Effects of suspended solids on larval walleye

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Effects of suspended solids on larval walleye

by

Todd Alan Phillips

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department: Animal Ecology
Major: Fisheries Biology
Major Professor: Robert C. Summerfelt

Iowa State University
Ames, Iowa
1996
This is to certify that the Master's thesis of

Todd Alan Phillips

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
# TABLE OF CONTENTS

**GENERAL INTRODUCTION**

- Goals and Objectives 2
- Thesis Organization 2
- References 3

**CHAPTER 1. LITERATURE REVIEW** 5

- Suspended Solids in Iowa 5
- Suspended Solids and Turbidity 5
- Effects of Suspended Solids on Primary Production and Aquatic Invertebrates 9
- Effects of Suspended Solids on Fish 10
- Effects of Suspended Solids on Fish Health 13
- Effects of Toxicants on Fish Gills 15
- Effects of Suspended Solids on Fish Gills 17
- Gill Development 18
- References 20

**CHAPTER 2. EFFECTS OF SUSPENDED SOLIDS ON LARVAL WALLEYE** 27

- Abstract 27
- Introduction 28
- Methods 29
- Results 36
- Discussion 52
- References 59

**CHAPTER 3. GILL DEVELOPMENT OF LARVAL WALLEYE** 63

- Abstract 63
- Introduction 64
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>65</td>
</tr>
<tr>
<td>Results</td>
<td>68</td>
</tr>
<tr>
<td>Discussion</td>
<td>77</td>
</tr>
<tr>
<td>References</td>
<td>80</td>
</tr>
<tr>
<td>GENERAL CONCLUSION</td>
<td>82</td>
</tr>
<tr>
<td>References</td>
<td>83</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>84</td>
</tr>
</tbody>
</table>
GENERAL INTRODUCTION

Agricultural runoff is a major factor affecting the water quality of Iowa's rivers and streams because 24 of 36 (66.7%) million acres of the state are planted in crops, mostly corn and soybeans, which are susceptible to erosion and runoff. The U.S. Geological Survey (USGS 1993; 1994) reported suspended sediment concentrations ranging from 3 to 3,180 mg/L in 1993 and 4 to 3,230 mg/L in 1994 in Iowa rivers and streams.

Suspended solids (SS), which vary in type, size, and concentration affect aquatic ecosystems in many ways. Turbidity from SS reduces light penetration, consequently primary production is reduced (Alabaster and Lloyd 1980; Lloyd et al. 1987). Decreased hatching success and survival of larval fishes (Auld and Schubel 1978) and decreased reaction distances and daily food consumption have been reported (Gardner 1981; Barrett et al. 1992).

Fish gills are a sensitive organ because the structure of the gills provides a large surface area for the movement of water (Heath 1995). Large flows of water (70 mL/min) have been observed to pass over the gills even in resting brook trout *Salvelinus fontinalis* (McKim and Goeden 1982). Therefore, fish gills receive substantial exposure to SS and it is not surprising that the delicate epithelia of the secondary lamellae are often the first system to be affected by toxicants (Heath 1995). Damage to fish gills by SS have been reported in rainbow trout *Oncorhynchus mykiss* (Goldes et al. 1986; 1988). Suspended solids have increased ventilation rates, gill flaring, and coughing of fish (Horkel and Pearson 1976; Berg and Northcote 1985).

Although the scope of research on SS is extensive, early life stages of fish are of special interest. Sublethal effects of SS on the early life stages of fish have not been studied because the brevity of the life stage and difficulty in providing adequate life support for larval fishes. The technology for raising larval fishes in the laboratory has been limited to a few species. However, Iowa State University has recently developed cultural techniques for raising larval walleye *Stizostedion vitreum* (Moore et al. 1994a, 1994b; Bristow and Summerfelt 1994) and
the success in raising larval walleye in laboratory environments presents an opportunity for experimental study of environmental contaminants. The walleye is a desirable species for measuring effects of SS on larval fish because it is regarded as an "extremely sensitive fish" to SS (Alabaster and Lloyd 1980).

Goals and Objectives

Two experiments were conducted. In the first experiment the objective is to evaluate the effects of chronic exposure of walleye from the prolarval to juvenile stage to SS concentrations that are typically found in Iowa rivers and streams. Gills of walleye exposed to SS (2.3 to 360 mg/L) were also examined for histological changes.

The objective of the second experiment is to describe the development of gill filaments and secondary lamellae in prolarval to juvenile walleye relative to critical developmental events. An understanding of gill development is critical to understanding changes in sensitivity to toxicants in the early life stages of fish.

Thesis Organization

This thesis consists of a general introduction, a literature review, two manuscripts prepared for publication in Transactions of the American Fisheries Society, and a general conclusion. The first manuscript will be submitted for publication under the authorship of Todd A. Phillips, who conducted and summarized the research, Robert C. Summerfelt, who provided supervision for the research and edited the manuscript, and Edwin C. Powell, who provided supervision and assistance in the histological procedures. The second manuscript will be submitted for publication under the authorship of Todd A. Phillips, who conducted and summarized the research, and Robert C. Summerfelt, who provided supervision for the research and edited the manuscript. The style used in this thesis follows that of the Transactions of the American Fisheries Society.
References


CHAPTER 1. LITERATURE REVIEW

Suspended Solids in Iowa

Suspended sediment concentrations in Iowa rivers and streams ranged from 3 to 3,180 mg/L (USGS 1993) from October 1992 to September 1993, and from 4 to 3,230 mg/L from October 1993 to September 1994 (USGS 1994). The mean maximum suspended sediment concentrations were 23% higher in 1993 than 1994, but mean minimum concentrations were 29% higher in 1994 than 1993 (Table 1). The higher maximum concentrations of suspended sediment in 1993 may be attributed to severe flooding that occurred during that year. In 1994, mean concentrations of SS in wadeable streams in Iowa ranged from about 10 mg/L in the Paleozoic Plateau to about 90 mg/L in the Loess Hills and Rolling Prairies subregion (Wilton 1996).

Suspended Solids and Turbidity

Suspended solids (SS) include clay, silt, and finely divided organic and inorganic matter and are defined as "the residue retained on a glass fiber filter dried to a constant weight at 103–105°C" (APHA et al. 1989). Because SS affect water clarity, turbidity is often used as a measure of SS. Because turbidity can be measured instantaneously with a meter, it is a more desirable measurement than SS which requires filtering a water sample through a weighed standard glass-fiber filter. Turbidity has been expressed in Jackson turbidity units (JTU), formazin turbidity units (FTU), and nephelometric turbidity units (NTU).

Turbidity is defined as “an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample” (APHA et al. 1989). Turbidity is caused by suspended matter including clay, silt, finely divided organic and inorganic matter, soluble colored organic compounds, and plankton and other microscopic organisms.
Table 1. Concentrations of suspended sediment (mg/L) in Iowa rivers and streams for the water years October 1992 to September 1993, and October 1993 to September 1994 (USGS 1993, 1994).

<table>
<thead>
<tr>
<th>Name (County)</th>
<th>Number of observations</th>
<th>Range in concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1993</td>
</tr>
<tr>
<td>Bloody Run Creek (Marquette)</td>
<td>365</td>
<td>3–2780</td>
</tr>
<tr>
<td>Mississippi River (McGregor)</td>
<td>365</td>
<td>3–211</td>
</tr>
<tr>
<td>Sny Magill Creek (Clayton)</td>
<td>365</td>
<td>4–2010</td>
</tr>
<tr>
<td>Iowa River (Marshalltown)</td>
<td>365</td>
<td>4–2600</td>
</tr>
<tr>
<td>Cedar River (Cedar Falls)</td>
<td>6</td>
<td>28–170</td>
</tr>
<tr>
<td>Iowa River (Wapello)</td>
<td>365</td>
<td>23–1390</td>
</tr>
<tr>
<td>Skunk River (Augusta)</td>
<td>365</td>
<td>10–2580</td>
</tr>
<tr>
<td>Hazelbrush Creek (Maple River)</td>
<td>365</td>
<td>12–3180</td>
</tr>
<tr>
<td>Raccoon River (Van Meter)</td>
<td>5</td>
<td>137–684</td>
</tr>
<tr>
<td>Elk Creek (Decatur City)</td>
<td>4</td>
<td>14–355</td>
</tr>
<tr>
<td>Total</td>
<td>2570</td>
<td></td>
</tr>
</tbody>
</table>

Mean minimum value: 24, 34
Minimum values (range): 3–137, 4–82
Mean maximum value: 1596, 1228
Maximum values (range): 170–3180, 118–3230
Lloyd et al. (1987) stated that turbidity can be used as an estimator of SS concentrations because there are definable relationships between turbidity and SS concentrations. United States Geological Survey (USGS) measurements of suspended sediment from the Cedar River, Iowa, showed a direct positive relationship between turbidity (NTU) and SS concentrations (mg/L; Figure 1). However, in a natural system, the composition of SS is heterogeneous including: clay, silt, finely divided organic and inorganic matter, and the relative composition changes temporally.

Many laboratory studies use clay to examine the effects of SS on fish. The relationship between red clay (mg/L), from Lake Superior, and FTU was defined by measuring the concentration of red clay (mg/L) and turbidity (FTU) in 33 water samples (Swenson and Matson 1976). Sigler et al. (1984) determined the relationship between NTU and bentonite clay (mg/L).

Clay contains silicon (Si), aluminum (Al) or magnesium (Mg), oxygen (O), and hydroxyl (OH) with various associate cations (Chamley 1989; Clauer and Chaudhuri 1995). The ions and hydroxyl groups are organized into two types of two-dimensional structures, called sheets. The two types of sheets are tetrahedral sheets and octahedral sheets. The general composition of tetrahedral sheets is T₂O₅ (T is the cation present). Silicon is the main cation associated with the tetrahedral sheet, but there are also varying amounts of Al or Fe⁺³ (Chamley 1989). The tetrahedral sheet is organized with silicon located at the center of the tetrahedral and oxygen anions forming the four corners. The octahedral sheets consist of oxygens located at the eight corners of the sheet with medium-sized cations (usually Al, Mg, Fe⁺², or Fe⁺³) at the center (Chamley 1989). The smallest structural unit of the octahedral sheet contains three octahedra and the sheet is classified as either trioctahedral or dioctahedral. Sheets are classified as trioctahedral if all three octahedra have octahedral cations at their center and the sheet is called dioctahedral if only two octahedra are occupied and one octahedra is vacant.
Figure 1. The relationship between SS (mg/L) and turbidity (NTU) in the Cedar River at Cedar Falls, Iowa based on data collected by the USGS (1993) from October 19, 1992 to August 17, 1993. Suspended solids averaged 83 mg/L with a range of 28–170 mg/L and turbidities averaged 22.4 NTU with a range of 3.4–55 NTU.
The assemblage of tetrahedral and octahedral sheets forms a layer. The two main types of layers are the 1:1 layer and 2:1 layer. The 1:1 layer consists of the assemblage of one tetrahedral sheet with one octahedral sheet and the 2:1 layer links two tetrahedral sheets in an external position with one octahedral sheet (Chamley 1989; Clauer and Chaudhuri 1995). The 1:1 layer is typical of the kaolinite group, but most clays, such as montmorillonite, belong to the 2:1 layer (Chamley 1989).

Bentonite, kaolin, and ball clay have been used in laboratory experiments to examine the effects of SS on fish. Kaolinite, Al\textsubscript{2}SiO\textsubscript{2}O\textsubscript{5}(OH)\textsubscript{4}, is a common product of weathering in soils and is characterized by a 1:1 layer (Chamley 1989). Kaolinite is also a common component in ball clays (Clauer and Chaudhuri 1995). Kaolinite clays are insoluble in water and are white or yellowish-white in appearance because they lack iron (Grim 1968; Stecher 1968). Bentonite is a highly colloidal hydrated aluminum silicate consisting mostly of montmorillonite (Stecher 1968). Montmorillonite, Na\textsubscript{0.33}(Al\textsubscript{1.67}Mg\textsubscript{0.33})Si\textsubscript{4}O\textsubscript{10}(OH)\textsubscript{2}, nH\textsubscript{2}O, is characterized by a 2:1 layer. It has the unique characteristic of swelling to several times its original volume when placed in water; the color of montmorillonite ranges from white to almost black (Grim 1968; Stecher 1968; Chamley 1989; Clauer and Chaudhuri 1995).

**Effects of Suspended Solids on Primary Production and Aquatic Invertebrates**

Suspended solids degrade basic water quality and aesthetics, and they can affect aquatic ecosystems in many ways. An extensive literature review found that many studies have evaluated the effects of SS, but do not provide chemical analysis and particle size of the SS used. In this literature review, chemical analysis and particle size will be reported unless no information concerning the SS was available.

Turbidity, caused by SS, reduces light penetration and primary production (Grobbelaar 1992). Lloyd et al. (1987) reported suspended sediment concentrations decreased plant production and abundance in rivers and streams. Hayes et al. (1992) observed no submerged
macrophytes in a lake containing 20–40 mg/L SS, but high concentrations of submerged macrophytes were observed in a lake containing 5 mg/L SS.

Aquatic invertebrates are affected when turbidity is sufficient to reduce light penetration and primary production. Also, feeding by filter-feeding invertebrates can be reduced by SS because their filter-feeding structures clog with SS. Ultimately, reduced feeding will decrease growth rates or increase death rates (Newcombe and MacDonald 1991).

Densities of lake zooplankton are reduced by turbidity (Lloyd et al. 1987; Kirk 1991). In glacially turbid lakes, zooplankton densities were found to be only 5% of those in clearer lakes (Lloyd et al. 1987). Algal ingestion by *Daphnia ambigua* was reduced in a 50–200 mg/L 95:5 mixture of illite:smectite clay with a mean particle size of 1 µm, but unaffected by a mean particle size of 0.6 µm (Kirk 1991).

**Effects of Suspended Solids on Fish**

Survival of larval fish establishes the year-class strength of the population. In soft and acidic waters, a major decline in fish populations has been attributed to direct mortality of embryo-larval fishes (Conklin et al. 1992). Laboratory studies indicate that larval fishes are less tolerant of SS than eggs or adults (Muncy et al. 1979) and suspended sediments can be detrimental to the hatching success and survival of larval fish. Auld and Schubel (1978) exposed fish eggs and larvae to sediment particles (1–4 µm) containing illite, chlorite, and kaolinite. They found that hatching success of white perch *Morone americana* and striped bass *Morone saxatilis* was reduced at concentrations of 1,000 mg/L of suspended sediment and survival of striped bass and yellow perch *Perca flavescens* larvae was significantly reduced at concentrations ≥ 500 mg/L illite, chlorite, and kaolinite. However, larval American shad *Alosa sapidissima* survival was reduced at concentrations of 100 mg/L illite, chlorite, and kaolinite.

Chronic exposure to concentrations of SS found in the natural environment may affect growth, development, and survival of fish. Rainbow trout *Oncorhynchus mykiss* exposed to
concentrations of 270 and 810 mg/L kaolin clay and diatomaceous earth for up to 6 months in the laboratory often died from disease (Herbert and Merkens 1961). Sigler et al. (1984) found that steelhead *Oncorhynchus mykiss* and coho salmon *Oncorhynchus kisutch* (30–65 mm) exposed to bentonite clay at levels of 25–50 NTU for 14 to 21 d in laboratory streams were smaller than fish in clear water. However, neither growth nor survival of larval lake herring *Coregonus artedii* were reduced when they were raised in tanks at concentrations of 28 mg/L red clay for 62 d (Swenson and Matson 1976).

Reduction in water clarity reduces visibility by fish that rely on their visual senses for feeding. Several studies have shown that the reaction distance of fish decreases in turbid water (Vinyard and O'Brien 1976; Berg and Northcote 1985; Boehlert and Morgan 1985; Barrett et al. 1992). The reaction distance, as defined by Crowl (1989), is "the greatest distance at which a predator can locate a prey." A decrease in reaction distance can have a negative effect on the fish because the chance of prey escaping, especially zooplankters, is much higher (Vinyard and O'Brien 1976).

The reaction distance of rainbow trout (87–185 mm standard length) decreased 20 and 55% in fish exposed to sand, silt, and fine organic matter at levels of 15 and 30 NTU, respectively, in artificial streams compared with fish exposed to low turbidities, 4–6 NTU (Barrett et al. 1992). Vinyard and O'Brien (1976), reported a significant decrease in the reaction distance of bluegill *Lepomis macrochirus* (65 mm total length) exposed to clay, in laboratory tanks, at a level of 30 JTU compared with fish exposed to low turbidities (1 JTU). Berg and Northcote (1985), using laboratory streams, reported the reaction distance of juvenile coho salmon decreased significantly in fish exposed to an angular sediment consisting of particles ranging from 0.02–0.06 mm at levels of 30 and 60 NTU compared with fish exposed to lower turbidities (0–20 NTU). In clear water (1–3 JTU), largemouth bass (280–300 mm total length) reaction distance increased linearly with increased prey movement and prey size, but in
somewhat turbid water (bentonite clay at levels of 17–19 JTU) the relationship was not linear (Crowl 1989).

If SS reduce reaction distance, it is not surprising to find an affect on daily food consumption. *Daphnia pulex* consumption (N/min) by bluegill (75.3 mm total length), in laboratory tanks, was reduced 21, 29, and 50% when exposed to bentonite clay at levels of 60, 120, and 190 NTU, respectively (Gardner 1981). Consumption of zooplankton, especially cyclopoid copepods and copepod nauplii, decreased when larval bluegill (12.5 mm total length) were exposed to bottom sediment at levels of 30–40 NTU at low light intensities (Miner and Stein 1993) and Johnston and Wildish (1982) reported that larval herring exposed to 20 mg/L of sediment consumed significantly less *Artemia* nauplii than fish in clear water (0 mg/L). Larval striped bass consumed 40% fewer copepods in 200 and 500 mg/L kaolin clay than larvae exposed to 0 and 75 mg/L kaolin clay, but consumption on *Daphnia pulex* was not reduced at any concentration of kaolin clay (Breitburg 1988). Breitburg (1988) suggested that reduced consumption rates may be the result of a decrease in reaction distance.

Although reduced feeding has been observed in some species of fish, other species increased feeding with increased turbidities. Maximum consumption of rotifers *Brachionus plicatilis* by Pacific herring *Clupea harengus pallasi* occurred at concentrations of 500 and 1,000 mg/L estuarine sediment and Mount Saint Helens volcanic ash (particles size <24.0 µm) compared with fish exposed to clear water, 0 mg/L (Boehlert and Morgan 1985). Vandenbyllaardt et al. (1991) reported that walleye (85 mm total length) consumption of fathead minnows *Pimephales promelas* was inhibited when exposed to 100 and 161 NTU Red River sediment in a 1-h feeding trial, but feeding was not inhibited at 121 NTU in a 4-h feeding trial.

Bristow and Summerfelt (1994) found that the proportion of tank populations of larval walleye that contained feed was significantly greater in turbid water (kaolinite with 76% of the particles <2.0 µm, at levels of 20 and 50 NTU) at 7-d posthatch in 3 of 4 experiments, and at
14-d posthatch, in 2 of 4 experiments compared with fish in clear water (<1.0 NTU). After 21- to 30-d posthatch, the lengths and weights of juvenile walleye raised in turbid water were significantly greater than in clear water. Bristow et al. (1996) also reported increased feed acceptance for 4-d posthatch walleye exposed to clay at 25 NTU compared with walleye in clear water (<1.0 NTU) or water colored blue with Aquashade (2 and 20 mg/L). After 21-d posthatch, the lengths and weights of walleye raised in turbid water (25 NTU) were significantly greater than in clear (<1.0 NTU) or colored water (2 and 20 mg/L Aquashade). Therefore, it is suggested that enhanced feeding in the presence of turbidity and SS occurs because a visual contrast between prey items is enhanced by SS (Boehlert and Morgan 1985; Bristow and Summerfelt 1994).

Effects of Suspended Solids on Fish Health

Short term exposures to SS have an abrasive effect on fish skin and gills, resulting from the continual passage of SS over the body. Abrasions on the skin and gills increases the risk of infection from microbial diseases. Herbert and Merkens (1961) found that rainbow trout survived short term exposure to concentrations of 270 and 810 mg/L kaolin clay and diatomaceous earth, but mortality from diseases increased after long term exposure (6 months). Redding et al. (1987) found that steelhead and coho salmon were able to survive concentrations of topsoil, kaolin clay, and volcanic ash as high as 2,000–3,000 mg/L for 7 to 8 d, but fish often developed infections by the bacterial pathogen *Vibrio anguillarum*. Redding et al. (1987) suggested that, although steelhead and coho salmon are able to survive high concentrations of SS, the growth and survival of the fish may be reduced by sublethal physiological stress. Another problem associated with the continual passage of SS over the body and gills of fish is that contaminated particles can provide an entry for toxicants when phagocytosed into the gills (Martens and Servizi 1993).
Studies have also shown that fish will avoid and emigrate from high concentrations of SS. Juvenile coho salmon significantly avoided water with levels of suspended sediment greater than 70 NTU (Bisson and Bilby 1982). Similar results were observed when steelhead and coho salmon emigrated from water containing bentonite clay at levels ranging from 100–300 NTU (Sigler et al. 1984). Sigler et al. (1984) suggested that increased fish emigration may occur if fish emerge from their eggs in waters with increased levels of turbidity and increased emigration downstream could result in reduced production if suitable habitat in downstream areas is not found by the emigrating fish.

Suspended solids have been reported to increase ventilation rates, gill flaring, and coughing of fish. Horkel and Pearson (1976) found that ventilation rates in green sunfish Lepomis cyanellus were 50 to 70% higher in fish exposed to bentonite clay at levels above 898 FTU at 15 and 25°C, respectively. Increased gill flaring was also observed in juvenile coho salmon exposed to angular sediment particles 0.02–0.06 mm at levels of 30 and 60 NTU (Berg and Northcote 1985). Increased coughing was observed in Arctic grayling Thymallus arcticus exposed to an inorganic suspended sediment with 90% of the particles <0.2 mm (McLeay et al. 1987). Heath (1995) suggested that gill flaring and coughing are methods used to clear foreign materials from the gill area by creating an abrupt change in buccal cavity pressure. Therefore, smaller fish may be more susceptible to SS than larger fish because smaller fish do not have the same capacity of larger fish to clear solid particles (Martens and Servizi 1993).

No relationship between the type or size of SS particles and the effects on fish was observed from the literature reviewed. However, some species of fish showed greater sensitivity than other species of fish exposed to the same type, size, and concentration of SS (Auld and Schubel 1978). Sensitivity of fish was also affected by the concentration and length of exposure to SS (Herbert and Merkens 1961; Auld and Schubel 1978; Gardner 1981; Breitberg 1988; Barrett et al. 1992).
Effects of Toxicants on Fish Gills

Few natural environments are free of contaminants of anthropogenic origin: fish are exposed to detergents, heavy metals, pesticides, fluctuations in pH, and SS. At appropriate concentrations, these substances are toxic to fish. The gills are the most vulnerable to exposure; therefore, gills have become a primary focal point concerning the effects of toxicants. Based on an extensive literature review of toxicants and other irritants, Mallatt (1985) suggested that fish gills are sensitive structures to toxicants and other irritants.

Gills are also good structures to examine because changes in their structure are relatively easy to detect. Pathological changes in gill epithelium include: epithelial lifting, hyperplasia, hypertrophy, and necrosis. Bulbing, clubbing, and fusion are common changes observed in secondary lamellae as well as increased mucus and chloride cell production. Lymphocyte and granulocyte invasion of subepithelial spaces have also been observed.

Changes in the tissues of gills have been observed in brown trout and rainbow trout exposed to the anionic detergent, sodium lauryl sulfate (Abel and Skidmore 1975; Abel 1976). Epithelial lifting and lymphocyte and granulocyte invasion of subepithelial spaces were observed in the gills of rainbow trout exposed to 100 mg/L of sodium lauryl sulfate (Abel and Skidmore 1975). Epithelial lifting and hypertrophy of epithelial cells were observed in gills of brown trout Salmo trutta exposed to concentrations of 18 and 32 mg/L sodium lauryl sulfate and death of pillar cells occurred at concentrations of 56 and 100 mg/L (Abel 1976).

Heavy metals are often released into rivers and streams by industrial activity. Because of this problem, affects on gills have been well documented. Epithelial lifting, lamellar fusion, and increased chloride cells were observed in the gills of winter flounder Pseudopleuronectes americanus exposed to copper concentrations of 1,000 µg/L and lamellar fusion and complete cell destruction were observed at concentrations of 3,200 µg/L copper (Baker 1969).

Epithelial lifting and lamellar fusion between adjacent lamellae were observed in the gills of rainbow trout exposed to 1.25 mg/L zinc for 96-h (Tuurala and Soivio 1982) and Skidmore...
and Tovell (1972) observed lamellar fusion and epithelial lifting in rainbow trout exposed to 40 mg/L zinc. Threespine stickleback *Gasterosteus aculeatus* exposed to zinc concentrations ranging from 0.5–1.0 mg/L were killed after 1–3 days of exposure and epithelial lifting, sloughing of cells, and lamellar fusion were observed in the gills (Matthiessen and Brafield 1973).

Increased numbers of chloride cells and epithelial lifting were observed in the gills of threespine sticklebacks exposed to concentrations of cadmium ranging from 0.5–6.0 mg/L compared with control fish (0.0 mg/L cadmium) and lamellar fusion was observed in the gills of threespine sticklebacks exposed to 6.0 mg/L (Oronsaye and Brafield 1984). Hypertrophy of the gill filaments and hyperplasia of the secondary lamellae were observed in the gills of the common mummichog *Fundulus heteroclitus* after 20 h of exposure to 50 mg/L cadmium (Gardner and Yevich 1970).

The effects of methyl bromide (CH$_3$Br), a fumigant used in horticulture, and permethrin, a synthetic pesticide, on the gill structure of fish have been reported (Segers et al. 1984; Kumaraguru et al. 1982). Segers et al. (1984) reported severe changes in the gills of common carp *Cyprinus carpio*, including complete disintegration of primary and secondary epithelial cells and pillar cell damage in the secondary lamellae, after exposure to 18.5 and 36 mg/L CH$_3$Br. The synthetic pyrethroid, permethrin, was also found to be highly toxic to fish. Rainbow trout were exposed to permethrin by adding the pesticide directly to their food and also by adding the pesticide to water (Kumaraguru et al. 1982). Exposure to permethrin in water resulted in epithelial lifting, hyperplasia, clubbing, and lamellar fusion, while exposure of permethrin in food resulted in clubbing of secondary lamellae and hypertrophy of epithelial cells.

The effects of pH changes on gill histology have been extensively studied in relation to acidification of lakes and rivers (Daye and Garside 1976; Leino and McCormick 1984; Karlsson-Norrgren et al. 1986; Tietge et al. 1988). The gills of brook trout *Salvelinus*
*Salvelinus fontinalis* exposed to pH concentrations below 5.6 and above 9.0 exhibited hypertrophy, increased mucus production, and epithelial lifting (Daye and Garside 1976). Leino and McCormick (1984) found that most histological effects on the gills of fathead minnows were observed at pH concentrations of 5.0 and 5.5 and consisted mainly of changes in numbers, distribution, and morphology of chloride cells.

The effects on fish exposed to a combination of low pH concentrations and elevated concentrations of aluminum have been examined because acidification results in an increased concentration for many metals (Karlsson-Norrgren et al. 1986; Tietge et al. 1988). Brown trout exposed to 200 µg/L aluminum at pH 5.5 showed epithelial cell hyperplasia and lamellar fusion between secondary lamellae (Karlsson-Norrgren et al. 1986). Tietge et al. (1988) observed increased hyperplasia and hypertrophy of chloride cells in brook trout exposed to low pH concentrations and aluminum compared with low pH concentrations only. Conklin et al. (1992) found that gills of larval brook trout exposed to soft, acidic water (4.4 mg/l as CaCO₃; pH 5.25) had more degenerating chloride cells. Hyperplasia of primary epithelium and lamellar fusion of filaments and secondary lamellae were also observed in fish exposed to soft, acidic water.

**Effects of Suspended Solids on Fish Gills**

Depending on type, size, concentration, and length of exposure, SS can be harmful to fish gills. Fish gills are a sensitive organ because the structure of the gills provides a large surface area for the movement of water (Heath 1995). In fact, one study found large flows of water (70 mL/min) passed over the gills in resting brook trout *Salvelinus fontinalis* (McKim and Goeden 1982). Therefore, the large volume of water passing over the gills increases the contact of the gill epithelia much more than the general body surface. The delicate epithelia of the secondary lamellae make the gills sensitive to SS and often the first system to be affected by toxicants (Heath 1995).
Negative effects of SS on fish gills have been reported many times (Herbert and Merkens 1961; Goldes et al. 1986; 1988; Magor 1988; Martens and Servizi 1993). Goldes et al. (1988) found that exposure of rainbow trout to the suspended clay kaolin caused lamellar fusion and inter-lamellar hyperplasia at high concentrations (4,887 mg/L). In a similar study, kaolin was found intracellularly in rainbow trout gills exposed to concentrations of 171, 1,017, and 4,887 mg/L after the first day of exposure (Goldes et al. 1986). Hypertrophy of epithelial cells of secondary lamellae and fusion between adjacent secondary lamellae occurred in gills of rainbow trout exposed to 270 mg/L kaolin clay and diatomaceous earth (Herbert and Merkens 1961). Gill lesions, including epithelial lifting and epithelial hyperplasia, were observed in juvenile coho salmon exposed to 132 mg/L suspended wood with particles ranging from 150–1,000 µm (Magor 1988).

Gill Development

The structure and histology of gills in adult fish have been well documented (Baker 1969; Abel 1976), but only a few studies have documented the development of the gill arches, gill filaments, and gill lamellae in larval fish (McDonald and McMahon 1977; El-Fiky et al. 1987; Osse 1989).

Before the development of the gills, the critical structure in gas exchange is the yolk sac that contains an extensive vitelline vasculature structure (McDonald and McMahon 1977; McElman and Balon 1979). As the gills begin to develop, they play an increasingly important role in gas exchange as the yolk sac diminishes (McDonald and McMahon 1977).

McDonald and McMahon (1977) described the development of the gills in Arctic char *Salvelinus alpinus* from hatching to 47-d posthatch. Gill filaments were observed to be unevenly distributed among the four gill arches when they were first observed at 2-d posthatch. Secondary lamellae first began to appear in these fish as early as 8-d posthatch and all larvae had secondary lamellae by 15-d posthatch. McDonald and McMahon (1977) suggest that,
during this developmental period, a transition from gas exchange at the gill filaments to gas exchange at the secondary lamellae occurs along with a transition from cutaneous to branchial gas exchange. The number of filaments increased from 257 filaments at 2-d posthatch to 544 filaments at 47-d posthatch, which is about 85% of the gill filaments present in a 1-year-old Arctic char.

El-Fiky et al. (1987) suggested that, in addition to the vitelline vasculature of the yolk sac, respiration of larval fish takes place through the superficial layer of red muscle fibers present immediately after hatching. They determined that respiration does not take place at the gills in the first few days after hatching because the gills are still in a rudimentary state. The importance of the red muscle layer was supported by measuring the total red layer muscle mass of fish immediately after hatching until the time the red layer was indistinguishable from the developing adult red muscles. El-Fiky et al. (1987) found that 12% of the total muscle mass of larvae at 3-d posthatch was made up by the red muscle layer and only 3 to 4% of the total muscle mass consisted of the red muscle layer at 40-d posthatch. Another observation that supports the importance of the red muscle layer was that the percentage of the red muscle layer that made up the total muscle mass decreased as development of the gills increased.

Osse (1989) reported that the skin is the major site of gas exchange throughout the embryonic and larval stages of the common carp. Cutaneous gas exchange provides the required amount of oxygen for aerobic metabolism at this time, therefore, the need for the structures involved in feeding to develop are much greater than the need for the gills to develop at this time. In addition, larval fish have physical limitations involving gill ventilation, therefore, the initial absence of gill filaments and secondary lamellae at hatching can be explained. Osse (1989) suggested that the median finfold may not be necessary for respiration because, in well aerated water, there is sufficient body surface area for gas exchange. It was also determined that the oxygen concentration at the boundary layer of the body wall is the most important parameter.
Because larval fish are less tolerant of chemicals than either eggs or adults (Muncy et al. 1979), the development of the gills is important in predicting the chemical concentrations that larval fish can tolerate. The sensitivity of walleye to elevated pH concentrations increases as they developed between 3- and 12-d posthatch (Bergerhouse 1992). Clayton and Summerfelt (in press) found that 80% of walleye 4-d old fish survived a 1-h exposure to 100 µL/L hydrogen peroxide treatment, but only 2% of 6-d old walleye survived the same treatment. These studies suggest that development of the gills, during this period, contributes to the increased sensitivity of larval fish to certain toxicants.

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CHAPTER 2. EFFECTS OF SUSPENDED SOLIDS ON LARVAL WALLEYE

A paper to be submitted to Transactions of the American Fisheries Society

Todd A. Phillips, Robert C. Summerfelt, and Edwin C. Powell

Abstract

Two experiments (E1 and E2) were conducted to determine the effects of suspended solids (SS) on survival, growth, and gill histology of prolarval to early juvenile walleye *Stizostedion vitreum*. A commercial aluminum-silicate clay was used as the source of solids. Walleye were exposed to SS concentrations ranging from 2.7–250 mg/L in E1 and 2.3–360 mg/L in E2. Because there was a linear relationship between turbidity and the concentration of SS (clay), turbidity, expressed in nephelometric turbidity units (NTU), was used throughout the experiments as a measure of SS concentrations. Other than turbidity, all environmental conditions and culture techniques were similar. In both experiments, survival-turbidity curves when fish were 28-d posthatch were parabolic: maximum survival was 46% at 164 NTU in E1, and 34% in E2 at 200 NTU. In both experiments, length-turbidity curves at 28-d posthatch were also parabolic. The maximum total length was 22 mm at 150 NTU in E1 and 27 mm at 200 NTU in E2. In both experiments, gills of 7 fish from each treatment at 28-d posthatch and 5 fish from each treatment at 42-d posthatch were examined for epithelial lifting, necrosis, lamellar fusion, hyperplasia, and clubbing. Gills were also examined for the presence of debris. No histological alterations were observed. However, small particles (about 39 µm) of debris, presumably food, but too large and of a different structure than clay, were observed between adjacent secondary lamellae in fish exposed to all turbidity levels. The findings indicate that chronic exposure of larval and early juvenile walleye to SS at concentrations as high as 360 mg/L are not harmful.
Introduction

Suspended solids (SS), which represent most of the nonpoint source pollution from farms, forests, and urban areas, account for about 60% of the total mass of pollutants in rivers and streams (EPA 1990). In Iowa, agricultural runoff is the major factor affecting the water quality of rivers and streams, because 24 of 36 (66.7%) million acres of the state are planted in row crops, mostly corn and soybeans, and this land is susceptible to erosion and runoff. U.S. Geological Survey (USGS 1993; 1994) reports on suspended sediment in Iowa rivers and streams indicated concentrations that ranged from 3 to 3,180 mg/L in 1993 and from 4 to 3,230 mg/L in 1994. In addition to degrading basic water quality and aesthetics, SS increase turbidity and affect aquatic ecosystems in many ways, including negative effects on zooplankton and primary production (Alabaster and Lloyd 1980; Lloyd et al. 1987; Kirk 1991; Grobbelaar 1992; Hayes et al. 1992).

Negative effects on survival, growth, feeding, reproduction, and behavior of fish are well documented (Vinyard and O’Brien 1976; Swenson and Matson 1976; Auld and Schubel 1978; Sigler et al. 1984; Vandenbyllaardt et al. 1991). A few studies examined the effects of SS on gill tissue (Herbert and Merkens 1961; Goldes et al. 1986; 1988; Martens and Servizi 1993). Gills are a sensitive organ because the structure of the gills provide a large surface area for the movement of water even in resting fish (Heath 1995). Large flows of water (70 mL/min) passed over the gills in resting brook trout *Salvelinus fontinalis* (McKim and Goeden 1982). Therefore, the large volume of water passing over the gills increases the contact of the gill epithelia much more than the general body surface. The delicate epithelia of the secondary lamellae make the gills sensitive to SS and often the first system to be affected by toxicants (Heath 1995). Therefore, it is critical to better understand the effects of SS on gills if the effects of SS are to be quantified. An extensive literature review by Muncy et al. (1979) indicated that larval fishes are less tolerant of SS than eggs or adults and that lethal levels of SS are dependent on the concentration, shape, particle size, and turbulence of SS.
Walleye Stizostedion vitreum is regarded as an "extremely sensitive fish" to SS (Alabaster and Lloyd 1980); thus, this species could be used as an indicator species to measure the effects of SS on fish populations. The objectives of this study are to evaluate the effects of chronic exposure of walleye, from prolarva to early juvenile, to concentrations of SS (2.3–360 mg/L) that are about 10% of maximum concentrations reported in Iowa rivers and streams (USGS 1993; 1994). Additionally, histopathological changes to the gills were examined.

Methods

Treatment

An aluminum silicate ball clay (Old Mine #4 Kentucky ball clay, Kentucky-Tennessee Clay Company¹, Mayfield, Kentucky) was used as the SS in this study. Ball clay is mostly kaolinite, with 72% of the particles <1.0 µm (Table 1). Kaolinite \([\text{Al}_2\text{SiO}_2\text{O}_5(\text{OH})_4]\) is a common product of weathering in soils (Chamley 1989) and has a white or yellowish-white appearance, when dry, because it has a low iron content (Grim 1968; Stecher 1968).

In experiment 1 (E1), six different turbidity levels (NTU) were used: clear (0.8), 12, 26, 54, 100, and 206. These turbidity levels correspond to estimated SS concentrations of 2.7, 15, 35, 70, 120, and 250 mg/L estimated from a standard curve showing the relationship between SS (mg/L) and turbidity (NTU) (Figure 1). In the experiments, turbidities were attained by pumping \(\approx 25 \text{ to } \approx 1,500 \text{ mL of an } 8 \text{ mg/L ball clay slurry to each of five } 277-\text{L tanks for } 20 \text{ s at } 15 \text{ min intervals } 24\text{-h/d.}

In experiment 2 (E2), six different turbidity levels (NTU) were used: clear (0.5), 91, 144, 182, 227, and 295. These turbidity levels correspond to estimated SS concentrations of 2.3, 115, 180, 220, 280, and 360 mg/L estimated from a standard curve showing the relationship between SS (mg/L) and turbidity (NTU) (Figure 1). The turbidities were attained by the same

¹Use of trade or manufacturer name does not imply endorsement.
Table 1. Physical and chemical properties of ball clay used as
the source of SS (Source: Kentucky-Tennessee Clay
Company, Mayfield, Kentucky).

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th>% Particle Size</th>
<th>Chemical Analysis %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific</td>
<td>24.4 Finer than</td>
<td>SiO$_2$ 55.9</td>
</tr>
<tr>
<td>Surface Area</td>
<td>10.0 $\mu$m 97</td>
<td>Al$_2$O$_3$ 27.2</td>
</tr>
<tr>
<td></td>
<td>5.0 $\mu$m 93</td>
<td>Fe$_2$O$_3$ 1.1</td>
</tr>
<tr>
<td>Soluble SO$_4$ med</td>
<td>1.0 $\mu$m 72</td>
<td>TiO$_2$ 1.2</td>
</tr>
<tr>
<td></td>
<td>0.5 $\mu$m 56</td>
<td>CaO 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MgO 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K$_2$O 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Na$_2$O 0.2</td>
</tr>
</tbody>
</table>
Figure 1. The relationship between SS (mg/L of ball clay) and turbidity (NTU). Solid circles are measured values from three replicate sets of clay standards. Dashed lines represent 95% confidence limits.
method used in E1, except that the ball clay slurry concentration was 14 g/L. The clay slurry was added to each of five tanks for 20 s at 12 min intervals 24-h/d.

In both experiments, turbidity was measured from samples taken from the culture tanks 2–3 times daily. Turbidity was measured with a 90° scattering light turbidimeter (HACH model 2100P, HACH Company, Loveland, Colorado). Turbidity measurements were used because more measurements were obtained each day. Therefore, it was preferable to use this method over the filtering method (APHA et al. 1989) used to measure SS.

Although only turbidity was measured, SS concentrations were calculated from a standard curve of the relationship between turbidity (NTU) and SS (mg/L). Concentrations of clay (mg/L) were measured using standard procedures for measuring SS (mg/L) (APHA et al. 1989) and a single relationship between turbidity and SS was used because the same clay type was used throughout both experiments.

**Scanning Electron Microscopy**

A JEOL U.S.A., Inc., model JSM-35 scanning electron microscope (SEM), 100–120 Å resolution, was used to examine the clay. The procedures involved attachment of a 13-mm brass disk to a 25 X 75 mm glass slide with double-sided tape. The non-adhesive side of a piece of silver tape was glued on the brass disk. The sample of clay was sputter coated with a 60:40 mixture of platinum:palladium in a vacuum (0.06 Torr), Polaron E5100 sputtering system, 35 mA for 4 min, with an argon atmosphere. After the clay was coated, the sample was examined by SEM. Photographs were taken of the clay at 1,000 X and 20,000 X magnification using Polaroid 665 positive/negative film.

**Fish**

In E1, eyed-eggs within 1 d of hatching were obtained from the Rathbun Fish Hatchery on February 10, 1995. Eggs were incubated in a standard hatching jar and only larvae that
hatched within a 24-h interval were used to maintain uniformity of age. Number of larvae were estimated gravimetrically (385 larvae/g), and stocked in the tanks when they were 3-d posthatch. Larvae were stocked at a density of 17.9 larvae/L (4950 larvae/277-L tank), the maximum density that could be achieved with the number of larvae available. The mean total length of 20 larvae, at hatching, was 7.1 ± 0.02 mm (mean ± SE).

In E2, eyed-eggs within 3 d of hatching were obtained from the London State Fish Hatchery, London, Ohio on April 4. Again, only larvae that hatched within a 24-h interval were used to maintain uniformity of age. Larvae were counted and stocked in the tanks when they were 1-d posthatch as described above. The average weight of larvae was 279 larvae/g and larvae were stocked at a density of 17.9 larvae/L to permit comparison of the results with E1. The mean total length of 20 larvae, at hatching, was 7.6 ± 0.04 mm (mean ± SE).

Culture Conditions

In both experiments, walleye were raised in 277-L cylindrical (77 cm diameter, 62 cm depth) fiberglass tanks with black sides and aqua-colored bottoms. Each tank had a centrally placed drain with a 5-cm PVC standpipe surrounded by a 15-cm PVC standpipe with a 710 µm mesh screen. Initially, tanks had a total inflow of 2.5 L/min (0.55 exchanges/h) and a circular flow of water. The inflow was distributed by a vertical bar at 2.0 L/min and a horizontal spray bar at 0.5 L/min. After 5 to 7 d, flow rate was increased to 5.0 L/min (1.1 exchanges/h) with a vertical bar flow of 4.5 L/min and 0.5 L/min through the horizontal spray bar.

Tanks were illuminated 24 h/d with 75-W flood lamps located about 1 m above the water. Light intensity was adjusted to provide 100 lx at the surface. Larvae were fed Fry Feed Kyowa B-400 and C-700 diets (Biokyowa Inc., Chesterfield, Missouri) 22 h/d throughout the study period. Larvae 3- to 11-d old were fed 100% B-400, then the food was gradually changed from B-400 to C-700 by feeding a 75:25 mixture of B-400:C-700 from 11- to 18-d posthatch, a 50:50 mixture from 18- to 21-d posthatch, a 25:75 mixture from 21- to 24-d
posthatch, and 100% C-700 after 24-d posthatch. Feeding was suspended for 2 h/d to clean the tanks. Tank hygiene was maintained by siphoning waste, feed, debris, and dead larvae from the tank bottoms each day. Dead larvae were counted in the siphoned debris. Drain screens were removed daily and sprayed clean with pressurized water.

**Water Quality**

In both experiments, temperature (± 0.1°C) was measured daily. Dissolved oxygen (DO) was measured to the nearest ± 0.1 mg/L using an oxygen-sensitive membrane electrode (polarographic) with a Yellow Springs model 56 meter. Total ammonia-nitrogen (TAN) was measured to the nearest ± 0.01 mg/L twice a week using the Nesslerization method (APHA et al. 1989) and a HACH DR/3000 spectrophotometer. Twice weekly using colorimetric methods and a DR/3000 spectrophotometer, concentrations of nitrite (diazotation method) and nitrate (low-range cadmium reduction method) were measured to the nearest 0.01 mg/L; chloride (mercuric thiocyanate method) was measured to the nearest 0.1 mg/L. The pH was measured to the nearest 0.1 mg/L twice a week using a standard combination electrode and meter standardized with pH 4.0, 7.0, and 10.0 buffers. Hardness was measured to the nearest 1 mg/L once a week using the Man Ver 2 burette titration method (HACH Company, Loveland, Colorado). Once weekly, total alkalinity and free CO₂ were measured to the nearest 1 mg/L by titration with 0.02N H₂SO₄ and 0.1N NaOH, respectively (APHA et al. 1989). Quality control samples (HACH Company, Loveland, Colorado) were analyzed along with water samples to verify the accuracy of the procedures used to measure TAN, nitrate, nitrite, and chloride. Measured concentrations for the externally supplied TAN, nitrate, nitrite, and chloride quality assurance samples were always within the certified 95% confidence interval.
**Fish Sampling**

In both experiments, 5 fish were randomly removed from each tank every day from 2 to 21-d posthatch, and 20 larvae were removed from each tank every 7 d to 42-d posthatch. Fish were removed from the tanks for measurements of length, observations of gas bladder inflation, presence of food, and deformities. However, in E1, no fish were examined in the 0.8 NTU treatment to 28- and 42-d posthatch and no fish exposed to 12 NTU to 42-d posthatch were examined because no fish survived (0%). In E2, no fish in the 0.5 NTU treatment could be examined at 42-d posthatch because none survived. Walleye were euthanized with 200 mg/L tricaine methanesulfonate (Finquel®) and observed microscopically for gas bladder inflation (GBI), total length (mm), presence of food in the gut, and deformities. After examination, fish were fixed in 10% buffered formalin for histological examination.

**Histological Analysis**

In both experiments (E1 and E2), gills of 7 fish from each treatment (except 0.8 NTU in E1 because no fish survived) were examined histopathologically at 28-d posthatch. At 42-d posthatch, 5 fish from each treatment (except 0.8 and 12 NTU in E1 and 0.5 NTU in E2 because no fish survived) were examined at 42-d posthatch. Only the head was processed for histological examination.

Tissues were dehydrated in a series of ethanol solutions, cleared in a 1:1 100% ethanol:cedarwood mixture, and infiltrated by passing the tissues through a series of paraffin solutions. After the tissues were processed through the last paraffin solution, they were embedded into paraffin, sectioned (8 µm), and placed on glass slides.

After the sections were dry, they were stained with hematoxylin and eosin (H and E). Light microscopy was used to examine the gills for necrosis, lamellar fusion, epithelial lifting, hypertrophy, hyperplasia, and clubbing. Gills were also examined for the presence of debris.
Statistical Analysis

In both experiments (E1 and E2) tank space was limited and no replication was possible, however, for water quality variables, the daily observations were used as repeated measures. A two factor ANOVA was used to assess differences among treatments for all water quality parameters: treatment (level of NTU) and time (water samples taken during both experiments) were the factors. Differences among treatments were considered statistically significant when \( P \leq 0.05 \). When the \( P \)-value of the ANOVA was significant, a simple linear regression was used to determine if a linear relationship existed between the water quality parameter and treatment. Simple linear regressions were used to determine the relationship between growth and turbidity from hatching to 28-d posthatch and the slope of the regression equation represented the growth rates. Turbidity at maximum survival and maximum total length was estimated by fitting the data to a least-squares model, using a second degree polynomial analysis. The optimal turbidities were the values for maximum survival and maximum total length from the curvilinear relationship. Histological analysis was quantified by examining the secondary lamellae on the first and third gill filaments for any histological alterations. Frequency of occurrence of specific histological changes was calculated as the number of occurrences/100 secondary lamellae for 7 fish from each treatment at 28-d posthatch and the number of occurrences/100 secondary lamellae for 5 fish from each treatment when fish were 42-d posthatch.

Results

Clay-Turbidity Relationship and SEM Characteristics of Clay

The linear relationship between concentration of clay (mg/L) and turbidity units (NTU) was positive \( (Y = 1.732 + 1.219X; r^2 = 0.99) \) (Figure 1). The coefficient of determination \( (r^2) \) was 0.99, which indicates that NTU provided an accurate estimate of SS (mg/L) prepared with clay.
Photographs at 1,000 X magnification show that the clay particles are mostly rounded with smooth edges; photographs at 20,000 X magnification show that the ball clay is made up of layers (Figure 2).

**Water Quality**

Other than turbidity and TAN, all environmental conditions and culture techniques were similar for both experiments. In E1, DO, temperature, pH, alkalinity, hardness, nitrate, nitrite, and chloride did not differ among treatments, but total ammonia nitrogen (NH₃-N; TAN) did (Table 2). There were positive linear relationships between turbidity and TAN and between number of fish at 28-d posthatch and TAN (Figure 3). Carbon dioxide values were always below detectable levels (0.4 mg/L).

In E2, except for TAN, statistically significant differences were not observed for temperature, DO, pH, alkalinity, hardness, nitrate, nitrite, and chloride among treatments (Table 3). Linear relationships were not observed between turbidity and TAN or number of fish at 28-d posthatch and TAN, but TAN values were higher in turbid water (91–295 NTU) than in clear water (0.5 NTU). Carbon dioxide values were always below detectable levels.

**Survival**

In E1, survival to 28-d posthatch was highest at 206 NTU and no walleye survived in clear water (0.8 NTU) after 22-d (Table 4). The maximum survival to 28-d was estimated to be 46% at 164 NTU using a second degree polynomial analysis (Figure 4). In E2, survival to 28-d posthatch was highest in the 182 NTU treatment then survival decreased at both increasing and decreasing turbidity levels (Table 4). The maximum survival to 28-d posthatch was estimated to be 34% at 200 NTU (Figure 5). Excluding data from the clear treatment (0.5 NTU), a simple linear regression through the remaining data points was not significant,
Figure 2. Scanning electron microscope (SEM) photographs of ball clay used in the experiments at 1,000 X (A) and 20,000 X (B).
Table 2. Water quality values (mean ± SE) for E1. A two factor ANOVA was used to determine differences among treatments.

<table>
<thead>
<tr>
<th>Treatment (NTU) (n=55)</th>
<th>Temp(^2) (°C) (n=26)</th>
<th>pH(^3) (n=8)</th>
<th>TAN (mg/L) (n=8)</th>
<th>DO (mg/L) (n=24)</th>
<th>Alk (mg/L) (n=4)</th>
<th>Hard (mg/L) (n=4)</th>
<th>Nitrate(^4) (mg/L) (n=8)</th>
<th>Nitrite (mg/L) (n=8)</th>
<th>Chloride (mg/L) (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>17.0</td>
<td>7.6</td>
<td>0.38±0.05</td>
<td>9.4±0.09</td>
<td>36.5±0.9</td>
<td>174±2.0</td>
<td>0.14</td>
<td>0.19±0.03</td>
<td>35.2±1.4</td>
</tr>
<tr>
<td>12</td>
<td>17.0</td>
<td>7.7</td>
<td>0.42±0.05</td>
<td>9.3±0.10</td>
<td>37.4±0.9</td>
<td>173±3.1</td>
<td>0.14</td>
<td>0.19±0.03</td>
<td>35.3±2.2</td>
</tr>
<tr>
<td>26</td>
<td>17.0</td>
<td>7.6</td>
<td>0.43±0.05</td>
<td>9.3±0.11</td>
<td>37.9±0.8</td>
<td>175±2.6</td>
<td>0.17</td>
<td>0.20±0.03</td>
<td>33.9±1.0</td>
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<tr>
<td>54</td>
<td>17.0</td>
<td>7.6</td>
<td>0.47±0.05</td>
<td>9.3±0.10</td>
<td>38.4±1.0</td>
<td>172±2.9</td>
<td>0.17</td>
<td>0.20±0.04</td>
<td>35.9±1.5</td>
</tr>
<tr>
<td>100</td>
<td>17.1</td>
<td>7.6</td>
<td>0.53±0.04</td>
<td>9.2±0.11</td>
<td>39.3±2.3</td>
<td>169±1.3</td>
<td>0.17</td>
<td>0.20±0.03</td>
<td>37.1±1.5</td>
</tr>
<tr>
<td>206</td>
<td>17.1</td>
<td>7.6</td>
<td>0.53±0.06</td>
<td>9.2±0.11</td>
<td>40.0±2.8</td>
<td>176±3.6</td>
<td>0.17</td>
<td>0.20±0.03</td>
<td>35.3±2.6</td>
</tr>
<tr>
<td>SE(^1)</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>1.3</td>
<td>2.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.73</td>
</tr>
<tr>
<td>P-value</td>
<td>0.98</td>
<td>0.18</td>
<td>0.001</td>
<td>0.10</td>
<td>0.50</td>
<td>0.50</td>
<td>0.10</td>
<td>0.89</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^1\) SE = (Error mean square/n)\(^{1/2}\).

\(^2\) SE for all groups was ± 0.3.

\(^3\) SE for all groups was ± 0.02.

\(^4\) SE for all groups was ± 0.02.
Figure 3. Simple linear regressions showing the relationship between TAN (mg/L) and turbidity (NTU) and between TAN (mg/L) and number of fish at 28-d posthatch in E1.
Table 3. Water quality values (mean ± SE) for E2. A two factor ANOVA was used to determine differences among treatments.

<table>
<thead>
<tr>
<th>Treatment (NTU) (n=71)</th>
<th>Temp(^2) (°C) (n=28)</th>
<th>pH (n=8)</th>
<th>TAN (mg/L) (n=8)</th>
<th>DO (mg/L) (n=27)</th>
<th>Alk (mg/L) (n=4)</th>
<th>Hard (mg/L) (n=4)</th>
<th>Nitrate(^3) (mg/L) (n=8)</th>
<th>Nitrite (mg/L) (n=8)</th>
<th>Chloride (mg/L) (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>17.1</td>
<td>7.6±0.07</td>
<td>0.25±0.07</td>
<td>9.1±0.10</td>
<td>34.3±0.3</td>
<td>134±1.0</td>
<td>0.35</td>
<td>0.72±0.04</td>
<td>40.0±2.0</td>
</tr>
<tr>
<td>91</td>
<td>17.0</td>
<td>7.5±0.07</td>
<td>0.31±0.06</td>
<td>8.9±0.11</td>
<td>33.8±0.5</td>
<td>134±0.5</td>
<td>0.35</td>
<td>0.73±0.04</td>
<td>39.8±2.0</td>
</tr>
<tr>
<td>144</td>
<td>17.1</td>
<td>7.5±0.07</td>
<td>0.34±0.06</td>
<td>8.9±0.10</td>
<td>34.0±0.4</td>
<td>133±0.6</td>
<td>0.35</td>
<td>0.73±0.04</td>
<td>40.8±1.0</td>
</tr>
<tr>
<td>182</td>
<td>17.1</td>
<td>7.6±0.04</td>
<td>0.34±0.05</td>
<td>8.9±0.10</td>
<td>33.5±0.3</td>
<td>133±0.5</td>
<td>0.36</td>
<td>0.72±0.03</td>
<td>41.0±1.1</td>
</tr>
<tr>
<td>227</td>
<td>17.1</td>
<td>7.5±0.06</td>
<td>0.32±0.05</td>
<td>8.9±0.10</td>
<td>34.3±0.3</td>
<td>134±0.5</td>
<td>0.35</td>
<td>0.73±0.03</td>
<td>40.5±0.8</td>
</tr>
<tr>
<td>295</td>
<td>17.1</td>
<td>7.6±0.06</td>
<td>0.37±0.06</td>
<td>8.9±0.10</td>
<td>33.8±0.5</td>
<td>132±0.5</td>
<td>0.35</td>
<td>0.73±0.03</td>
<td>41.4±1.0</td>
</tr>
<tr>
<td>SE(^1)</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.22</td>
<td>0.6</td>
<td>0.01</td>
<td>0.01</td>
<td>0.70</td>
</tr>
<tr>
<td>P-value</td>
<td>0.99</td>
<td>0.46</td>
<td>0.001</td>
<td>0.828</td>
<td>0.15</td>
<td>0.12</td>
<td>0.88</td>
<td>0.86</td>
<td>0.60</td>
</tr>
</tbody>
</table>

1 SE = (Error mean square/n)\(^{1/2}\).

2 SE for all groups was ± 0.1.

3 SE for all groups was ± 0.02.
Table 4. Survival of larval walleye to 28-d posthatch in E1 and E2.

<table>
<thead>
<tr>
<th>Turbidity (NTU)</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean¹</td>
<td>95% C.I.</td>
<td>Survival (%)</td>
<td>Mean²</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>0.8</td>
<td>(0.7,1.0)</td>
<td>0.0</td>
<td>0.5</td>
<td>(0.4,0.6)</td>
</tr>
<tr>
<td>12</td>
<td>(11.0,13.5)</td>
<td>10.6</td>
<td>91</td>
<td>(87.4,94.0)</td>
</tr>
<tr>
<td>26</td>
<td>(24.1,27.1)</td>
<td>18.4</td>
<td>144</td>
<td>(138.2,150.6)</td>
</tr>
<tr>
<td>54</td>
<td>(51.6,55.6)</td>
<td>27.5</td>
<td>182</td>
<td>(172.9,191.1)</td>
</tr>
<tr>
<td>100</td>
<td>(95.1,105.7)</td>
<td>36.9</td>
<td>227</td>
<td>(216.4,238.4)</td>
</tr>
<tr>
<td>206</td>
<td>(198.8,213.1)</td>
<td>43.3</td>
<td>295</td>
<td>(285.3,304.3)</td>
</tr>
</tbody>
</table>

¹55 observations were used to calculate the mean for each turbidity measure.

²71 observations were used to calculate the mean for each turbidity measure.
Figure 4. The relationship between survival of walleye to 28-d posthatch and turbidity in E1.

The regression line is a second degree polynomial.
Figure 5. A quadratic relationship between survival of walleye to 28-d posthatch in E2. The regression line is a second degree polynomial. The dashed line is the simple linear regression between survival and turbidity treatments, except 0.5 NTU group.
suggesting no significant difference in survival of fish raised in 91 to 295 NTU water (Figure 5).

**Total Length and Growth Rate**

In E1, mean lengths of 28-d old fish ranged from 19.2 mm for fish exposed to 26 NTU to 21.8 mm for fish exposed to 100 NTU (Table 5). Maximum total length of 28-d old fish was estimated to be 22 mm at 150 NTU (Figure 6). The slope of the regression equations represented the mean growth rates in each treatment (Figure 7). Growth rates were about 0.25 mm/d for fish in clear water (0.8 NTU), 0.43 mm/d for fish in 12–26 NTU, and 0.50 mm/d for fish in 54–206 NTU.

In E2, mean lengths of 28-d posthatch walleye ranged from 18.1 mm for fish exposed to 0.5 NTU to 26.4 mm for fish exposed to 227 NTU (Table 5). The maximum total length of 28-d old fish was estimated to be 27 mm at 200 NTU (Figure 8); however, a simple linear regression, excluding data from the clear treatment (0.5 NTU), showed no significant differences in total length for fish raised in 91 to 295 NTU water (Figure 8). Growth rates, derived from slopes of the regression equations, were 0.40 mm/d for walleye in clear water (0.8 NTU) and 0.66–0.68 mm/d for walleye exposed to 91–295 NTU (Figure 9).

**Gas bladder inflation and feed acceptance**

In E1, feed was first observed in the gut of 6-d old walleye raised in 206 NTU. All 7-d old walleye had begun to feed in all treatments, except the lowest treatment (0.8 NTU). Gas bladder inflation (GBI) occurred in walleye raised in turbid water (12–206 NTU) when fish were 9-d old and occurred in fish in clear water (0.8 NTU) on day 10. Nearly 100% of 28-d old fish in all of the turbid water tanks were feeding and GBI had occurred; however, survival was 0% in the clear water tank (Table 6).
Table 5. Mean total lengths (mm ± SE) of 20 fish measured for 7-, 14-, 21-, and 28-d old walleye in E1 and E2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>7 d (mm) (mean ± SE)</th>
<th>14 d (mm) (mean ± SE)</th>
<th>21 d (mm) (mean ± SE)</th>
<th>28 d (mm) (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 (NTU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>8.9 ± 0.04</td>
<td>10.8 ± 0.18</td>
<td>12.3 ± 0.22†</td>
<td>††</td>
</tr>
<tr>
<td>12</td>
<td>9.1 ± 0.03</td>
<td>12.7 ± 0.11</td>
<td>14.8 ± 0.13</td>
<td>19.3 ± 0.24</td>
</tr>
<tr>
<td>26</td>
<td>9.1 ± 0.04</td>
<td>13.2 ± 0.09</td>
<td>15.2 ± 0.13</td>
<td>19.2 ± 0.28</td>
</tr>
<tr>
<td>54</td>
<td>9.1 ± 0.02</td>
<td>13.6 ± 0.10</td>
<td>16.7 ± 0.14</td>
<td>20.4 ± 0.32</td>
</tr>
<tr>
<td>100</td>
<td>9.1 ± 0.05</td>
<td>13.6 ± 0.10</td>
<td>16.6 ± 0.12</td>
<td>21.8 ± 0.28</td>
</tr>
<tr>
<td>206</td>
<td>9.2 ± 0.06</td>
<td>13.6 ± 0.12</td>
<td>16.6 ± 0.15</td>
<td>21.4 ± 0.21</td>
</tr>
<tr>
<td>E2 (NTU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>9.5 ± 0.07</td>
<td>12.5 ± 0.16</td>
<td>16.1 ± 0.28</td>
<td>18.1 ± 0.33</td>
</tr>
<tr>
<td>91</td>
<td>10.5 ± 0.12</td>
<td>15.9 ± 0.14</td>
<td>20.8 ± 0.16</td>
<td>26.3 ± 0.25</td>
</tr>
<tr>
<td>144</td>
<td>10.7 ± 0.11</td>
<td>16.6 ± 0.13</td>
<td>21.2 ± 0.21</td>
<td>25.7 ± 0.32</td>
</tr>
<tr>
<td>182</td>
<td>10.5 ± 0.13</td>
<td>16.1 ± 0.14</td>
<td>21.5 ± 0.28</td>
<td>25.1 ± 0.25</td>
</tr>
<tr>
<td>227</td>
<td>10.9 ± 0.09</td>
<td>16.4 ± 0.11</td>
<td>21.1 ± 0.22</td>
<td>26.4 ± 0.41</td>
</tr>
<tr>
<td>295</td>
<td>10.9 ± 0.07</td>
<td>15.6 ± 0.20</td>
<td>21.2 ± 0.20</td>
<td>25.4 ± 0.31</td>
</tr>
</tbody>
</table>

†Because of poor survival, only 13 fish were measured in this treatment group.
††Total mortality
$Y = 18.381 + 0.048X - 1.629E^{-4}X^2; \ r^2 = 0.95$

$P = 0.049$

Figure 6. The relationship between mean total length of walleye to 28-d posthatch in E1.
Figure 7. The relationship between total length of walleye to 28-d posthatch in E1. The slopes of the regression equations represent mean growth rates.
Figure 8. A quadratic relationship between total length (mm) of walleye to 28-d posthatch in E2. The regression line is a second degree polynomial. The dashed line is a simple linear regression showing the relationship between total length (mm) of walleye to 28-d posthatch and turbidity treatments, excluding 0.5 NTU.
Figure 9. The relationship between total length (mm) of walleye to 28-d posthatch in E2. The slopes of the regression equations represent mean growth rates.
Table 6. Gas bladder inflation (GBI) and feeding of larval walleye reared in clear and turbid water in E1 and E2.

Data for observations of 20 fish on 7-, 14-, 21-, and 28-d posthatch.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>7 d posthatch</th>
<th>14 d posthatch</th>
<th>21 d posthatch</th>
<th>28 d posthatch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% GBI)</td>
<td>(% Food)</td>
<td>(% GBI)</td>
<td>(% Food)</td>
</tr>
<tr>
<td>E1 (NTU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>45</td>
<td>100</td>
<td>65</td>
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<tr>
<td>26</td>
<td>0</td>
<td>60</td>
<td>100</td>
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<td>100</td>
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</tr>
<tr>
<td>206</td>
<td>0</td>
<td>65</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

| E2 (NTU)  |               |                |                |                |                |                |
| 0.5       | 20            | 20             | 80             | 100            | 80             | 95             | 60             |
| 91        | 100           | 100            | 100            | 100            | 100            | 100            | 100            |
| 144       | 100           | 95             | 95             | 100            | 100            | 100            | 100            |
| 182       | 100           | 100            | 100            | 100            | 100            | 100            | 100            |
| 227       | 100           | 100            | 85             | 100            | 100            | 95             | 100            |
| 295       | 95            | 95             | 95             | 75             | 100            | 100            | 90             |

† Because of poor survival, only 13 fish were measured in this treatment group.
†† Total mortality.
In E2, walleye raised in all levels of turbidity (91–295) began feeding at 4-d posthatch, but walleye raised in clear water (0.5 NTU) did not begin feeding until 7-d posthatch. GBI occurred in walleye raised in turbid water (91–295 NTU) at 5-d posthatch, and occurred in fish raised in clear water (0.5 NTU) on day 6. At 7-d posthatch, only 20% of the walleye in clear water were observed to have feed in their gut, and only 20% had inflated gas bladders. However, 95–100% of the walleye in the turbid water tanks had feed in their gut and inflated gas bladders on day 7. After 28-d posthatch, 100% of the fish in turbid water had feed in their gut and GBI had occurred; 80% of the fish in clear water had feed in their gut, and 95% had inflated gas bladders (Table 6).

**Gill histology**

Secondary lamellae are composed of pillar cells and lacunae (spaces between pillar cells in which red blood cells flow). The basement membrane is located between the pillar cells and epithelial cells; chloride cells and mucus cells are located at intervals along the epithelial cell layer at the base of the secondary lamellae (Figure 10).

In E1, no gill tissue damage was observed in 28- or 42-d old fish, but debris was observed between adjacent secondary lamellae (Table 7). In E2, no evidence of damaged gill tissue was observed in 28-d old fish (Table 8). Hyperplasia was observed in 1 of 5 fish exposed to 182 NTU at 42-d posthatch and debris was observed between adjacent secondary lamellae at 28- and 42-d posthatch (Table 8; Figure 10). All fish observed with debris between secondary lamellae had normal gill histology (Figure 10).

**Discussion**

In both experiments, water quality parameters for all treatment groups were within acceptable ranges for culturing fish. Other than TAN, no water quality parameters differed significantly in E1 and E2. Variations in TAN were positively correlated with survival rates in
Figure 10. Gill structure of (A) a 42-d old walleye with normal gill histology exposed to 295 NTU with debris between adjacent secondary lamellae and (B) a 42-d old walleye with hyperplasia exposed to 91 NTU.
Table 7. Frequency of occurrence (number of histological changes/100 secondary lamellae) of histological changes in gills of walleye in E1. Seven fish from each treatment were examined at 28-d posthatch and 5 fish were examined at 42-d posthatch.

<table>
<thead>
<tr>
<th>Treatment (NTU)</th>
<th>Days posthatch</th>
<th>Epithelial Lifting</th>
<th>Necrosis</th>
<th>Lamellar Fusion</th>
<th>Debris</th>
<th>Hyperplasia</th>
<th>Clubbing</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>28</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>0.8</td>
<td>42</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>12</td>
<td>28</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>12</td>
<td>42</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>26</td>
<td>28</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>54</td>
<td>28</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>54</td>
<td>42</td>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>100</td>
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<td>0.0</td>
<td>0.0</td>
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<tr>
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<td>0.0</td>
</tr>
<tr>
<td>206</td>
<td>42</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

† No survival.
Table 8. Frequency of occurrence (number of histological changes/100 secondary lamellae) of histological changes in gills of in E2. Seven fish from each treatment were examined at 28-d posthatch and 5 fish were examined at 42-d posthatch.

<table>
<thead>
<tr>
<th>Treatment (NTU)</th>
<th>Days posthatch</th>
<th>Epithelial lifting</th>
<th>Necrosis</th>
<th>Lamellar fusion</th>
<th>Debris</th>
<th>Hyperplasia</th>
<th>Clubbing</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>28</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.5</td>
<td>42</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>91</td>
<td>28</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>91</td>
<td>42</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>144</td>
<td>28</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>144</td>
<td>42</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>182</td>
<td>28</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>182</td>
<td>42</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.1</td>
<td>5.7</td>
<td>0.0</td>
</tr>
<tr>
<td>227</td>
<td>28</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>227</td>
<td>42</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>295</td>
<td>28</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>295</td>
<td>42</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

† No survival.
the turbid water treatments (12–295 NTU). There was no survival 0.8 NTU to 28-d posthatch in E1.

Sensitivity of fish exposed to the same type, size, and concentration of SS is species specific (Auld and Schubel 1978). Sensitivity is also dependent on the concentration and length of exposure to SS (Herbert and Merkens 1961; Auld and Schubel 1978; Gardner 1981; Breitberg 1988; Barrett et al. 1992). Auld and Schubel (1978) found that striped bass *Morone saxatilis* and yellow perch *Perca flavescens* were able to tolerate high concentrations of suspended sediment containing illite, chlorite, and kaolinite (1–4 µm particles) collected from the Chesapeake Bay (~500 mg/L), but survival of American shad larvae *Alosa sapidissima* decreased when concentrations were 100 mg/L.

Bristow and Summerfelt (1994) and Bristow et al. (1996) reported that turbidities up to 50 NTU, produced with clay of the same type used in the present study, enhanced survival of larval walleye. Survival was 35% in turbid water (23.8 NTU) compared with 6.6% and 1.9% in clear and blue colored water, respectively (Bristow et al. 1996). In the present study, a survival-turbidity curve suggested a peak survival of 46% at 164 NTU in E1 and 34% at 200 NTU in E2. Excluding fish in the clear water (0.8 NTU) treatment in E2, there was no difference in survival and total length of walleye raised in 91 to 295 NTU.

Although effects of SS and turbidity on the growth of fish have been examined before, results differ (Swenson and Matson 1976; Sigler et al. 1984; Bristow and Summerfelt 1994; Bristow et al. 1996). In addition to the type of clay used in the present study, bentonite and kaolin have also been used to examine the effects of SS on fish (Auld and Schubel 1978; Gardner 1981; Sigler et al. 1984). Kaolin is similar to the clay used in the present study, in that, it is a hydrous aluminum silicate consisting mostly of kaolinite, it is insoluble in water and white or yellowish-white in appearance because it has a low iron content (Grim 1968; Stecher 1968; Clauer and Chaudhuri 1995). Bentonite is a highly colloidal hydrated aluminum silicate consisting mostly of montmorillonite. It has the unique characteristic of swelling to several
times its original volume when placed in water and ranges in color from white to almost black
(Grim 1968; Stecher 1968; Clauer and Chaudhuri 1995).

Sigler et al. (1984) used a bentonite and kaolinite clay to create turbid water to evaluate
growth effects on steelhead *Oncorhynchus mykiss* and coho salmon *Oncorhynchus kisutch*. Steelhead and coho salmon exposed to clear water had greater weights and lengths than fish in
turbid water (11–49 NTU). Growth of larval lake herring *Coregonus artedii* was not affected
by exposure to 1–28 mg/L red clay derived from sediment samples from Lake Superior
(Swenson and Matson 1976). Growth of larval walleye was enhanced at 20–50 NTU created
by ball clay (Bristow and Summerfelt 1994; Bristow et al. 1996). The mean total length of
walleye exposed to turbid water (23.8 NTU) was 22.3 mm compared with 16.7 and 15.1 mm
for fish exposed to clear or blue colored water, respectively, and the mean weight of walleye at
21-d posthatch was 270–380% greater for fish in turbid water than clear of blue colored water
(Bristow et al. 1996). In the present study, growth rates of walleye increased when higher
turbidity levels were used (12–295 NTU) and the maximum total length of 28-d old walleye
was estimated to be 22 mm at 150 NTU in E1 and 27 mm at 200 NTU in E2.

Feeding response of fish in solutions with high concentrations of SS depends on the
species, length of exposure, and type of SS used (Gardner 1981; Johnston and Wildish 1982;
Boehlert and Morgan 1985; Vandenbyllaardt et al. 1991; Miner and Stein 1993; Bristow and
Summerfelt 1994; Bristow et al. 1996). Feeding rates (number of prey/min) of bluegill
*Lepomis macrochirus* exposed to bentonite clay decreased at increasing turbidity levels (60–
190 NTU) (Gardner 1981). Vandenbyllaardt et al. (1991) reported that 85-mm walleye
feeding on fathead minnows *Pimephales promelas* was inhibited in water made turbid with Red
River sediment at levels of 100 and 161 NTU in a 1-h feeding trial, but feeding was not
inhibited at 121 NTU in a 4-h feeding trial. Maximum feeding of larval pacific herring *Clupea
harengus pallasi* on rotifers *Brachionus plicatilis* occurred at concentrations of 500 and 1000
mg/L estuarine sediment and Mount Saint Helens volcanic ash consisting of particles <24.0 µm
compared with fish exposed to clear water, 0 mg/L (Boehlert and Morgan 1985). Larval walleye exposed to clay, at turbidities of 20 to 50 NTU, began feeding earlier and had higher survival and faster growth than fish raised in clear water (Bristow and Summerfelt 1994; Bristow et al. 1996). Bristow et al. (1996) found that larval walleye were feeding by 4-d posthatch in turbid water, but not in clear or blue colored water and at 7-d posthatch 95% of fish were feeding in turbid water compared with 13% and 12% in clear and blue colored water, respectively. In E1 and E2, larval walleye began feeding earlier in turbid water with levels ranging from 12–296 NTU than fish in clear water (<1.0 NTU).

Boehlert and Morgan (1985) and Bristow and Summerfelt (1994) suggested increased turbidity levels may increase feeding of larval fish by providing a visual contrast of prey items. It is also possible that feed particles may be better illuminated by the light scattering effect of turbidity (Bristow and Summerfelt 1994).

In this study, larval walleye had higher rates of GBI when they were exposed to turbidity levels ranging from 12–296 NTU than fish raised in turbidities <1.0 NTU. Similar results were observed in walleye exposed to turbidity levels up to 50 NTU (Bristow and Summerfelt 1994; Bristow et al. 1996). It has been determined that the major inhibitor of GBI in larval walleye is oil on the surface of the water (Chatain and Ounais-Guschemann 1990; Barrows et al. 1993). As suggested by Bristow and Summerfelt (1994), it is possible that clay may have had some affinity for the oil in the water because kaolinite material is often used to remove oil (Stecher 1968).

No histological changes were observed in 28-d old walleye exposed to turbidities ranging from 12–295 NTU. However, 1 of 5 fish exposed to 182 NTU for 42 d had mild hyperplasia, but not at higher levels of turbidity. Although mild hyperplasia was observed in one fish, this seems to have been an isolated incident unrelated to SS concentrations.

Although no histological changes were observed in the gills of walleye exposed to 0.5–295 NTU, debris was located between adjacent secondary lamellae of some fish. The occurrence
was small (0.4–1.2 debris/100 secondary lamellae). The large size of the debris (about 39 µm) is much greater than clay because 97% of the ball clay is finer than 10 µm. The particles were more likely to be fragments of the microparticulate feed.

The findings indicate that chronic exposure of larval and early juvenile walleye to clay (SS) at concentrations as high as 360 mg/L are not harmful. Feeding and gas bladder inflation commences earlier, and growth was faster in turbid compared with clear water. To the degree that this study is relevant to events in turbid streams, we conclude that poor year class strength of walleye, a species previously characterized as highly sensitive to SS, cannot be attributed to SS when food is abundant. However, in nature, food may not be as abundant as in the laboratory, and further research is needed to determine if SS may result in poor year class strength because of starvation during the critical period when larvae shift from endogenous to exogenous feeding.

References


CHAPTER 3. GILL DEVELOPMENT OF LARVAL WALLEYE

A paper to be submitted to Transactions of the American Fisheries Society

Todd A. Phillips and Robert C. Summerfelt

Abstract

The development of gill filaments and secondary lamellae of larval walleye *Stizostedion vitreum* from hatching to 21-d posthatch is described. Total number of gill filaments were counted on the second gill arch of 5 fish each day from hatching to 21-d posthatch, except on days 7, 14, and 21 when 20 fish were examined. Length of gill filaments and number of secondary lamellae/gill filament were determined on the first, third, fifth, and seventh gill filaments on the most ventral part of the gill arch. There were strong positive relationships between cumulative temperature units (TU, °C) and total number of gill filaments, lengths of gill filaments, and number of secondary lamellae/gill filament. Gill filaments were first observed at 3-d posthatch (47 TU), and the first secondary lamellae were observed at 10-d posthatch (163 TU). There were strong positive relationships between total length and number of gill filaments, between total length and length of gill filaments, and between length and number of secondary lamellae/gill filament. Rapid development of gill structures took place at the end of the prolarval stage when larvae made the transition from yolk sac respiration to branchial respiration. Rapid development of secondary lamellae occurred at the end of the postlarval I stage (disappearance of the oil globule), when larvae switched from an endogenous energy source (oil globule) to exogenous feeding. Other studies on larval walleye suggested that increased sensitivity to certain toxicants occurs during the transition from yolk sac respiration to branchial respiration. This hypothesis is supported by the substantial increase in
growth and number of both gill filaments and secondary lamellae in the first 21 d of walleye development.

Introduction

Balon (1984) hypothesized that early ontogeny of larval fishes is a saltatory process; i.e., “a sequence of rapid changes in form and function alternating with prolonged intervals (steady states) of slower development during which complex structures are prepared for the next rapid change.” This is an important hypothesis, because larval fish are less tolerant of chemicals than either eggs or adults (Muncy et al. 1979). This sensitivity could be caused by rapid development of the gills of larval fish; gills are sensitive to many types of environmental contaminants including: heavy metals, organic pesticides, temperature, and pH (Mallatt 1985). Fish gills are a sensitive organ because the structure of the gills provides a large surface area for the movement of water (Heath 1995) and high amounts of water passing over the gills provides an opportunity for toxicants to pass over the gills. The delicate epithelia of the secondary lamellae make the gills sensitive to toxicants and often the first system to be affected by toxicants (Heath 1995).

Increased toxicity of hydrogen peroxide and elevated pH have been observed in walleye as they develop from prolarvae to postlarvae II (Bergerhouse 1992; Clayton and Summerfelt in press). Clayton and Summerfelt (in press) found that 6-d posthatch walleye were much more sensitive to hydrogen peroxide than 4-d posthatch walleye.

Although studies suggest that gill ontogeny is an important factor contributing to increased sensitivity to toxicants, few studies have documented the development of the gill arches, gill filaments, and gill lamellae in larval fish (McDonald and McMahon 1977; El-Fiky et al. 1987; Osse 1989). The objectives of this study are to determine if gill development in larval walleye is a saltatory process and to determine the critical events of gill development.
Methods

Terminology

Terminology for walleye development follows that given by Li and Mathias (1982): prolarva, postlarva I, and postlarva II. The prolarval stage is the yolk sac stage. The postlarval I stage begins with the disappearance of the yolk sac and continues with the beginning of exogenous feeding and gas bladder inflation. The beginning of the postlarval II stage is marked by the disappearance of the oil globule, and by the development of the first loop in the intestine, the differentiation of the gill rakers, and the development of fin rays on the base of the caudal fin. Juvenile fish have fin rays on the median fins, increased pigmentation, formation of the pylorus and blind sacs of the stomach, and gill rakers begin to function in filtering.

Fish

Eyed-eggs were obtained from the London State Fish Hatchery, London, Ohio on April 4, 1995. Eggs were incubated for 3-d before hatching began in a standard hatching jar and only larvae that hatched within a 24-h interval were used to maintain uniformity of age. Number of larvae were estimated gravimetrically (279 larvae/g), and stocked in the tank when they were 1-d old. Larvae were stocked at a density of 17.9 larvae/L (4950 larvae/277-L tank), the maximum density that could be achieved with the number of larvae available. The mean total length of 20 larvae, at hatching, was 7.1 ± 0.02 mm (mean ± SE).

Culture Conditions

Walleye were raised in turbid water at 17.0 ± 0.1°C. Turbid water was used because growth and survival of walleye to 21- to 30-d posthatch is significantly greater in turbid water (16.1 to 49.7 NTU) than in clear water (0.3 NTU) (Bristow and Summerfelt 1994). Bristow and Summerfelt (1994) found that culture water that was made turbid by the addition of a small
volume of a clay slurry every 20 min significantly increased feed acceptance at 7- and 14-d posthatch, and gas bladder inflation, lengths, weights, and survival of walleye to 21- to 30-d posthatch. We used a turbidity level of 91 NTU. Turbidity was attained by pumping =700 mL of a clay slurry consisting of 8 g/L of ball clay (Old Mine #4 Kentucky ball clay, Kentucky-Tennessee Clay Company¹, Mayfield, Kentucky) into the tank for 20 s at 15 min intervals 24-h/d. The clay is a hydrous aluminum silicate consisting mostly of kaolinite with 72% of the particles <1.0 µm, and 93% < 5 µm.

Walleye were raised in a 277-L cylindrical (77 cm diameter, 62 cm depth) fiberglass tank with black sides and an aqua-colored bottom. The tank had a centrally placed drain with a 5-cm PVC standpipe surrounded by a 15-cm PVC standpipe with 710-µm mesh screen. Initially, the tank had a total inflow of 2.5 L/min (0.55 exchanges/h) and a circular flow of water. The inflow was distributed by a vertical bar at 2.0 L/min and a surface spray of 0.5 L/min. Surface spray has been found to be essential for clearing oil and debris from the tank surface which helps obtain high gas bladder inflation (GBI) rates (Moore et al. 1994). After 5 to 7 d, the flow rate was increased to 5.0 L/min (1.1 exchanges/h) with a vertical bar flow of 4.5 L/min and a horizontal spray bar at 0.5 L/min.

The tank was illuminated 24 h/d with a 75-W flood lamp located about 1 m above the water surface. Light intensity was adjusted to provide 100 lx at the water surface. Walleye were fed Fry Feed Kyowa B-400 and C-700 diets (Biokyowa Inc., Chesterfield, Missouri) 22 h/d. Fish were fed 100% B-400 from 3- to 11-d posthatch, then gradually switched from B-400 to C-700 by feeding a 75:25 mixture of B-400:C-700 from 11- to 18-d posthatch, a 50:50 mixture was fed from 18- to 21-d posthatch, a 25:75 mixture was fed from 21- to 24-d posthatch, and 100% C-700 after 24-d posthatch. Feeding was suspended for 2 h/d to clean the tank. Tank hygiene was maintained by siphoning waste, feed, debris, and dead fish from the tank bottom.

¹Use of trade or manufacturer name does not imply endorsement.
and the drain screen was removed daily and sprayed clean with pressurized water. Dead fish were counted in the debris siphoned from the tank.

**Fish Sampling**

Every day, 5 walleye were randomly removed from the tank, but 20 fish were removed when fish were 7-, 14-, and 21-d old. Fish removed from tanks were euthanized using 200 mg/L tricaine methanesulfonate (Finquel®). Total length, yolk sac length, and oil globule diameter were measured using a dissection microscope at 0.46 to 4 X magnification and an ocular micrometer (0.01 mm) that was calibrated with a stage micrometer. These measurements allowed determination of the day when: the yolk sac and oil globule disappeared, GBI began, and first feeding occurred. After examination, the fish were fixed in 10% buffered formalin for examination of gill development.

**Analysis of Gill Filaments**

The second gill arch was dissected from 5 walleye daily for the first 21 d, except on days 7, 14, and 21 when the gills from 20 walleye were examined. The total number of gill filaments were counted by examining the gill arch microscopically at 10 X magnification. The number of secondary lamellae/gill filament on the first, third, fifth, and seventh gill filaments were counted, and the length of each filament was measured using an ocular micrometer (0.01 mm) at a magnification of 10 X.

**Statistical analysis**

Simple linear regressions were used to describe the relationship between cumulative temperature units, TU (sum of daily temperature °C) and total length, total number of gill filaments, and length of gill filaments. Relationships between total length and number of gill filaments, and between total length and length of gill filaments were described with a linear
regression. Fourth degree polynomial regressions were used to describe developmental changes in TU and number of secondary lamellae/gill filament and between total length (mm) and number of secondary lamellae/gill filament. Total TU to complete yolk sac and oil globule absorption were estimated from a single linear regression line. Regressions were considered to be statistically significant when the P-value for the regression coefficients were ≤0.05.

Results

The first prolarvae observed with open mouths were 3-d old (47 TU), but all fish had open mouths at 4-d (64 TU) (Figure 1). Feed was first observed in 4-d old larvae at 4-d (64 TU), and 100% of 7-d old postlarvae I (113 TU) were feeding. The first fish with an inflated gas bladder was 5-d old (96 TU), and all 7-d old (113 TU) postlarvae I had inflated gas bladders. Walleye measured 10.0 ± 0.0 mm (Table 1) at 5-d (81 TU) when the yolk sac disappeared (Figure 2). The oil globule disappeared when larvae were 10-d old (163 TU; Figure 2), at a length of 13.2 ± 0.13 mm (Table 1). Fish were 7.6 ± 0.2 mm (mean ± SE) at hatching and grew to 20.8 ± 0.04 mm (mean ± SE) at 21-d (354 TU). There was a strong linear relationship between TU and total length ($r^2 = 0.99$; Figure 3). The mean growth rate was 0.039 mm/TU from hatch to 21-d (Figure 3).

There was a strong positive rectilinear relationship between TU and total number of gill filaments ($r^2 = 0.98$) and between total number of gill filaments and total length ($r^2 = 0.98$) (Figure 4). Gill filaments first appeared in the mid-region of the gill arch in 3-d old (47 TU) prolarvae, 9.0 ± 0.04 mm (Figure 5; Table 2). The number of gill filaments increased from 5 to 8 (60%) from 3- to 5-d posthatch (47–81 TU), and increased from 8 to 29 (263%) from 5- to 8-d posthatch (81–129 TU). Gill filaments were present from the mid-region to the ventral region of the gill arch until 8-d, and filaments appeared in the dorsal region of the gill arch at 9-d (146 TU). There was a strong positive relationship between TU and gill filament length ($r^2 = 0.98$) and gill filament length and total length ($r^2 = 0.98$) (Figure 6). Length of the gill
Figure 1. Schematic summary of critical developmental events in walleye to 21-d posthatch.
Table 1. Relationship among developmental events in walleye to 21-d posthatch.

<table>
<thead>
<tr>
<th>Days posthatch</th>
<th>Temperature units (°C)</th>
<th>No. fish sampled</th>
<th>Total length (mean ± SE) (mm)</th>
<th>Yolk sac length (mean ± SE) (mm)</th>
<th>Oil globule diameter (mm) (mean ± SE)</th>
<th>Mouth open (%)</th>
<th>Fish feeding (%)</th>
<th>Gas bladder inflation (%)</th>
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<td>8</td>
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<td>11.8 ± 0.22</td>
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<td>146.1</td>
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<td>21</td>
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<td>20</td>
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</table>
Figure 2. Absorption of the yolk sac (81 TU) and oil globule (163 TU) of larval walleye.

Five fish were measured each day and 20 fish were measured at hatching and 7-d posthatch.
Figure 3. Relationship between total length and TU in larval walleye from hatching to 21-d posthatch: disappearance of the yolk sac (A) occurred at 81 TU (5-d) and the oil globule (B) at 163 TU (10-d).
Figure 4. Relationship between number of gill filaments on the second gill arch and TU of 5 fish on each day from 1- to 21-d posthatch at 17.0 ± 0.1°C and the relationship between mean number of gill filaments and total length (mm) of walleye from 1- to 21-d posthatch. Number of gill filaments increased rapidly after (A) yolk sac absorption, 81 TU and (B) oil globule absorption, 163 TU. Dashed lines represent 95% confidence limits for the regression line.
Figure 5. Photographs of (A) a 3-d old prolarval walleye gill with developing gill filaments and (B) a 10-d old postlarval II walleye gill with developing secondary lamellae.
Table 2. Relationship among developmental events in walleye to 21-d posthatch.

<table>
<thead>
<tr>
<th>Days posthatch</th>
<th>Temperature units (°C)</th>
<th>No. fish sampled</th>
<th>Total length (mean ± SE)</th>
<th>No. gill filaments (no. ± SE)</th>
<th>Length of gill filaments (µm) (mean ± SE)</th>
<th>No. secondary lamellae/gill filament (mean ± SE)</th>
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Figure 6. Relationship between length (µm) of the first, third, fifth, and seventh gill filaments on the ventral-most part of the second gill arch and TU of 5 fish examined each day to 21-d posthatch and the relationship between length of gill filaments (µm) and total length (mm) of walleye to 21-d posthatch. Length of gill filaments increased rapidly after the oil globule was absorbed, 163 TU (A). Dashed lines represent 95% confidence limits for the regression line.
filaments increased from 37 to 156 µm (a 322% increase) from 3- to 10-d posthatch and from 156 to 508 µm (a 226% increase) from 10- to 18-d posthatch.

A strong positive relationship existed between TU and the number of secondary lamellae/gill filament \( (r^2 = 0.99) \) and between number of secondary lamellae/gill filament and total length \( (r^2 = 0.99) \) (Figure 7). Secondary lamellae began to differentiate from the gill filaments in 9-d old (146 TU) fish. Secondary lamellae were first observed in 10-d old (163 TU) fish on gill filaments located in the mid region of the gill arch (Figure 5; Table 2). Gill filaments in 12-d old (198 TU) fish were present in all regions of the gill arch.

**Discussion**

Secondary lamellae first appeared on gill filaments of 3-d old prolarval *Rutilus rutilus* (El-Fiky et al. 1987). Larval walleye did not begin to open their mouth until they were 3-d old (20%), and all fish had open mouths at 4-d. Osse (1989) stated that the absence of gill filaments and secondary lamellae in prolarval fish can be explained by the physical limitations involving gill ventilation. His calculations showed that 6–7 mm common carp *Cyprinus carpio* larva could not obtain sufficient oxygen from water passing through the mouth (Osse 1989). Osse (1989) also suggested that development of structures used for feeding is more critical than development of gills at this time because cutaneous gas exchange is sufficient for aerobic metabolism. Feeding commenced one day after the mouth opened. Although development of the gill filaments paralleled that of the mouth opening and first feeding, there were no secondary lamellae (i.e., functional respiration surface) until 9- to 10-d posthatch. Therefore, the gills cannot be effective in gas exchange the first few days after hatching because the mouth is not open and a functional surface is not present.

It has been reported that the yolk sac and a superficial red layer of muscle are responsible for gas exchange in larval fish (El-Fiky et al. 1987; McDonald and McMahon 1977; McElman and Balon 1979). El-Fiky et al. (1987) found a close correlation between the percentage of the
Figure 7. Relationship between number of secondary lamellae/gill filament on the first, third, fifth, and seventh gill filaments on the ventral-most part of the second gill arch and TU of 5 fish examined each day to 21-d posthatch and the relationship between number of secondary lamellae/gill filament and total length (mm) of walleye to 21-d posthatch. Lines were fitted with a 4th degree polynomial.
superficial layer of red muscle and the development of the gills of *Rutilus rutilus*. At 3-d posthatch, the superficial layer of red muscle made up 12% of the total amount of muscle, but it was only 3% at 40-d posthatch.

Although a superficial layer of red muscle contributes to gas exchange in some fish, many biologists think the extensive vitelline vasculature structure of the yolk sac is the most important respiratory structure of larval fish (El-Fiky et al. 1987; McDonald and McMahon 1977; McElman and Balon 1979). Our study shows that walleye lack functional gill structure to serve aerobic respiration. Walleye behavior suggests a functional role for the yolk sac. Prolarvae walleye suspend themselves vertically at the water surface, often with the abdominal surface of the yolk sac and oil globule pressed to the surface film (Krise and Meade 1986). However, as the gills begin to develop, fish begin swimming horizontally and do not stay suspended at the water surface (Krise and Meade 1986). McDonald and McMahon (1977) found that as the yolk sac diminished, the gills in Arctic char *Salvelinus alpinus* played an increasingly important role in gas exchange.

Variations in rate of gill development can be explained by the theory of saltatory ontogeny (Balon 1984). Development was not a gradual accumulation of small changes but a sequence of rapid changes in form and function. Rapid development of gill structures took place after prolarval respiration stopped. Rapid development of the secondary lamellae took place after the oil globule disappeared. A 263% increase in the number of gill filaments occurred between 5- (81 TU) and 8-d (129 TU) posthatch, which was immediately after the yolk sac was fully absorbed. The lengths of gill filaments increased 137% from 10-d posthatch (163 TU) to 18-d posthatch (301 TU), and the number of secondary lamellae/gill filament increased from 1 to 17 in the same interval, which coincided with full absorption of the oil globule.

Understanding of developmental events helps explain changes in sensitivity to toxicants. The transition from yolk sac respiration to gill respiration can result in an increased sensitivity to pH changes (Bergerhouse 1992) and hydrogen peroxide (Clayton and Summerfelt in press).
Bergerhouse (1992) reported less than 10% mortality of prolarval (3-d posthatch) walleye exposed to pH 10.0, but mortalities exceeded 50% in postlarval II (12-d posthatch) walleye exposed to the same pH. Clayton and Summerfelt (in press) found that 80% of 4-d old prolarval walleye survived a 1-h exposure to 100 mL/L hydrogen peroxide, but only 2% of 6-d old postlarval I walleye survived the same treatment. The number of gill filaments increased 71% in walleye between 4- (64 TU) and 6-d (96 TU) old and 600% between 3- (47) and 12-d (198 TU). Therefore, the results from our study support the idea that increased sensitivity of fish to certain toxicants may occur with gill development.

References


GENERAL CONCLUSION

Although affects of suspended solids (SS) on aquatic life have been reported many times, the investigators usually failed to report the size, shape, and chemical composition of the SS used in the experiments. Sometimes, they used material sediments which are heterogeneous mixtures of inorganic and organic substances. Therefore, it is difficult to compare results among experiments and future studies should report the properties of SS so comparisons among experiments can be made.

Larval walleye do not seem to be sensitive to SS at concentrations up to 360 mg/L. No histological changes were observed and survival, growth, gas bladder inflation, and feed acceptance were higher in walleye exposed to SS ranging from 16 to 360 mg/L compared with walleye in clear water (2.3–2.7 mg/L). These observations parallel those reported by Bristow and Summerfelt (1994) who evaluated concentrations of the same clay at turbidity levels of 16.1 to 49.7 NTU. The facts demonstrate that turbidity enhances the feeding of larval walleye. Boehlert and Morgan (1985) and Bristow and Summerfelt (1994) suggest that feeding is enhanced because it creates a visual contrast between the prey items and turbid water.

Balon (1984) hypothesized that early ontogeny of larval fishes is a saltatory process, i.e., “a sequence of rapid changes in form and function alternating with prolonged intervals (steady states) of slower development during which complex structures are prepared for the next rapid change.” The results from the present study support this hypothesis because the first appearance of gill filaments occurred immediately after the mouth opened and the yolk sac was fully absorbed and secondary lamellae first appeared immediately after the oil globule disappeared. These events suggest that the gills play an increasingly important role in respiration as the yolk sac and oil globule are absorbed and support the findings of McDonald and McMahon (1977) that showed the gills in Arctic char Salvelinus alpinus played an increasingly important role in gas exchange as the yolk sac diminished.
The development of gill filaments and secondary lamellae, in prolarval to juvenile walleye, is critical in determining when walleye become sensitive to toxicants. Studies reported that the transition from yolk sac respiration to gill respiration can result in an increased sensitivity to pH changes (Bergerhouse 1992) and hydrogen peroxide (Clayton and Summerfelt in press). The present results support this hypothesis because the gills undergo extensive development from the prolarval to postlarval II stage.

References
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