coefficient of determination (r^2) was 0.99 for the absorption-concentration curve and 0.95 for the absorption-oil thickness curve. Both procedures were highly reproducible, but, it is evident from the standard curves that there is considerable variation when the estimated thicknesses were less than 0.3 μm. This did not cause any difficulties when sampling culture tanks because oil layers were typically between 0.4 μm and 0.7 μm thick.

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Figure 1. Oil sampling and extraction apparatus.

Figure 2. Optimum wavelength for herring, menhaden, and cod oils. All standards were 30 ppm fish oil in TCE.

Figure 3. Standard curve of oil in solvent concentrations. Concentrations are 10, 20, 30, and 100 ppm.

Figure 4. Standard curve of absorbance and oil thickness values. Oil thicknesses are 0.1, 0.3, 0.5, 0.7, and 1.0 μm.

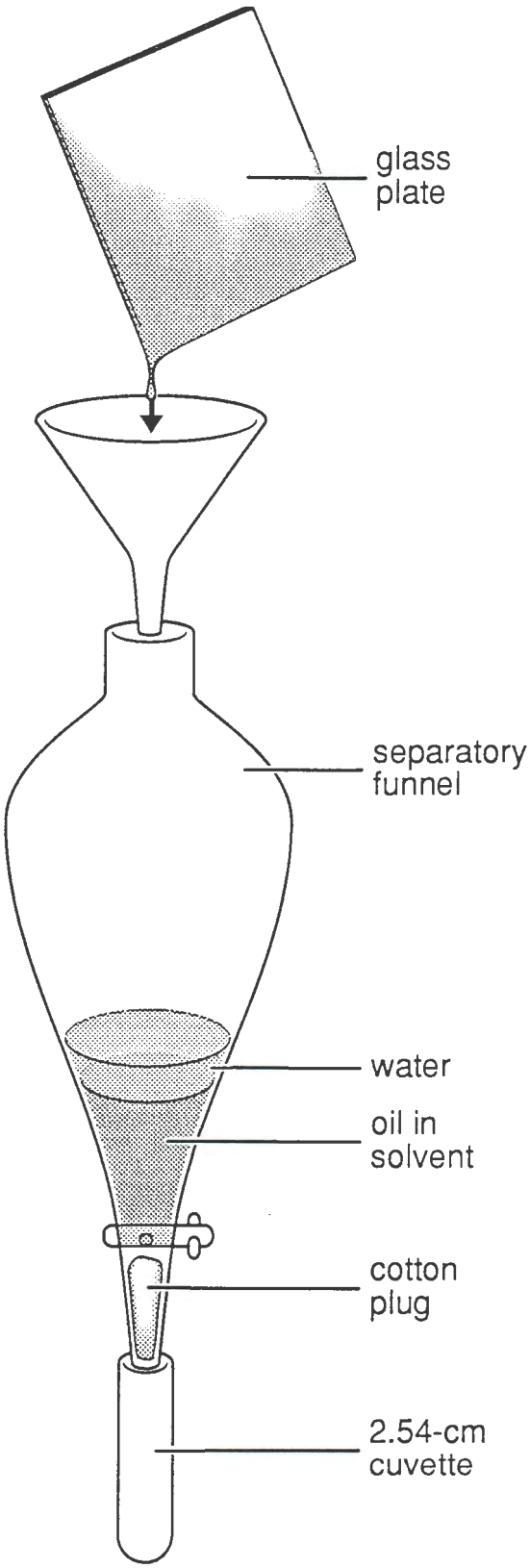
glass plate
separatory funnel
water
oil in solvent
cotton plug
2.54-cm cuvette

Figure 1.

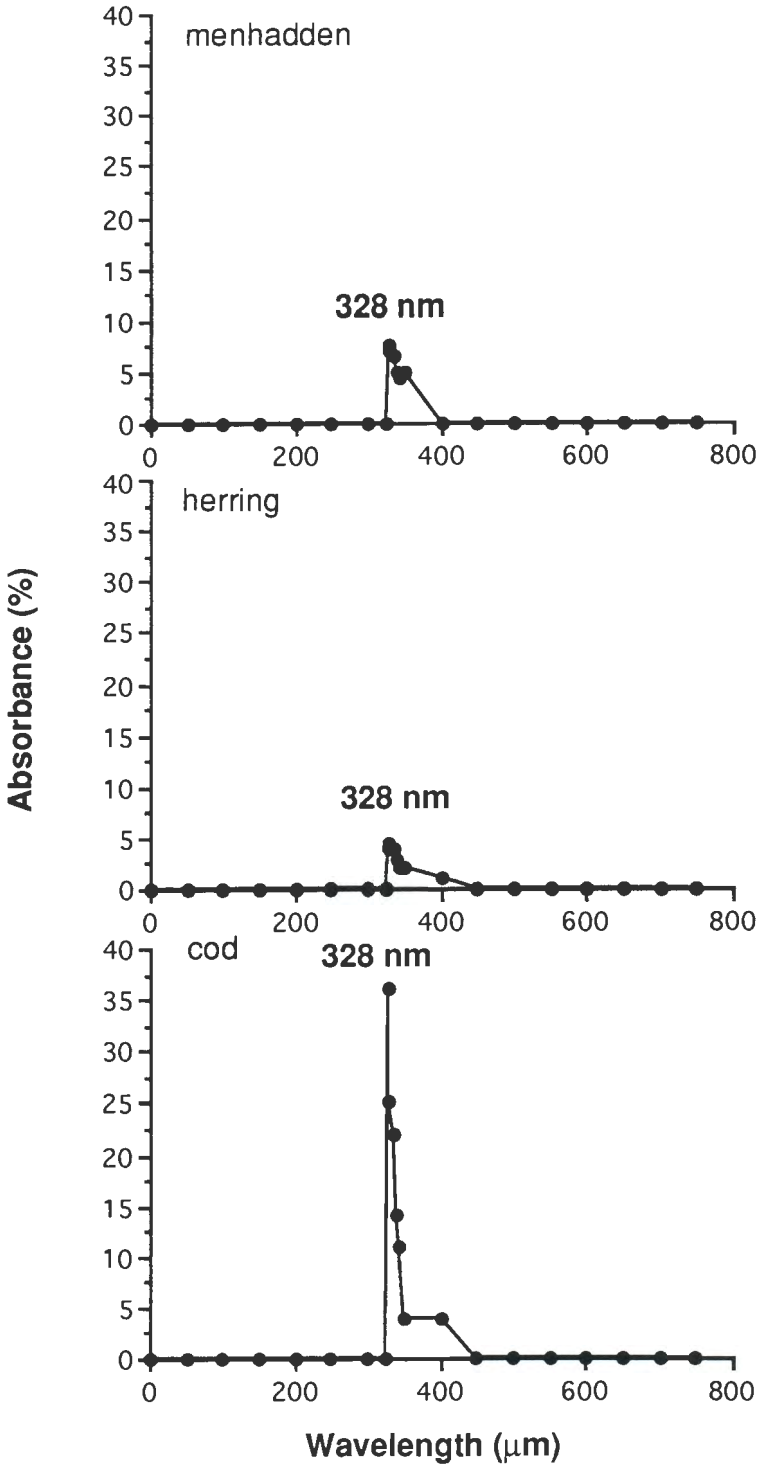
menhadden
328 nm
herring
328 nm
cod
328 nm
Absorbance (%)
Wavelength (μm)
0
5
10
15
20
25
30
35
40
200
400
600
800

Figure 2.

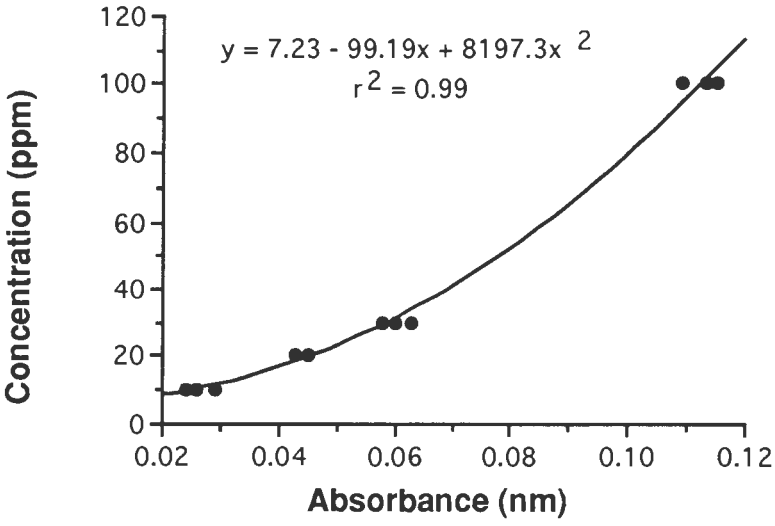
y = 7.23 - 99.19x + 8197.3x 2
r2 = 0.99
Concentration (ppm)
120
100
80
60
40
20
0
0.02
0.04
0.06
0.08
0.10
0.12
Absorbance (nm)

Figure 3.

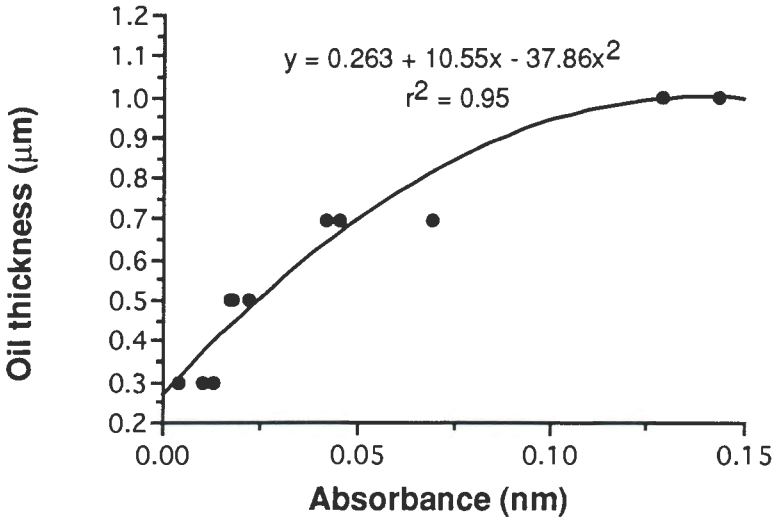
y = 0.263 + 10.55x - 37.86x2
r2 = 0.95
Oil thickness (μm)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.00
0.05
0.10
0.15
Absorbance (nm)

Figure 4.

PAPER II.

ON THE ORIGIN OF OIL FILMS IN TANKS USED FOR THE MASS CULTURE OF LARVAL WALLEYE

ABSTRACT

An oil layer on the water surface of the culture tank is considered the cause for noninflation of the gas bladder of larval walleye (Stizostedion vitreum) reared in intensive culture on formulated feed. It has been presumed that the origin of the oil is from the formulated feed introduced to the system; but, this has not been substantiated. An experiment was conducted to determine whether the oily microlayer originates from the fish or the feed. Six 157-L culture tanks were stocked with 5-day-old walleye fry at a density of 30 fry/L (4,710 fry/tank). Fish in three tanks were fed formulated feed, and fish in the other three tanks were not fed. Oil thickness in tanks was measured in all tanks before stocking or adding feed and again when the fish were 7, 9, 11, 14, 16, and 18 days posthatch. Oil thickness without fish or feed (i.e., the background concentration in water supply) was estimated to be 0.114 μm (SE = 0.004). Maximum thickness (0.292 μm) of the oily surface film occurred in the fish and feed treatment when an oil globule was still present in the fish. The findings indicate that, in the critical period when gas bladder inflation occurs, at least 64.7% of the total oil thickness, exclusive of the background levels, is derived from the oil globule released from walleye fry and 35.3% from feed introduced to the culture system.

INTRODUCTION

Mass culture of larval walleye, Stizostedion vitreum, in an intensive-culture environment on formulated feed has not been practical because survival rates have been too low, typically 20% to 21 days posthatch (Krise and Meade, 1986; Kindschi and MacConnell, 1989). The major factor reducing survival has been the failure of the fry to inflate the gas bladder (Colesante et al., 1986; Barrows et al., 1988; Kindschi and MacConnell, 1989; Summerfelt, 1991). If they survive past the larval stage, fry without gas bladders exhibit decreased growth rates and lordotic skeletal malformities and are more susceptible to stress (Chapman et al., 1988; Chatain and Ounais-Guschemann, 1990). Other species experiencing this larval culture problem are striped bass, Morone saxatilis, red sea bream, Pagrus major, and gilt-headed bream, Sparus auratus (Doroshev and Cornacchia, 1979; Kitajima et al., 1981; Chatain and Ounais-Guschemann, 1990).

The factors involved in gas bladder inflation in walleye are not completely understood. The most widely accepted explanation for noninflation of the gas bladder in an intensive culture environment is that an oily microfilm at the air-water interface of culture tanks prevents the larvae from penetrating the surface to gulp air (Barrows et al., 1993; Chatain and Ounais-Guschemann, 1990). Walleye are physostomous as larvae and, although experimental observations are lacking, penetration of the air-water interface seems to be necessary to initiate inflation of the gas bladder. Kitajima et al. (1981) demonstrated that larvae of the red sea bream would not inflate the gas bladder when their access to the water surface was blocked by a layer of liquid paraffin, and Chapman et al. (1988) stated that striped bass reared in aquaria with glass ceilings did not inflate their gas bladders.

The most prominent explanation for this physical barrier at the water surface is the oily surface layer observed to form in culture tanks. Colesante et al. (1986) suggested that this oil film originated from the formulated feed introduced to the culture system. The W-16 feed used at that time was especially oily. Chapman et al. (1988) speculated that the oil may originate from food and feces or be released from oil globules of dead fish. Our observations suggest that oil on the surface of walleye culture tanks is related to the dissolution of the oil globule.

We used a procedure developed for the sampling and quantification of oil surface microlayers in larviculture tanks to determine whether the oil present in walleye culture tanks originates from the feed introduced to the system or from the fish themselves.

METHODS

Oil standards and sampling methodology

The methodology used to sample and quantify oil microlayer thicknesses in this experiment is described by Boggs and Summerfelt (unpublished). The method employs a glass plate and solvent to sample the oil film from the tank surface, and a colorimetric procedure to quantify the concentrations of oil and solvent. Oil thickness is estimated by comparing oil thicknesses on aquaria prepared with cod oil.

The culture system

The culture system consisted of six cuboidal tanks each with a 157-L capacity. All tanks were equipped with a center standpipe drain and a circular flow pattern. The culture system was operated as a single pass, flow through system with one exchange per hour. The water was dechlorinated by using activated carbon filters, and sodium sulfite was added to neutralize any residual chlorine not removed by the carbon filter. The pH was maintained at approximately 7.8. Water temperature was maintained between 15 and 16° C throughout the experimental period.

Walleye were obtained as eyed-eggs from London Fish Hatchery, London, Ohio, and incubated at 15° C in standard 3-L cylindrical hatching jars until hatching was complete. Fry were stocked in all tanks at a density of 30 fry/L when the fry were 5 days old. Fry feed Kyowa B-400 and B-700 (Biokyowa, Inc., Chesterfield, MO)[1] were fed to fish in three of the culture tanks (feed treatment) whereas fish in the three tanks were not fed (no-feed control)(Table 1).

Data collection

Oil film samples were taken 3 days a week beginning when the fish were 7 days old (posthatch) and terminating on the 18th day posthatch. Two samples were taken

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[1] Use of trade or manufacturer names does not imply endorsement.

from every tank each sampling day, and the mean of these two measurements, the tank mean, was used in the analysis. The tanks were also sampled before the introduction of the fry to determine the thickness of any oil present without fish or feed (i.e., oil derived from the water supply).

Tanks were cleaned and dead fish counted daily . Fish were examined on days 12 and 16 posthatch for inflation of the gas bladder and measurements of the oil globule. At termination of the experiment, fish in all tanks were counted to determine survival, and 100 fish from each tank were examined for inflation of the gas bladder.

Analysis

The tank means for the oil thicknesses in feed and no-feed treatments were plotted against days posthatch and described with a rectilinear regression. Differences between treatment means on every sampling day (3 replicates per treatment) were analyzed by an unpaired t-test. Also, the mean oil thicknesses for each treatment were analyzed for three oil globule status categories (6 replicates per category): present (days 7 and 9), transitional (days 11 and 14), and absent (days 16 and 18) by one factor ANOVAs and Duncan's multiple comparison test. Statistical differences were considered significant at the 5% level ($P \leq 0.05$).

RESULTS

Oil thickness comparisons

Background oil thickness in the culture tanks before the introduction of feed or fry averaged 0.114 μm (SE = 0.004). The oil must originate from the massive plumbing and pumping system of the city and university before the water reaches the laboratories. The laboratory does not use air compressors or oil lubricated pumps.

In the six comparisons, when fry were 7, 9, 11, 14, 16, and 18 days posthatch, only on day 7 posthatch was the oil thickness significantly greater in the treatment given feed than in the control treatment (Table 2).

Oil layer thickness differed significantly between treatments on day 7 posthatch when the oil layers in the feed treatment were greater than those in the no-feed control ($P = 0.009$). All other treatment differences throughout the experimental period were not statistically significant.

In both treatments, oil layers were significantly thicker in the oil-globule-present stage than during the transitional stage or after oil globule absorption. There were no significant differences in oil thickness between the transitional and absent status categories in any of the treatments (Table 2).

Oil thickness was measured in all tanks before stocking or adding feed, and these values were subtracted from oil layer estimates on days 7 and 9 posthatch. By comparing oil thicknesses in the feed and control treatments, it was possible to estimate that 54.5 % of the oil of the oil thickness was derived from the fish on day 7 posthatch and 64.7 % on day 9 posthatch.

Fry performance comparisons

The incidence of gas bladder inflation was greater in the no-feed control (36.3 %) than in the feed treatment (21.7 %). The survival in the feed treatment (14.7%) was not significantly different than that observed in the control treatment (8.7%) (Table 2).

Oil globule diameter measurements of 30 fry at hatching indicated an average diameter of 0.91 mm (SE = 0.012). The oil globule diameter decreased to an average of 0.36 mm (SE = 0.008) by day 12 posthatch, and it was completely absorbed by day 16 posthatch (Figure 2). Oil globule diameter measurements on day 12 posthatch indicated fry in the feed treatment had significantly larger oil globules than fry in the no-feed control ($P = 0.016$).

Dead fish counts in both treatments increased until termination of the experiment.

DISCUSSION

In both treatments, maximum oil layer thickness was observed on days 7 or 9 posthatch. This is consistent with a preliminary experiment performed in this laboratory during the 1992 culture season. Oil thicknesses differed significantly only between the tank treatments on day 7 posthatch. After this time, oil thicknesses averaged 0.06 μm (SE = 0.006). The uniformity of oil thicknesses in both treatment groups suggests that the oil layer is mainly derived from the fish, not the formulated feed.

When fry development was stratified into three oil-globule-status categories, oil globule present (days 7 and 9 posthatch), transitional (days 11 and 14 posthatch), and oil globule absent (days 16 and 18 posthatch), an analysis of variance (ANOVA) indicated highly significant differences in oil layer thickness among these categories (Table 2). Duncan's multiple comparison test indicated that oil layers were significantly thicker during the early stage of fry development when the oil globules were the largest. Oil thickness on the tank surface declined as the oil globule was absorbed. Because both feeding amounts and the incidence of dead fish in the culture tanks increased as the experimental period progressed, this suggests the oil globule as the point of origin of most of the material responsible for the formation of the oil layer.

Oil layer thicknesses in the feed treatment were thicker than the no-feed control on days 7 and 9 posthatch, and the oil layers subsequently declined to very similar levels by day 11 posthatch. Measurements of the diameter of the oil globules made on day 12 posthatch indicated fry in the no-feed control had significantly smaller oil globules than fry in the feed treatment. Intuitively, fry in the no-feed control

treatment were likely to be metabolizing more of the oil than fry with an exogenous feed, leaving less oily material to gather on the tank surface.

In a previous study conducted in this laboratory in tanks with an upflow design (Loadman et al. 1989), a significant increase in gas bladder inflation was found in three tanks equipped with a surface spray (GBI averaged 37.4%) as compared with three tanks without a surface spray (GBI averaged 18.3%). However, survival in both groups was less than 2.0%. We attribute the increase in survival observed in the present study to the use of a circular flow in the tanks, a flow-through rather than a recycle system, and changes in feeding regime (increased feed at 5 to 10 d posthatch) when fish begin to utilize exogenous energy sources.

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Table 1.— Feeding amounts and ratios for the feed treatment throughout the experimental period.

Fish age (days posthatch)	Feed ratio B-400:B-700	Quantity (g/1000 fry)
3	100:0	1.5
4-5	100:0	3.0
6-9	75:25	8.0
10-12	75:25	7.2
13-15	50:50	7.0
16-18	25:75	8.0

Table 2.— Comparisons of mean oil thickness (μm) by treatment and by oil-globule-status categories. Category means that do not share a letter in common are significantly different at the 5% level (Duncan's multiple comparison test).

Days posthatch	Oil thickness (μm)[a]		t-Test	
	Feed	Control	t-Value	P-value
Background oil w/o fish or feed	0.114	0.114		
Oil globule present (1)				
7	0.292	0.150	4.732	0.009
9	0.278	0.170	1.880	0.133
Category 1:	0.285[x]	0.160[x]		
Transitional (2)				
11	0.069	0.089	-0.632	0.562
14	0.040	0.073	-1.248	0.280
Category 2:	0.055[y]	0.081[y]		
Oil globule absent (3)				
16	0.047	0.050	-0.149	0.889
18	0.077	0.085	-0.229	0.829
Category 3:	0.062[y]	0.068[y]		
ANOVA				
F-value	106.26	15.93		
P-value	0.002	0.025		

[a] Values adjusted by subtracting average background level (i.e., oil thickness before introduction of fish or feed.)

Table 3.— Comparisons of gas bladder inflation (GBI), survival, total length (TL), growth rate (GR), and feed present in gut (FP) in each treatment when fish were 19 days old.

	Treatment		t-Test	
	Feed	No feed	t-Value	P-value
GBI (%)	21.7	36.3	-1.023	0.364
Survival (%)[1]	14.7	8.7	1.515	0.204
TL (mm)	10.5	9.7	2.451	0.070
GR (mm/d)[2]	0.022	0.018	2.460	0.069

[1]Survival percentages were adjusted by using the arcsin percentage transformation.

[2]Specific growth rate = $\frac{\ln L_1 - \ln L_0}{\Delta T}$, where $L_0 = 7.1$

Figure 1. Formation of the oil layer in the feed and no-feed treatments. Each data point represents the mean of two oil thickness measurements.

Figure 2. Rates of absorption of the oil globule by the walleye fry. Each data point represents 30 fish.

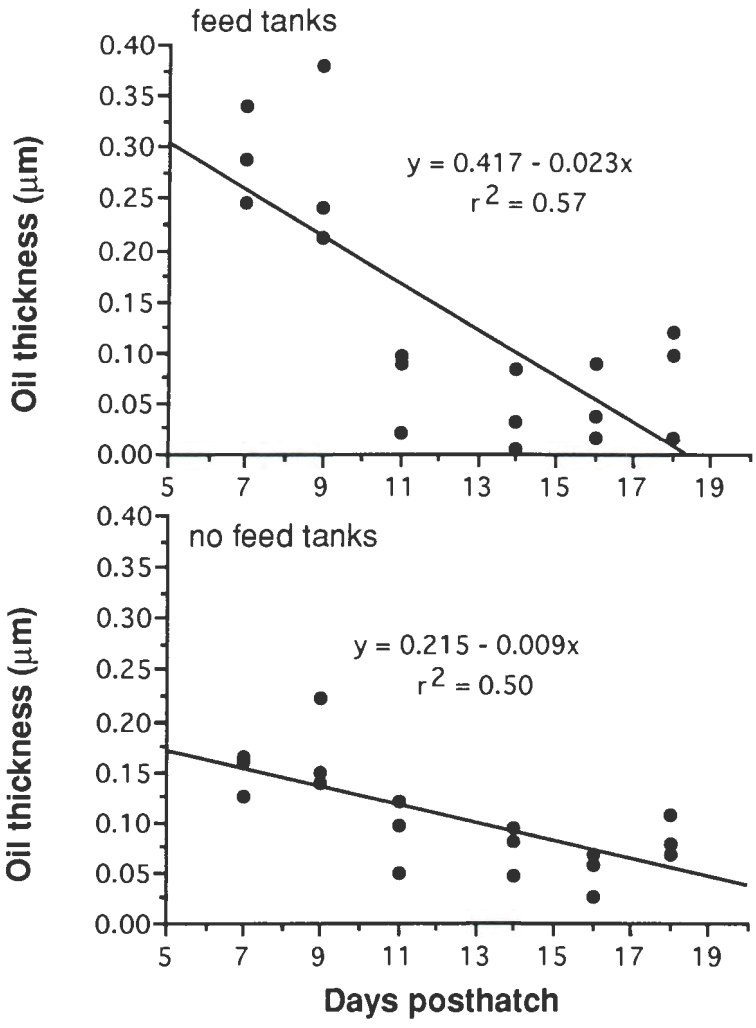
feed tanks
y = 0.417 - 0.023x
r2 = 0.57
no feed tanks
y = 0.215 - 0.009x
r2 = 0.50
Oil thickness (μm)
Oil thickness (μm)
Days posthatch
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
5
7
9
11
13
15
17
19

Figure 1.

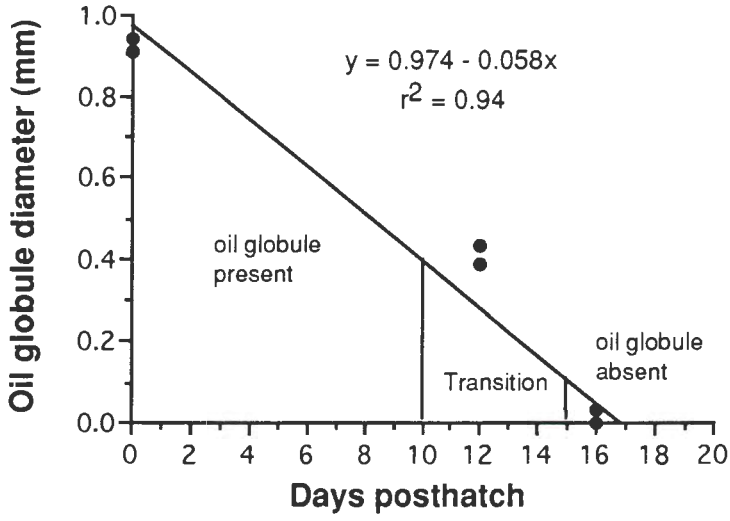
Oil globule diameter (mm)
1.0
0.8
0.6
0.4
0.2
0.0
y = 0.974 - 0.058x
r2 = 0.94
oil globule
present
Transition
oil globule
absent
0
2
4
6
8
10
12
14
16
18
20
Days posthatch

Figure 2.

PAPER III.

FATTY ACID ANALYSIS AND COMPARISON OF LIPIDS EXTRACTED FROM LARVAL WALLEYE AND FROM CULTURE TANK SURFACES

ABSTRACT

Lipids extracted from 7 day old walleye (Stizostedion vitreum) and lipids sampled from the water surface of culture tanks were analyzed for fatty acid composition using gas chromatography. Ten of eleven fatty acids detected in lipids from the larvae were present in water surface samples. Fatty acids identified from samples included seven saturated hydrocarbon chains (14:0, 15:0, 16:0, 17:0, 18:0, 20:0, and 22:0), two monoenes (16:1 and 18:1), and two polyenes (18:2 and 18:3). Only one fatty acid, docosanoic acid (22:0), was detected in samples from the larvae and not detected in surface samples. The percent composition of constituent fatty acids from the two sources were not significantly different ($P > 0.05$). The similarity in fatty acid composition of the lipids from the two sources suggests that oil responsible for forming oil films on the surface of culture tanks originates from the larvae.

INTRODUCTION

Noninflation of the gas bladder (NGB) in intensively cultured larval walleye (Stizostedion vitreum) is considered a major problem in culturing this species (Barrows et al. 1988; Summerfelt 1991). Fish without an inflated gas bladder swim poorly, exhibit decreased growth rates, lordotic skeletal malformities, and are more susceptible to stress (Chapman et al. 1988; Chatain and Ounais-Guschemann 1990).

The factors involved in gas bladder inflation in walleye are not completely understood, but environmental and developmental hypotheses have been explored (Battaglene and Talbot 1990; Bulak and Heidinger 1980). The most widely accepted explanation for NGB in intensive culture is that an oily microfilm at the air-water interface of culture tanks presents a physical barrier that prevents the larvae from penetrating the surface film to gulp air for first filling of the gas bladder. The origin of this oily material has not been determined. Colesante et al. (1986) and Barrows et al. (1988) suggested the oil originated from the formulated feed introduced to the culture system. Chapman et al. (1988) speculated that the oil may originate from food and feces or released from the oil globules of dead fish. Boggs and Summerfelt (unpublished) related oil layer thicknesses in culture tanks to larval development; oil thickness on the surface of culture tanks decreased as the oil globule diminished in size.

We used gas chromatography technology to compare constituent fatty acids of lipids extracted from larval walleye and sampled from the surface of culture tanks. Similarities in fatty acid composition would suggest the oil originates from the fish rather than formulated feed.

METHODS

The culture system consisted of three 37.9-L glass aquaria filled with 31.25-L of water. Water was supplied to each aquaria by a subsurface inflow at a rate of 0.5 exchanges per hour. Water exited through a surface drain covered by 710-µm Nitex® mesh. A previous study performed in this laboratory indicated screen material retained oil surface microlayers and would not allow them to exit surface drains.

Walleye were obtained as eyed-eggs from London Fish Hatchery, London, Ohio and incubated at 16° C in standard 3-L cylindrical hatching jars. Hatching jars emptied into a 157-L holding tank where larvae were held until stocking to eliminate contamination of culture tanks with oils from hatching eggs. Larvae were stocked at a density of 150/L (approximately 4950 larvae/tank) when the larvae were 1 d old and cultured at 17° C until 7 d posthatch. Larvae were not fed at any time during the experimental period and aquaria were covered to prevent airborne material from contaminating the culture tanks. At 7 d posthatch, 30 larvae were removed from each tank and examined for gas bladder inflation and measurements of total length and oil globule diameter.

Lipids were extracted from 7 d posthatch larval walleye using a methodology described by Folch et al. (1957). Whole larvae were homogenized with 2:1 chloroform:methanol solution and filtered through fat-free paper into a separatory funnel. The solution was mixed with 0.2 times its volume of water and the lower layer drained into a centrifuge tube, then centrifuged for two minutes at 2400 rpm. After centrifugation, the upper layer and any interfacial fluff was siphoned off and the lower layer and remaining rinsing fluids were made into one phase by the addition of 1 ml of methanol.

Lipids were sampled from the surface of culture tanks containing 7 day-old walleye by touching a circular piece of wire screen (1000-μm mesh) gently to the surface of the culture tanks and dipping it into 30-ml of chloroform in a glass petri dish. The lipids that attached to the wire screen were eluted with the chloroform wash. The screen was repeatedly touched to the water surface and rinsed in the petri dish until the oil layer on the surface of the culture tank was visibly reduced and a 30-ml sample of oil and chloroform was obtained.

Samples from larvae and tank surfaces were evaporated using a warm water bath and nitrogen gas and prepared for analysis by the addition of sodium methoxide in methanol and hexane. After 30 min with gentle mixing every 10 min, 0.8 ml of water and 1.5 ml of hexane were added and the hexane layer was analyzed using a Hewlett Packard 5890A gas chromatograph fitted with a hydrogen flame detector and 15-M Durabond capillary column with a 0.25 μ film thickness. Fatty acids in samples were identified by comparison to a standard analyzed under identical conditions.

RESULTS

Fatty acids identified from the samples included seven saturated methyl esters (14:0, 15:0, 16:0, 17:0, 18:0, 20:0, and 22:0), two monoenes (16:1 and 18:1), and two polyenes (18:2 and 18:3). The saturated esters comprised 34.19% of the total fish sample and 36.32% of the total surface sample. Monoenes contributed 31.64% and 21.78% to the total lipid sample from the fish and from the tank surface, respectively. Polyenes comprised 18.87% of the fish sample and 26.92% of the surface sample. Oxidation products composed 15.0 to 15.3% of the total lipid sample from the surface and fish sources, respectively (Table 1). Only one fatty acid, docosanoic acid (22:0), was detected in samples from the larvae and not detected in surface samples. All other constituent fatty acids were detected in both samples. Chi^2 comparisons of percent composition of constituent fatty acids from the two sources were not significantly different at the 5% level.

At the termination of the experiment (7 days posthatch), walleye larvae averaged 9.3 mm (SE = 0.05) in length, oil globule diameter averaged 0.5 mm (SE = 0.02) and gas bladder inflation was 5%. There was no mortality in the culture tanks until 5 d posthatch and mortality was low throughout the experimental period (< 2%).

DISCUSSION

By hatching the walleye larvae into a holding tank before stocking and not feeding the larvae during the experimental period, we were able to eliminate lipids and other perivitelline material released from hatching eggs and formulated feeds as possible sources of oils contributing to the formation of the oil surface microlayer. By 3 d posthatch, daily observations indicated a visible oil layer on the surface of the culture tanks. This layer became more pronounced as the experimental period progressed. Mortality was very low throughout the experimental period (< 2%) suggesting that dead fish were not responsible for the majority of the oil. Lipids extracted from larvae and from the surface of culture tanks were very similar in the types and concentrations of fatty acids. It seems the majority of the oil responsible for the formation of the oil surface microlayer in culture tanks is originating from live walleye larvae.

Webster and Lovell (1990) analyzed fatty acids in total lipids from 1 day-old striped bass (Morone saxatilis) and reported saturated fatty acids comprised 14.08%, monoenes comprised 50.18% and polyenes comprised 31.0% of the total lipid sample. Eldridge et al. (1983) analyzed fatty acids from the oil globule of unhatched striped bass eggs. Fatty acids from this source were predominantly monoenes (63.6%) and polyenes (27.2%) while saturated fatty acids composed only 8.5% of the total sample.

The results of this study differ substantially from the fatty acid composition of lipids sampled from larval walleye and from culture tank surfaces. However, larvae in this experiment were not fed at any time during the 7 d experimental period which could have affected total lipid amounts and composition. Martin et al. (1984) stated that striped bass larvae starved for two days exhibited a dramatic decrease in total

known fatty acid levels when compared to fed larvae. It is possible that not feeding the walleye larvae during the experimental period caused some fatty acids to decrease to nondetectable levels. Differences in lipid composition among species are well documented and could also account for some of the differences between lipids sampled from striped bass larvae and from walleye larvae.

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Table 1. Component fatty acids of lipids (%) sampled from larval walleye and from the surface of culture tanks. All differences in fatty acid composition and concentration were non-significant at the 5% level (Chi square comparisons).

	Source	
	Fish	Tank surface
Saturated (total %)	(34.19)	(36.32)
14:0	1.53	1.67
15:0	1.66	1.64
16:0	11.76	12.61
17:0	11.67	15.1
18:0	2.13	4.1
20:0	1.9	1.2
22:0	3.54	—
Monoenes (total %)	(31.64)	(21.78)
16:1	12.45	6.55
18:1	19.19	15.23
Polyenes (total %)	(18.87)	(26.92)
18:2	11.57	21.52
18:3	7.3	5.4
Oxidation products (total %)[1]	(15.3)	(14.98)

[1]Unidentifiable carbon chains.

PAPER IV.

EVALUATION OF TWO METHODS FOR REMOVAL OF OIL FILMS FROM THE SURFACE OF TANKS USED FOR THE CULTURE OF LARVAL WALLEYE

ABSTRACT

The presence of an oil layer on the water surface is considered to be the cause for noninflation of the gas bladder of larval walleye (Stizostedion vitreum) reared in intensive culture on formulated feed. A water-surface-spray and an oil trap were compared to determine their effectiveness for reducing the thickness of the oil layer during the critical period of gas bladder inflation (5-15 days posthatch) and for enhancing gas bladder inflation (GBI). The experimental design included three treatments (surface-spray, oil trap, and a control with no oil removal apparatus). Oil thickness was measured in all tanks when the fish were 7, 9, 11, 14, 16, and 18 days old. Maximum oil layer thickness (0.290 - 0.192 μm) in all treatments occurred when fish were 7 and 9 days old, when their oil globule was at maximum diameter. The thickest oil layers typically occurred in the control, and minimum thickness in the treatment with the surface spray. When the fish were 18 days old, differences in GBI among treatments were highly significant ($P < 0.001$); GBI was 62.3% for fish in the spray treatment, 41.7% for fish reared in tanks with the oil trap, and 21.7% in the control. Small differences in oil thickness among treatments were large enough to produce a significance difference in GBI.

INTRODUCTION

Mass culture of larval walleye, Stizostedion vitreum, in an intensive culture environment on formulated feed has not been practical because survival rates have been too low, typically 20% to 21 days posthatch (Krise and Meade 1986; Kindschi and MacConnell 1989). However, viability rates as high as 60.8% to 25 d posthatch have been reported (Moore et al. 1994). The major factor reducing survival has been the failure of the fry to inflate the gas bladder (Colesante et al. 1986; Barrows et al. 1988; Kindschi and MacConnell 1989; Summerfelt 1991). The factors involved in gas bladder inflation in walleye are not completely understood. The most widely accepted theory for noninflation of the gas bladder in an intensive culture environment is that an oily microfilm at the air-water interface of culture tanks forms a physical barrier that prevents the larvae from penetrating the surface to gulp air. Colesante et al. (1986) suggested that this oil film originated from the formulated feed introduced to the culture system. Chapman et al. (1988) suggested that the oil originates from food and feces, or released from oil globules of dead fish. Regardless of the origin of the oil, failure to remove or reduce it will result in poor gas bladder inflation and, subsequently, poor survival.

Several approaches have been used to address the oil film problem. Chatain and Ounais-Guschemann (1990) evaluated two types of water sprays, and an air blower-surface trap system to remove oil from the surface of culture tanks and reported the blower-trap system was the only system to improve inflation rates without affecting growth. Lim (1993) used a triangular oil skimmer in conjunction with an air jet system to reduce the oil film in tanks used for the larval culture of greasy grouper, Epinephelus tauvina, and the brown-marbled grouper, E. fuscoguttatus. Oil absorbent cloth has been used in an attempt to reduce oil films in larval culture tanks

(Friedmann and Bates 1988). Van Olst et al. (1990) found the sorbent cloth to be ineffective in improving the gas bladder inflation in larval striped bass (Morone saxatilis). Summerfelt (1991) reported that sorbent cloth did not improve gas bladder inflation (GBI) in intensively cultured larval walleye but walleye reared in tanks with a surface exit for the oil film had higher inflation rates than tank designs with a subsurface drain. Barrows et al. (1993) reported 98.4% GBI in walleye reared in culture tanks equipped with a surface water spray and 51.7% GBI in walleye reared in tanks without the spray. Moore et al. (1994) reported that larval walleye had substantially higher rates of survival, gas bladder inflation and viability in tanks with a water surface spray than when reared without a spray.

Oil layer thicknesses on tanks equipped with a water surface spray and an air blower and trap apparatus were sampled and quantified in order to compare these two methods for removal of oil films from larval walleye culture tanks. We collected oil samples from walleye fry culture tanks with a procedure that had been developed for the collection of microfilms from the ocean's surface (Harvey and Burzell 1972), and also used to collect water surface insecticide samples from prairie ponds (Muir et al. 1992). A spectrophotometric procedure was used for the analysis of oil in water.

METHODS

Oil standards and sampling methodology

The methodology used to sample and quantify oil microlayer thicknesses in this experiment is described in Boggs and Summerfelt (unpublished). A glass plate and solvent was used to sample the oil film from the tank surface and a colorimetric procedure used to quantify the concentrations of oil and solvent. Oil thickness was estimated by comparison with a standard curve prepared with cod oil.

The culture system

The culture system consisted of 12, 157-L cuboidal tanks. All tanks were equipped with a center standpipe drain and a circular flow pattern. The tanks were provided with one exchange of water per hour; the system was single-pass to prevent an accumulation of oils. The water was dechlorinated using activated carbon filters and the addition of sodium sulfite to remove any residual chloramines. Water pH was maintained at approximately 7.8.

Walleye were obtained as eyed-eggs from London Fish Hatchery, London, Ohio and incubated at 15°C in standard 3-L cylindrical hatching jars until hatching was complete. Fry were stocked in all tanks on day 5 posthatch at a density of 30 fry/L (4710 fry/tank). Water temperature was maintained between 15 and 16°C throughout the experimental period. Fry feed Kyowa B-400 and B-700 (Biokyowa Inc., Chesterfield, Mo.)[1] , a microparticulate formulation, were fed in amounts and ratios that were dependent on fish age (Table 1).

Three tanks were equipped with a water spray at a 45° angle to the tank surface and perpendicular to the water flow (Moore et al. 1994). Three tanks were equipped with oil traps consisting of Nalgene® tubing in a spiral configuration similar to that

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[1] Use of trade or manufacturer names does not imply endorsement.

used by Chatain and Ounais-Guschemann (1990). The trap was attached to the screen that covered the standpipe. Air was blown across the water surface and pushed the surface film into the trap where it could be manually removed. The control treatment consisted of three tanks with no oil removal apparatus.

Oil film samples were taken three days a week starting at day 7 posthatch and terminating day 18 posthatch. Two samples were taken from every tank each sampling day and the mean of these two measurements, the tank mean, used in the analysis. The tanks were also sampled before the introduction of the fry to determine if any oil was already present in the system.

Tanks were cleaned and dead fish counted daily. Fish were sampled twice during the experimental period and examined for inflation of the gas bladder and measurements of the oil globule. At termination of the experiment, fry in all tanks were counted to determine survival and 100 fry from each tank were examined for inflation of the gas bladder. Fifty fry from each tank were also measured to evaluate differences in total length and specific growth rate. A size at hatch of 7.1 mm was used for the specific growth rate calculation (Siegwarth and Summerfelt 1993).

Analysis

The tank means for the oil thicknesses in all treatments were plotted against days posthatch and described with a linear curve. An analysis of variance (ANOVA) was used to examine differences between treatment means on every sampling day. Also, the mean oil thicknesses for each treatment were analyzed for three oil globule status categories: present (days 7 and 9), transitional (days 11 and 14) and absent (days 16 and 18) by one factor ANOVAs using Duncan's multiple comparison test. Differences were considered significant at the 5% level ($P \leq 0.05$).

RESULTS

Oil thickness comparisons

In all treatments, maximum oil layer thickness was on day 7 or day 9 posthatch, thickness decreased on day 11 posthatch and by 14 days posthatch, oil thickness was similar in all treatments. Oil layer thicknesses were similar in all treatments on sample days 14, 16 and 18. There were no significant differences in oil layer thickness among treatments throughout the experimental period.

Comparisons of the three oil globule status categories indicated that in all treatments, oil layers were significantly thicker when the oil globule was present in the fry than during the transitional stage and after the oil globule had been absorbed. There were no significant differences between the transitional and absent status categories in any of the treatments (Table 2).

Fry performance comparisons

Among all fry performance comparisons, only the differences in GBI were statistically significant (Table 3). The highest incidence of gas bladder inflation (62.3%) was in the spray treatment followed by the trap treatment (41.7%), and the control (21.7%). The best overall survival (16.6%) was in the trap treatment followed by the control (14.7%) and the spray treatment (13.9%), but differences in survival among treatments were not significant (Table 3). Feed acceptance, as measured by the presence of food in the gut (FP, Table 3) was greatest in the trap treatment (69.7%) followed by the control (64.7%), and the spray treatment (54.3%), but these differences were not significant. Likewise, differences in total length and specific growth rate when the fish were 19 days old were small and not significant (Table 3).

Oil globule diameter measurements of thirty fry at hatching had a mean diameter of 0.91 mm (SE = 0.012). The oil globule diameter decreased to an average of 0.36

mm (SE = 0.008) by day 12 posthatch, and by day 16 posthatch the oil globule had been completely absorbed by most of the fry.

Counts of dead fish in all treatments increased from 10 to 18 days posthatch with the exception of the counts in the spray tanks on day 7 posthatch. On that day, 1,197 fry died in one spray tank due to insufficient inflow causing the count for that day to be much higher than in the other tanks.

DISCUSSION

Although oil layer thickness did not differ among treatments, rates of gas bladder inflation were significantly greater in the spray and trap treatments, indicating a positive influence on gas bladder inflation. Except for gas bladder inflation, however, none of the fry performance characteristics differed significantly among treatments. Chatain and Ounais-Guschemann (1990) stated the only oil removal system they evaluated that effectively increased gas bladder inflation while not adversely affecting growth rates was an air blower and trap. Our study indicates that both water-surface-sprays and blower and trap apparatus have a positive effect on gas bladder inflation while having little effect on growth rates or acceptance of feed.

We divided the absorption of the oil globule by the fry into three oil-globule-status categories to relate oil thickness to larval development. This analysis revealed that oil layers were significantly thicker during the early stage of fry development when the oil globules were the largest. Oil layers subsequently declined as the oil globules were absorbed. Because both feeding amounts and the incidence of dead fish in the culture tanks increased as the experimental period progressed, this suggests that the oil globule, rather than the fish food, was the major source for the oil on the tank surface.

According to McElman and Balon (1979), walleye reared at 15°C will inflate their gas bladders sometime between days 7 and 14 posthatch. In this experiment, oil layers were at their thickest during that critical period. Although the oil thickness in tanks with water spray and trap apparatus did not differ significantly from the control tanks, the fry in these tanks exhibited the highest incidence of gas bladder inflation. These data suggest that even small differences in oil thickness were large enough to significantly affect gas bladder inflation.

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Figure 1. Tank design and flow patterns for the control (A), the spray (B), and the trap (C) treatments.

Figure 2. Formation of the oil layers in all tank treatments. Each data point represents the mean of two oil thickness estimates.

Figure 3. Rates of absorption of the oil globule by the walleye fry. Each data point represents 30 fish.

Figure 4. Comparison of dead fish collected from the culture tanks between 8 and17 days posthatch.

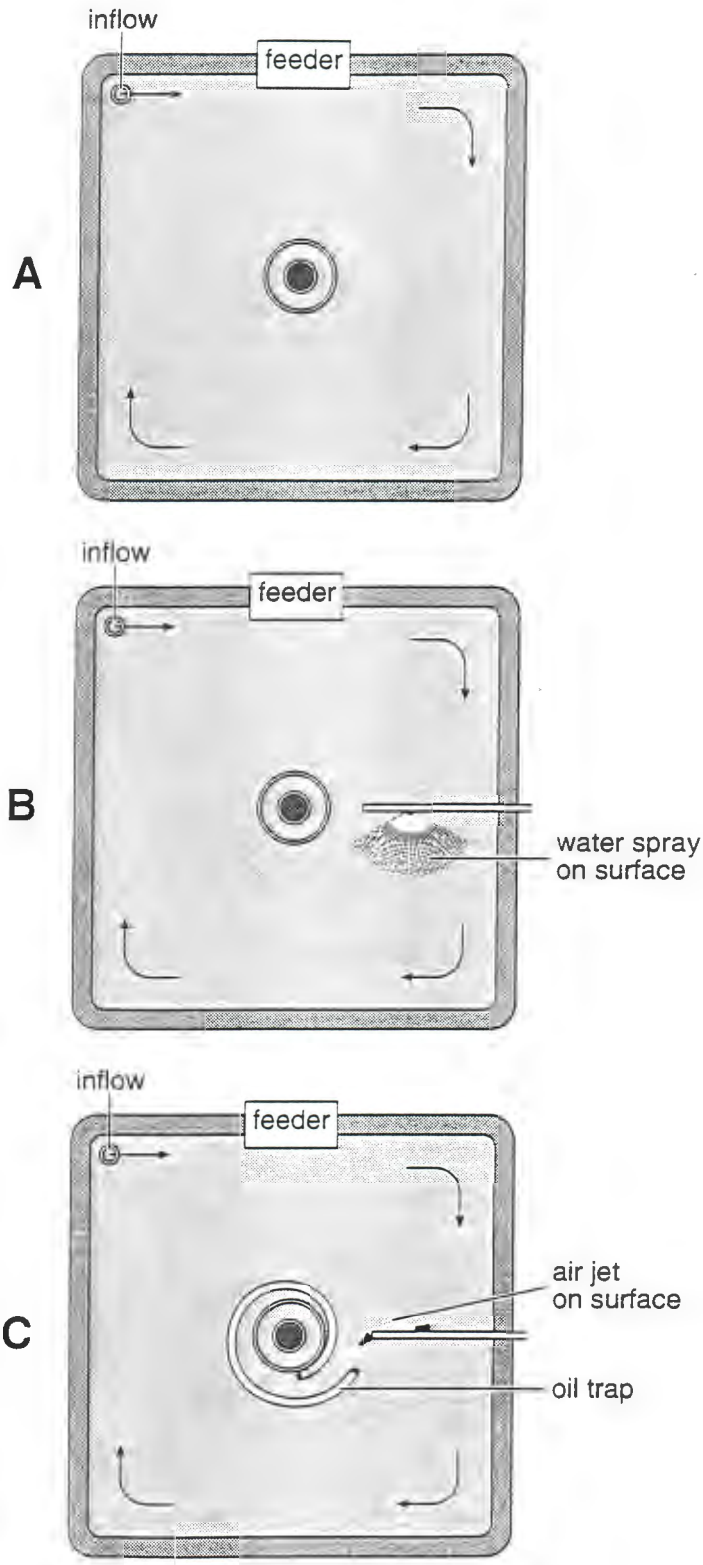
inflow
feeder
A
inflow
feeder
B
water spray
on surface
inflow
feeder
air jet
on surface
C
oil trap

Figure 1.

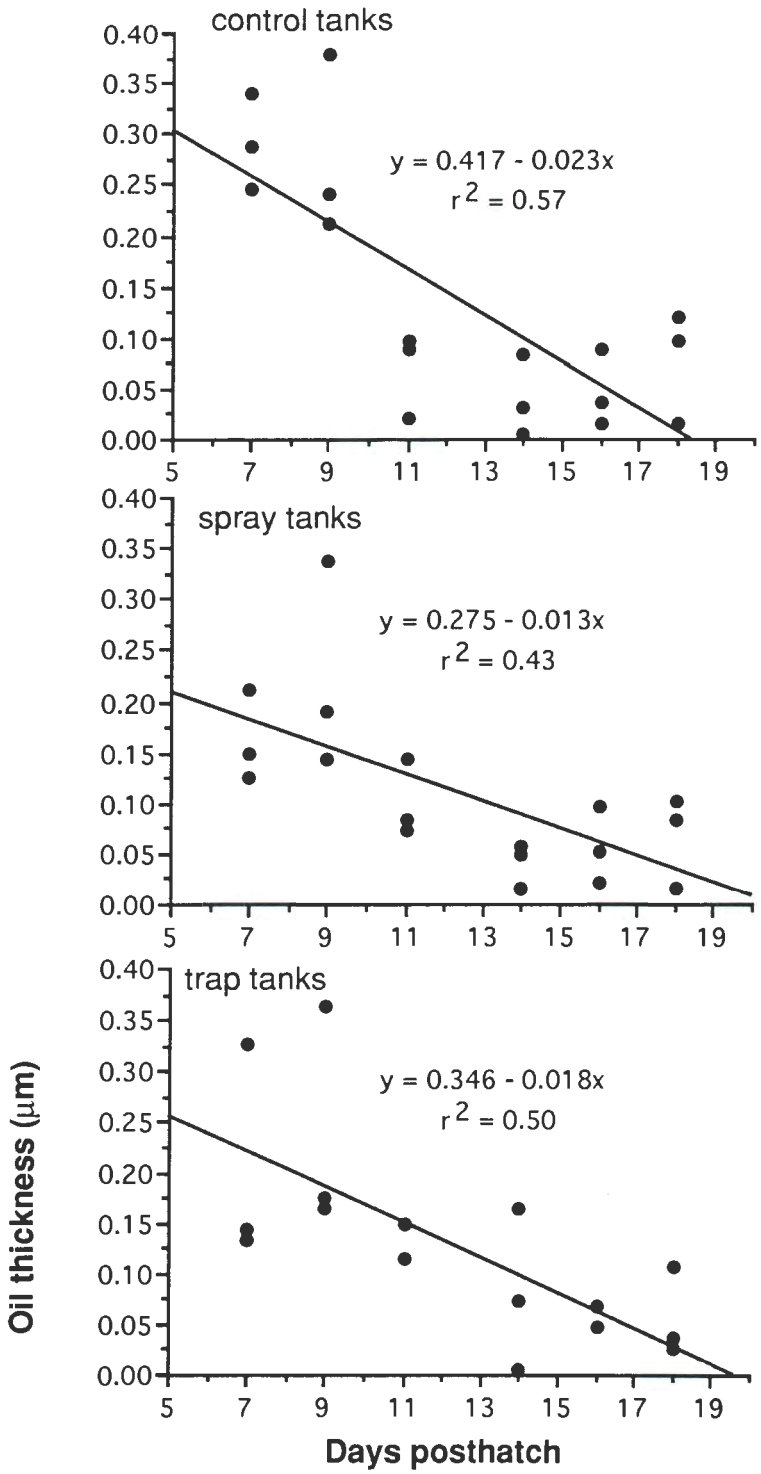
control tanks
y = 0.417 - 0.023x
r2 = 0.57
spray tanks
y = 0.275 - 0.013x
r2 = 0.43
trap tanks
y = 0.346 - 0.018x
r2 = 0.50
Oil thickness (µm)
Days posthatch
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
5
7
9
11
13
15
17
19

Figure 2.

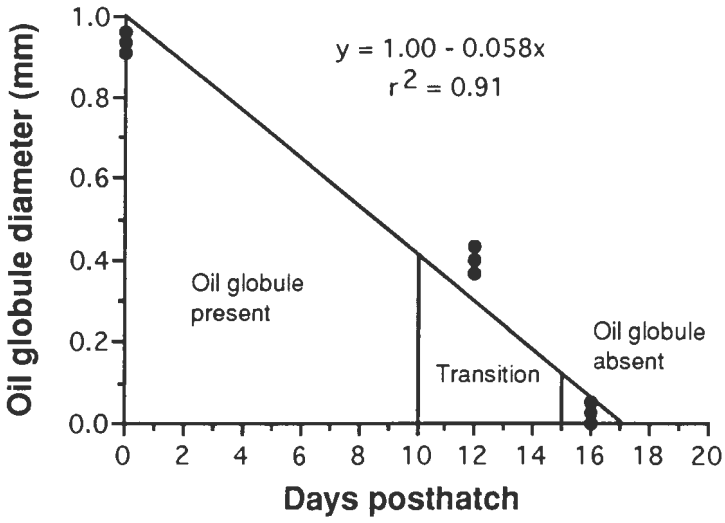
Oil globule diameter (mm)
1.0
0.8
0.6
0.4
0.2
0.0
y = 1.00 - 0.058x
r2 = 0.91
Oil globule
present
Transition
Oil globule
absent
0
2
4
6
8
10
12
14
16
18
20
Days posthatch

Figure 3.

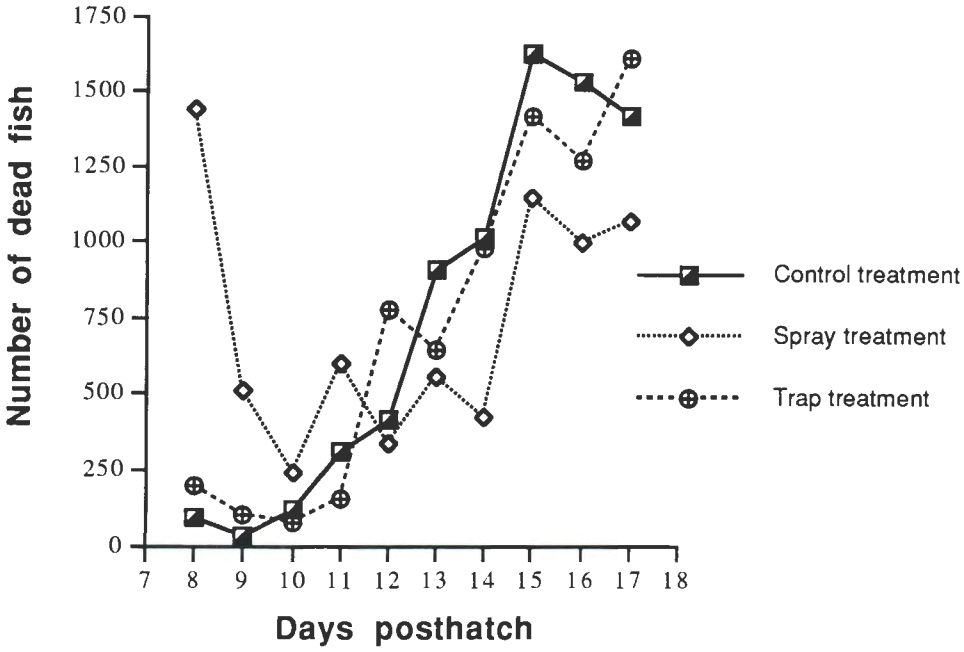
Number of dead fish
1750
1500
1250
1000
750
500
250
0
7
8
9
10
11
12
13
14
15
16
17
18
Days posthatch
Control treatment
Spray treatment
Trap treatment

Figure 4.

Table 1.— Feeding amounts and ratios used for all treatments throughout the experimental period.

Fish age (day posthatch)	B-400:B-700	Quantity (g/1000 fry)
3	100:0	1.5
4-5	100:0	3.0
6-9	75:25	8.0
10-12	75:25	7.2
13-15	50:50	7.0
16-18	25-75	8.0

Table 2.— Comparisons of mean oil thickness (μm) by treatment and by oil globule status categories. Category means which do not share a letter in common are significantly different at the 5% level (Duncan's multiple comparison test).

Days posthatch	Oil thickness (μm)[a]			ANOVA	
	Control	Spray	Trap	F-Value	P-value
Background oil w/o fish or feed	0.114	0.114	0.114		
Oil globule present (1)					
7	0.302	0.162	0.202	2.605	0.153
9	0.278	0.223	0.235	0.242	0.792
Category 1:	0.290^{x}	0.192^{x}	0.218^{x}		
Transitional (2)					
11	0.069	0.100	0.137	2.896	0.132
14	0.040	0.041	0.081	0.592	0.583
Category 2:	0.054^{y}	0.071^{y}	0.109^{y}		
Oil globule absent (3)					
16	0.047	0.057	0.039	0.187	0.834
18	0.077	0.067	0.057	0.127	0.883
Category 3:	0.062^{y}	0.062^{y}	0.048^{y}		
ANOVA					
F-value	92.79	8.76	19.69		
P-value	0.002	0.055	0.018		

[a] Values adjusted by subtracting average oil background level.

Table 3.— Comparisons of gas bladder inflation (GBI), survival, total length (TL), growth rate (GR) and feed present in gut (FP) by treatment when fish were 19 days old. Means which do not share a letter in common are significantly different at the 5% level (Duncans multiple comparison test).

	Treatment			ANOVA	
	Feed	Spray	Trap	F-value	P-value
GBI (%)	21.7[a]	62.3[b]	41.7[c]	86.54	0.0001
Survival (%)[1]	14.7[a]	13.9[a]	16.6[a]	0.285	0.761
FP (%)	64.7[a]	54.3[a]	69.7[a]	1.42	0.312
TL (mm)	10.5[a]	10.3[a]	10.2[a]	0.736	0.518
GR (mm/d)[2]	0.022[a]	0.021[a]	0.020[a]	0.693	0.536

[1]Survival percentages were adjusted using the arcsin percentage transformation.

[2]Specific growth rate = $\frac{\ln L_1 - \ln L_0}{\Delta T}$ where $L_0 = 7.1$.

PAPER V.

THE EFFECTS OF SCREEN MATERIAL ON THE EXIT OF OIL FILMS FROM LARVAL CULTURE TANKS

ABSTRACT

Eight different screen types and two mesh sizes were evaluated for the ability to allow an oil surface microlayer to pass out of larval fish culture tanks. All screens were strongly lipophilic, and oil would not pass through the screens without the use of a solvent. The two screen types most commonly used in walleye culture systems, stainless-steel 1000-μm and the Nitex® 710-μm and 1000-μm mesh openings were among the most retentive (lipophilic) of the screen types. Painted Nitex® 710 μm and 1000 μm and the Tetko® polyester monofilament were the least retentive of the fabric types. There were no consistent differences in the degree of oil retention between 710-μm and 1000-μm mesh size or percent of open area (41-53%).

INTRODUCTION

Failure to inflate the gas bladder during the larval stage is the dominant obstacle in the culture of many physoclistous fish species. In physoclistous fish, the external connection to the gas bladder, the pneumatic duct, is functional for only a short time during the larval stage of the fish. Fishes without an inflated gas bladder have experienced decreased growth rates and increased susceptibility to stress and cannibalism (Doroshev and Cornacchia 1979; Chatain and Ounais-Guschemann 1990; Battaglene and Talbot 1990; Summerfelt 1991).

The most widely accepted explanation for noninflation of the gas bladder (NGB) is that a microfilm of oil on the surface of culture tanks prevents the larvae from penetrating the surface to gulp air. Colesante et al. (1986) suggested that the oil film originated from the formulated feed introduced to the culture system. Chapman et al. (1988) suggested that the oil may come from food and feces or released from oil globules of dead fish. We found that oil thickness on the surface of walleye (Stizostedion vitreum) culture tanks coincided with the dissolution of the oil globule (Boggs and Summerfelt, unpublished).

Various techniques have been devised to alleviate the oil-film problem. Barrows et el. (1988), under the assumption that the oil originated from feed introduced to the tanks, used a plastic ring to contain the spread of the feed and oil film. Oil-sorbant cloth was tested to evaluate the possibility that it could reduce oil films in culture systems (Friedmann and Bates 1988). Van Olst et al. (1990) found that this strategy was ineffective in improving gas bladder inflation of larval striped bass. Summerfelt (1991) reported that an oil sorbent cloth did not improve gas bladder inflation in intensively cultured larval walleye.

The method that has shown the most promise in improving gas bladder inflation in an intensive culture environment has been the use of a surface water spray to homogenize the oil film in the water column or to push the oil film to one side of the tank and down a surface drain (Barrows et al. 1993; Moore et al. 1994). Barrows et al. (1993) reported that inflation rates of larval walleye were significantly greater when reared with a water spray (98.4%) than without a spray (51.7%).

The physical effect of the surface spray on the oil film has not been explained. We do not know whether the surface spray disrupts the oil film or homogenizes it into particles small enough to pass through the screen. The small mesh size of the screen material needed to retain larval fish seems to inhibit the surface exit of the oil films. The lipophilic nature of the screen material causes it to retain and prevent the oil from passing through it. The goal of our research was to determine whether certain screen materials may be better at allowing an oil surface microlayer to pass out of the culture tanks

We evaluated eight different screen types and two mesh sizes (13 samples) to compare their ability to pass oil.

METHODS

Screen evaluation procedure

Thirteen screen samples were evaluated (Table 1). These synthetic fibers were soaked in water for 24 h to eliminate stretch. The screening was then cut into 16-cm squares and mounted over 100-mm sections of 75-mm PVC pipe and glued in place with PVC cement. Two screen types, Nitex® 710 and 1000, were also painted flat black with Color Creation® indoor/outdoor spray paint (Wal-mart Stores Inc., Bentonville, Ark.) to simulate screens used in our culture system. We referred to these as screen mounts.

Each screen mount was placed over a 100-mm polypropylene funnel, and a 350-ml aliquot of a 20 ppm cod oil in water solution was poured from a glass beaker through the screen mount and retrieved in a 500-ml separatory funnel. Oil adhering to the glass beaker was removed with a 35-ml aliquot of TCE and quantified. The screen was removed, and an aliquot of 35 ml of TCE was used to rinse any remaining solution into the separatory funnel. The screen mount was then placed over another funnel and rinsed with a 20-ml aliquot of TCE, which drained into a 30-ml spectrophotometric cuvette. This was repeated three times, and the rinse collected in separate cuvettes; these were designated as 1st, 2nd, and 3rd washes.

After collection of the oil samples rinsed from the screen material, the separatory funnel containing the 350-ml sample and the 35-ml aliquot of TCE was shaken for 1 min and allowed to stand for 10 min. After separation, the lower organic layer was drained through a #40 Whatman filter paper and into a 30-ml spectrophotometric cuvette.

The four samples obtained from each screen type were analyzed for oil in water concentration by using a spectrophotometer (Bausch and Lomb Spectronic 70) and

a standard curve. This was repeated three times for each of the screen types and their oil retentions were compared.

Procedure for measuring oil in water concentration

The solvent 1,1,1 trichloroethane was used to separate oil from water, and a colorimetric procedure (HACH Company 1989; 427-429) was used to quantify the concentrations of oil in TCE by comparison with a standard curve prepared with a food-grade cod oil.

To select the wavelength that gives the greatest spectrophotometric absorbance, each oil was diluted with the TCE to form a series a standard solutions of different concentrations. To determine the wavelength of the greatest absorbance, absorptivities were measured from 0 to 800 nm in 50-nm increments. A reagent blank containing pure TCE was used to null the spectrophotometer. A sample cell containing a known concentration of oil and TCE was then placed in the spectrophotometer, and the absorption recorded. The wavelength was increased in 50-nm increments, and the percentage of light absorption was recorded at each wavelength. When the area of maximum absorbance was located, the increments were decreased to 5 nm and then to 1 nm until the wavelength that allowed the greatest amount of absorption was located. This wavelength would allow the greatest amount of sensitivity for that particular oil.

Once the optimum wavelength was determined, the standard concentration curve was derived by analyzing several standard solutions of different concentrations and relating their spectrophotometric values with concentration (ppm). Standard curve data were collected on five separate occasions with standards of 10 ppm, 20 ppm, 30 ppm, 100 ppm, and 200 ppm.

RESULTS

Optimum wavelength and standard curves

A wavelength of 328 nm was the maximum value determined for the cod oil . A single peak at 328 nm was found for the cod oil, and no secondary peaks were found throughout the rest of the spectrum.

Standard curves relating oil concentration (ppm) with absorption values were converted to log-log form to develop a linear regression. The coefficient of determination (r^2) for the five standard curves ranged from 0.868 to 0.904.

Evaluation of screen types

The amount of oil recovered in the separatory funnel after the filtrate had passed through the screens was undetectable. Thus, without a solvent, no oil would pass through any of the screen types. Even three washes of TCE did not allow recovery of all oil from the screens. The percentage of the total amount recovered from the screens ranged from 55.4% (polyester monofilament 1000) to 16.6% (stainless steel 1000), the most lipophilic of all screen types.

The spray-painted black Nitex® 1000 and 710 had considerably higher percentages of recovery than the unpainted Nitex® 1000 and 710; however, it is possible that enough paint particles were removed by the TCE to affect analysis (i.e., a positive reading). To evaluate this possibility, painted Nitex® 1000 and 710 were rinsed with TCE without oil being previously administered. The spectrophotometric analysis indicated that extraneous particles were responsible for some of the recovery percentage. The corrected total amount of oil recovered for the black

Nitex® 1000 is 0.00465 ml or 31.0% and 0.00508 ml or 33.8% for the black Nitex® 710. These corrected values do not change the ranking of the two fabrics (Table 3) but do indicate that the paint on the screens enhances the performance of the screens compared with the unpainted Nitex® fabric.

DISCUSSION

Both stainless-steel and Nitex® nylon screens are commonly used for retaining walleye fry in rearing tanks. Colesante et al. (1986) used a stainless-steel or "coated bronze" screen with openings less than 200 μm or a "standard" window screen with 10 meshes per inch. Barrows et al. (1988) used a stainless steel screen with 600 μm mesh and Kindschi and Barrows (1991) used Nitex® nylon screen and recommended a maximum mesh size of 710 μm for retention of walleye fry.

Our evaluation of screens indicated that oil in water solutions does not pass through screens used in fry rearing tanks. All fabric types retained oil, although some types were more lipophilic than others. The two screen types most commonly used in walleye culture systems, stainless-steel 1000 and the Nitex® 710 and 1000, were among the most retentive of the fabric types tested. The painted Nitex® 710 and 1000 and the Tetko® polyester monofilament 1000 were the least retentive of the fabric types. The results of this evaluation indicate that it is unlikely that the size of the mesh or the percentage of open area dictates the ability of the screen to retain oil; there were no discernible patterns between the degree of oil retention and the mesh size and percent of open area. It is also unlikely that the size of the mesh opening was responsible for the degree of retention of the fabric type. The 710-μm screens did not retain more oil than the 1000-μm screens. The most probable factor influencing the oil retention of the screens is the lipophilic character of the material used to fabricate the screen. The painted Nitex® fabrics were among the least retentive of the screen types, whereas the unpainted Nitex® fabrics were among the most retentive.

On the basis of these findings, the authors recommend using screen material that has been spray-painted to facilitate the passage of oily material out of the culture tanks, daily cleaning of the screen material in the culture system to remove any oily material, and upgrading mesh size as the fish grow. Also, based on the high retention rates of typical screen materials, it seems that successful walleye culture (i.e., with high gas bladder inflation) must depend on systems that clear the oily surface film because screens small enough to retain walleye fry are likely to retain most of the oil in the culture tanks.

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Table 1.- Type, size, and mesh characteristics of screens.

screen type	mesh opening (microns)	mesh count (#/cm)	thread dia. (microns)	open area (%)
Tetko polyester 1000	1000	6.65/cm	500	45
Tetko flourtex 1000	1000	6.65/cm	500	45
Tetko flourtex 710	710	10.2/cm	270	53
Tetko Nitex 1000	1000	6.65/cm	500	44
Tetko Nitex 710	710	9.4/cm	350	45
Tetko polyester monofilament 1000	1000	6.65/cm	500	45
Tetko polyester monofilament 710	710	9.4/cm	350	45
McMaster-Carr nylon 1000	1000	*	500	44
McMaster-Carr polypro 1000	1000	*	400	41
McMaster-Carr polypro 710	710	*	500	45
stainless steel 1000	1000	11/cm	380	44
black Nitex 1000[1]	1000	9.4/cm	*	*
black Nitex 710[1]	710	6.65/cm	*	*

[1] Painted flat black with Color Creation® indoor/outdoor spray paint (Wal-mart Stores Inc., Bentonville, Ark.)

Table 2.- Oil concentration (ppm) in the TCE washes of screens after the passage of a 50-ppm oil in water mixture. Values are means of three washes (SD).

screen type	1st TCE wash	2nd TCE wash	3rd TCE wash
flourtex 1000	77.0 (43.6)	42.3 (38.7)	45.7 (40.9)
flourtex 710	73.6 (24.9)	52.6 (53)	51.5 (18.5)
polyester mono 1000	135.7 (132.1)	106.3 (158.7)	106.3 (158.5)
polyester mono 710	66.6 (49.3)	21.6 (45)	43.4 (5.4)
Nitex 1000	58.7 (24.5)	33.3 (44.5)	7.3 (26.5)
Nitex 710	76 (50.5)	43.3 (38.5)	3.8 (25.8)
black Nitex 1000	122.3 (40.8)	109.7 (54)	63.2 (28)
black Nitex 710	134.3 (58.3)	111 (66.8)	74.8 (54.2)
nylon 1000	71.3 (61.2)	54 (19.7)	37.5 (37.8)
polyester 1000	65.3 (42)	36.6 (43.2)	44.5 (39.7)
polypro 1000	63 (52.4)	38.6 (54.4)	13.1 (49.3)
polypro 710	95.6 (78.1)	59.6 (23.2)	20.1 (42.5)
stainless steel 1000	43.3 (35.2)	51.6 (9.1)	1.4 (31.5)

Table 3.- Oil recovered from beaker and screens in consecutive TCE washes.

screen type	mls recovered (% recovered) 1st wash	2nd wash	3rd wash	Beaker	Total
polyester mono 1000	.00271 (15.5)	.00213 (12.2)	.00213 (12.2)	.00273 (15.6)	.00970 (55.4)
black Nitex 710	.00269 (15.4)	.00222 (12.7)	.00150 (8.6)	.00132 (7.5)	.00773 (44.2)
black Nitex 1000	.00245 (14.0)	.00219 (12.5)	.00126 (7.2)	.00126 (7.2)	.00716 (40.9)
flourtex 710	.00147 (8.4)	.00105 (6.0)	.00103 (5.9)	.00131 (7.4)	.00468 (27.7)
polypro 710	.00191 (10.9)	.00119 (6.8)	.00040 (2.3)	.00131 (7.5)	.00481 (27.5)
flourtex 1000	.00154 (8.8)	.00085 (4.8)	.00091 (5.2)	.00118 (6.7)	.00448 (25.5)
nylon 1000	.00143 (8.2)	.00108 (6.2)	.00075 (4.3)	.00101 (5.8)	.00427 (24.5)
polyester mono 710	.00133 (7.6)	.00043 (2.5)	.00087 (5.0)	.00154 (8.8)	.00417 (23.8)
polyester 1000	.00131 (7.5)	.00073 (4.2)	.00089 (5.1)	.00108 (6.2)	.00401 (23.0)
Nitex 710	.00152 (8.7)	.00087 (5.0)	.00008 (0.4)	.00133 (7.6)	.00380 (21.7)
polypro 1000	.00126 (7.2)	.00077 (4.4)	.00026 (1.5)	.00116 (6.6)	.00345 (18.7)
Nitex 1000	.00117 (6.7)	.00067 (3.8)	.00015 (0.8)	.00094 (5.4)	.00293 (16.7)
stainless steel 1000	.00087 (5.0)	.00103 (5.9)	.00003 (0.2)	.00096 (5.5)	.00289 (16.6)

Figure 1. Screen mount and oil sampling apparatus.

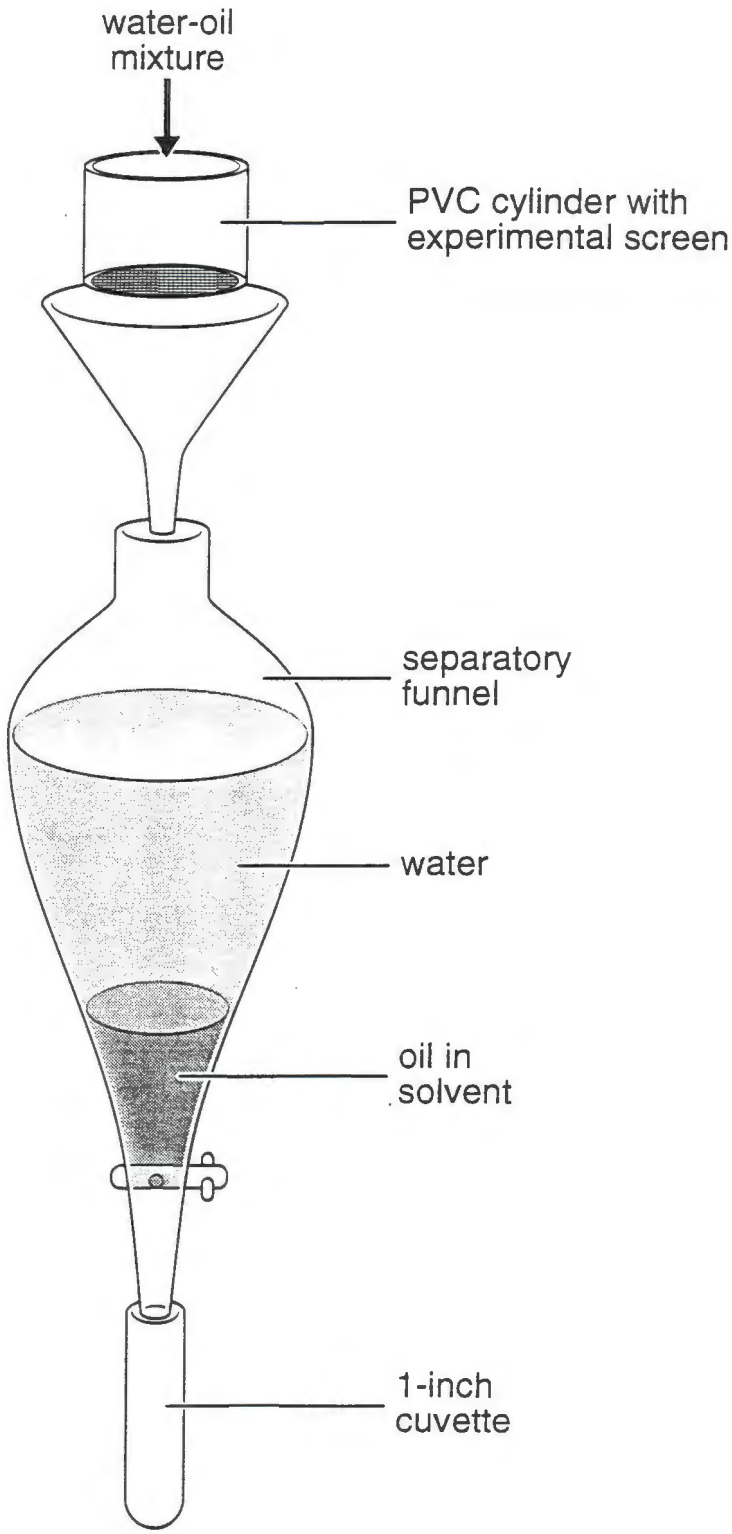
water-oil
mixture
PVC cylinder with
experimental screen
separatory
funnel
water
oil in
solvent
1-inch
cuvette

Figure 1.

GENERAL SUMMARY

The research summarized in the preceding papers suggests that the majority of the oily material responsible for the formation of the oil surface microlayer is originating from the walleye larvae, however, feed may also contribute to the development of the oil surface microlayer. Both the water surface spray and the air blower and trap apparatus reduced oil layer thicknesses during the critical period when gas bladder inflation must take place (7 to 14 days posthatch). Inflation rates for the spray and trap treatments were significantly higher than a control treatment with no oil removal apparatus. Screen materials used for covering drains in intensive culture systems are strongly lipophilic and retain oily material in culture tanks; spraypainting screens seems to facilitate the passage of oil through screens and out of culture systems.

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