Behavior of larval walleye

Phillip Warren Rieger

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Behavior of larval walleye

by

Phillip Warren Rieger

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Animal Ecology
Major: Fisheries Biology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1995
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GENERAL INTRODUCTION

Dissertation Organization

This dissertation is organized into five papers. I am the primary author and my major advisor, Dr. R.C. Summerfelt, is the secondary author for all five papers. I conceived the overall project in concert with Dr. Summerfelt and drew upon both of our previous experiences with larval fish to guide the study design and content. This research was supported by funding from an American Fishing Tackle Manufacturers Association Boehme Fellowship and the Iowa Agriculture and Home Economics Experiment Station project 2982. The Department of Animal Ecology provided laboratory space and I obtained cinemagraphic assistance from the Iowa State University Office of Biotechnology Image Analysis Facility.

Each of the five papers follows the general format required by the journal to which submission is proposed. However, submission formats have been modified to allow inclusion of tables, figures, and plates at the most convenient location as soon after referenced within the body of each paper. This format provides a document that would resemble the paper when published, rather than in submission form which requires all tables, figures, and plates to be enclosed at the end of the text, each on a separate sheet of paper.

The five papers are preceded by a literature review related to the goal of the research which is to obtain greater fundamental knowledge of larval walleye behavior with emphases on the developmental events of gas bladder inflation, first feeding, and cannibalism.

The sequence of the five papers in the dissertation were arranged from general to specific topics. The first paper, "Developmental behavior of larval walleye," describes developmental behavior aspects of larval walleye. This first paper presents an overview of larval walleye behavior as derived from qualitative and quantitative findings of the various experiments.
The second paper, "Microcinemagraphic evidence of physostomous gas bladder inflation in walleye, *Stizostedion vitreum* larvae," is a short paper that provides evidence for the hypothesis that larval walleye ingest air to fill their gas bladder. Larval walleye are observed penetrating the water surface and air bubbles are observed in the gut where they seem to be transported into the gas bladder.

The next two papers address more specific research studies: "The influence of turbidity on larval walleye, *Stizostedion vitreum* behavior and development in tank culture," and, "The effects of temperature on larval walleye behavior, development, and viability in tank culture."

These two studies include experimental design to determine how temperature or turbidity influence larval walleye behavior and then relate behavior to effects on critical developmental events. The last paper, "The nature and significance of cannibalistic behavior in larviculture of walleye," like the first, draws information from various observations throughout several experiments and general studies. It assumes my findings on turbidity and temperature have addressed questions on the mechanisms of gas bladder inflation and first feeding, then examines the problem of cannibalistic behavior. The last paper shows that, when obfuscat ing physical problems affecting behavior and development are eliminated, aggressive behavior remains a serious impairment to successful intensive walleye larviculture. It provides fundamental knowledge of aggressive behavior in larval walleye which can be a guide to further studies toward resolution of this problem.
Literature Review

Opportunity exists for new technological and biological knowledge to expand aquaculture in the United States. There is an increasing trend throughout the world towards intensification of fisheries for food and recreation (Welcomme 1994). Against the background of increasing demand and more or less constant or decreasing supply of fisheries products, attention has turned to aquaculture to replenish natural aquatic habitats and for production of food through aquaculture. In contrast to world fish and shellfish catches, which have declined by 5.1% since 1989, aquaculture production of fish and shellfish has increased by 10.9% during the same period (Tacon 1994). Tank culture of larval fish has been shown to be a cost effective and reliable method to supplement and enhance production of many fish species (Van Olst et al. 1990).

Walleye (Stizostedion vitreum) popularity as a food and sport fish makes it an excellent candidate for aquaculture. Walleye fisheries, once an important aspect of the Great Lakes fishery, are now closed in most of their original range to commercial exploitation in the United States. Habitat degradation and overfishing are thought responsible for large declines in walleye stocks in the earlier half of this century (Regier et al. 1969). Replacement of natural stocks has focused on artificial propagation. In 1983 and 1984 one billion walleye fry were stocked throughout North America for recreational fisheries management (Connover 1986). Currently, walleye culture is focused on artificial propagation of wild fish captured during the annual spawning season, pond culture of the larvae to small fingerling size (35-50 mm), and training of pond-reared fingerlings to formulated feed after rearing to advanced size (100-150 mm). Tank culture of larvae could circumvent the pond culture phase. Because of this, many agencies have been attempting to develop intensive culture techniques for walleye larvae for several decades.
Survival of walleye from hatch to fingerlings in intensive culture was typically less than 10% until the 1990's (Beyerle 1979; Loadman et al. 1989; Nickum and Stickney 1993). Problems with intensive walleye culture have been associated with gas bladder inflation (GBI) (Colesante et al. 1986; Barrows et al. 1988; Summerfelt 1991; Nickum and Stickney 1993), adaptation to exogenous feeding on formulated feed (Kindschi and MacConnell 1989; Loadman et al. 1989; Nagel 1991), and cannibalism (Beyerle 1975; Cuff 1977; Li and Mathias 1982; Loadman et al. 1989). These developmental problems are shared by culturists of other species including whitefish (Coregonus spp.), striped bass (Morone saxatilis), red sea bream (Pagus major), spotted seatrout (Cynoscion nebulosus), Dolphin (Coryphaena hippurus), European sea bass (Dicentrarchus labrax), Australian bass (Macquaria novemaculeata), and gilt-headed bream (Sparus auratus) (Braum 1967; Doroshov and Comacchia 1979; Katajima et al. 1981; Tucker 1988; Owstowski 1989; Battaglene and Talbot 1990; and, Chatain and Ounais-Gushmann 1990).

Walleye Development.

Walleye development is a saltatory process as described by Nelson (1968), McElman and Balon (1979), and Li and Mathias (1982). Saltatory development is characterized by abrupt functional changes in form and function of the developing organism which result in a new environmental relationship. A diversity of terminology has been developed and used by various researchers describing saltatory development during the early life of walleye. Nelson (1968), in a study of embryo and larval characteristics of walleye, followed the terminology of Hubbs (1944) who recognized two posthatch stages in fishes before juvenile: the "prolarva", which retains a yolk sac; and the "postlarva", which has absorbed the yolk sac but is still Unlike the juvenile stage. Li and Mathias (1982) elaborated Nelson's (1968) terminology, by subdivided the postlarva into a "postlarva I" stage, characterized by mixed
nutrition (oil and prey), and "postlarva II", characterized by exogenous feeding, no oil globule, and rapid structural change in the digestive system. Balon (1975) also divides the posthatch period into three main phases based on morphological characteristics: an "eleutheroembryo" (free embryo), which is free from the egg and is equivalent to prolarva; an early larval phase, the "propterygiolarva", characterized by undifferentiated fin folds; and a later larval phase, the "pterygiolarva", characterized by fin rays forming. McElman and Balon (1979) used ecomorphological criteria to further subdivide the three main developmental phases into additional steps to emphasize the saltatory aspects of walleye development: the eleutheroembryo phase was subdivided into two steps with the second being characterized by transfer to gill respiration, termination of surface suspension, increase in active swimming, and rapid yolk depletion; and the propterygiolarva phase was also subdivided into two steps, the second of which was marked by an increasingly predacious behavior and gas bladder inflation. McElman and Balon (1979) did not follow development beyond the propterygiolarva phase in this study. Krise and Meade (1986), from a review of previous studies, used four stages in a descriptive summary of various changes in walleye development: "Stage I", surface suspension; "Stage II", initiation of gill respiration; "Stage III", initiation of feeding; and, "Stage IV", initiation of cannibalism. Auer (1982), in a comprehensive guide to larval fishes of the Great Lakes Basin, used "yolk-sac larvae" to refer to the phase of development from hatch to complete absorption of yolk, and "larvae" to refer to the phase of development from absorption of yolk to development of a full complement of adult fin rays and absorption of fin fold. Sigurdson (1994) used protocol set up by Darryl Schneider at the Larval Fish Laboratory in Colorado which describes "protolarval", "mesolarval", and "metalarval" stages. Many authors simply refer to all posthatch, pre-juvenile walleye as "fry" (Nickum and Stickney 1993; Bristow 1994).
The juvenile period begins when development of the digestive system, gills, fins, and other structural components of the fish become almost adult in character — the appearance of pyloric caeca is often used to signal the beginning of the juvenile period.

Critical Periods.

Of particular concern to walleye larviculture is an occurrence of extreme mortality of undefined cause(s) during the period of mixed endogenous and exogenous nutrition (postlarvae I). Nickum (1978) stated that the population may decline to near zero during a period of only a few days. This coincidence of high mortality with a developmental event is referred to as a critical period (Li and Ayles 1981; Browman 1989). Browman (1989) in a review of critical periods in fish, stated that this period was an ontogenetic event which required "... spatial and temporal overlap between appropriate genetic and/or environmental input and the developing organism." Li and Mathias (1982) stated that "An understanding of the causes of this critical period, and a search for the means to suppress it, are essential elements in the improvement of walleye culture methods." Developmental events occurring just prior to or during this period include gas GBI, first feeding, and cannibalism. Much of walleye larviculture research has focused on the contribution of these three critical events to poor walleye viability in larviculture.

Because of the coincidence of this critical period to the disappearance of the oil globule, an energy/nutrient deficit has been hypothesized as causing starvation (Nickum 1978) or providing impetus for cannibalism (Krize and Meade 1986). Li and Mathias (1982) found that most cannibalism and mortality in walleye occurred during a short 6 to 10 day interval, beginning with the transition to postlarvae I, and ending during the postlarva II period; they hypothesized that mortality during this period was caused primarily by a combination of cannibalism and starvation. Loadman et al. (1986) suggested that injuries caused by
unsuccessful cannibalistic attacks among larval walleye were a greater source of mortality than successful cannibalism. Failure of the larvae to inflate their gas bladders may also contribute to the mortality by causing lordotic deformities, poor first feeding success leading to starvation, or by presenting weaker, smaller prey for cannibalistic individuals (Summerfelt 1991).

In summary, GBI, initiation of feeding, and cannibalism are critical developmental events that have been individually and collectively regarded as major causes of high mortality in walleye larviculture. Research, however, has failed to show a direct linkage between overall mortality and any of these specific causes. Studies of strategies to resolve gas bladder inflation or first feeding problems, although resolving a singular problem, have not resolved this high mortality event.

I propose that researchers have been unsuccessful in resolving mortality during the critical period because there is a lack of fundamental understanding of the interrelationships of developmental events and larval behavior. No studies have been found that comprehensively address larval walleye behavior. McElman and Balon (1979) suggested that these developmental events were dynamic behavioral transitions intimately related to nutrient reserves and energy expenditures, and that their developmental synchrony was an integral component of the saltatory nature of walleye development. Blaxter (1970) stated that during critical periods, the organism is "... particularly sensitive to deprivation or abnormal experience, something that may be of significance in aquacultural rearing of fish and invertebrates." Environmental variables that substantially influence larval walleye behavior and development include temperature and illumination (Smith and Koenst 1975; McElman and Balon 1979; Colesante 1982; Raisanen 1982; Krise and Meade 1986). Temperature is related to energy expenditures, rate of development, and activity level. Illumination affects distribution, orientation, and movement of larvae. Understanding the relationships of larval
behavior to developmental events and to these environmental variables may provide the fundamental biological knowledge needed to resolve the high mortality observed in larviculture of walleye.

My objectives are to observe and describe the behavioral aspects of developmental events (GBI, first feeding, and cannibalism) and their interrelationships to each other and to variations in temperature and illumination.

*Gas Bladder Inflation (GBI).*

The gas bladder, also known as swim or air bladder, is an organ in fish that primarily functions to regulate hydrostatic balance. The gas bladder develops as a pouch from the foregut. The biological significance, architecture, and physiology of the gas bladder is discussed thoroughly by Steen (1970). Fishes that pass air from the gut, through a pneumatic duct into the gas bladder to maintain hydrostatic balance are called physostomous; fishes that lack a pneumatic duct, but remove gas from the blood with a rete mirabile are called physoclistous (Moyle and Cech 1988). The physoclistous mechanism is more derived and occurs primarily only in Perciformes and other most recently evolved fish orders (Moyle and Cech 1988). In many physoclistous Perciforms, including walleye (Percidae), striped bass (Percichthyidae), and bluegill (*Lepomis macrochirus*) and green sunfishes (*Lepomis cyanellus*) (Centrarchidae) a pneumatic duct has been found during the embryonic and larval phases (Duwe 1955; Doroshov et al. 1981; Marty 1995). Denial of surface access during the larval period has been shown to preclude GBI in walleye (Kindschi and MacConnell 1989) and other physoclist including the three spined stickleback (*Gasterosteus aculeatus*), striped bass, and gilt-headed bream (*Sparus auratus*) (von Ledebur 1928, cited by Tait 1960; Chapman et al. 1988; Chatain and Ounais-Guschemann 1990). The only physical evidence that the larval pneumatic duct functions to pass air to the gas bladder, and is not just an
embryonic recapitulation of the more primitive form, is a photograph by Van Olst et al. (1990) which shows several microbubbles (0.01 mm in diameter) inside the pneumatic duct of a striped bass larva. In contrast, larvae of more derived percomorph physoclists, (Sarotherodon mossambica) and (Hemichromis bimaculata) (Cichlidae), lack a pneumatic duct during the larval phase and have been shown to inflate their gas bladders without surface access (McEwen 1940; Doroshev and Cornacchia 1979).

When fishes with a pneumatic duct during the larval period fail to initially fill the gas bladder by a physostomous mechanism, the gas bladder will degenerate and never fill (Doroshov and Cornacchia 1979; Marty 1995). Without inflated gas bladders, fish have decreased growth and survival (Chatain 1987), increased spinal deformities (Kitajima et al. 1994), and are unusable for stocking purposes (Kindschi and Barrows 1993). Non-inflation of the gas bladder (NGB) during the larval phase is particularly common in intensive culture of walleye (Summerfelt 1991) and other physoclistous species such as striped bass and giltheaded bream (Doroshov and Cornacchia 1979; Chatain and Ounais-Guschemann 1990). Hypotheses of the etiology of NGB and potential resolutions are diverse; for striped bass, pathogenic, environmental, hormonal, developmental and genetic factors have been suggested (Hadley et al. 1987; Brown et al. 1988; Chapman et al. 1988; Van Olst et al. 1990). Less research has been conducted in reference to walleye gas bladder etiology.

Improved GBI rates in walleye and other species have been attributed to many culture techniques (Table 1). These techniques have each seemed to contribute to better inflation rates. Gas bladder inflation intensive culture of walleye, however, has remained inconsistent. Nickum and Stickney (1993) reported that GBI rates above 1% were rare in tank culture; but Moore et al. (1994), Barrows et al. (1993), and Friedman and Bates (1986) have observed GBI success greater than 90% in tank culture.
Table 1. A summary of strategies used to improve gas bladder inflation in larval fish culture.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friedman &amp; Bates (1986)</td>
<td>striped bass</td>
<td>absorbent cloth &amp; surface spray</td>
</tr>
<tr>
<td>Hadley et al. (1987)</td>
<td>striped bass</td>
<td>lower rearing temperatures</td>
</tr>
<tr>
<td>Barrows et al. (1988)</td>
<td>walleye</td>
<td>feeding ring to contain oil film</td>
</tr>
<tr>
<td>Brown et al. (1988)</td>
<td>striped bass</td>
<td>maternal hormonal therapy</td>
</tr>
<tr>
<td>Chapman et al. (1988)</td>
<td>striped bass</td>
<td>oxygen supersaturation</td>
</tr>
<tr>
<td>Van Olst et al. (1990)</td>
<td>striped bass</td>
<td>salinity and aeration</td>
</tr>
<tr>
<td>Bushman (1992)</td>
<td>walleye</td>
<td>pH manipulation</td>
</tr>
<tr>
<td>Bristow &amp; Summerfelt (1994)</td>
<td>walleye</td>
<td>turbidity</td>
</tr>
<tr>
<td>Moore et al. (1994)</td>
<td>walleye</td>
<td>surface spray</td>
</tr>
</tbody>
</table>

The most convincing evidence indicates that access to a clean air/water interface is the most critical factor to GBI (Summerfelt 1991). An oil film, released by larvae, in concert with normal surface tension, may form a barrier sufficient to prevent most of the larvae from gulping air for transport through the pneumatic duct into the developing gas bladder (Boggs 1994). If the larvae do not penetrate the surface and gulp air before a critical developmental time, the opportunity for physostomous first filling may be lost. The use of the surface spray and air blower and oil trap, which produced some of the highest GBI to date in intensive culture, were designed to remove this oil film from the water surface.

Marty et al. (1995) provides the first description of the anatomy and development of the walleye gas bladder and pneumatic duct. They suggest that a "window" of physostomous physiology exists only prior to differentiation of the gut by the pyloric sphincter. Separation
of the opening of the pneumatic duct from the opening of the common bile duct at a common lumen occurs at about 12 days posthatch. Marty et al. (1995) proposed that the separation of the common bile duct from the opening of the pneumatic duct at 12 days posthatch would preclude physostomous GBI because the mixing of bile as a surfactant with ingested air bubbles would not occur thereafter. This hypothesis is supported by observations of 0.01 mm bubbles occurring along with larger bubbles ranging in size up to 0.5 mm in diameter in the gut of striped bass by Van Olst et al. (1990) which indicate that a surfactant may assist in bubble cleavage and sustain microbubbles small enough for transport through the pneumatic duct. An oil film on the water surface might, rather than inhibiting GBI as a barrier to air access, when gulped along with the air from the surface, interfere with the bile surfactant and preclude cleavage of air bubbles into microbubbles for transport through the pneumatic duct.

Even in the absence of an oil film, the surface tension of any body of water is well recognized as a natural interfacial barrier between air and water. Usinger (1956), in reference to aquatic insects, stated "To an organism of small size, this air-water interface can be an impenetrable barrier, a surface on which to rest, or a ceiling from which to hang suspended." The same characteristics may be as applicable to an 8 to 10 mm fish larva. It has been noted that larval walleye "suspend" from the surface tension during the prolarva phase (McElmon and Balon 1979). The health, vitality, and behavior of the larval walleye may be critical to a larva's ability to break this surface barrier and gulp air for the developing gas bladder. Environmental factors affecting larval activities, such as turbulence, temperature, and illumination, are therefore important considerations to GBI in walleye larviculture. The interrelationships of these variables complicate resolution of GBI and other developmental issues.
First Feeding.

Walleye must initiate exogenous feeding before the oil globule has been absorbed to sustain the energy requirements of swimming, growth, and organogenesis. At water temperatures of about 16°C to 19°C, first feeding is reported to begin during 6 to 10 days posthatch (Bristow 1994). Conversion to exogenous feeding on prepared feed is considered the main bottleneck to successful walleye larviculture in intensive systems (Nickum and Stickney 1993).

It is generally assumed that for most carnivorous fish species with small larvae, the use of live food is necessary for a short time after hatching to ensure high survival and growth rates (Person - Le Ruyet 1989). A tank culture feeding strategy based on live prey, however, is rather expensive, requires more labor and/or complex equipment, and the nutritional content of live prey are difficult to control. Several commercial feeds, potentially suitable for walleye larvae, are available. Nevertheless, early attempts to culture walleye fingerlings exclusively on dry diets were unsuccessful (Colesante et al. 1986). Culture strategies, such as subsurface water currents to keep food particles in suspension, continuous feeding, overfeeding, and use of gustatorially "attractive" feeds can improve the success of conversion to exogenous feed using prepared feed (Colesante et al. 1986; Loadman et al. 1989; Nagel 1991).

Poor results in first feeding of carnivorous species in aquaculture environments is common. Inadequate knowledge of feeding behavior of fish larvae has been recommended as the cause of these poor results (Person-Le Ruyet 1989). In addition, the bioenergetics and physiology of walleye during this critical phase are poorly understood. Nickum (1978) described a problem termed the "dwindles", where larvae become listless and eventually die, even larvae that appear to be consuming prepared food. This condition may be related to developmental asynchrony of larval bioenergetics, digestive histogenesis, behavior, or
perhaps to deterioration of feed quality. Nickum (1978) proposed using lower rearing temperatures to mitigate such problems. Greater attention to larval walleye feeding behavior and its relationships to environmental factors, GBI, and cannibalism may resolve these problems.

**Cannibalism.**

Many researchers have observed cannibalism to be a potentially significant problem in larviculture (Cuff 1977; Nickum 1978; Beyerle 1975; Li and Mathias 1982). Hecht and Pienaar (1993), in a review of cannibalism in fish larviculture, noted the occurrence of cannibalism in at least fifteen families of cultured fishes. Their review indicated that cannibalistic behavior was affected by food availability, population density, refuges, water clarity, and light intensity.

Lewis et al. (1981), having studied intensive culture methods for striped bass larvae for many years, concluded that cannibalism could easily account for losses of up to 90% of original stock if strict measures were not implemented to mitigate contributory factors. Colesante et al. (1986) stated that only a small percent of walleye larvae in a group were "inherently" cannibalistic, and that once these were removed, additional cannibals rarely developed; they also observed that dispersal of walleye with illumination and tank color strategies reduced cannibalism. Bristow (1994) found that cannibalism may have a genetic component; he observed higher incidence of cannibalism in tanks with walleye progeny from Minnesota than in tanks with progeny from other midwestern states (e.g., Ohio, Kansas, and Iowa). Loadman et al. (1989) suggested that maintenance of a water current, into which the larval walleye would orient, could lessen cannibalism by reducing front-to-side encounters between individuals. Van Olst et al. (1990) observed that cannibalism in striped bass culture continued throughout the first 30 days of development (until the fry could be successfully
graded), and that the extent of cannibalism appeared to be related to size differences. Paller and Lewis (1987) also correlated increased size differences with reduced survival. Tucker (1988) found that after spotted seatrout fry adapted to prepared feed, attempted cannibalism decreased sharply. Nutritional demands may play a role in stimulation of cannibalistic behavior. No studies have, however, fully demonstrated techniques that reduce larval walleye cannibalism in intensive culture, although Bristow and Summerfelt (1994) showed a reduction of cannibalism in turbid water.

At densities used in intensive culture (30-50 larvae per liter), walleye larvae are exposed to frequent contact with other larvae of the same age, although environmental factors such as water circulation and illumination may reduce this contact. Any, even seemingly minor, size differences within a population in intensive culture may significantly contribute to cannibalism. Presumably, the greater the size differences the greater the potential for cannibalism. Studies have shown, however, that walleye larvae are known to consume other walleye larvae of the same size (Cuff 1980; Bristow 1994). Li and Mathias (1981) termed the situation where walleye larvae of the same age cannibalize each other as "cohort-cannibalism" to distinguish it from cannibalism where a significant difference in age and size is a factor. The most significant contributors to cannibalism within a population seem to be adaptation to exogenous food and the occurrence of significant numbers of individuals without inflated gas bladders. These factors may greatly enhance size differences, variability in larval motility, and aggressive behavior within a population.

Loadman et al. (1986) suggested that attacks resulting in seizure of other larvae, but not in ingestion, may be a significant source of mortality in walleye larviculture. This hypothesis has not yet been further studied. Although cannibalism may not be responsible for more than 10 to 20% of mortalities in walleye larviculture, other cohort aggression injuries may be
responsible for high mortality experienced in intensive walleye larviculture. Further study of
this phenomenon is warranted.

Water Temperature.

Temperature is the most important environmental variable affecting the rate of fish
development. Morphological development of larval fish is commonly measured, not strictly
by time, but in temperature units (TU); where \(TU = \text{number of days posthatch} \times \text{average temperature in degrees Celsius (Krise and Meade 1986)}\). Larval rearing
temperature has long been recognized as influencing energy expenditures and nutrient
assimilation. Appetite may be suppressed at low temperatures, and disease may result from
water temperature that is too warm.

In many studies of walleye culture, temperature was not controlled (i.e., ambient
temperature was used) and in some other cases, the temperature was not reported. Nickum
and Stickney (1993) stated that no controlled, replicated studies have been conducted to test
the effect of temperature on the feeding behavior of walleye larvae. Several researchers have,
however, arrived at conclusions regarding temperature based on circumstantial evidence, or
decided to use a particular temperature regime for unstated reasons, as summarized in
Table 2.

Nickum (1978) concluded that higher temperatures (i.e., > 20°C) may cause the larvae to
exhaust endogenous reserves before they are able to adapt to prepared feed, perhaps
accounting for the massive starvation and cannibalism observed. His perceptions were,
however, not based on controlled or replicated studies.
Table 2. Temperature regimes used or recommended by various investigators of larval walleye culture.

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>TEMPERATURE (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li &amp; Mathias (1972)</td>
<td>20°</td>
</tr>
<tr>
<td>Smith &amp; Koenst (1975)</td>
<td>21°</td>
</tr>
<tr>
<td>Hokanson (1977)</td>
<td>increasing by 1° per day</td>
</tr>
<tr>
<td>Nickum (1978)</td>
<td>less than 20°</td>
</tr>
<tr>
<td>McElman &amp; Balon (1979)</td>
<td>15°</td>
</tr>
<tr>
<td>Colesante et al. (1986)</td>
<td>11.5° - 18.3°</td>
</tr>
<tr>
<td>Summerfelt et al. (1991)</td>
<td>14.7° - 19°</td>
</tr>
<tr>
<td>Moore et al. (1994)</td>
<td>14.5° - 25°</td>
</tr>
</tbody>
</table>

Rearing temperatures commonly used by walleye culturists, although causing no direct mortality, influence metabolism, behavioral and the rapidity of developmental events. McElman and Balon (1979) stated that a relationship between oil globule size and a dynamic behavioral transition suggests that energy expenditures occurring during ontogeny are related to appropriate developmental synchrony. Excessive metabolism from higher temperatures may overtax the physiological ability of the organism to undergo proper histogenesis and/or behavioral development; and conversely, lower temperatures may not provide activity levels sufficient to accomplish critical events such as GBI and first feeding in the intensive culture environment. Lower temperatures may also lessen aggressive behavior leading to cannibalism and larval injuries by reducing activity levels of the larvae.

A temperature manipulation strategy which provides appropriate activity levels, does not over-tax metabolic demands, and enhances conversion to exogenous feeding, may require use of alternative temperatures for different developmental events.
Illumination.

Illumination influences activity, sensory abilities, and phototaxis of larval walleye. The interrelationships of these factors may influence GBI, conversion to exogenous feeding, and cannibalism. Many researchers have noted that larval walleye are photopositive during the first 20-30 days of life (Nickum 1978; Colesante et al. 1986; Krise and Meade 1986). Corrazza and Nickum (1981) and Colesante et al. (1986) observed that utilization of darker tank sides and uniform lighting more evenly distributed walleye larvae throughout the tank. Uniform distribution of larvae may reduce encounters between larvae, and reduce cannibalism. Proper illumination strategies may evenly distribute larvae across the surface of the water, rather than allowing concentration along the sides, thus facilitating the larvae's opportunity to gulp air necessary for gas bladder inflation and more uniform distribution of larvae may reduce competition for food (thus decreasing size differences).

Raisanen (1982) evaluated walleye survival in covered and uncovered tanks; survival and food ingestion were lower in covered tanks. Chesney (1989) found that reduced light intensity inhibited growth and forage rates of striped bass fry. Ostrowski (1989) found that overall survival of dolphin averaged 50% in black tanks and 25% in uncolored (tan) tanks; he attributed the better contrast of feed to the black tanks with better feeding. These findings suggest that visual cues, affected by illumination strategies, are important to larval feeding ability. Loadman et al. (1986) reported reduced cannibalism in walleye larvae during periods of darkness.

There is evidence of illumination effects on larval walleye and other species, but little attention is generally given to this parameter as a management tool for larviculture. Although various illumination strategies have been used in previous studies (Table 3), few have experimentally compared photoperiod or illumination variables in walleye larviculture.
Table 3. Illumination regimes recommended or used by various researchers during larval walleye culture.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Photoperiod</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woynarovich (1960)</td>
<td>pike-perch*</td>
<td></td>
<td>less than 400</td>
</tr>
<tr>
<td>McElman &amp; Balon (1979)</td>
<td>walleye</td>
<td>12</td>
<td>11 - 350</td>
</tr>
<tr>
<td>Nickum (1979)</td>
<td>walleye</td>
<td>16</td>
<td>uniform</td>
</tr>
<tr>
<td>Kindschi &amp; MacConnell (1989)</td>
<td>walleye</td>
<td>24</td>
<td>480 - 600</td>
</tr>
<tr>
<td>Summerfelt et al. (1991)</td>
<td>walleye</td>
<td>16 - 22</td>
<td>550 - 1,100</td>
</tr>
</tbody>
</table>

* Stizostedion lucioperca

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DEVELOPMENTAL BEHAVIOR OF LARVAL WALLEYE

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Key words: Fish larvae, Fish development, Fish behavior

Synopsis

We used high resolution cinematography to observe larval walleye, Stizostedion vitreum activities in 80-ml and 3-l laboratory aquaria. This technique enabled us to directly examine behavior related to walleye development. The relationship of behavior to critical developmental events of gas bladder inflation, first-feeding, and cannibalism were of particular interest. Descriptions of behavior include swimming speed, swimming style, and spatial distribution, as well as predatory interactions with food and other larvae. Four discrete saltatory phases of behavior were observed: surface suspension, gas bladder inflation, first-feeding, and fully exogenous feeding. Descriptions of these behavioral phases are compared to physiological and ecomorphological descriptions of walleye development by other authors.

Introduction

During ontogenetic periods an organism is particularly sensitive to denial or abnormal experience (Blaxter 1970). Browman (1989) in a review of ontogenetic critical periods in fish, stated that the critical period was an ontogenetic event which required "... spatial and temporal overlap between appropriate genetic and/or environmental input and the developing organism." The concept of a critical period of larval fish development was introduced by Hjort (1914) to refer to a period of increased mortality associated with the transition from endogenous to exogenous nutrition. McElman & Balon (1979) proposed the saltatory nature of walleye development. According to this theory, development proceeds gradually through each period until a point is reached when a rather abrupt change in habits is possible. McElman & Balon (1979) suggested that gas bladder inflation (GBI), first feeding, and cannibalism were dynamic behavioral transitions intimately related to nutrient reserves and energy expenditures, and developmental synchrony were integral components of the saltatory nature of walleye, *Stizostedion vitreum*, development. These events may be critical to survival of walleye larvae (Li & Mathias 1982; Kindschi & Barrows 1993). Successful passage through early life stages is influenced by factors affecting, and affected by behavior.

We used cinematography to provide *in situ* observations of larval walleye in small laboratory aquaria. Our objectives focused on how behavior facilitates, or is changed by critical developmental events, and how behavior relates to the saltatory development of walleye larvae. Various play-back options of video recordings of small populations of larvae were used to obtain quantitative estimates of swimming speed, cohort aggression, and distribution. Other information, such as feeding success, GBI, and details of feeding behavior were also estimated from analyses of high power cinematography of small groups of larvae. Daily
cannibalism and mortality in the populations were used to relate behavioral changes to ecological and morphological aspects of development.

Terminology used to describe developmental phases of walleye and fish in general vary considerably (Table 1). Some workers consider the embryonic period to end upon hatching, and others believe it to last until the young begin to feed themselves. Hubbs (1944) proposed one of the least complicated sets of terms with two larval stages: the prolarva, which retains a yolk sac, and the postlarva, which has absorbed the yolk sac but is still unlike the juvenile stage. Li & Mathias (1982) divide the postlarva stage into two additional stages to represent those that still retain an oil globule, and those that are fully exogenous; e.g., postlarva I & II. McElmon & Balon (1979) provide additional steps within saltatory phases of development. Many workers simply refer to all posthatch, pre-juvenile, walleye as fry (Nickum & Stickney 1993; Bristow 1993). All studies generally agree that the larval period ends when development of the digestive system, gills, and other structural components of the fish become almost adult in character. Moyle & Cech (1988) state that the larval period ends when the axial skeleton is formed and the embryonic median fin-fold is gone. Li & Mathias (1982) use the appearance of pyloric caeca to signal the beginning of the juvenile period and reported this event to occur at 18 to 20 days posthatch when reared at 18°C to 20°C.

Because our study focused on developmental behavior, we use behavioral terminology to characterize phases of development. We use only the general term larvae to refer to posthatch individuals observed in our study to avoid confusing other developmental terminology with our behavioral descriptions. We relate our developmental behavior characterizations to the ecomorphological phases and steps provided in McElman & Balon (1979), three stages described from feeding and nutritional criterion of Li & Mathias (1982), and the stages based on physiology and behavior of Krise & Meade (1986).
Table 1. Terminology used to categorize stages of early development of walleye, or fishes in general. References with * specifically describe walleye development, other references describe fish development in general.

<table>
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<tr>
<th>Reference</th>
<th>Developmental Terms</th>
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<tr>
<td>Hubbs (1944)</td>
<td>prolarva &amp; postlarva</td>
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<tr>
<td>Nelson (1968)*</td>
<td>followed Hubbs (1944)</td>
</tr>
<tr>
<td>Balon (1975)</td>
<td>eleutheroembryo, propterygiolarva, &amp; ptergyiolarva</td>
</tr>
<tr>
<td>McElman &amp; Balon (1979)*</td>
<td>same as Balon (1975) plus additional steps</td>
</tr>
<tr>
<td>Weihs (1981)</td>
<td>yolk-sac larva &amp; larva</td>
</tr>
<tr>
<td>Li &amp; Mathias (1982)*</td>
<td>prolarva, postlarva I, &amp; postlarva II</td>
</tr>
<tr>
<td>Krise &amp; Meade (1986)*</td>
<td>Stage I, surface suspension; Stage II, gill respiration; Stage III, initiates feeding; Stage IV, cannibalism.</td>
</tr>
<tr>
<td>Sigurdson (1994)*</td>
<td>protolarva, mesolarva, &amp; metalarva</td>
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All temporal descriptions used in our study are expressed in temperature units (TU) to provide standardization of event timing for walleye reared at different temperatures. A TU is defined as the product of daily temperatures above 0°C and the number of days posthatch (Krise & Meade 1986). Our observations were of larvae reared at 17.5°C and 20°C from hatching until 300 TU.
Methods and Materials

Culture Methods.— Our study was conducted during the spring seasons of 1993 and 1994. Fertilized walleye eggs were obtained from the Iowa Department of Natural Resources and hatched at water temperatures of 16°C. A laboratory was constructed to rear larvae in aquaria suitable for in situ cinematography. In 1993, 545 larvae were reared in four 3-l aquaria at a water temperature of 17.5°C; and, in 1994, 641 larvae were reared in six 3-l aquaria at a water temperature of 20°C. Water delivery to each aquarium was regulated to provide one replacement per hour. Water temperatures were maintained by thermostat controlled sump chillers. Temperatures in each aquaria were verified daily with a hand-held mercury thermometer. Turbidity levels between 20 and 30 NTU (nephrometric turbidity units) were maintained in culture water to mitigate the reflection of light from aquaria sides and prevent phototaxis of larvae to the tank edges. Excessive tank-edge orientation of larvae has been shown to inhibit GBI and first feeding success (Bristow & Summerfelt 1994; Rieger 1995). During this study, > 98% GBI and feeding success was observed in > 95% of all individuals from all populations at 300 TU. Fluorescent lights were located approximately 60 cm above the aquaria. Lighting panels were screened with white paper to diffuse and reduce the illumination to about 240 lux at the water surface. A photoperiod of 14 h light: 10 h dark was used. Black plastic sheeting enclosed the culture chamber to prevent all but direct overhead lighting. During each day of the rearing period, 10 to 12 larvae were removed from the larger 3-l aquaria by ladle and placed in 80-ml aquaria for high magnification observations. Following this cinematography, all larvae were placed back into the 3-l aquaria from which they were removed. Larvae were regularly fed Kyowa B-400 (Biokyowa Inc., Chesterfield, MO, USA) and occasionally, in the 80-ml aquaria, they were offered live Daphnia pulex to provide observations of live prey feeding behavior.
Cinematography.— Cinematography equipment included a Sony CCD color video camera equipped with a low magnification zoom macro lens and a high magnification zoom microscopic lens. Observations were recorded on a Sony Beta VCR which provides high resolution recording with various replay options to enhance observations and analyses of recorded information. A Zeiss SEM-(IBAS; 16 bit) image analysis system was used to produce photographic prints from selected video frames to represent descriptive aspects of behavior.

Video recordings were made from the following perspectives:

(1) High magnification, side-view microscopic recordings of individual larvae in the 80-ml aquaria to show detailed activities and morphological information of individual larvae;

(2) Medium magnification, side-view recordings of small groups (8 to 12 larvae) in the 80-ml aquaria for detailed views of interactions among larvae and with prepared feed and live prey; and,

(3) Lower magnification, overhead and side view recordings of the larger 3-l rearing containers were used to observe behavior of larger populations (up to 150 larvae at once) in the 30-l rearing environment.

The black plastic sheeting which enclosed the culture environment also enclosed the camera within the culture chamber during photography. During overhead observations of the 3-l aquaria, however, the aquaria were removed from the recirculating system and placed in a black box open only from above to allow overhead photography but prevent any light from entering the aquaria except from directly above. During these overhead observations, a 2 cm grid background was placed under the aquaria to provide a spatial reference for later analyses of larval distribution and movements.
Behavioral Measurements. — Quantitative descriptions of walleye behavior were derived from analyses of slow-motion digital-time display of the videos of daily overhead recordings of each 3-l aquaria. In 1993, each of the four aquaria were photographed for 8 minutes per day, providing 32 minutes of recording per day of the entire population. In 1994, each of the six aquaria were photographed for 5 minutes each per day, providing 30 minutes of recording per day of the entire population.

The number of cannibalistic attacks was determined from analyses of the recordings. Slow motion, reverse, digital-time display, and stop motion capabilities of the VCR allowed each attack to be individually monitored from beginning to end, even when simultaneous attacks were in progress. A successful attack was defined as a strike that resulted in seizure of the fin or body of another larva that lasted for at least two seconds. An attack rate was calculated by dividing the number of attacks by the number of larvae in the population at the time of the observation period times 100 (i.e., \( \% \ 8-\text{min}^{-1} \)). Hourly attack rates (\( \% \ h^{-1} \)) were estimated by the product of this ratio and the number of sample periods in one hour (e.g., \( \% \ 8-\text{min}^{-1} \times 7.5 = \% h^{-1} \)). This rate represents the percentage of the population making cannibalistic attacks per hour.

Swimming speeds were calculated from the overhead recordings. Each day three larvae were selected from the recordings of each 3-l aquaria. By using slow-motion replay and digital-time display options of the VCR, a 15 second path of each larva was traced on a mylar sheet placed over the monitor screen. A map measurer was used to determine the distance each larva swam during the 15 second period. This distance was converted to speed (cm sec\(^{-1}\)).

Daily mortalities and larvae cannibalized were determined from counting individual dead larvae when cleaning the aquaria each day. Debris siphoned from each aquaria was carefully sorted. Whole larvae and cannibalism remains were recorded. Because walleye larvae were never seen to ingest an entire fish (i.e., the head was always emitted after ingestion of the rest
of the body) mortality from cannibalism was ascertained by counting head capsules in the aquaria debris. The number of cannibalisms in progress at the time of recording were subtracted from the next day's number of cannibalistic remains to prevent counting a cannibalism twice. Daily mortality rates and cannibalism were obtained from all culture aquaria during the study by dividing the mortality and cannibalism counts by the number of fish in the population on the same date.

Results

Based on our observations, four discrete phases of behavior are characterized:

(1) Surface suspension behavior (hatch to 40 TU): larvae either swim to the surface where they attempt to attach to the surface tension, or drift to the bottom, where they rest or return to the surface. Larvae are strongly photopositive.

(2) Gas bladder inflation behavior (40 to 100 TU): larvae swim with constant, rapid, anguilliform movements at the water surface and make frequent attempts to penetrate the surface tension.

(3) First-feeding behavior (100 to 240 TU): larvae swim in a slower, more subcarangiform mode, have less surface orientation, and exhibit a rover-predator behavior with frequent attacks on food or other larvae.

(4) Fully exogenous (after 240 TU): larvae become adept at feeding and become photonegative; activity levels decrease, feeding behavior becomes more 'lie-in-wait', and cannibalistic attacks cease.
Many of the terms used in defining these behavior modes (i.e., anguilliform, subcarangiform, rover-predatory, and lie-in-wait) are described in Moyle and Cech (1988).

**Surface Suspension Phase (0 - 40 TU).—** During this initial phase, the larvae are most often sinking slowly to the bottom, swimming weakly against the surface at a 45° to 60° angle, or resting while attached at the surface or on the bottom of the container. Although both an oil globule and yolk sac were present during this phase, the larvae are negatively buoyant. While suspended by attachment to the surface tension, swimming movements cease and the larvae are able to rest. If the water surface was not disturbed (i.e., no aeration or water impingement to cause turbulence) the larvae often remain attached to the surface tension where they seem to rest at the water surface for many minutes at a time. When turbulence was present, the larvae's attachment is easily disrupted, and they either continue to swim against the surface, which seems to be an attempt to reestablish attachment, or sink to the bottom.

**Gas Bladder Inflation Phase (40 - 100 TU).—** During this phase the larvae develop stronger swimming abilities; their angle toward the surface interface decreases to about 30°, their swimming speed increases and they spend much more time swimming, rarely sinking and resting during lighted periods. The larvae exhibit the highest average swimming speed (nearly 4 cm per sec at 20° C) observed during the larval period (Fig. 1). The larvae are highly phototactic; they are usually at the surface if the illumination is directly overhead, but are easily attracted to the tank edges or in any direction toward an illumination source or object which reflects light. The larvae continually 'bite' at the water surface tension while swimming against it with an anguilliform (i.e., eel-like) motion. This behavior seems to provide the mechanism for the larvae to penetrate the water surface interface and gulp air for physostomous GBI.
First-Feeding Phase (100 - 240 TU).— This phase seems to be initiated by GBI. The larvae's behavior seems to change from one that facilitates GBI to one that facilitates first-feeding. The larvae have some buoyancy control, phototactic behavior lessens, and they no longer swim rapidly against the water surface. The larvae also begin to use the entire water column and assume a more subcarangiform swimming style. Average swimming speed steadily declines from about 3 cm\(^{-1}\) to 1.5 cm\(^{-1}\) during this phase (Fig. 1). The larvae responded to the presence of food as evident from attacks on particulate feed or cohorts. A roving predatory search pattern is recognized by frequent starts and stops during swimming movements as the larvae continuously move about in the aquaria. Attacks on other larvae become very frequent during this phase. Attack rates show that cannibalistic attacks occur only during this phase (Fig. 2). The cannibalistic attack rate intensifies at about 180 TU, then recedes to zero by the end of this phase.

Fig. 1. Mean swimming speeds (cm sec\(^{-1}\)) of walleye larvae reared in 3-l aquaria at 20°C. Error bars represent 95% confidence intervals.
Attack behavior against non-living prepared feed, *Daphnia*, or other larvae are similar. This behavior includes four discrete elements: fixation, tracking, an 'S'-shaped strike posture, and a high speed open mouthed strike. Feeding and aggressive behavior during the first-feeding phase follows a well defined pattern (Fig. 3):

1. A larvae exhibits fixation a potential prey item and tracks the prey movements, slowly closing the gap to achieve a striking distance (Fig. 3 A);

2. Upon tracking to a strike distance (usually about 0.5-1.0 cm) the larvae will assume an 'S-shaped' strike posture, coiling the trunk from the mid body to the caudal fin into a semi-circle (Fig. 3 B);

3. The strike is made at high velocity with the mouth wide open (Fig. 3 C) — analyses of slow-motion cinematography indicate a larvae may achieve a speed of about 15 cm sec$^{-1}$ during the strike.

Fig. 2. Attack rates (% of fish in population attacked per hour) for walleye larvae reared in six 3-l aquaria.
Fig. 3. Three-phase feeding behavior common during First-Feeding Phase: (A) larva fixates on prey (arrow) and closes to strike distance; (B) strike pose exhibited with 'S-shaped' body posture (as indicated in this side view by the curling of the caudal region away from the camera); (C) an open-mouthed high velocity strike; and, (D) *Daphnia* can be seen in mouth.
Initial attempts by the larvae to capture prepared feed particles as they slowly sank in the water column or live *Daphnia* did not seem very successful - although not measured, it seemed like a large percent of strikes during early feeding attempts were unsuccessful in engulfing the prey. After a few days, feed particles and smaller *Daphnia* were more easily consumed by attacking larvae. The three-phased feeding behavior was very similar whether the larvae were attacking prepared feed particles, live *Daphnia*, or other larvae. With prepared feed, the particles are followed downward as they sink, usually for 2-3 cm before making the strike. With *Daphnia* the pursuit distance is usually less than two cm, but the *Daphnia* movements are very erratic causing the larvae to frequently pause and reposition before a strike is made. When tracking other larvae, the pursuit often covers greater distances. Observations of tracking other larvae for more than 10 cm were not uncommon; sometimes larvae would pursue another larva for over 20 cm.

*Fully Exogenous Phase (240 + TU).*— The larvae are successful at obtaining feed particles or live *Daphnia* and are fully exogenous by 240 TU. Their swimming speed and style begin to conform to more of a lie-in-wait predator behavior. The larvae no longer exhibit the rover-predator behavior. The four phase, fixation - tracking - S-posture - strike, feeding behavior observed in the first feeding phase is absent. The larvae simply wait until prey or feed particles happen to pass nearby where they make a close-range strike - a lie-in-wait behavior. Swimming speeds now average about 1.5 cm sec\(^{-1}\) and the larvae are in position near the bottom of the rearing containers. Cannibalistic attacks are absent, although occasional 'nips' are observed that do not result in seizures of other larvae. Positioning in the water column is performed almost entirely with gas bladder function and pectoral fin movements, accompanied by an occasional twitch of the caudal fin to move around within the container.
Discussion

*Behavioral Phases.*— We observed that walleye larvae exhibit saltatory behavioral phases of development which are easily related to previous characterizations of walleye development (Table 2). McElman & Balon (1979) described at least five developmental steps using ecomorphological criterion to define their phases and steps. Li & Mathias (1982) described three stages of development related to feeding and nutritional factors and morphological development. Krise & Meade (1986), used a combination of morphological, physiological and behavioral criterion to describe four developmental stages, two of which were concurrent.

In the present study, behavioral characteristics derived from cinematographic observations were related to specified critical developmental events (i.e., GBI, first-feeding, and cannibalism) to describe phases of behavior.

Although these descriptions of developmental phases were based on observations conducted over a wide time period (i.e., from 1979 to 1994), by different observers with different culture facilities, and from differing perspectives, they express notable similarities. Three of the reports describe an initial 'surface suspension' behavior immediately upon hatching; they all report initiation of feeding at 100 TU; and, they all report cannibalistic activity not beginning until after 100 TU (i.e., cohort cannibalism commences with onset of feeding).

There are, however, notable differences in timing of development among these four studies. We observed a discrete end to the surface suspension behavior by 40 TU, while other studies report this behavior ending later. We measured a definite decline in cohort aggression and cannibalism by 240 TU, while two other studies did not note an end to cannibalism, although Li & Mathias (1982) observed cannibalism also occurring during a discrete period about the same as ours.
Table 2. A comparison of four descriptions of walleye development.

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<tbody>
<tr>
<td>0</td>
<td>F1: surface suspension,</td>
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<td></td>
<td>SURFACE SUSPENSION PHASE:</td>
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<td></td>
<td>reduced vitelline circulation,</td>
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<td>swim to surface &amp; drift to bottom.</td>
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<td>20</td>
<td>pectoral fins moving, mouth open, blood circulation to gills.</td>
<td>PROLARVA: yolk &amp; oil globule present, swim at 30° to vertical, mostly vertical motion, digestive &amp; feeding characteristics develop.</td>
<td></td>
<td>GBI PHASE: very rapid anguilliform swimming, always at surface @ 30° angle to horizontal, frequent attempts to penetrate surface tension with snout.</td>
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<td>40</td>
<td>F2: gill respiration, end surface suspension, increase active swimming, rapid yolk depletion.</td>
<td>STAGE I: gill respiration, fish swim horizontally, end surface suspension.</td>
<td></td>
<td>FIRST FEEDING PHASE: surface orientation wanes, larvae use entire water column, swim speed steadily declines and becomes more carangiform, rover-predator behavior cannibalistic attacks begin and peak mid phase, 3-phased attack (pursuit,'S'-shaped pose, &amp; strike).</td>
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<td>60</td>
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<td>STAGE II: gill respiration, fish swim horizontally, end surface suspension.</td>
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<td>80</td>
<td></td>
<td>STAGES III &amp; IV (concurrent) STAGE III: initiation of feeding &amp; cannibalism, yolk absorbed, food in stomach, stress syndrome occurs. IV: cannibalistic attacks, gas bladder inflation in some, but not all fish, starving fish resort to Stage I behavior.</td>
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<td>100</td>
<td>PP1: begin larval period, begin mixed nutrition, rapid oil globule absorption.</td>
<td>POSTLARVA I: disappearance of yolk, feeding begins, little change in digestive system, swim at 30° to horizontal &amp; more strongly.</td>
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<td>120</td>
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<td>STAGES III &amp; IV (concurrent) III: initiation of feeding &amp; cannibalism, yolk absorbed, food in stomach, stress syndrome occurs. IV: cannibalistic attacks, gas bladder inflation in some, but not all fish, starving fish resort to Stage I behavior.</td>
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<td>200</td>
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<td>POSTLARVA II: disappearance of oil globule, rapid change in digestive system, gill rakers form, swim horizontally.</td>
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<td>220</td>
<td>PP 2: gas bladder inflation, feed on larger food particles, reduction in number of larvae</td>
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<td>240</td>
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<td>260</td>
<td>by extensive cannibalism.</td>
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<td>280</td>
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<td>STAGE IV: initiation of feeding &amp; cannibalism, yolk absorbed, food in stomach, stress syndrome occurs. IV: cannibalistic attacks, gas bladder inflation in some, but not all fish, starving fish resort to Stage I behavior.</td>
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41
Using daily estimates of average swimming speed, we showed a discrete pattern to swimming activity, which coupled with distribution and qualitative style observations provide a complete description of the transition of swimming style through the various developmental phases. In the other studies, mention is made of changes in swimming angle and increases in swimming activity, but they did not describe the pattern we observed.

Gas bladder inflation seemed to vary in occurrence among the four studies. Krise & Meade (1986) reported GBI in "some but not all fry" after the onset of cannibalism. McElman & Balon (1979) did not mention GBI success but noted that GBI occurred well after initiation of feeding and marked the transition to the proopterygiolarval phase, step 2 at about 235 TU. Li & Mathias (1982) do not mention GBI.

Beyerle (1979) reported a 'stress syndrome behavior,' where the larvae swim continuously in a circular motion as if swarming or schooling without feeding, and move to the sides of the tank and face the tank wall. Krise & Meade (1986) note this stress syndrome as occurring in Stage III. This 'stress syndrome behavior' describes our observed GBI behavior, indicating a high proportion of larvae without inflated gas bladders in the population well after yolk absorption. The description of larvae moving to the sides of the tank describe the larvae's phototactic behavior during this phase in the absence of turbidity to diffuse and scatter the light and prevent reflection from tank edges (Bristow & Summerfelt 1995; Rieger 1995). In our study, GBI was > 98% at the end of the study (measured by anesthetizing and examining all surviving larvae through a dissecting scope). We believe that GBI triggered the initiation of first-feeding behavior, which included cannibalistic behavior. As such, we think that poor GBI percentages would likely retard developmental behavior in a proportion of the population. When poor GBI rates occur in a population, GBI behavior (i.e., swimming speed and style, surface orientation, and surface biting) would thus continue in part of the population well after other individuals with GBI may have terminated such behavior. This mixture of
developmental progress in individuals would prevent an observer from noticing the saltatory changes in behavior that correspond to morphological development. Thus, it is possible that our high GBI success allowed us to observe and understand the saltatory nature of developmental behavior in larval walleye.

**Feeding and Cannibalistic Behavior.** — Several conclusions can be made regarding larval walleye feeding behavior:

1. First-feeding behavior develops immediately after GBI;
2. First-feeding behavior is typical of a rover-predator, with constant swimming throughout the aquaria, with a predatory attack behavior consisting of fixation, tracking, an 'S-shaped' body posture for strike, and a high velocity strike;
3. First-feeding behavior occurs from about 100 TU to 240 TU, and is followed by a change in behavior where the larvae become more lie-in-wait, abandoning the tour-phase attack sequence used during the first-feeding phase;
4. The four-phase attack behavior is used on non-living feed particles, live *Daphnia*, and other larvae; and,
5. Cannibalistic behavior occurs only during the 100-240 TU first-feeding phase.

In our observations of populations with > 98% GBI, nearly all larvae were observed to have been feeding at the end of 260 TU. After initiation of the first-feeding phase, it was rare to see a larva without feed in the gut, and there was no indication of starvation. It seemed that Kyowa B-400 was more easily consumed by the larvae than live *Daphnia* and that GBI was a key factor in adaptation to prepared feed. No larvae were ever observed feeding on floating feed or feed that was lying on the bottom of the aquaria. This observation supports other research conclusions that techniques to improve suspension of non-living feed particles, or constant, over-feeding throughout the entire larval phase would improve first-feeding success.
and improve growth throughout the larval period in aquaculture (Nickum 1978; Krise & Meade 1986). Our findings also show that offering of feed is not necessary until GBI has begun to appear in a significant proportion of the population, and further describe the behavioral characteristics that accompany this readiness. Of particular importance is the finding that cohort aggression, with its potential relationship to high mortalities, occurs only during a discrete period while the larvae are still acquiring feeding skills — this tendency to attempt to feed on other larvae begins to wane by about 220 TU, and disappears entirely by 260 TU. Knowledge of this behavioral characteristic should assist aquaculture researchers in developing strategies to mitigate high mortalities during the larval period through appropriately timed culture techniques.

Acknowledgments

Financial support for this study was provided by a Boehme Fellowship grant from the American Fishing Tackle Manufacturers Association and funding from the Iowa Agriculture and Home Economics Experiment Station, project 2982. Image analysis and cinematographic assistance was provided by the Iowa State University Office of Biotechnology Image Analysis Facility. Laboratory space and miscellaneous supplies were provided by Iowa State University Department of Animal Ecology.
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MICROCINEMAGRAPHIC EVIDENCE OF PHYSOSTOMOUS GAS BLADDER INFLATION IN WALLEYE, 
*Stizostedion vitreum* LARVAE

A paper to be submitted for publication in the Journal of Fish Biology

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*Key words:* *Stizostedion vitreum*; fish development; gas bladder inflation; fish behaviour.

*Abstract.*—High resolution microcinematography demonstrates that larval walleye, *Stizostedion vitreum* penetrate the air-water interface. This observation supports the assumption that some physoclistous fish gulp air for first filling the gas bladder. Furthermore, air bubbles were observed in the gut. Muscular contractions were observed that positioned and pressed a microbubble against the ventral edge of the gas bladder where it disappeared within a few minutes. This observation supports the assumption that gulped air is transmitted through the pneumatic duct to the gas bladder. These observations provide the first direct evidence of gas bladder inflation in a larval perciform fish.

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I. INTRODUCTION

Initial gas bladder inflation (GBI) in larval walleye, *Stizostedion vitreum*, is believed to require a physostomous mechanism. Physostomous GBI requires a pneumatic duct to transport gulped air to the gas bladder. Walleye, however, are physoclistous as adults. Physoclistous GBI does not require a pneumatic duct, but obtains air from the blood with a gas gland (the *rete mirablia*). It is hypothesized that the pneumatic duct is present only in the larval form to initiate gas bladder inflation; this duct is presumed transitory and the opportunity for first filling of the gas bladder is only a few days of larval life.

Histological evidence of a pneumatic duct which functions to pass air from the gut into the gas bladder has been found in the larvae of *S. vitreum* (Marty *et al.* In Press) as well as other percomorph physoclists including *Lepomis cyanellus* and *L. macrochirus* (Duwe 1955) and *Morone saxitalis* (Doroshev *et al.* 1981). Denial of surface access during the larval period has been shown to preclude GBI in *S. vitreum* (Kindschi & MacConnell 1989) and other physoclists including *Gasterosteus aculeatus* (von Ledebur 1928, cited by Tait 1960), *M. saxitalis* (Chapman *et al.* 1988), and *Sparus auratus* (Chatain & Ounais-Guschemann 1990). In these fishes, the pneumatic duct degenerates late in the larval period and if physostomous inflation has not occurred, the fish is without a functional gas bladder. It seems that if physostomous inflation does not occur during the larval period, then the gas bladder degenerates. However, larvae of more derived percomorph physoclists, *Sarotherodon mossambica* and *Hemichromis bimaculata* (Cichlidae), lack a pneumatic duct and have been shown to inflate their gas bladders without surface access (McEwen, 1940; Doroshev & Cornacchia 1979). Without inflated gas bladders, fish have decreased growth and survival (Chatain 1987), increased spinal deformities (Kitajima *et al.* 1994), and are unusable for stocking purposes (Kindschi & Barrows 1993). Gas bladder malfunction during the larval period is a considerable obstacle to culture of many species, including
Macquaria novemaculeata (Battaglene & Talbot 1990), S. auratus (Chatain & Ounais-Gushemann 1990), *M. saxatilis* (Bennett *et al.* 1987), and *S. vitreum* (Colesante *et al.* 1986).

Numerous studies have been conducted to develop techniques to improve GBI in larviculture of *S. vitreum*, *M. saxatilis*, and *S. auratus* including tank design, lighting, feeding technique, hormonal therapy, pH manipulation, water inflow method, salinity, and aeration, blowers or oxygen supersaturation (Comacchia & Colt 1984; Barrows *et al.* 1988; Brown *et al.* 1988; Chatain & Ounais-Guschemann 1989; Van Olst *et al.* 1990; Bushman 1992; Barrows *et al.* 1993; Moore *et al.* 1994). Techniques that attempt to enhance access through the surface-water interface seem the most successful (Chatain & Ounais-Guschemann 1990; Summerfelt 1991; Moore *et al.* 1994). This indicates that penetration of the surface tension is the primary obstacle to GBI in culture of these species. Usinger (1956), in reference to aquatic insects, stated 'To an organism of small size, this air-water interface can be an impenetrable barrier, a surface on which to rest, or a ceiling from which to hang suspended.' Larval *S. vitreum* have been observed to 'suspend' from the surface tension during the first two or three days posthatch and to swim actively at a 30° angle to the horizontal at the water surface for several days thereafter (McElmon & Balon 1979; Krise & Meade 1986; Rieger 1995). Rieger (1995) has recently shown that larval walleye swimming speeds are during a three or four day interval immediately preceding GBI, and that the larvae appear to be biting at the water surface during this time. These findings indicate that larval walleye behavior includes innate mechanisms of surface tension penetration for access to air. Van Olst *et al.* (1990) have shown micrographs of air bubbles in the gut and pneumatic duct of *M. saxatilis*, and observed muscular mastication of gut bubbles in the vicinity of the pneumatic duct opening into the gut. Visual evidence of gut bubbles in *S. vitreum* or any other physoclistous larvae, however, have not been reported. And visual evidence of surface penetration has never been reported for any physoclistous larvae.
We used high resolution microcinematography to provide in situ and in vivo observations of *S. vitreum* to describe larvae both penetrating the surface tension and the fate of an air bubble in the gut below the gas bladder.

II. METHODS AND MATERIALS

Our study was conducted during the spring of 1993. Fertilized *S. vitreum* eggs were obtained from Rathburn State Fish Hatchery, Iowa, USA. Eggs were hatched in MacDonald jars at water temperatures of 16°C, then newly hatched larvae were transferred into a laboratory culture system.

A laboratory culture system was constructed to rear walleye larvae in aquaria suitable for in situ microcinematography. A recirculating culture system provided one replacement of water per hour to each aquaria. Overhead fluorescent lights were located approximately 60 cm above the aquaria. These lights were screened with white paper to diffuse and reduce illumination to 240 lux to provide lighting appropriate for microcinematography. Groups of 100 to 150 larvae were reared in 3-L aquaria and smaller 80-ml (10 cm by 8 cm by 1 cm) aquaria were used to observe larvae with microcinemagraphic equipment. Each day of the rearing period, groups of 8 to 12 larvae were removed from the larger 3-L aquaria by ladle and placed in 80-ml aquaria for in situ cinematographic observations. Groups of 20 to 30 larvae were also removed, anesthetized with Finquel (tricaine methane sulfonate) and placed in a petri dish for in vivo observations.

Cinematography equipment included a Sony CCD color video camera equipped with a high magnification zoom microscopic lens. Observations were recorded with a Sony Beta VCR which provides high resolution recording with various replay options to enhance observations and analyses of recorded information. A Zeiss SEM-(IBAS; 16 bit) image analysis system was used to produce photographic prints from selected video frames to represent descriptive
aspects of larval activities. Slow motion, reverse, digital time display, and stop motion capabilities of the VCR allowed the activities of each larvae in each aquaria to be analyzed from sample observation periods.

III. RESULTS

Penetration of the surface tension was verified in one larva. This event was discovered only upon review of the recordings at one fourth actual recording speed. The entire event occurs in less than two seconds and is not easily recognized at normal speed. Slow speed and frame-by-frame analyses revealed details of the event (Figure 1 A-D). Although ingestion of an air bubble, with its subsequent transfer into the gut of the larva, is not shown in these photographs, the recording clearly illustrates penetration of the surface tension by the snout with extension of most of the mouth into the air. Frame by frame analysis of the event in slow motion with digital time display reveals that surface tension penetration occurs for 0.5 seconds. Slow motion video replay further reveals that during this penetration period the larva is apparently suspended by the surface tension — no swimming movements occur during the 0.5 seconds of penetration. A significant body movement occurs both just before the larva penetrates the surface tension, and just before the larvae exits the penetration, indicating that the surface tension is a formidable barrier to penetration from either direction.

From observations of anesthetized larvae, several larvae were found to contain small air bubbles in the gut. Only one of these recordings showed active manipulation of a bubble by muscular contractions and eventual disappearance of the bubble in the vicinity of the opening of the pneumatic duct. A few frames of this sequence are shown in Figure 2. In this specimen, the air bubble was observed for about seven minutes before it disappeared at the ventral edge of the gas bladder. During this time there was considerable movement of gut
muscles that moved the bubble from its original location in the foregut (Figure 2 A) into a
pocket in the gut along the ventral edge of the gas bladder (Figure 2 B). From this position,
muscular contractions of the gut pushed the bubble against the ventral edge of the gas bladder
(Figure 2 C), and these contractions deformed the spherical shape of the bubble, appearing to
almost cleave the bubble. After several minutes of mastication at this location, the bubble was
observed as diminishing in size; then, within a few seconds, the bubble disappeared entirely
(Figure 2 D). A pneumatic duct could not be seen in the recording. Opaque tissues may have
prevent us from observing the pneumatic duct, or the pneumatic duct may be located on the
other side of the gas bladder. When this video recording is replayed at four times normal
speed, the shape of the gas bladder can be seen as deformed by the muscular contractions that
are pushing the bubble against the lower edge of the bladder; i.e., the ventral portion of the
gas bladder is somewhat flattened by the contractions.
Figure 1. *In situ* observations, from microcinematography, of a walleye larva penetrating surface tension: (A) the larva approaches surface; (B) the larva begins to push against interface with rapid movements of the caudal fin; (C) the larva at moment of penetration; (D) after penetration, caudal movements cease and the larva is momentarily suspended by surface tension for 0.5 sec. Entire sequence from A-D occurred in 1.5 sec.
Figure 2. *In vivo* observations, from microcinematography, of an air bubble in larval walleye gut: (A) bubble is first seen in lower gut; (B) four minutes later the bubble is moved through a constriction into a pocket just below the gas bladder, and muscular contractions deform bubble into a non-spherical shape; (C) muscular contractions continue to deform bubble and push it against ventral edge of gas bladder — bubble seems to have diminished in size while in this position; (D) bubble has disappeared entirely about three minutes after moving into the pocket below gas bladder.
IV. CONCLUSIONS

Doroshev & Cornacchia (1979) and Marty et al. (1995) demonstrated the occurrence of a pneumatic duct in *M. Saxitalis* and *S. vitreum* larvae, but there was no direct evidence that the duct was more than a phylogenetic recapitulation of the more primitive physostomous form. Only circumstantial evidence that denial of surface access precluded GBI in these species supports the hypothesis that larvae gulp air and pass an air bubble through the pneumatic duct to inflate the gas bladder. Our high resolution *in situ* microcinematography demonstrates that *S. vitreum* larvae can penetrate the air-water interface with their snout where they suspend in a position that could facilitate ingestion of air. We further show with *in vivo* microcinematography that air bubbles are found in the gut. Bubbles in the gut are positioned and pushed against the ventral edge of the gas bladder. These activities seem to reduce the size of the air bubble to the diameter of the pneumatic duct. These observations suggest that the air bubbles disappear because they are passed into the gas bladder.

A comparison of our *in vitro* observations with histological micrographs of the gas bladder and pneumatic duct anatomy in larval walleye by Marty et al. (1995) indicates that the disappearance of the air bubble in our video occurred at the exact location of the opening of the pneumatic duct into the gut lumen. Van Olst et al. (1990) showed 0.1 mm air bubbles in the pneumatic duct of *M. saxitalis*, but did not observe these bubbles in a prior state. We have found no studies that visually demonstrate any other physostomous mechanisms for any other physoclistous species. Our study appears to be the first visual substantiation of a physoclist larva performing surface penetration, and the first showing a gut bubble disappearing at the opening to the pneumatic duct.

Our study shows limited proof of physostomous activity — only two specimens are presented with this evidence. However, these observations support other evidence of
physostomous mechanism in larval physoclists which include: histological evidence of pneumatic ducts (Doroshev et al. 1981; Marty et al. In Press); evidence that GBI does not occur with denial of surface access (von Ledebur 1928; Chapman et al. 1988; Kindschi & MacConnell 1989; Chatain & Ounais-Guschemann 1990); and, the visual evidence of microbubbles in the pneumatic duct of M. saxitalis (Van Olst et al., 1990). Our study also demonstrates the value of high resolution microcinematography to in vitro and in vivo investigations of larval fish behaviour and physiological mechanisms. Further studies of this kind are recommended to confirm our observations for other species.

ACKNOWLEDGMENTS

Financial support for this study was provided by a Boehme Fellowship grant from the American Fishing Tackle Manufacturers Association and the Iowa Agriculture and Home Economics Experiment Station (project 2982). Image analysis and cinematographic assistance was provided by the Iowa State University Office of Biotechnology Image Analysis Facility. Laboratory space and miscellaneous supplies were provided by Iowa State University Department of Animal Ecology.
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THE INFLUENCE OF TURBIDITY ON LARVAL WALLEYE, *Stizostedion vitreum*, BEHAVIOR AND DEVELOPMENT IN TANK CULTURE

A paper to be submitted for publication in Aquaculture

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ABSTRACT

Behavior and development of larval walleye, *Stizostedion vitreum*, reared in clear and turbid water in laboratory aquaria are compared from hatch to 17-days. Significant differences ($P \leq 0.05$) in distribution and swimming speed were observed. In clear water, larvae showed a strong association with aquaria sides, whereas in tanks with turbid water, larvae avoided aquaria sides. Larvae in turbid water had greater average swimming speeds, larger size, and greater gas bladder inflation (GBI) than larvae cultured in tanks with clear water. There was no significant difference in survival or cannibalism between the two treatments, but temporal display of daily values showed mortality and cannibalism were diminishing in turbid water, while still increasing in clear water at the end of the study. Larval viability (survival $\times$ GBI) was three times greater in turbid water than in clear water (19.0% and 5.7%, $P \leq 0.05$). Better performance and viability in turbid water are attributed to the changes in larval distribution and swimming behavior.

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INTRODUCTION

Problems with first feeding and non-inflation of the gas bladder have long been recognized as major constraints to successful larviculture of walleye, *Stizostedion vitreum* (Nickum, 1978; Colesante et al., 1986; Barrows et al., 1988; Kindschi and MacConnell, 1989; Nagel, 1991). The National Task Force for Public Fish Hatchery Policy (1974) identified the inability to rear larvae of species like striped bass, *Morone saxitalis*, and walleye on artificial diets as the most critical bottleneck in the national fish culture program (Nickum, 1978).

Failure of the gas bladder to inflate during the larval phase is a problem with intensive culture of many physoclistous species of fish including *Pagrus major*, *Dicentarchus labrax*, *Sparus auratus*, and *Macquaria novemaculeata*, as well as striped bass and walleye (Doroshov and Comacchia, 1979; Kitajima et al., 1981; Battaglene and Talbot, 1990; Chatain and Ounais-Guschemann, 1990; Summerfelt, 1991). Without inflated gas bladders, fish have reduced growth and survival (Chatain, 1987), increased incidence of spinal deformities (Kitajima et al., 1994), and they are unusable for stocking (Kindschi and Barrows, 1993) or for growth to a size suitable for the commercial food-fish market.

Numerous strategies have been tested to improve gas bladder inflation (GBI) in larviculture of physoclistous species (Table 1). These techniques have, however, rarely provided GBI above 50%. The highest GBI percentages were found in systems using surface spray. Friedman and Bates (1986) reported 96% GBI in striped bass, and Moore et al. (1994) achieved GBI as high as 100% in walleye. These results, however, were not initially replicable by other culturists using identical culture systems (Van Olst et al., 1990; Bristow and Summerfelt, 1994).
Table 1. Summary of techniques recommended or tested to improve gas bladder inflation striped bass and walleye larvae.

<table>
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<tr>
<th>Reference</th>
<th>Species</th>
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<tr>
<td>Friedman &amp; Bates (1986)</td>
<td>striped bass</td>
<td>absorbent cloth &amp; surface spray</td>
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<td>Hadley et al. (1987)</td>
<td>striped bass</td>
<td>lower rearing temperatures</td>
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<td>Barrows et al. (1988)</td>
<td>walleye</td>
<td>feeding ring to contain oil film</td>
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<td>Brown et al. (1988)</td>
<td>striped bass</td>
<td>maternal hormonal therapy</td>
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<td>Chapman et al. (1988)</td>
<td>striped bass</td>
<td>oxygen supersaturation</td>
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<td>Van Olst et al. (1990)</td>
<td>striped bass</td>
<td>salinity and aeration</td>
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<td>Bushman (1992)</td>
<td>walleye</td>
<td>pH manipulation</td>
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<td>Bristow &amp; Summerfelt (1994)</td>
<td>walleye</td>
<td>turbidity</td>
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<tr>
<td>Moore et al. (1994)</td>
<td>walleye</td>
<td>surface spray</td>
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A review of those studies with the greatest % GBI revealed that flow-thru water from natural sources had been used (Friedman and Bates, 1986; Moore et al., 1994); whereas in other studies that had used identical rearing techniques, yet achieving lower % GBI, recycled dechlorinated tap water was used (Van Olst et al., 1990; Bristow and Summerfelt, 1994). A review of water quality data for Moore et al. (1994) compared to similar rearing conditions at Iowa State University revealed a large difference in turbidity of culture water between the two locations. At ISU, the turbidity of dechlorinated tap water was nearly zero, whereas sand-filtered lake water used by Moore et al. (1994) averaged 11.2 nephelometric turbidity units (NTU). Experiments by Bristow and Summerfelt (1994) to test the effects of turbidity on performance of larval walleyes at ISU resulted in significantly greater GBI, growth, and
survival in larvae reared at NTU between 40 and 50, compared to larvae reared in clear water. Turbidity did not appear to alter any other water quality parameters. It was hypothesized that a difference in behavior may have caused the performance differences. Preliminary observations of larval walleye behavior in turbid water indicated that there may be less of an attraction of the larvae to the tank sides than in clear water, but high turbidity hindered visual observations of the larvae in the 278-liter cylindrical culture tanks used by Bristow and Summerfelt (1994).

The objective of this study was to examine behavioral characteristics that might influence developmental performance within aquaria containing either clear water or turbid water. Small aquaria were used to rear walleye so that cinematography could be used to determine distribution and swimming speed for larvae in the rearing containers. Developmental performance was evaluated in terms of % GBI, feeding success, growth, cannibalism, and survival of walleye larvae reared in clear and turbid water. Performance differences were related to behavioral observations in the treatments.

METHODS AND MATERIALS

Culture Methods.

This study was conducted in 1993 with fertilized walleye eggs obtained from the Iowa Department of Natural Resources, Rathburn fish hatchery. Eggs were hatched at water temperatures of about 16°C, then larvae were immediately transferred into eight 3-liter aquaria; four aquaria each for clear water and turbid water treatments. Each 3-liter aquaria was stocked with 150 to 175 newly hatched larvae. Larvae were fed Kyowa B-400 (BioKyowa, Inc., Chesterfield, MO, USA) four times each day at 2 grams per aquaria per feeding. Water supply
was recycled within each system and the flow rate was controlled to provide one replacement of water per hour to each aquaria. Water temperature was maintained at 17.5°C (± 0.5°) with thermostatically controlled chillers in each of two 250-liter sumps. Water temperature in each aquaria was verified daily with a hand-held mercury thermometer. Overhead fluorescent lights were placed approximately 60 cm above the aquaria. Lighting panels were screened with white paper to soften and reduce the illumination to 240 lux of intensity at the water surface. Black plastic sheeting enclosed the culture chamber to prevent all but direct overhead lighting. The photoperiod was 14 h light (0800 to 2000) and 10 h dark.

Turbidity was created in one culture system (4 aquaria) by adding clay (Old Mine #4 Kentucky ball clay, Paoli Clay Company, Paoli, WI, USA) to the sump of one recycle system, while the other system used untreated water. Clay was added once or twice daily to maintain levels between 30 to 40 NTU. Turbidity and temperatures were measured twice daily at 0900 and at 1600. Turbidity levels in the non-turbid system were always less than 1 NTU.

**Cinematography.**

Cinematography equipment included a Sony CCD color video camera and a Sony Beta VCR which provides high resolution recording with various replay options to enhance observations and analyses of recorded information.

On 2-d through 15-d posthatch, between 1300 and 1500 h, all eight aquaria were filmed for eight minutes each from directly overhead to monitor the movements and distribution of each larval walleye within the aquaria. During overhead recordings the aquaria were placed in a black box open only from above to allow overhead photography but prevent any light from entering the aquaria except from directly above. A 2 cm grid background was placed under the aquaria to provide a spatial reference for later analyses of larval distribution and movements within the aquaria.
Performance Variables.

Performance was evaluated using measurements of GBI, standard length (to show growth), cannibalism, and survival and viability of larvae surviving to the end of the study at 17-d posthatch. Viability, the product of survival and GBI, is a measure of the percent of surviving larvae that have GBI — larvae surviving without GBI are not considered viable since they are unsuitable for stocking or grow out for foodfish. Surviving larvae from each aquaria were anesthetized with tricaine methane sulfonate, placed in a petri dish, and examined under a dissecting scope at 17-d posthatch. Gas bladder inflation and standard lengths for each larvae were recorded. Cannibalism and mortality of larvae in each aquaria were determined daily from observations made when the aquaria were cleaned. Debris from each aquaria was carefully siphoned into a dish and examined for dead and cannibalized larvae (all cannibalized larvae were determined from the presence of larval heads in the debris because walleye larvae do not consume the head of same-age larvae during cannibalization). Cannibalization (fish with a fish in its mouth) was observed in the video recordings. The number of cannibalizations in progress during filming were subtracted from the next day’s head capsule count when calculating daily cannibalism to prevent counting a cannibalism twice. By adding the cumulative number of daily mortalities and cannibalization, plus the number of survivors on 17-d posthatch, the number of larvae stocked in each aquaria was accurately verified.

Behavioral Measurements.

Calculations of larval distribution and swimming speed were made daily from 2-d through 15-d posthatch. From each daily 8-min recording, the video replay was stopped three times at two min intervals (i.e., at 2, 4, and 6 min within the 8-min recording) to provide an instantaneous display of larval positions within aquaria. At each of these displays, the
occurrence of larvae observed within each of the 2-cm cells underlying the aquaria were recorded. The grid underlying the aquaria contained 96 cells; 36 cells were along the edge of the aquaria, and 60 cells were inside this edge-cell perimeter (i.e., nonedge cells). Larvae distribution was expressed as the percent in nonedge cells. The percent in nonedge cells was calculated as the number of larvae observed in nonedge cells divided by the total number of larvae counted in that observation.

From the same video locations (i.e., beginning at 2, 4, and 6 min within each 8-min sample recording), a 15-sec period of cinematography was replayed in slow motion (i.e., at one fourth real time) to trace the swimming paths of selected larvae. At the beginning of this 15-sec interval, three larvae were selected as specimens for these tracings. Larval selection was based on the location of larvae within the aquaria at the beginning of the 15-sec period: one larva was chosen from the left center of the aquaria, one from the center, and one from the right center. The swimming path of each of these three larva was traced with a colored marker on a mylar sheet overlaying the video monitor. Each larva's path was traced with a different color to maintain the identity of each larva's path. A map measurer was used to measure the swimming distance for each larva. From these measurements, swimming speeds (cm • sec\(^{-1}\)) of the larvae were calculated.

**Experimental Design.**

The percent occurrence of larvae in nonedge cells and swimming speeds of larvae from the three samples per aquaria per day were averaged and each aquarium mean was an experimental unit (i.e., \(n = 4\) per treatment). Mean distribution and swimming speeds were determined for each day of the study as well as for groups of days that correspond to larval stages: prolarva = hatch to 5-d, postlarva I = 6-d through 10-d, and postlarva II = 11-d through 16-d (Li and Mathias, 1972). Analysis of covariance (ANCOVA) was used to determine if differences
between larval distribution and swimming speed in turbid and clear water were significant at the 5% level \((P \leq 0.05)\). The covariance of age interaction with distribution or swimming speed were used to determine if differences were independent of changes in larval age. If age interaction was not significant (i.e., \(P > 0.05\)), the interaction was removed from the ANCOVA model to show the significance of the influence of turbidity on behavior without age interaction (Abacus Concepts, 1991). Covariance calculations were made only for larval stage comparisons since there would be no change in age within daily comparisons. Daily distribution and swimming speed means (± 95% confidence intervals) were also plotted to graphically display temporal trends in these behavioral characteristics. Larval distribution was also compared to a uniform distribution to determine if there was an edge or nonedge association of larvae. A uniform distribution would be 62.5% nonedge which is the percentage of nonedge cells underlying the aquaria \((60 \cdot 96^{-1} \cdot 100)\).

A two-tailed unpaired t-test was performed on the means of overall tank performance means (i.e., GBI, standard length, cannibalism, survival, and viability to 17-d posthatch) to determine whether differences between treatments (i.e., again, tanks were the experimental units, \(n = 4\) per treatment) were statistically significant (i.e., \(P\)-value ≤ 0.05).

RESULTS

*Larval Distribution.*

On every day of the study, larvae in turbid water were found in nonedge locations at a rate greater than expected with uniform distribution (Fig. 1). On all days, and for all three stages of development, there was a significant difference in distribution between larvae in turbid and clear water (Table 2). In turbid water, larval distribution seemed to remain relatively constant throughout the three larval stages; there was only a slight, but steady, decline from 85.3% nonedge for prolarvae, to 82.2% during the postlarva I stage, then to 79.9% during the
postlarva II stage. Larvae reared in clear water, however, showed greater changes in distribution with age. Larvae in clear water had the lowest nonedge association during the prolarvae stage (36.8% nonedge). A trend toward uniform distribution of larvae in clear water was observed during the postlarva I stage (starting at 32% at 6-d, and increasing to 67% at 10-d), but during the postlarvae II stage this trend reversed and distribution of larvae in clear water was becoming increasingly edge associated (declining from 61% at 11-d to 46.2% at 15-d).

During the postlarva I stage, the ANCOVA showed that the covariate of age did not have a significant influence on the differences in distribution of larvae in clear or turbid water ($P = 0.225$). During other stages, however, age did have a significant interaction on the effect of water clarity on distribution ($P < 0.05$). Age was undoubtedly significant as a covariate due to the increase then decrease of nonedge distribution described for larvae in clear water during the postlarvae I and II stages.

![Graph](image)

Fig. 1. Distribution of walleye larvae reared in turbid and clear water with 95% CI. Dotted line at 62.5% represents uniform distribution (the % of nonedge cells below the aquaria).
Table 2. Distribution of walleye larvae (± standard error) with F and P values for the significance of the difference between turbid and clear values and for age interaction.

<table>
<thead>
<tr>
<th>Age in Days</th>
<th>% Nonedge in clear water % Nonedge in turbid water</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>35.5 ±5.8</td>
<td>82.8 ±2.5</td>
</tr>
<tr>
<td>3</td>
<td>35.7 ±2.9</td>
<td>86.0 ±1.5</td>
</tr>
<tr>
<td>4</td>
<td>32.2 ±3.4</td>
<td>85.2 ±7.7</td>
</tr>
<tr>
<td>5</td>
<td>43.5 ±8.9</td>
<td>87.0 ±1.3</td>
</tr>
<tr>
<td>All prolarvae</td>
<td>36.8 ±2.8</td>
<td>85.3 ±1.0</td>
</tr>
<tr>
<td>Age interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>32.0 ±3.8</td>
<td>82.5 ±2.2</td>
</tr>
<tr>
<td>7</td>
<td>50.2 ±9.6</td>
<td>86.5 ±1.0</td>
</tr>
<tr>
<td>8</td>
<td>50.2 ±7.4</td>
<td>82.0 ±0.4</td>
</tr>
<tr>
<td>9</td>
<td>52.0 ±7.2</td>
<td>78.0 ±2.6</td>
</tr>
<tr>
<td>10</td>
<td>67.2 ±5.4</td>
<td>81.8 ±1.9</td>
</tr>
<tr>
<td>All postlarvae I</td>
<td>50.4 ±3.8</td>
<td>82.2 ±1.0</td>
</tr>
<tr>
<td>Age interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>61.0 ±3.8</td>
<td>86.0 ±0.7</td>
</tr>
<tr>
<td>12</td>
<td>53.5 ±4.1</td>
<td>80.8 ±2.1</td>
</tr>
<tr>
<td>13</td>
<td>48.2 ±4.2</td>
<td>76.8 ±2.2</td>
</tr>
<tr>
<td>14</td>
<td>47.2 ±2.2</td>
<td>78.8 ±3.8</td>
</tr>
<tr>
<td>15</td>
<td>46.2 ±1.8</td>
<td>77.0 ±1.1</td>
</tr>
<tr>
<td>All postlarvae II</td>
<td>51.3 ±1.8</td>
<td>79.9 ±1.3</td>
</tr>
<tr>
<td>Age interaction</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Swimming speed.

Swimming speeds of larvae reared in turbid water were significantly greater than of larvae reared in clear water for all three developmental stages (Table 3). Developmental trends in swimming speed followed the same general pattern as distribution: in turbid water swimming speeds were relatively constant, with a slight decline throughout the study; whereas, in clear water swimming speeds were lowest during the prolarva stage, then increased to speeds nearly as high as for larvae in turbid water by the end of the postlarva I stage, then again declining during the postlarva II stage (Fig. 2).
Table 3. Swimming speeds (cm sec\(^{-1}\)) of walleye larvae (± standard error) with \(P\) values for the significance of the difference between turbid and clear water and for age interaction.

<table>
<thead>
<tr>
<th>Age in Days</th>
<th>Swim Speed clear water</th>
<th>Swim Speed turbid water</th>
<th>ANOVA F value</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.83 ±0.31</td>
<td>2.64 ±0.16</td>
<td>5.47</td>
<td>0.058</td>
</tr>
<tr>
<td>3</td>
<td>1.15 ±0.09</td>
<td>2.38 ±0.14</td>
<td>57.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4</td>
<td>1.84 ±0.14</td>
<td>2.75 ±0.20</td>
<td>13.91</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5</td>
<td>1.90 ±0.22</td>
<td>2.82 ±0.12</td>
<td>13.51</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>All prolarvae</td>
<td>1.68 ±0.12</td>
<td>2.65 ±0.08</td>
<td>43.24</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age interaction</td>
<td></td>
<td></td>
<td>2.11</td>
<td>0.159</td>
</tr>
<tr>
<td>6</td>
<td>1.24 ±0.19</td>
<td>2.84 ±0.12</td>
<td>50.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>7</td>
<td>2.21 ±0.27</td>
<td>3.02 ±0.14</td>
<td>7.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>8</td>
<td>2.63 ±0.36</td>
<td>2.66 ±0.35</td>
<td>0.01</td>
<td>0.947</td>
</tr>
<tr>
<td>9</td>
<td>1.98 ±0.22</td>
<td>2.95 ±0.07</td>
<td>17.81</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>10</td>
<td>2.60 ±0.20</td>
<td>2.40 ±0.03</td>
<td>1.09</td>
<td>0.338</td>
</tr>
<tr>
<td>All postlarvae I</td>
<td>2.13 ±0.16</td>
<td>2.28 ±0.09</td>
<td>13.51</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age interaction</td>
<td></td>
<td></td>
<td>1.55</td>
<td>0.222</td>
</tr>
<tr>
<td>11</td>
<td>2.04 ±0.10</td>
<td>2.10 ±0.13</td>
<td>0.11</td>
<td>0.751</td>
</tr>
<tr>
<td>12</td>
<td>1.84 ±0.23</td>
<td>2.41 ±0.23</td>
<td>3.08</td>
<td>0.129</td>
</tr>
<tr>
<td>13</td>
<td>2.10 ±0.15</td>
<td>2.32 ±0.23</td>
<td>0.68</td>
<td>0.442</td>
</tr>
<tr>
<td>14</td>
<td>1.59 ±0.20</td>
<td>2.39 ±0.17</td>
<td>9.39</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>15</td>
<td>1.82 ±0.18</td>
<td>2.00 ±0.24</td>
<td>0.21</td>
<td>0.669</td>
</tr>
<tr>
<td>All postlarvae II</td>
<td>1.88 ±0.18</td>
<td>2.24 ±0.09</td>
<td>8.54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age interaction</td>
<td></td>
<td></td>
<td>0.93</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Fig. 2. Swimming speeds of walleye larvae reared in turbid and clear water with 95% CI.
As with distribution comparisons, differences in swimming speed were especially pronounced during the prolarvae stage when the average swimming speed for larvae in turbid water was 58% higher in turbid water than in clear water (2.65 cm sec\(^{-1}\) compared to 1.68 cm sec\(^{-1}\), \(P = 0.0001\)). During the postlarvae I stage the differences seemed to break down: mean swimming speeds in clear water had increased to 2.13 cm sec\(^{-1}\), while in turbid water swimming speeds had declined slightly to 2.28 cm sec\(^{-1}\). These differences during the postlarvae I stage, however, were still significant \((P = 0.0008)\). During the postlarvae II stage, swimming speeds continued to decline slightly in clear water (from 2.04 at 11-d to 1.82 cm sec\(^{-1}\) at 15-d); while swimming speed leveled for larvae in turbid water (starting at 2.1 at 11-d and ending at 2.0 cm sec\(^{-1}\) at 15-d). The ANCOVA showed that age had no significant interaction with differences in swimming speeds in clear and turbid water \((P > 0.05)\) for prolarva and postlarvae I developmental stages, but that age interaction was significant during the postlarva II stage \((P < 0.05)\).

**Developmental Performance.**

Both % GBI and larval size were significantly greater in turbid water than in clear water at 17-d posthatch (Table 4). Mean GBI was 86% in turbid water and 23% in clear water. Mean standard length of larvae in turbid water was 12.2 mm, and 9.7 mm for larvae in clear water. There was no significant difference in cannibalism or survival of larvae. The trend in daily cannibalism and mortality values, however, indicated that cannibalism and mortality of larvae in turbid water had diminished by the end of the study, while they were still increasing in clear water on day 16 (Fig. 3). Although differences in overall survival in turbid and clear water were not significant, viability of larvae in turbid water was significantly greater than for larvae in clear water at the end of the study (19% and 5.7%, \(P < 0.05\)) because of the substantially higher % GBI in turbid water.
Table 4. Performance of larval walleye reared to 17-d posthatch in turbid and clear water (where N = the number of larvae stocked into each treatment). All values are means of fish populations reared in four 3-L tanks (± standard error).

<table>
<thead>
<tr>
<th>Treatment (W)</th>
<th>GBI(%)</th>
<th>Size(mm)</th>
<th>Cannibalism(%)</th>
<th>Survival(%)</th>
<th>Viability(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear (585)</td>
<td>23.2 ± 3.9</td>
<td>9.7 ±0.2</td>
<td>27.7 ±2.5</td>
<td>25.4 ±2.4</td>
<td>5.7 ±0.7</td>
</tr>
<tr>
<td>Turbid (641)</td>
<td>85.9 ±8.5</td>
<td>12.2 ±0.2</td>
<td>21.1 ±1.7</td>
<td>22.1 ±0.9</td>
<td>19.0 ±2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>8.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2.2</td>
<td>0.75</td>
</tr>
<tr>
<td>1.3</td>
<td>0.24</td>
</tr>
<tr>
<td>5.7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Fig. 3. Temporal trends in daily cannibalism and mortality of larval walleye reared in turbid and clear water.
DISCUSSION

In neither turbid nor clear water was distribution uniform within the aquaria. In turbid water the larvae avoided the tank edges and clear water they were attracted to the tank edges. We hypothesize this difference is attributed to phototactic response of the larvae. In clear water the light passes through the water uninterrupted until reflecting from the tank edge; even a black background would therefore appear shiny from an underwater viewer. The phototactic larvae seem to be highly attracted to this reflection, causing the significant association with the aquaria edges in clear water observed in our study. Turbidity, however, causes reflective scattering of light in the water column, diminishing or eliminating the reflection of light from the tank edge. Nonedge distribution in turbid water may result from the larvae avoiding the darkness of the aquaria background when reflection of light from the aquaria side is eliminated.

Previous studies have associated illumination and tank color with distribution changes and improved performance of fish larvae. Nickum (1978) reported that walleye fry were so attracted to the sides of light-colored tanks that they ignored all forms of feed, whereas fry in dark-colored tanks were more evenly distributed and fed better; Nickum noted that uniform, or internal lighting near the center of the tank improved acceptance of feed. Ostrowski (1989) found that overall survival of dolphin, *Hippurus coryphaena*, averaged 50% in black tanks and 25% in uncolored (tan) tanks; Ostrowski attributed the better contrast of feed to the black tanks with better feeding which resulted in higher survival. Malison and Held (1991) reported that using internal tank lighting improved habituation of yellow perch, *Perca flavescens*, to prepared feed. None of these authors evaluated GBI, swimming speed, or larval distribution, although they all suggested better feeding resulted from either improved
distribution or visual contrast. Our study verified that darker aquaria backgrounds (created by reducing reflection of light from the aquaria sides) does influence distribution; that darker backgrounds cause larvae to avoid the aquaria sides. The significantly greater length of larvae reared in turbid water further indicates that nonedge distribution may improve first-feeding success in larval walleye.

Beyerle (1979) reported a "stress syndrome behavior" of walleye larvae reared in tanks, where the larvae swim continuously in a circular motion as if swarming or schooling without feeding, and move to the sides of the tank and face the tank wall; Beyerle attributed high mortalities to this energy consuming, non-feeding behavior. Beyerle's description of stress syndrome behavior is similar to the association of larvae with the tank edge we observed in clear water aquaria.

In turbid water, both distribution and swimming speed were relatively constant throughout the study; but, in clear water, there was an increase in both nonedge distribution and swimming speed during the postlarvae I stage, followed by a decrease in both characteristics during the postlarva II stage. Even though all larvae chosen for swimming speed measurements were located near the center of the aquaria at the beginning of the 15 sec measurement period, swimming speeds were significantly less for larvae in clear water than for larvae in turbid water, and the difference in swimming speed was most pronounced when differences in distribution were the greatest. These patterns in the relationship of distribution to swimming speed indicate that the presence of the shiny aquaria edge, which was inferred to influence distribution, may have also influenced swimming speed, even of larvae not exhibiting an edge association. The disorientation described by Beyerle (1979) may have been caused by the presence of a light reflection in tank edges, which, as indicated in the present study, resulted in both diminished swimming speed and tank-edge distribution. The
reduced nonedge distribution and swimming speed (i.e., disorientation) seems to have resulted in reduced developmental performance.

Our study found no significant difference in overall cannibalism or survival between larvae reared in turbid and clear water (Table 4). Temporal display of cannibalism and mortality values from daily counts, however, indicate that there were differences in the timing of these factors (Fig. 3). Cannibalism and mortality appeared earlier in the turbid aquaria, beginning at 7-d and 9-d respectively, both increasing to a maximum at 13-d then showing a definite decline for 16-d larvae. In clear water, however, cannibalism and mortality did not begin until several days after beginning in the turbid water, beginning at 10-d and 11-d respectively, and both still increasing for 16-d larvae. Li and Mathias (1982) also observed a discrete window of cannibalism in larval walleye that began on 6-d posthatch, increased and peaked on days 8 - 10, then declined to zero by 14-d (Li and Mathias used rearing temperatures of 19°C to 22°C, thus accounting for earlier cannibalism than shown in our study). If the temporal trends we observed in cannibalism and mortality would have continued to occur over a 8 - 9 day period in our clear water aquaria as were observed in the turbid water, then cannibalism and mortality totals for clear water aquaria may have significantly exceeded those found in the turbid water aquaria.

Hecht and Pienaar (1993) observed in larviculture of African catfish, *Clarias gariepinus*, a species that occurs naturally in turbid conditions, that turbidity significantly reduced the incidence of territorial behavior and cannibalism. Loadman et al. (1986) suggested that cannibalism may be reduced when larval walleye are dispersed throughout the water column. Scrutiny of the temporal differences in cannibalism (Fig. 3) in relation to the temporal differences in distribution (Fig. 1) of our study suggests a hypothesis for the relationship of cannibalism and water clarity.
Our data show that in turbid water, nonedge distribution occurred throughout the study and cannibalism began early in the postlarvae I stage; cannibalism then increased and declined to zero for 16-d larvae. In clear water, however, cannibalism did not begin until the beginning of the Postlarvae II stage which coincided with larval distribution changing from significantly edge associated to more uniform distribution (Fig. 1). Then, in clear water, as cannibalism increased during the postlarva II stage, the trend in distribution reversed and larvae became increasingly edge associated again. This reversal of distribution in the clear water may have been related to the onset of cannibalism. Cannibalism may occur predominantly among nonedge larvae. It seems that when the larvae in clear water were significantly edge associated, cannibalism (and probably feeding) was not occurring; but when the population became more evenly distributed, cannibalism began. We think that as cannibalism began, those larvae that were showing nonedge association were both prey and predator; whereas larvae still exhibiting an edge association were less engaged in cannibalistic activity. The percentage of nonedge larvae may have then begun to decline because nonedge larvae were being killed by cannibalistic behavior. Simultaneously, larvae still exhibiting edge association were being less affected by cannibalism, and they became an increasingly higher percentage of the surviving population, resulting in the reversal of distribution trend to an increasingly edge associated population during the postlarva II stage.

This deduction suggests that turbidity actually increases cannibalism because edge-associated larvae are not preying (on feed or other larvae). This result, however, seems short-lived, as evidenced by the rise in cannibalism and mortality near the end of our study. We think that when the larvae in clear water begin to lose an edge association they begin to prey on each other; however, because larvae in clear water have not been feeding they are nearly starved. Li and Mathias (1982) and Loadman et al. (1986) observed that larval walleye were more cannibalistic when deprived of adequate feed. Larvae in clear water thus
may have a greater cannibalistic drive than the larvae in turbid water once they disassociate from the tank edge and begin predatory behavior because they have a greater stimulus for predatory behavior.

It seems therefore, that turbid water may decrease the overall incidence of cannibalism in tank culture, not directly because of better dispersal within the aquaria as Loadman et al. (1986) suggested, but because of an earlier adaptation to exogenous feeding in turbid water; because they begin their cannibalistic behavior earlier, and in conjunction with first feeding behavior, they are less starved and their cannibalism stimulus is not as strong as for larvae in clear water which begin cannibalization after a longer period of not feeding. Populations of larval walleye in clear water would therefore show a delayed, but more pronounced, incidence of cannibalism than larvae in turbid water. Our study, however, was not conducted for a long enough period of time to show the entire effect of cannibalism on the populations in clear water.

In summary, our study provides the following findings and hypotheses of the influence of turbidity on larval walleye behavior and associated effects on performance in tank culture:

(1) Loss of reflective tank sides precludes phototactic attraction of larvae to the tank sides; a dark background now repels the larvae from the edge and they evenly distribute within the other areas of the tank at the water surface.

(2) Distribution away from the tank edge improves larval orientation and swimming performance which exposes the larvae to the water surface and sinking feed particles.

(3) Better access to the water surface, coupled with better swimming performance, improves the larvae's ability to penetrate the surface tension and perform GBI.

(4) Better exposure to sinking feed allows the larvae to first-feed more successfully.
(5) Cannibalism (like feeding) may occur less among edge-associated larvae, and thus occurs later with larvae in clear water when those larvae begin to show less on an association with the tank edges.

(6) Because the larvae in clear water show significant edge association until the late postlarvae I stage, and nonedge distribution is necessary for feeding success, exogenous deprivation has occurred in this population; when their edge association wanes, they are underfed, creating a greater stimulus for cannibalism than of larvae in turbid water where the larvae are better fed due to a nonedge distribution throughout the larval period.

The result of these behavioral issues is better GBI, better growth, and higher viability of walleye reared in turbid water. This study confirms the results of Bristow and Summerfelt (1994) that the production of walleye juveniles in tank culture can be greatly enhanced by using clay to induce turbidity in the rearing water. This study further suggests that the influence of turbidity on distribution and swimming speed is the cause of better developmental performance. Similar methods are probably applicable to the culture of other phototaxic species, especially those in which GBI, first feeding and cannibalism problems inhibit culture success.

ACKNOWLEDGMENTS

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Domesticated Broodstock and intensive fingerling culture techniques for commercial
ABSTRACT. Larval walleye behavior and development at 14°C, 17.5°C, and 20°C are described. Swimming speeds, cannibalism, mortality, gas bladder inflation (GBI), and growth were determined from daily observations and from examination of all larvae surviving to the accumulation of 300 TUs. Larvae were reared at densities of 20-50/L but analysis of covariance (ANCOVA) using density as the covariate did not reveal any interaction between density and temperature for any performance variable. Growth and cannibalism were significantly greater for fish raised at 17.5°C and 20°C than for fish raised at 14°C, but differences in survival and GBI were not significant among the three temperature treatments. Viability (the product of survival and GBI) was, however, significantly greater at 20°C and 17.5°C than at 14°C. Swimming speed, cannibalism, and mortalities had different temporal patterns at each of the three temperatures corresponding to accelerated development at higher temperatures. In tank culture, a 17°C to 20°C rearing temperature seems to enhance passage of larvae through critical developmental events, resulting in higher viability which means improved yield per unit of tank space.
INTRODUCTION

The popularity of walleye, *Stizostedion vitreum*, as a sport fish and the high market value of imported walleye fillets make walleye an excellent candidate for application of biotechnology to expand production for sports fisheries and commercial food-fish. In 1983 and 1984 one billion walleye larvae were stocked throughout North America (Connover 1986). Mortality of larvae stocked in natural waters is, however, reported to exceed 90% (Loadman et al. 1986). Intensive culture on artificial diets could provide an alternative to pond culture with minnows for raising walleye to lengths greater than 150 mm for stocking. Many early attempts to raise walleye on artificial diets in intensive culture met with limited success (Colby et al. 1979; Loadman et al. 1989). Survival to 30 days was typically less than 7% (Nickum 1978). A review of more recent research reveals survival of walleye through the larval period in tank culture as high as 71%; however, survival still rarely exceeds 50%, and more often is less than 15% (Moore et al. 1994).

Of particular concern to walleye larviculture is the occurrence of high mortality of undefined causes during the larval phase — populations may decrease substantially during a period of only a few days. This period of high larval mortality occurs in both cultured and natural populations. In Oneida Lake, New York, mortality of larval walleye was 95% over a two-week period (Noble 1972). Li and Ayles (1981) termed this mortality event a "critical period". The critical period does not begin immediately upon hatching, but when larvae make the transition from yolk sac to feeding. Because of the coincident of this period to the disappearance of the oil globule, an energy/nutrient deficit or poor adaptation to first feeding has been hypothesized as causing starvation (Kindschi and MacConnell 1989; Loadman et al. 1989; Nagel 1991). The contribution of cannibalism, although not shown to exceed 10% of the total mortality, is considered a contributory factor (Doepke 1970; Beyerle 1975; Cuff
Cannibalism may be related to nutrient deficiencies (Loadman et al. 1986; Li and Mathias 1982). Higher temperatures significantly increased attack rates of larval walleye on zooplankton indicating a correlation between temperature and feeding activity level (Johnston and Mathias 1994); temperature may likewise influence cannibalism in walleye larviculture. Poor gas bladder inflation (GBI) is also suspected to contribute to poor viability in walleye larviculture (Nickum 1978; Colesante et al. 1986; Barrows et al. 1988; Summerfelt 1991). Although studies of culture techniques have resolved problem with GBI using surface spray (Barrows et al. 1988; Moore et al. 1994) and use of turbid rearing water significantly improves growth of walleye larvae reared in tanks (Bristow and Summerfelt 1994; Rieger 1995), overall survival rarely exceeds 50%. Research has failed to show a direct linkage between overall mortality and any of these developmental variables.

Energy expenditures, nutrient reserves, and developmental rate during the first 30 days posthatch are related to the rearing temperature (Smith and Koenst 1975; McElman and Balon 1979; Raisanen 1982; Krise and Meade 1986). Among the several studies on culture of larval walleye, temperature conditions have varied considerably; often temperature was not controlled (i.e., ambient temperature was used), and unmeasured daily variations may have superimposed any temperature effects on their results. None of the previous studies have focused on temperature effects on behavior, developmental performance, or viability of walleye larvae. Several researchers have, however, arrived at conclusions regarding temperature, or they have recommended a particular temperature regime (Table 1).

Nickum (1978) concluded from several years of research that success of larval culture of walleye was poor at 20°C. Nickum and Stickney (1993) noted that although no controlled, replicated studies had been conducted to test the effects of temperature on the feeding behavior of walleye fry, it seemed reasonable to suggest using 10°C to 15°C temperatures —
the temperatures of the natural environment during walleye larval development. Data provided in Moore et al. (1994) during a study of tank design and water flow patterns, showed variability in rearing success at different temperatures in a succession of trials over the same spring: tanks with temperatures of 14.5°C to 16.1°C had average survival rates of 16.4%; tanks using water temperatures of 14.5°C to 18.9°C had average survival rates of 34.2%; and, water temperatures of 18.9°C to 25°C provided an average of 68.2% survival. In that study the increasing temperatures resulted from rising ambient water temperatures of the water intake supply, and increasing temperatures seemed to improve larval survival.

Table 1. Temperature regimes used or recommended by various investigators for intensive walleye larviculture.

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>TEMPERATURE (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith &amp; Koenst (1975)</td>
<td>21°</td>
</tr>
<tr>
<td>Hokanson (1977)</td>
<td>increasing by 1° per day</td>
</tr>
<tr>
<td>Nickum (1978)</td>
<td>less than 20°</td>
</tr>
<tr>
<td>McElman &amp; Balon (1979)</td>
<td>15°</td>
</tr>
<tr>
<td>Li &amp; Mathias (1972)</td>
<td>20°</td>
</tr>
<tr>
<td>Colesante et al. (1986)</td>
<td>11.5° - 18.3°</td>
</tr>
<tr>
<td>Summerfelt et al. (1991)</td>
<td>14.7° - 19°</td>
</tr>
<tr>
<td>Moore et al. (1994)</td>
<td>14.5° - 25°</td>
</tr>
</tbody>
</table>

It is possible that the developmental events (i.e., gas bladder inflation, cannibalism, and first feeding) can be favorably manipulated with rearing temperatures. A temperature regime strategy which provides appropriate activity levels, does not over-tax metabolic demands and developmental events, and enhances conversion to exogenous feeding, may require use of alternative temperatures for different phases of development. Lower temperatures may mitigate cannibalistic behavior by reducing activity levels and nutrient demands of the larvae, whereas higher temperatures may, for the same reasons, increase appetite and acceptance of formulated feed.
Our study was designed to test the influence of temperature on larval development and viability by comparing GBI, larval length, cannibalism, and survival at a range of rearing temperatures to a constant temperature unit endpoint (300 TUs). Walleye larvae were reared at 14°C, 17.5°C, and 20°C. These three regimes represent the range of temperatures that have been used by various researchers (Table 1). Cinematography was used to observe effects of temperature on larval swimming speed during each day of the study.

METHODS AND MATERIALS

Culture Methods

The study was conducted in 1993 and 1994: walleye were reared at 17.5°C in 1993 and simultaneously at both 14°C and 20°C in 1994. Four aquaria were used at 17.5°C with an average stocking density of 53 larvae per liter, whereas three aquaria were used at 14°C with an average stocking density of 21 larvae per liter, and three aquaria were used at 20°C with an average stocking density of 30 larvae per liter. Differences in stocking density in 1994 were not intentional, but the result of loss of larvae at 14°C from impingement in overflow screens during the first 48 hours posthatch. Because of differences in density among the three accidental density treatments, statistical analyses of treatment means was done by analysis of covariance (ANCOVA) using density as the covariate. Rearing conditions other than temperature and stocking density were identical during both years for all three temperature systems.

Eyed-eggs were obtained from Iowa Department of Natural Resources. Eggs were hatched in MacDonald jars at 16°C. Immediately upon hatching, larvae were transferred into 3-L glass aquaria suitable for in situ cinematography. Mean standard length of larvae was
7.5 mm (±0.5 mm) at the time of stocking. The aquaria were supplied with recycled water at one replacement per hour. Water temperatures of 14°C, 17.5°C and 20°C were maintained with thermostatically controlled chillers in the sump tank of each system. Temperatures were verified daily with a hand-held mercury thermometer. Turbidity was maintained between 30 to 40 NTU with regular additions of clay (Old Mine #4 Kentucky ball clay, Paoli Clay Company, Paoli, WI, USA) to the water to reduce the reflection of light from aquaria sides and prevent phototaxis of larvae to the tank edges (Bristow and Summerfelt 1994; Rieger 1995). Fluorescent lights were located 60 cm above the aquaria. The lighting panels were covered with white paper to diffuse and reduce the illumination to 240 lux at the water surface. The culture chamber was enclosed with black plastic sheeting to eliminate all but direct overhead lighting. The photoperiod consisted of 14 h light and 10 h dark. Larvae were fed a microparticulate feed, Kyowa B-400 (BioKyowa, Inc., Chesterfield, MO, USA) at a rate of 2 grams per aquaria, four times per day.

Cinematography and Swimming Speed

Cinematography equipment included a Sony CCD color video camera and a Sony Beta VCR which provides high resolution recording with various replay options to enhance observations and analyses of recorded information. During video recording, the aquaria were placed in a black box open only from above to allow overhead photography but prevent any light from entering the aquaria except from directly above. During these overhead observations, a 2-cm grid background was placed under the aquaria to provide a spatial reference for later analyses of larval movements. Slow motion, reverse, digital time display, and stop motion capabilities of the VCR allowed individual larvae to be monitored during sample observation periods.
Swimming speeds were derived from 15-second subsamples of the daily overhead video recordings. Video recordings were made for 5-8 min intervals per aquaria per day between 1300 and 1500 h. At three preselected times during each recording period (e.g., 2, 4, and 6 minutes from the beginning during an 8-min recording), the motion was stopped and three larvae from different parts of the aquaria were selected as specimens: one larvae from the center of the aquaria, one from the left center, and one from the right center. The recording was observed at a slow replay speed (one fourth real time) and the movement path of each specimen was traced with a marker on a transparent mylar sheet placed over the monitor. The recording was returned to the same time of origin to obtain tracings of each of the three larvae from simultaneous times. Each specimen was traced with a different color to allow continuous identification of each larva. The length of the path of each specimen was measured with a map measurer. The swimming speed in cm/sec of each larva was calculated from the length of the swimming path (cm) divided by the duration of the recording (15 sec).

Performance Variables

Daily mortality and cannibalization were determined by counting individual dead and cannibalized larvae when cleaning the aquaria each day. Debris siphoned from each aquaria was carefully sorted and whole larvae and remains (head capsules) were recorded. Because walleye larvae were never seen to ingest an entire individual (i.e., the head was always emitted after ingestion of the rest of the body) cannibalistic remains were easily distinguished from other mortalities in the aquaria debris. Total daily cannibalism was determined for each aquaria from the counts of cannibals at the time of video recordings (i.e., fish with a fish in their mouth) plus the number of head capsules found in the aquaria. The number of cannibalizations in progress during each daily video recording were subtracted from the next
day's head capsule counts when calculating daily cannibalism to prevent counting a
cannibalization twice. From these data, accurate percent daily mortality and percent daily
cannibalism were obtained by dividing the mortality and cannibalism counts by the number in
the population on that date. Overall survival of larvae was calculated by dividing the number
of surviving larvae by the number stocked. Viability represents the percentage of surviving
larvae with an inflated gas bladder (% survival X % GBI). Viability seems like an
appropriate descriptive term of the success of a treatment because surviving larvae without an
inflated gas bladder by 300 TU are not viable because they would not live if stocked in
natural waters and are not suitable for grow out as food fish.

Percent GBI and standard lengths (to represent growth) were determined by examination
of all larvae surviving to termination of the rearing periods. To have comparable data, fish in
all treatments were reared to the same temperature end point: each rearing period was
terminated as closely as possible to 300 temperature units (TU), with TU = the number of
days times the rearing temperature in °C. The 20°C treatment was terminated on 15-d
posthatch (300 TU); the 17.5°C treatment on 17-d posthatch (298 TU); and, the 14°C
treatment on 21-d posthatch (294 TU). The surviving larvae were anesthetized with
Finquel® (tricaine methane sulfonate), and examined through a dissecting scope to determine
standard lengths and GBI.

**Experimental Design**

Comparisons of performance for the three temperatures were made by analysis of
covariance (ANCOVA) for fish length, total cannibalism, GBI, survival, and viability of
larvae at 300 TU. Growth comparisons were determined from differences in standard length
at the end of the study. Each aquarium was an experimental unit (i.e., n=3 for 14°C and 20°C, and n=4 for 17.5°C).

Because stocking densities varied among temperature treatments, statistical analysis was done by ANCOVA with density as a covariate to determine whether density differences influenced temperature effects. A (temperature X density) interaction P-value was used to determine whether density had a significant effect on each performance variable. If the interaction was not significant (i.e., \( P > 0.05 \)), the interaction of density was removed from the ANCOVA model to determine the influence of temperature on the parameters when corrected for density effects (Abacus Concepts 1991).

The ANCOVA provided mean values, standard errors, and an F-test of the significance of the influence of temperature on differences. Fisher's LSD test was used to make pairwise comparisons where the ANCOVA showed a significant temperature effect (i.e., \( P < 0.05 \)). Pairwise differences were determined as significant at the 95% level (i.e., \( P < 0.05 \)).

Daily % cannibalism and % mortality means were graphed to obtain a visual display of temporal trends in cannibalism and mortality at the three different temperatures. For these daily presentations, cannibalism and mortalities remained as separate values (i.e., cannibalism mortalities were not included in daily mortality counts), whereas when measuring overall survival for performance comparisons, cannibalism and other mortalities were combined in the accounting process.

Comparisons of swimming speed among treatments were also made with both temporal graphs and ANCOVA. Because behavior of larval walleye was suspected to change during different stages of development (i.e., prolarva, postlarva I, and postlarva II), statistical comparisons were made of mean swimming speeds for each developmental stage. We used TU as a guide to the amount of time required for transition from one stage to the next: the prolarva stage occurs from 0 through 100 TU; postlarva I = 120 through 200 TU; and,
postlarva II = 220 through 300 TU (Li and Mathias 1982). An ANCOVA was used to determine the significance of any influence of temperature on differences in mean swimming speed among treatments. As with performance comparisons, density was used as a covariate to control for any influence of density on swimming speeds and pairwise comparisons were made with Fisher's LSD. Daily mean swimming speeds for each temperature were also plotted with time to display temporal trends in swimming speed for each temperature treatment.

RESULTS

Performance Comparisons

The density covariate interaction did not significantly influence comparisons among temperature treatments for any performance comparison (Table 2). Therefore, the interaction of temperature X density was removed from the ANCOVA model in statistical analyses of the effect of temperature on performance (i.e., the Temp wo Int P-value was used to determine the significance of the influence of temperature in Table 2).

Survival and GBI were not significantly influenced by temperature. Although GBI for 20°C was 98.0%, compared to 85.9% and 74.7% for 17.5°C and 14°C respectively, these differences were not significant (P = 0.24) because of the large variation among tanks within the treatments (Table 2). Survival ranged from 15% to 22% (P = 0.07). Because the ANCOVA for the main treatment was not significant, pairwise comparisons were not performed for these parameters.

Temperature significantly influenced growth, cannibalism, and viability (Table 2). Pairwise comparisons showed that standard length of larvae at 300 TU was significantly greater for fish raised at higher temperatures (17.0 mm at 20°C, 12.2 mm at
17.5°C, and 10.5 mm at 14°C, \( P < 0.05 \) (Table 3). Viability was significantly greater at 20°C compared with 14°C (21.6% and 10.7%, \( P = 0.0293 \)), but viability at 17.5°C (19.0%) was not significantly different from either the 14°C or 20°C treatment groups. Although temperature did not significantly influence GBI or survival independently, the multiplicative effect of GBI and survival was sufficient to make viability differences significant.

Cannibalism was significantly lower at 14°C than at either of the higher temperatures (\( P = 0.0014 \)), whereas, there was no difference in cannibalism between fish reared at 17°C and 20°C (\( P = 0.74 \)).

Temporal trends from daily counts of mortality and cannibalization show that the intervals of cannibalistic behavior and mortalities were apparently completed at 17.5°C and 20°C at 300 TU; whereas, at 14°C, cannibalization and mortality were still increasing upon termination of the study (Figure 1).

Table 2. Performance of larval walleye reared at three temperatures to 300 TU posthatch where \( n \) (aquaria) are experimental units. All values are means (± standard error). Analyses are derived from ANCOVA using fish density (N/L) as a covariate: Temp w Int = with (temperature x density) interaction; Interaction = the (temperature x density) interaction; and, Temp wo Int = after removal of interaction.

<table>
<thead>
<tr>
<th>Temp (^{\circ})C</th>
<th>n</th>
<th>Density (N/L)</th>
<th>Length (mm)</th>
<th>Cannibalism (%)</th>
<th>GBI (%)</th>
<th>Survival (%)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0°</td>
<td>3</td>
<td>30</td>
<td>17.0±0.43</td>
<td>22.1±2.64</td>
<td>98.0±2.00</td>
<td>22.0±3.46</td>
<td>21.6±3.49</td>
</tr>
<tr>
<td>17.5°</td>
<td>4</td>
<td>53</td>
<td>12.2±0.19</td>
<td>21.1±1.73</td>
<td>85.9±8.54</td>
<td>22.0±1.08</td>
<td>19.0±2.34</td>
</tr>
<tr>
<td>14.0°</td>
<td>3</td>
<td>21</td>
<td>10.5±0.07</td>
<td>7.0±1.63</td>
<td>74.7±8.51</td>
<td>15.0±4.04</td>
<td>10.7±2.14</td>
</tr>
</tbody>
</table>

\( P \)-value Temp w Int = 0.0001 0.0021 0.1877 0.2113 0.0652

\( P \)-value Interaction = 0.3566 0.8625 0.8181 0.9196 0.8934

\( P \)-value Temp wo Int = 0.0001 0.0120 0.2403 0.0699 0.0124
Table 3. Pairwise comparisons of differences in performance (Diff.) for walleye larvae reared at three temperatures (°C) with Fisher's LSD for parameters that showed a significant temperature effect in ANCOVA (i.e., $P \leq 0.05$ in Table 2).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Length</th>
<th>Cannibalism</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diff.</td>
<td>Diff.</td>
<td>Diff.</td>
</tr>
<tr>
<td></td>
<td>$P$-value</td>
<td>$P$-value</td>
<td>$P$-value</td>
</tr>
<tr>
<td>20.0° with 17.5°</td>
<td>4.85</td>
<td>0.97</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.7395</td>
<td>0.5096</td>
</tr>
<tr>
<td>20.0° with 14.0°</td>
<td>6.49</td>
<td>15.13</td>
<td>10.87</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.0014</td>
<td>0.0293</td>
</tr>
<tr>
<td>17.5° with 14.0°</td>
<td>1.64</td>
<td>14.17</td>
<td>8.29</td>
</tr>
<tr>
<td></td>
<td>0.0027</td>
<td>0.0014</td>
<td>0.0615</td>
</tr>
</tbody>
</table>

Figure 1. A temporal comparison of daily cannibalism and mortality percentages for walleye larvae reared at three temperatures (°C).
Swimming Speed Comparisons

The ANCOVA of swimming speeds show significant effects of temperature at all three developmental stages. The (temperature x density) interaction was not significant for any developmental stage (Table 4). Pairwise comparisons showed that the greatest difference in swimming speeds occurred during the prolarva stage when larvae reared at 20°C had a significantly higher mean swimming speed than larvae at either 17.5°C or 14°C (3.77, 2.65, and 2.22 cm/sec respectively, \( P < 0.05 \)) (Table 5). During other developmental stages, swimming speed differences were less distinct.

Table 4. Swimming speeds (cm/sec) (± standard errors) for walleye larvae reared at three temperatures by developmental stages showing the ANCOVA of density interaction and the temperature influence on swimming speed with the interaction removed (Temp. w/o Int.).

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Temperatures (Means ± SE)</th>
<th>Density Interaction</th>
<th>Temp. w/o Int.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.0°C</td>
<td>17.5°C</td>
<td>20.0°C</td>
</tr>
<tr>
<td>Prolarva</td>
<td>2.22 ±0.094</td>
<td>2.65 ±0.083</td>
<td>3.77 ±0.157</td>
</tr>
<tr>
<td>Postlarva I</td>
<td>1.94 ±0.133</td>
<td>2.79 ±0.088</td>
<td>2.52 ±0.157</td>
</tr>
<tr>
<td>Postlarva II</td>
<td>1.17 ±0.130</td>
<td>2.23 ±0.090</td>
<td>1.29 ±0.124</td>
</tr>
</tbody>
</table>

Table 5. Fisher's LSD pairwise comparisons of differences (Diff.) in swimming speed (cm/sec) for walleye larvae reared at three temperatures.

<table>
<thead>
<tr>
<th>Comparisons (Diff. &amp; P-values)</th>
<th>14.0° with 17.5°</th>
<th>14.0° with 20.0°</th>
<th>17.5° with 20.0°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolarva</td>
<td>0.428 ( P=0.0037 )</td>
<td>1.552 ( P=0.0001 )</td>
<td>1.124 ( P=0.0001 )</td>
</tr>
<tr>
<td>Postlarva I</td>
<td>0.833 ( P=0.0001 )</td>
<td>0.579 ( P=0.0011 )</td>
<td>0.253 ( P=0.1391 )</td>
</tr>
<tr>
<td>Postlarva II</td>
<td>1.069 ( P=0.0001 )</td>
<td>0.125 ( P=0.5394 )</td>
<td>0.944 ( P=0.0001 )</td>
</tr>
</tbody>
</table>
Larvae reared at 20°C showed a substantial change in swimming speed during the study. Swimming speeds of larvae in 20°C water declined from 3.77 cm/sec (mean prolarva speed) to 1.29 cm/sec (mean postlarva II speed) (Figure 2); most of this change occurred during the postlarva I stage when swimming speed steadily declined from 3.2 cm/sec on 5-d to 1.2 cm/sec on 10-d. This change was not observed for the other temperatures: mean swimming speeds at 17.5°C rose slightly during the postlarva I stage, then decreased slightly during the postlarva II stage with a total decline of only 0.56 cm/sec; and, swimming speeds at 14°C showed a gradual decline throughout the study from 2.22 cm/sec to 1.17 cm/sec with a total decline of 1.05 cm/sec.

Figure 2. Average daily swimming speeds (cm/sec, with standard error bars) for walleye larvae reared at three temperatures.
DISCUSSION

We observed a significantly higher swimming speed of prolarvae, as well as a significantly greater length (i.e., growth rate) at 300 TU for larvae reared at 20°C compared to larvae reared at 14°C or 17.5°C. Johnston and Mathias (1994) reported a higher attack rate of walleye larvae on zooplankton at higher water temperatures in a study of walleye larvae feeding at 15°C, 18.5°C, and 22°C temperatures. Higher temperatures may provide a greater level of activity (measured as swimming speed in our study) during development of first feeding behavior. This greater activity level may accelerate first feeding success through a higher attack rate which then leads to greater growth rates during the larval period.

Our study also indicates that higher temperatures accelerate cannibalistic behavior. Li and Mathias (1982) found that cannibalism of larval walleye reared at temperatures from 19°C to 22°C occurred between 5-d and 14-d posthatch, peaking on 9-d and 10-d. Our results, like those of Li and Mathias (1982) show a discrete period of cannibalism. In the present study, at 20°C most cannibalism occurred between 5-d and 10-d posthatch; at 17.5°C the period of cannibalism was from 8-d to 16-d posthatch; and, at 14°C, cannibalism did not begin until 16-d and was still ongoing at 300 TU. These temporal differences show that cannibalistic behavior, like activity levels and growth, is accelerated by higher temperatures.

Although the period of cannibalism was five days at 20°C and eight days at 17.5°C, total cannibalization was not different between these two temperatures (22.1% and 21.1% respectively, \( P = 0.74 \)). The similarity in total cannibalism at 17.5°C and 20°C is due to higher daily % cannibalism values at 20°C, when as much as 10% of the population was cannibalized on 8-d posthatch, and on both 7-d and 9-d, over 7% of the population was cannibalized. At 17.5°C, however, the highest % daily cannibalism was 7% on 13-d, and cannibalization never exceeded 6% on any other day. Although the total cannibalism that
occurred by the end of the study was significantly lower at 14°C than for the two higher temperatures, a continuation of the study beyond 300 TU would have likely increased total cannibalism at 14°C because % daily cannibalism seemed to have been increasing upon termination of the study at 300 TU; whereas at the higher temperatures cannibalism seemed to have been over.

The trends in percent daily mortality followed the same pattern as cannibalism, indicating a retarded interval of mortality at lower temperatures. The daily mortality of larvae at 14°C was relatively low (<7%/d) until the beginning of cannibalism on 16-d, at which time mortality began to increase and was 30%/d on 19-d and 20-d. If the present study had continued beyond 300 TU, total survival may have been greater at higher temperatures even though within the 300 TU period, differences were not significant.

Higher temperatures seemed to improve GBI, but differences were not significant because of high variability between aquaria. Viability, however, which is the product of GBI and survival, was significantly greater at 20°C than at 14°C (21.6% compared to 10.7%).

In conclusion, we found evidence that rearing temperatures of 20°C would increase performance and viability of walleye larvae in intensive culture. Higher temperatures seem to accelerate passage through the critical period because of a greater initial activity level which improves first feeding success. Cannibalism and mortality seem to occur at a greater rate at higher temperature, but for a shorter time. Further studies to develop an optimum temperature management strategy that accelerates passage of larvae through the critical periods and improves GBI and first feeding success without significantly increasing cannibalism or other mortalities may prove valuable in further development of intensive walleye larviculture.
ACKNOWLEDGMENTS

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THE NATURE AND SIGNIFICANCE OF CANNIBALISTIC BEHAVIOR IN LARVICULTURE OF WALLEYE

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Abstract.—Microcinematography was used to record behavior of larval walleye (Stizostedion vitreum) in 3-L laboratory aquaria at 17.5°C and 20°C. Cannibalistic attack behavior was nearly identical to predatory attack behavior by larvae on live Daphnia and particles of prepared feed. Predatory behavior had a distinct pattern which included fixation, tracking, strike posture, and a high speed burst with mouth open. Ingestion of sibling larvae resulted only from successful seizure of the caudal fin from which the prey was gradually swallowed up to the prey's head. Most cannibalistic attacks, however, involved seizure of the pectoral fin, opercle, trunk, or head of the prey. Pectoral fin seizures were the most common, accounting for 79% of all seizures observed. Successful cannibalization represented only 1.9% of the total number of seizures recorded. Over the 15 to 17-d posthatch interval, cannibalistic behavior among larvae occurred only in a discrete 5-8 day period. At both temperatures there was a strong correlation of daily mortality and occurrence of cannibalism (r = 0.97 and 0.82), and of daily mortality and cannibalistic attack rates (r = 0.66 and 0.83). The findings suggest that injuries from cannibalistic attacks were a greater cause of larval mortality than actual cannibalism.

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Mortalities among larval walleye (*Stizostedion vitreum*) can be caused by cannibalism in tank (Cuff 1977; Li and Mathias 1982; Loadman et al. 1986) and pond culture (Dobie 1956; Li and Ayles 1981; McIntyre et al. 1987) as well as in natural lakes (Doepke 1970). Cannibalism is also a problem for larviculturists of many other piscivorous fish species including striped bass (*Morone saxitalis*), spotted seatrout (*Cynoscion nebulosus*), and dolphin (*Coryphaena hippurus*) (Doroshov and Cornacchia 1979; Tucker 1988; Owstowski 1989). The review by Hecht and Pienaar (1993) identified the occurrence of cannibalism in fifteen cultured species of fish.

Before 1980 survival of larval walleye in tank culture was typically less than 1% (Nickum 1986). In spite of improvements in survival, variation between trials by the same researchers is substantial. Examples of such variability include Barrows et al. (1988) from 0.5 to 12.3%; Summerfelt (1991) 4.6 to 43.4%; Zitzow (1991) 13.8 to 25.5%; Moore et al. (1994) 3.8 to 68.2%; and, Bristow and Summerfelt (1994) 5.0 to 61.2%. Li and Mathias (1982) stated that an understanding of the causes of the high mortalities in walleye larviculture, and a search for the means to suppress it, are essential elements in the improvement of walleye culture methods. They found that high mortalities occur during the period of conversion from endogenous to exogenous feeding. The coincidence of the occurrence of mortalities with the first-feeding stage of development suggests that mortalities result from cannibalism, starvation, or both (Nickum and Stickney 1993). Poor adaptation of larval walleyes to non-living prepared feed has been a recognized problem (Colesante et al. 1986). Nickum and Stickney (1993) stated that immediate failure of the larvae to accept either live or dry feed has been followed by cannibalism, "tail-biting", and early starvation.
Kindschi and MacConnell (1989) also suggested that poor first feeding success and nutrient deficiencies contribute to mortality.

Noninflation of the gas bladder has also been inferred as responsible for high mortalities in larviculture of walleye (Nickum 1978; Colesante et al. 1986; Barrows et al. 1988; Summerfelt 1991; Nickum and Stickney 1993). Additionally, noninflation of the gas bladder has been proposed as contributing to cannibalism (i.e., larvae without an inflated gas bladder would be more vulnerable to predation by larvae that have an inflated gas bladder and superior swimming ability) (Krise and Meade 1986; Summerfelt 1991). Loadman et al. (1986) concluded that injuries from attacks that did not result in ingestion of the prey were a significant cause of mortality in walleye larviculture.

Our objectives are to describe characteristics of cannibalistic behavior in larval walleye and attempt to show the relationship between cannibalistic behavior and mortality. Our findings suggest a strong correlation of cannibalistic behavior and mortality of walleye in larviculture environments.

**Methods and Materials**

*Culture Methods.*— Fertilized walleye eggs were obtained from the Iowa Department of Natural Resources. Eggs were hatched in MacDonald jars at 16°C and immediately transferred into 3-L glass aquaria suitable for *in situ* microcinematography. The study was conducted over a two year period. In 1993, 641 larvae were stocked in four aquaria (53 larvae/L) and reared at 17.5°C; in 1994, 545 larvae were stocked in six aquaria (30 larvae/L) and reared at 20°C. Water delivery to each aquarium was regulated to provide one replacement per hour from a recirculating system. Temperatures were maintained with thermostatically controlled sump
chillers. Water temperatures in each aquaria were verified daily with a hand-held mercury thermometer.

Turbidity was maintained between 30 to 40 NTU with the addition of clay (Old Mine #4 Kentucky ball clay, Paoli Clay Company, Paoli, WI, USA) to the water to mitigate the reflection of light from aquaria sides to prevent phototaxis of larvae to the tank edges (Bristow and Summerfelt 1994; Rieger 1995). Fluorescent lights, located 60 cm above the aquaria, were covered with white paper to diffuse and reduce the illumination to 240 lux at the water surface. The culture area was enclosed with black plastic sheeting to eliminate all but direct overhead lighting. The photoperiod was 14 h light: 10 h dark. Larvae were fed a microparticulate feed (Kyowa B-400; Biokyowa, Inc., Chesterfield, MO).

The study was terminated at 15-d posthatch for larvae reared at 20°C and at 17-d posthatch for larvae reared at 17.5°C. The two rearing periods allowed for differences in developmental rate due to differences in rearing temperatures. Each study was terminated at 300 temperature units (TU), where TU = the number of days multiplied by the number of degrees Celsius (i.e., also known as degree days).

**Cinematography.**— Cinematography was performed with a Sony CCD color video camera equipped with a low power zoom macro lens and a high magnification zoom microscopic lens. Observations were recorded on a Sony Beta VCR which provides high resolution recording with various replay options to enhance observations and analyses of recorded information. A Zeiss SEM-(IBAS; 16 bit) image analysis system was used to produce prints from selected video frames to represent descriptive aspects of behavior.

Cinematography was performed of entire populations in each 3-L aquaria, and of smaller numbers (8 - 12 larvae) at higher magnification in 80-ml aquaria. On each day of the rearing period, the 3-L aquaria were filmed from directly overhead for 5-8 minute intervals. During these overhead observations, the 3-L aquaria were placed in a black box open only from
above to allow overhead photography but prevent any light from entering the aquaria except from directly above. A 2 cm grid background was placed under the 3-L aquaria to provide a spatial reference for later analyses of larval movements. All cinematography was performed between 1300 and 1500 hrs. Eight to twelve larvae were removed from the larger 3-L aquaria by ladle and placed in the 80-ml aquaria for more detailed observations of larval interactions with food and other larvae. Live *Daphnia pulex* were occasionally added to the smaller aquaria to provide observations of feeding behavior and the effect of live food on cannibalistic behavior. The 80-ml aquaria were viewed from a side view while enclosed in the black plastic covered culture environment. Following cinematography in the 80-ml aquaria, these larvae were returned to the 3-L aquaria from which they were removed.

_Behavioral Measurements._— Cannibalistic attacks were quantified by study of the 5-8 min daily overhead cinematographic recordings of each aquaria. Slow motion, reverse, digital time display, and stop motion capabilities of the VCR allowed each attack to be individually monitored from beginning to end, even when simultaneous attacks were in progress. Analyses of these recordings provided information on the number, duration, and seizure sites of cannibalistic attacks. "Nipping" and "biting", terms often used in reporting observations of cannibalistic behavior of larval fish, were not considered seizures. For the purpose of defining a successful attack, a seizure is a strike of a larva that results in the grasp of the fin or body of another larva that lasts for at least two seconds. The sum of all observed seizures divided by the number of larvae in the aquaria on that day provided the attack per larva for each sample period (i.e., %/8-min). The attack rate was expressed as percent of the population per hour (%/h), which is the %/8-min value x 7.5 (the number of 8 min samples per hour).

Microcinematographic recordings of small groups of larvae in the 80-ml aquaria were reviewed for qualitative information of cannibalistic behavior. Slow motion replay options
enhanced analyses of individual cannibalistic attacks and allowed detailed descriptions of characteristics of predatory behavior. These observations were not included in the quantitative analyses.

*Population Dynamics.* Larval mortalities were determined from daily counts of dead and cannibalized larvae in debris siphoned from each aquaria. Both whole dead larvae and head capsules were recorded. Because the larvae were never seen to ingest an entire individual (i.e., the head was always emitted after ingestion of the rest of the body) cannibalistic remains were easily identified in the aquaria debris by counting head capsules.

To maintain distinction between mortalities identified as resulting from cannibalization, from unknown causes, or a combination of the two, the following terms are used: mortality = the number of larvae found in aquaria for which the cause of death could not be ascertained (i.e., an entire dead larvae); cannibalism = the number of larval remains (i.e., head capsules) found in the aquaria for which the cause of death was attributed to cannibalism; overall mortality = the sum of mortality and cannibalism represents the mortality from all causes combined. To maintain distinction between daily and total mortality and cannibalism, the following terms are used: daily cannibalism or daily mortality refers to the values obtained for each day of the study, whereas total cannibalism or total mortality refers to the amount of cannibalism or mortality accumulated throughout the study (i.e., the sum of daily values); likewise, total overall mortality refers to the accumulated sum of both mortality and cannibalism throughout the study.

Daily cannibalism was determined from the number of cannibalizations at the time of recording (i.e., fish with a fish in their mouth) plus the number of cannibalistic remains (head capsules) found in the aquaria on that day. In this way cannibalization in progress was considered as part of the cannibalism that had occurred since the previous day. The number
of cannibalizations in progress were subtracted from the next day's head capsule counts when calculating cannibalism to prevent double-counting a cannibalization.

Verification of the number of larvae stocked into each aquaria was obtained by adding the number of larvae remaining in each aquaria at the end of the study to the cumulative daily overall mortalities. Daily populations were determined by subtracting each daily overall mortality from the population from the succeeding day.

The percent total mortality and percent total cannibalism for each aquaria were calculated by dividing the total number of mortalities and the total number of cannibalizations accumulated at the end of each study by the number of larvae stocked into each aquaria. Daily cannibalism and daily mortality were also calculated by dividing the number of dead larvae and the number of cannibalisms found each day by the number of larvae present in the aquaria at the start of that day (%/d).

**Experimental Design.**—The cannibalism and mortality data were analyzed to provide mean % total mortality and mean % total cannibalization (± standard error) for each year of the study (i.e., at 17.5°C and 20°C). Individual aquaria were the experimental units (i.e., n=4 at 17.5°C and n=6 at 20°C). A t-test was performed to determine if there were significant differences in mortality or cannibalism between the two temperatures. The level of significance was P ≤ 0.05.

The %/d mortality, %/d cannibalism, and %/hr cannibalistic attacks were graphed to show the temporal relationship of these variables at each temperature. Regression analyses of %/d mortality with both %/d cannibalism and %/hr cannibalistic attacks were performed to show the relationships between these variables. Correlation coefficients for each of these combinations of variables were calculated to show the strength of the relationships.
Results

*Mortality, cannibalism, and attack rates.*— There was no difference in total mortality or total cannibalism between larvae reared at 17.5°C and 20°C during the two years of this study ($P = 0.3388$ and $0.2741$) (Table 1). Total overall mortality was 76.7 - 77.9%. Cannibalism accounted for 25.9 - 27.1% of the total overall mortality. The incidence of cannibalistic attacks, cannibalism, and mortality occurred simultaneously during a 8-10 day period of larval development (Figure 1). For larvae reared at 17.5°C, the onset of these variables occurred a few days later, and for a longer period of time, than for larvae reared at 20°C. The incidence and intensity of mortality, cannibalism, and attack rates followed a similar temporal pattern (Figure 2). The correlations between mortality and cannibalism were strong ($r = 0.97$, $P < 0.001$, at 17.5°C; and $r = 0.82$, $P < 0.0003$, at 20°C) (Figure 3). The correlations of mortality with attack rate, although not as strong as with cannibalism, were highly significant ($r = 0.66$, $P < 0.008$, at 17.5°C; and $r = 0.83$, $P < 0.0002$, at 20°C).

Table 1. Mean total mortality and cannibalism ($\pm$ standard error), total overall mortality (total mortality + total cannibalism), and percent of mortality due to cannibalism (cannibalism + overall mortality x 100) for walleye larvae reared at 17.5°C and 20°C in 3-L aquaria, where $n$ = number of aquaria and $N$ = total number of larvae stocked at each temperature with $P$-values for significance of difference between temperatures.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>n</th>
<th>N</th>
<th>Mortality (% of N)</th>
<th>Cannibalization (% of N)</th>
<th>Overall mortality (% of N)</th>
<th>Mortality due to cannibalism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.5°</td>
<td>4</td>
<td>641</td>
<td>56.8 ± 1.47</td>
<td>21.1 ± 1.73</td>
<td>77.9</td>
<td>27.1</td>
</tr>
<tr>
<td>20.0°</td>
<td>6</td>
<td>545</td>
<td>59.8 ± 2.14</td>
<td>16.9 ± 2.62</td>
<td>76.7</td>
<td>25.9</td>
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<tr>
<td>$P$-value</td>
<td></td>
<td></td>
<td>0.3388</td>
<td>0.2741</td>
<td></td>
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</tr>
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</table>
Figure 1. Mean daily mortality (%/d), daily cannibalism (%/d), and attack rates (%/h) for walleye larvae reared in 3-L aquaria at 17.5°C and 20°C. Mortality and cannibalism values are the percent of larvae in the population that died or were cannibalized on given days (e.g., 34% of population died on 9-d in 20°C study); attack rates are estimates of the percent of population seized in a cannibalistic attacks per hour from analyses of 5-8 minute per day cinematography of each aquaria.
Figure 2. Linear regression with 95% confidence bands of mortality in relation to cannibalism and attack rates for larval walleye reared at 17.5°C and 20.0°C, where %/d = the percentage of the population affected per day and %/hr = the percentage of the population affected per hour. The %/d mortality is for larvae found dead in aquaria (i.e., does not include larvae cannibalized).
Predatory Behavior. — Predatory behavior of larvae was similar whether the prey was non-living sinking feed particles, live Daphnia, or other larvae. Attack behavior consisted of a four-phase sequence: (1) fixation on the prey; (2) tracking (pursuit and closure to a strike distance of 5-10 mm); (3) assumption of an 'S'-shaped strike posture; and, (4) the strike (an open-mouthed burst at high speed). Fixation on the prey was noted when the attention of the larvae would focus (fix) on the prey, ignoring other potential prey even when alternative prey were closer to the predator. The S-shaped strike posture always occurred prior to the strike, regardless of the prey; often, a predator would assume the S-posture several times while readjusting the strike distance in preparation to attack a moving target. Tracking (pursuit and closure) distances were usually at least 2 cm, and often more than 10 cm. Larvae were observed pursuing other larvae for up to 20 cm in a semi-circle around the aquarium.

Most cannibalistic attacks were initiated with the predator tracking the prey from behind; however, the pectoral fin was most often the site of the strike, accounting for 79.6% of the total number of seizures observed (Table 2). Except for a 2-min duration of the one opercle attack, pectoral attacks also had the longest average duration of 39 seconds compared to an average seizure duration of 34 seconds. The longest seizure duration observed of a pectoral fin was for 5 minutes. A recording was made of a pectoral seizure which resulted in the pectoral fin being completely torn from the prey's body about 5 sec after seizure.

Usually the attack was made on another larvae that was swimming normally in the aquaria, although occasionally the prey was motionless or against the aquaria edge (Figure 3). During the cinematography observations, two attacks resulting in successful cannibalization were witnessed (both caudal attacks). Successful cannibalization represented only 1.9% of the total seizures witnessed. Caudal seizures, 12% of the total, were always made from behind a moving prey (Figure 4), and, surprisingly, only 2 out of 13 caudal
seizures resulted in ingestion of the prey. The average duration of caudal seizures (except those resulting in ingestion) was only 9 seconds. Occasionally heads-on confrontations were observed (Figure 5). In these confrontations, it was often unclear which of the two larvae was going to attack — sometimes they would both assume an S-shaped posture, but only one would make the strike. Sometimes in heads-on confrontations, one larva would seem to be backing away, giving the impression that the prey anticipated an attack from the other larva. Only 6% of the total number of seizures observed resulted in seizure of the head (Table 2).

Table 2. Location and duration of cannibalistic seizures from observations of larval walleye reared in ten 3-L aquaria, 4 at 17.5°C and 6 at 20°C, from hatch to 300 TU posthatch. Number of larvae stocked = 641 at 17.5°C and 545 at 20°C. Data are based on observations from a total of 62 minutes per day of cinematography (32 min/d at 17.5°C and 30 min/d at 20°C) conducted from hatch to 300 TU (15-17 days posthatch).

<table>
<thead>
<tr>
<th>Location of Seizure</th>
<th>Number of Seizures</th>
<th>Percent of Total Seizures</th>
<th>Average Duration (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17.5° 20° both</td>
<td>17.5° 20° both</td>
<td></td>
</tr>
<tr>
<td>Pectoral fin</td>
<td>37 49 86</td>
<td>69 89 79</td>
<td>34 42 39</td>
</tr>
<tr>
<td>Caudal fin*</td>
<td>12 1 13</td>
<td>22 2 12</td>
<td>9 NA 9</td>
</tr>
<tr>
<td>Head</td>
<td>3 3 6</td>
<td>6 5 6</td>
<td>28 38 33</td>
</tr>
<tr>
<td>Trunk</td>
<td>1 2 3</td>
<td>2 4 3</td>
<td>5 105 72</td>
</tr>
<tr>
<td>Opercle</td>
<td>1 0 1</td>
<td>2 0 1</td>
<td>120 NA 20</td>
</tr>
<tr>
<td>Total</td>
<td>54 55 109</td>
<td>27 40 34</td>
<td></td>
</tr>
</tbody>
</table>

* Two caudal seizures resulted in ingestion (successful cannibalization): these seizures are not included in the duration calculations but are included in the number of seizures recorded.
Figure 3. Side view of a pectoral fin attack by a 12-d walleye larva in an 80-ml aquarium: (A) the predator has approached to within striking distance of the prey, which is in a motionless vertical position against the aquarium edge (arrow); (B) the open-mouthed high speed strike results in seizure of the pectoral fin (upper right); and, (C) 5-sec later, the prey has been pulled away from the tank edge firmly in the grip of the attacking larva (upper left).
Figure 4. Side view of a cannibalistic attack by a 10-d walleye larva observed in an 80-ml aquaria: (A) after tracking for about 4 cm, an 'S'-shaped strike posture is assumed; (B) an open-mouthed high speed strike (the blurry appearance of both the attacker and the prey larva is evidence that the prey is also moving at high speed in this frame, indicating an escape response); (C) the prey's caudal fin has been seized; and, (D) after about three seconds, during which the pair made a complete circle, the prey is on the verge of escape.
Figure 5. Dorsal view of a cannibalistic attack between d-14 posthatch walleye larvae in a 3-L aquaria. These video frames show aspects of a heads-on confrontation: (A) a larva has assumed the 'S'-shaped strike posture toward the head of the other larva; (B) an open-mouthed high speed strike directed at the head of the prey; and, (C) seizure of the prey's head.

Discussion

The observations described here indicate that cannibalistic behavior is a significant cause of mortality in larviculture of walleye. Verified cannibalism accounted for 26 - 27% of the total mortality we observed. Less than 2% of the cannibalistic seizures we observed, however, resulted in successful engulfment of the prey. Engulfment was witnessed from only caudal fin seizures, and only 2 out of the 13 caudal fin seizures observed resulted in successful engulfment of the prey. Most seizures were of the pectoral fin and lasted for an average duration of 39 seconds. Our microcinematography showed dying larvae that had damaged pectoral and caudal fins and one larva was observed to have a pectoral fin completely pulled from its body by another larva during a cannibalistic seizure. These
observations suggest that injuries due to seizures that do not result in cannibalism may be responsible for much of the mortality not directly attributed to cannibalism that occurs during the larval period. The parallel patterns of larval mortality, cannibalism, and attack incidence we observed in both years of the study (Figure 1) further strengthens the suggestion of a relationship between cannibalistic behavior and mortality.

The period of cannibalistic behavior seems to last for only about 10 days (between 5-d and 15-d posthatch) at 17.5°C to 20.0°C. Li and Mathias (1982) also observed a discrete period of cannibalization and mortality during larval walleye development (between 6-d and 16-d day posthatch at 19°C - 22°C rearing temperatures). They concluded that starvation seemed to be the major cause of the mortality, and that cannibalism was the larvae's response to near starvation. That conclusion apparently resulted from their data showing that mortalities not accounted for by cannibalization occurred at a rate of about 40%/d, whereas verified cannibalization accounted for only 5 -15%/d. More recently, Johnston and Mathias (1993) concluded that cannibalism was a major cause of mortality in pond-reared walleye larvae and they found no support for starvation as a source of mortality when zooplankton densities were ≥ 50/L.

Loadman et al. (1986) was the first to study cannibalistic attacks among walleye larvae that did not result in ingestion of the prey. They concluded that trunk attacks among larval walleye may be a greater source of mortality than the more commonly observed tail attacks (they categorized all seizures as tail or trunk). Our observations substantiate the findings of Loadman et al. (1986). In our study, attacks resulting in prey seizures, which averaged 34 seconds, occurred at a rate in excess of 10% of the population per hour for much of the 10-day period of high mortality. Loadman et al. (1986) found that non-engulfment attack seizures averaged 20 seconds at a rates exceeding 10%/hr. Loadman et al. (1986) did not used video recordings for their observations, thus they may have underestimated seizure
durations and the number of seizures of short duration because the exact moment of the attack would be difficult to determine, and multiple simultaneous attacks may have gone unnoticed. Loadman et al. (1986) calculated that, because of the relatively short duration for non-cannibalistic seizures (only 20 seconds), compared with the several hours required for an ingested larvae to be digested and the head emitted, that an observer would be 50 times more likely to observe a cannibalization in progress than seizures that resulted in the escape of the prey. This contrast would account for the lack of attention previously given to the potential significance of attack seizures other than cannibalizations to mortality of walleye in tank culture.

Cuff et al. (1979) suggested that larval walleye will prey preferentially on conspecifics even though in the natural environment they feed on zooplankton. McIntyre et al. (1987), found that juvenile and larval walleye in aquaria with no alternate prey consumed 41% of the other walleye present; when zooplankton were present, losses to cannibalism dropped to 28% (slightly more than the 17-21% overall cannibalization observed in our study); and, in the presence of fathead minnows (Pimephales promelas), cannibalism disappeared among juvenile walleye. These observations indicate a behavioral propensity for other fish as a prey item among juvenile walleyes. We observed well-fed larval walleye pursuing other larvae for considerable distances in the presence of Daphnia and prepared feed particles in the water column. This prey preference may be the result of a stronger visual impression made by another fish compared with that of Daphnia or microparticulate feed particles. Wootton (1990) stated that size, contrast and movement are in most situations the most important prey characteristic for a predatory fish hunting by sight and that reaction distances increase with an increase in visual contrast between the prey and the background. Other fish seem to provide a more attractive visual stimulus to walleye larvae than zooplankton, and perhaps fathead minnows are easier prey than other walleye juveniles.
Sibling larvae that are less developed may, likewise, be easier prey than healthy larvae. Krise and Meade (1986) and Summerfelt (1991) predicted that poor gas bladder inflation may contribute to cannibalism by providing more vulnerable prey for cannibalization by fish that had inflated their gas bladders. Our observations indicated that in many instances, walleye larvae are aware of, and do implement escape responses to attacking larvae. In the present study, a quick burst of speed by a prey exposed to a caudal attack allowed the prey to escape cannibalism although a caudal seizure lasting several seconds was made by the predator (Figure 4). Northern pike, Esox lucius, larvae have also been shown to exhibit an escape reaction from conspecifics that adopt the S-shaped attack posture (Giles et al. 1986). In contrast, the larvae that was attacked while motionless against the aquarium side, shown in figure 3, appeared to make no attempt to elude capture and became firmly grasped by the attacker. In this instance, the prey larvae was not actively swimming, it did not have an inflated gas bladder, and it had no feed in its gut; whereas the attacker had an inflated gas bladder, had feed in its gut, was larger, and was actively swimming prior to the attack. In this case, a pectoral fin seizure did not result in engulfment of the prey, but considerable injury may have been done to the prey.

Although the implications of cannibalism in aquaculture are negative, cannibalism is a feature not only of captive, but of wild populations (Doepke 1970), where it may be of adaptive significance both for its potential regulatory effects on population abundance and for its contribution to the food source in prey poor environments (Smith and Reay 1991). Polis (1981) suggested that in the short term where food is temporarily insufficient to support a larval population, cannibalism can ensure that at least some larvae would survive. This may be important to walleye larvae when there is a mismatch between the timing of first-feeding and the development of adequate zooplankton densities in lakes. Conspecifics represent a diet of high nutritional quality and cannibalism is often associated with high growth rates,
particularly during the larval period (Nickum 1978; Li and Mathias 1982). Rapid growth rates during the larval period may decrease susceptibility to predation and increase survival of sibling larvae. Cannibalism, or deaths from injuries from cannibalistic attacks, to larvae exhibiting retarded or relatively poor developmental performance may improve stocks of piscivorous fish by removing less fit individuals from the population.

Methods or strategies to suppress cannibalism in tank culture might be successful through mitigation of the apparent visual preference of larval walleye to other fish larvae even in the presence of prepared feed. A culture strategy using colored lighting or camouflage backgrounds might reduce the perceptibility of larvae, yet not effect, or perhaps even improve the visibility of prepared feed particles in a tank culture environment. Hecht and Pienaar (1993) found that turbidity reduced cannibalism in larviculture of African catfish, *Clarias gariepinus*, a species that thrives under turbid conditions in the wild. Bristow and Summerfelt (1995) indicated that cannibalism in walleye larviculture may be less in turbid water than in clear water but they found no difference in cannibalism between larvae reared in colored or uncolored water. Rieger (1995), however, found no significant difference in cannibalism between walleye larvae reared in clear compared to larvae reared in turbid water (27.7% compared to 21.1% respectively, *P* = 0.75). Rieger (1995), however, observed a difference in the timing of the incidence of cannibalism between clear and turbid water with cannibalism occurring sooner and for a shorter period of time in turbid water than in clear water.

Tank culture offers the advantages of greater control of the culture environment and the use of prepared feed to rear the larvae to a desired size before stocking in natural waters or for grow out as food fish. For practical reasons, however, larvae are stocked at high densities (30-60/L). Cannibalistic behavior, it would seem, is the major bottleneck to
successful tank culture at high densities. Research of methods to suppress cannibalistic behavior is desirable to further increase the advantages of tank culture of walleye larvae.

Acknowledgments

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References


CONCLUSIONS

Walleye larviculture has made substantial advances in the last decade, but there are still unresolved causes of mortality in tank culture. Gas bladder inflation (GBI), first feeding, and cannibalism have been considered the sources of mortality. No studies have, however, clearly shown any, or any combination, of these events as the single cause of high mortality. Historically, larviculture studies have focused on trying new techniques to improve GBI or first feeding. Investigations have focused on removal of surface oil to improve air access for GBI, and GBI has improved dramatically in recent years through the use of surface spray and turbidity. Various feeding strategy studies have been based on the presumption that poor first feeding was a significant contributor to mortality; solutions focus on overfeeding continuously to ensure availability of feed to the larvae. Cannibalism, although noted as a contributing problem, has not been well studied. Nearly all of these studies based their results and conclusions on population dynamics data — how many larvae live, inflate, and feed. No research had been conducted specifically to observe how the larvae accomplish critical events. No in-depth studies had been performed of larval walleye developmental behavior.

To my knowledge, this study was the first to use high resolution microcinematography to obtain qualitative and quantitative observations of larval fish behavior. This approach to larviculture study has provided new kinds of information of larval walleye development. My study has shown how the larva perform gas bladder inflation and first-feeding; it provides a better understanding of the nature and significance of cannibalistic behavior; and, it augments previous developmental studies with descriptions of the behavioral repertoire of saltatory development. In addition to fundamental knowledge, I have also shown the value of two overlooked culture strategies in improving gas bladder inflation and first feeding.
success: the use of turbidity to affect nonedge distribution, and the use of higher temperatures to enhance larval vigor during the critical gas bladder inflation and first feeding phase. Through behavioral study emphases, I not only demonstrated that these strategies work to improve GBI and first feeding, essentially eliminating them as factors of high mortality, but I also show how they facilitate these developmental events. The use of microcinematography provided the first quantitative evidence that cannibalistic behavior may have far greater impact on larval success than other critical events; and, in light of the GBI and first feeding successes of my studies on turbidity and temperature, that this behavior appears to be the only remaining bottleneck to intensive walleye larviculture. Further studies of techniques to mitigate cannibalistic behavior will guide improvement in intensive walleye larviculture.

In conclusion, the following culture recommendations for intensive larviculture are based on a synopsis of my overall study results:

(1) A recycle intensive culture system should be maintained with about 30 NTU of turbidity, induced with clay, to eliminate tank edge association; and,

(2) Temperature control should provide about 20°C water temperature by day 3 posthatch, and maintain this temperature until first feeding is apparent in most of, or all of, the population, which should occur by day 7.

Future research should focus on feeding and cannibalistic behavior and these studies should perhaps be studied together. Studies of cannibalistic behavior should not consider cannibalism alone as the problem for which mitigation is desired, but also consider mortalities due to attack injuries. Recommendations for future studies include the following:

(1) A study of feeding and lighting regime manipulations to determine if a strategy of short, lighted feeding periods throughout the day, rather than continuous feeding and
lighting, might provide suitable first feeding conditions, yet mitigate cannibalistic behavior by lessening the "free time" available to larvae for aggressive activities; and, (2) A study of temperature manipulations that, while providing high temperatures for vigorous GBI and first feeding activity, may use lower temperatures to reduce activity levels following adaptation to prepared feed and thus reduce cannibalistic behavior, or alternatively, increase temperatures above 20°C to accelerate development past the cannibalistic phase, then lower temperature to reduce incidence of disease potential.

It is noteworthy that my study results, and thus the recommendations above, are most likely applicable to other physoclistous, piscivorous, phototactic species such as striped bass, snook, dolphin, sea bream, or spotted trout.

The value of high resolution microcinematography to investigate fundamental biological phenomenon is obvious. The in situ and in vivo observations of the physostomous GBI mechanisms are examples. Digitized recordings with various play back and image analyses options provide demonstrative visual exhibits of surface tension penetration and gut bubble movement, mastication, and dissolution. The nature and significance of cannibalistic behavior would not have been possible without microcinemagraphic recordings. Never before have studies of larval behavior been able to replay an event, from beginning to end, in slow motion. High resolution microcinematography should enable future researcher to obtain new knowledge of behavior, physiology, or ecology of organisms previously studied primarily only by traditional population dynamics.