



Tennessee Valley Authority, Post Office Box 2000, Soddy Daisy, Tennessee 37384-2000

February 10, 2009

State of Tennessee
Department of Environment and Conservation
Chattanooga Environmental Field Office
Division of Water Pollution Control
State Office Building, Suite 550
540 McCallie Avenue
Chattanooga, Tennessee 37402-2013

Attention: Richard Urban, Ph.D., Environmental Field Office Manager

Dear Dr. Urban:

TENNESSEE VALLEY AUTHORITY (TVA) - SEQUOYAH NUCLEAR PLANT (SQN) --
NPDES PERMIT NO. TN0026450 - SHAD COMPLAINT RESPONSE

On Friday February 6, 2009 at 0905 Ann Hurt of SQN's Environmental Staff received an email from Leetha Abazid stating:

"I need a letter on TVA letterhead mailed to the Chattanooga Field Office stating what actually occurred, including dates, temperatures, number and species affected DO readings, and location. We had received a complaint from a resident of the county stating that many species of fish including threadfin shad were dead at the pond near the wing wall (intake bay) at SQNP. Please have this letter to TDEC by February 11, 2009."

This correspondence is in response to that email.

Sequoyah has not observed any dead fish in the Intake Forebay, notwithstanding the presence of shad impinged on the traveling screens. Monitoring in boats and visual inspections confirm this (see attached photos). During the period of December 1, 2008 and February 6, 2009 there were very cold water temperatures in the Tennessee River. The attached chart (Attachment 1) shows adequate dissolved oxygen, cold river temperatures, and high condenser cooling water (CCW) differential pressure (DP).

Occurrences of high CCW DP are directly related to drops in river temperature. Differential pressure monitoring is used to measure the buildup of waterborne material on the screens and is required to ensure safe, unobstructed operation of the CCW system. On these days, the cause of the high DP events was the buildup of lethargic shad on the intake screens. To reduce the differential pressure, the shad are removed from the intake screens by a CCW backwash system. The numbers of shad have varied episodically from dozens to thousands.

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MRA

The fish impinged on the CCW traveling screens were primarily threadfin shad. The lethargy of these fish is caused by cold-stress from river temperatures below 50°F. Threadfin shad are very sensitive to low water temperature and do not feed or move extensively when the temperature falls below 50°F. Due to this natural wintertime phenomenon, shad are unable to swim or maintain their position in the water column and cannot resist the flow of water in the withdrawal zone for the plant intake pumping station. Fost (2006) reported that threadfin shad began to exhibit reduced or impaired swimming performance at 7.5°C (45.5°F). The shad that are suffering from cold-stress are pulled against the CCW traveling screens, and subsequently removed by a CCW backwash system. Similar events with lethargic fish are occurring throughout the Valley due to arctic cold fronts that have cooled water temperatures in Tennessee's reservoirs. On 2/5/09, the 24 hour average water temperature at the bottom of the Sequoyah skimmer wall dropped below 42°F. Until water temperatures increase above 50°F shad will be susceptible to cold-stress.

Enclosed are the following two documents. The first is TVA Sequoyah Nuclear Plant NPDES Permit No. TN0026450 316(b) Monitoring Program Fish Impingement at Sequoyah Nuclear Plant During 2005 through 2007. This document was originally sent to Mr. Paul E. Davis, Director and the Chattanooga Environmental Assistance Center on 12/19/2007. The second, EPRI - The Role of Temperature and Nutritional Status in Impingement of Clupeid Fish Species, discusses the episodic impingement of large numbers of certain fish species, particularly in the winter, at many power plant cooling water intake structures.

If you have any questions or need additional information, please contact Ann Hurt at (423) 843-6714 or myself at (423) 843-6700.

Sincerely,



Stephanie A. Howard
Principal Environmental Engineer
Signatory Authority for
Timothy P. Cleary
Site Vice President
Sequoyah Nuclear Plant

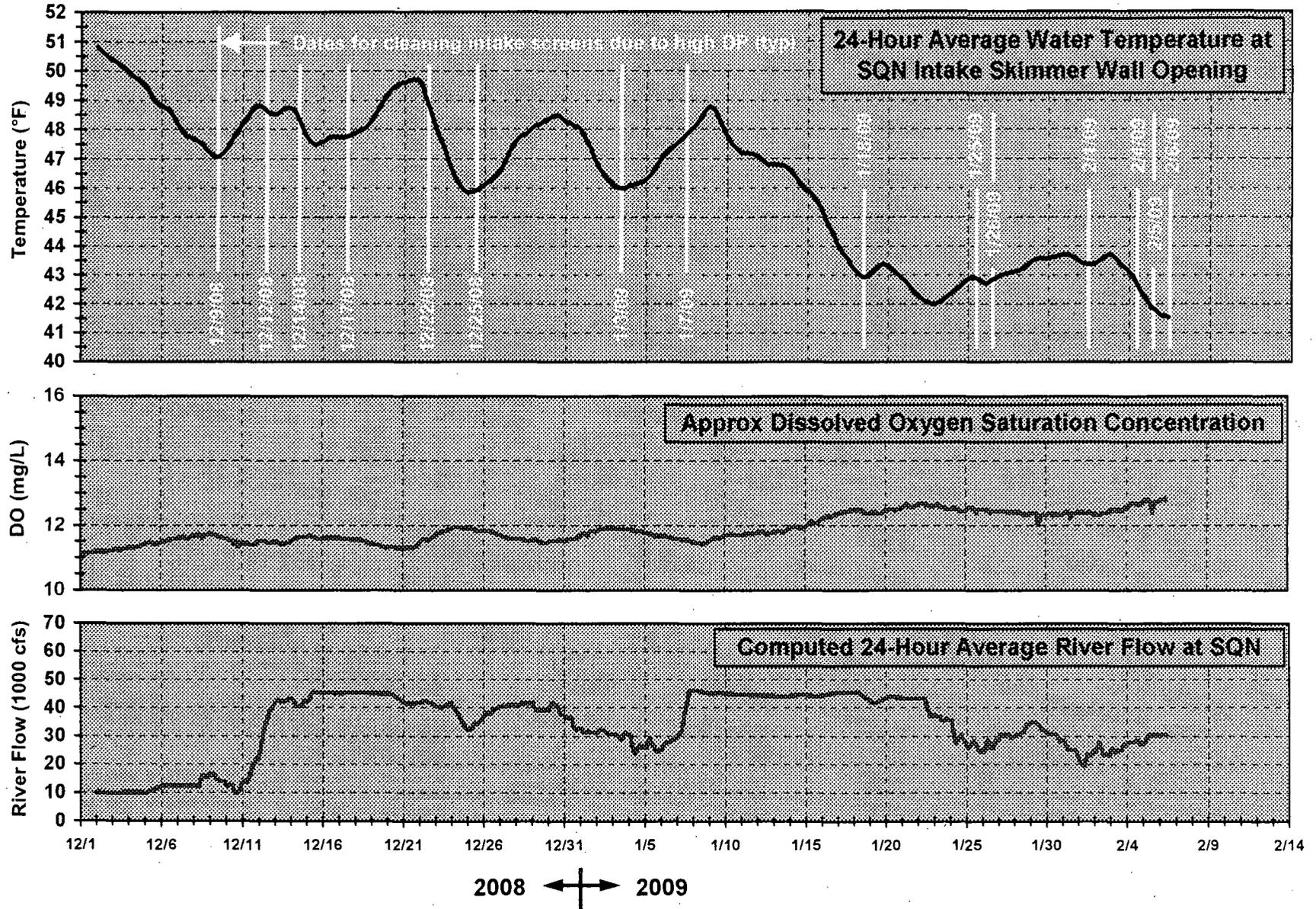
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cc (Enclosures):

U.S. Nuclear Regulatory Commission
ATTN: Document Control Desk
Washington, D.C. 20555

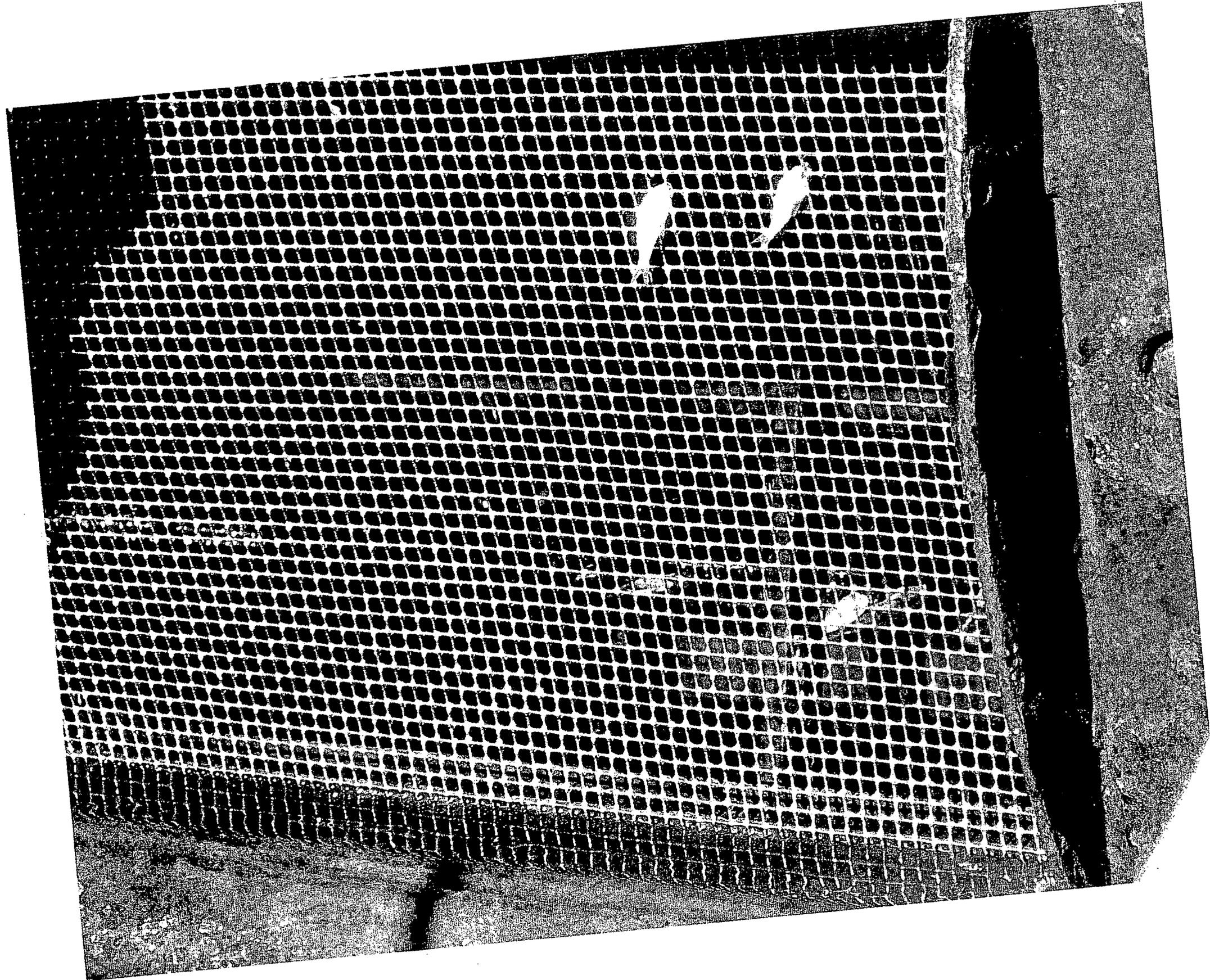
Fost, B. A. 2006. Physiological & Behavioral Indicators of Shad Susceptibility to Impingement at Water Intakes. M. S. Thesis, University of Tennessee, Knoxville. 45pp.

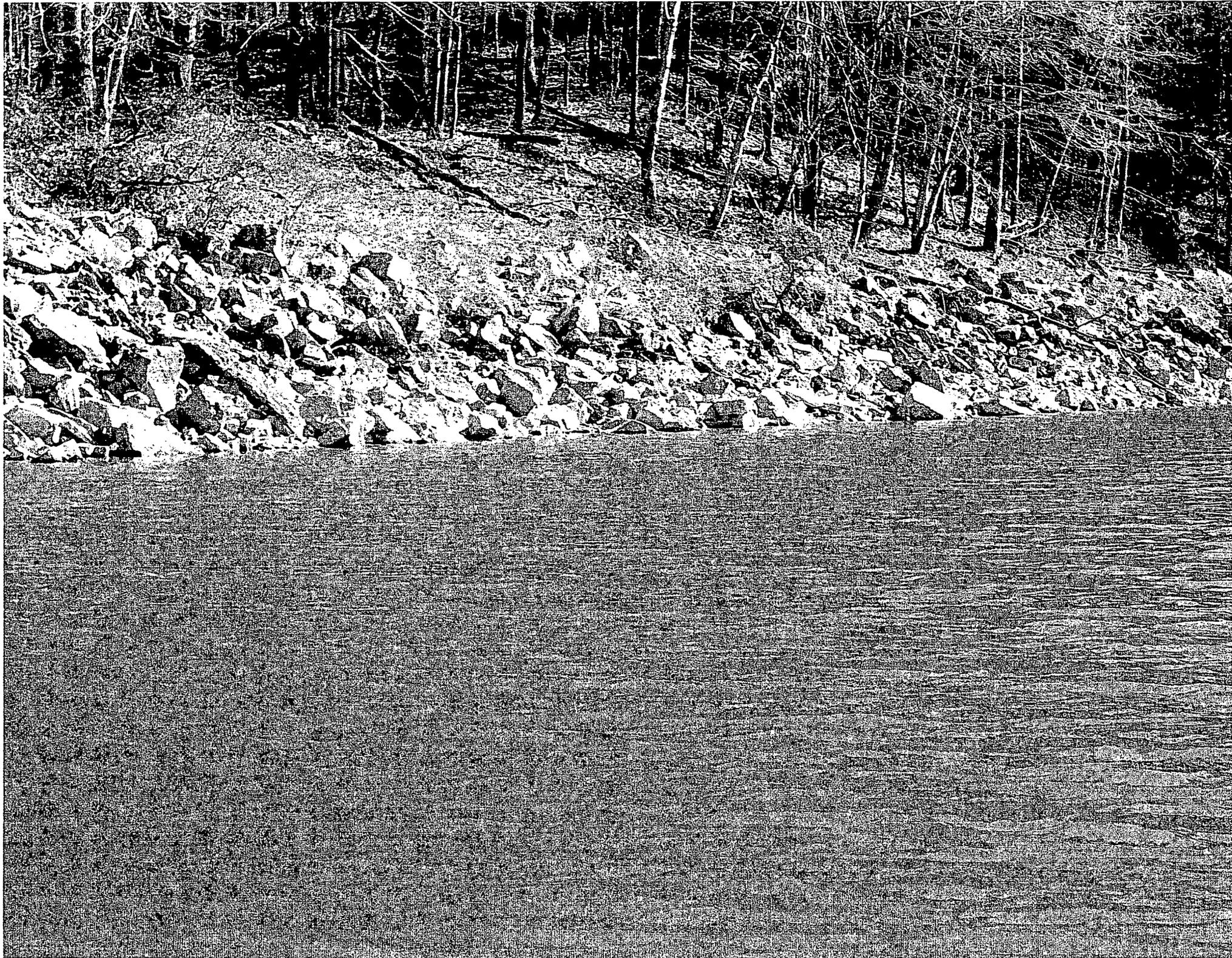
ATTACHMENT 1

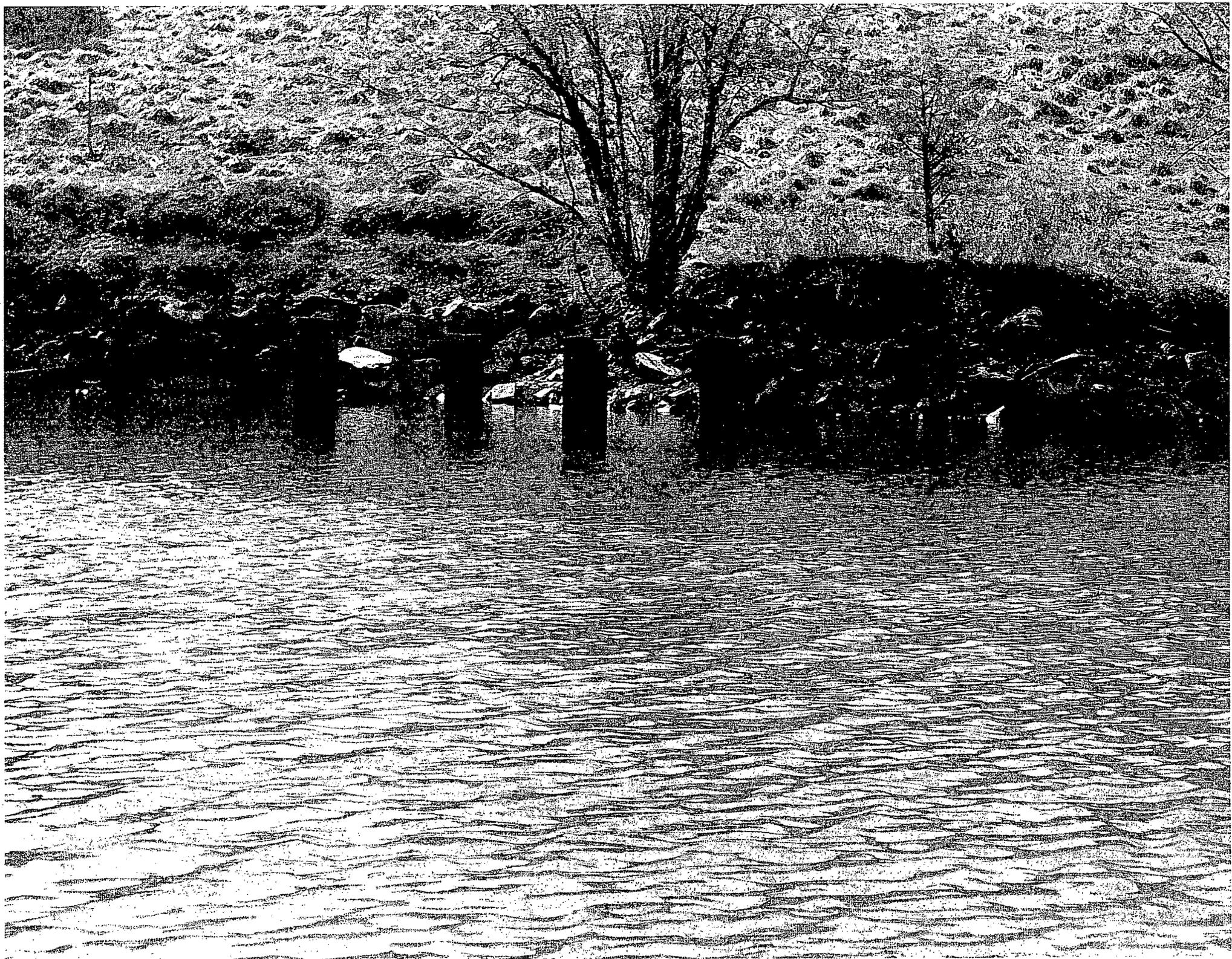
Water temperature, dissolved oxygen, river flow, and dates for cleaning intake screens due to high DP at Sequoyah Nuclear Plant from December 1, 2008 through February 6, 2009





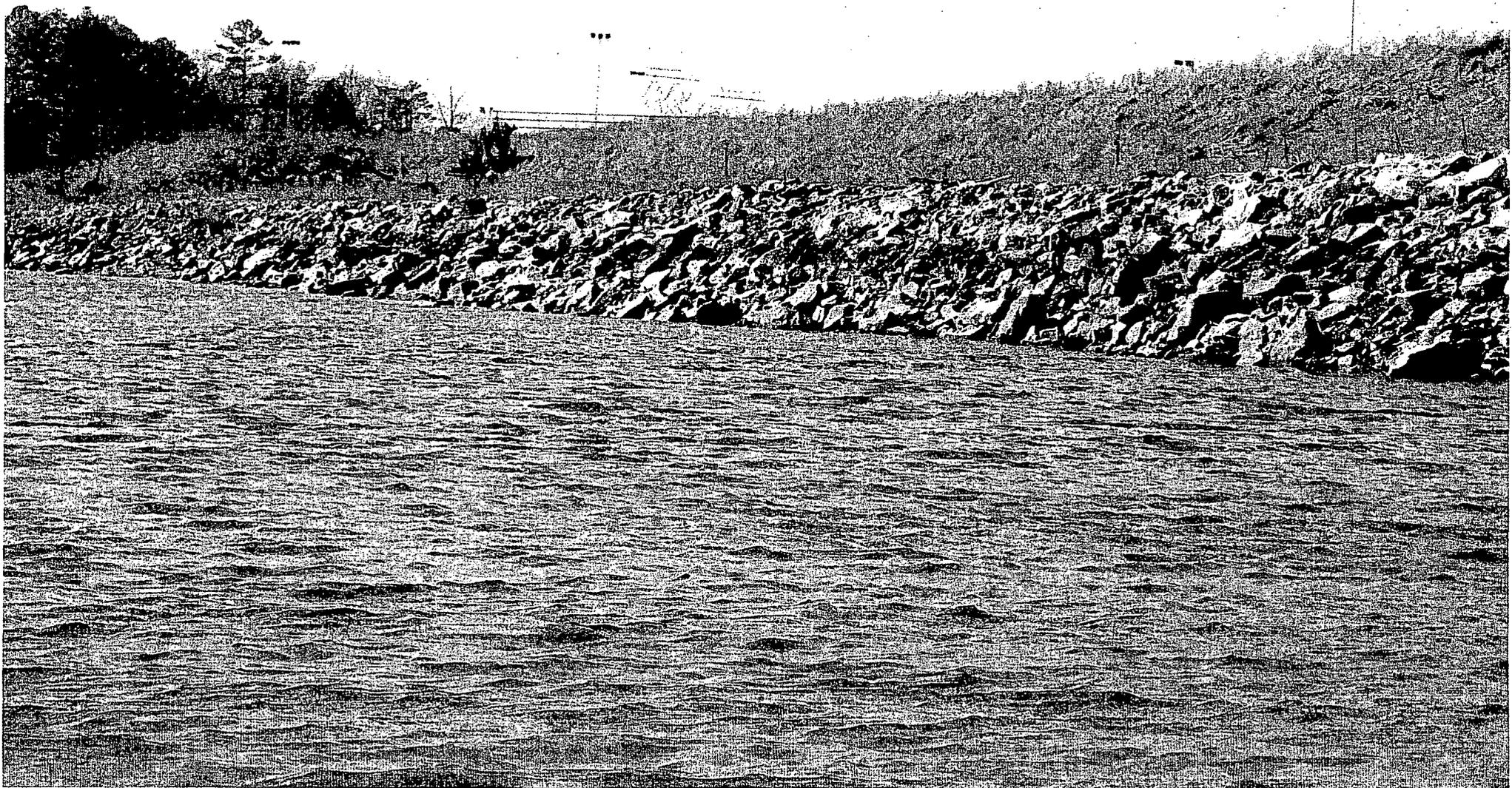














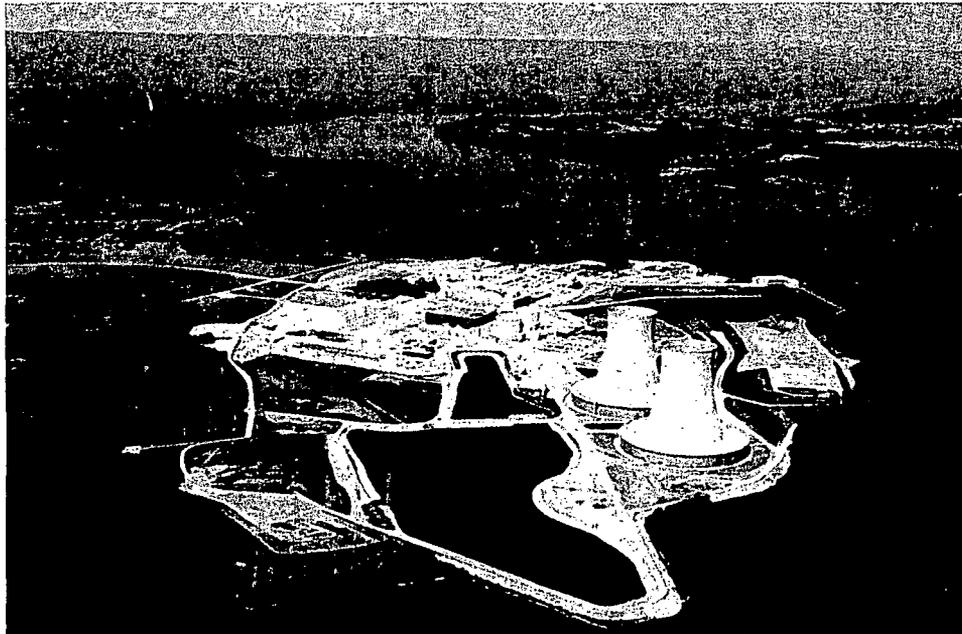




TENNESSEE VALLEY AUTHORITY

**SEQUOYAH NUCLEAR PLANT
NPDES PERMIT NO. TN0026450
316(b) MONITORING PROGRAM**

**FISH IMPINGEMENT AT
SEQUOYAH NUCLEAR PLANT
DURING 2005 THROUGH 2007**



ENVIRONMENTAL STEWARDSHIP AND POLICY

2007

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LIST OF ACRONYMS

AM&M	Aquatic Monitoring and Management
CCW	Condenser Cooling Water
CWA	Clean Water Act
EA	Equivalent Adult
EPA	Environmental Protection Agency
EPRI	Formerly known as the Electric Power Research Institute
MW	Megawatt
PIC	Proposal for Information Collection
PF	Production Foregone
RFAI	Reservoir Fish Assemblage Index
SQN	Sequoyah Nuclear Plant
TDEC	Tennessee Department of Environment and Conservation
TRM	Tennessee River Mile
TVA	Tennessee Valley Authority

Introduction

Sequoyah Nuclear Plant (SQN) withdraws condenser cooling water (CCW) from the Tennessee River and is subject to compliance with the Tennessee Water Quality Act and the federal Clean Water Act (CWA). Section 316(b) of the CWA requires the location, design, construction, and capacity of cooling water intake structures to reflect the best technology available for minimizing adverse environmental impact.

Impingement mortality is a potential mechanism for adverse impacts and is defined as the condition in which fish and/or shellfish are trapped or impinged against an intake screen and often killed in the process. In response to the Environmental Protection Agency (EPA) issuance of a 2004 rule for implementing Section 316(b), a rule subsequently suspended in 2007, and in accordance with a Proposal for Information Collection submitted to Tennessee Department of Environment and Conservation (TDEC) in 2005, Tennessee Valley Authority (TVA) conducted impingement monitoring at SQN to update the impingement database for potential intake effects. This report presents impingement mortality data collected from the CCW intake screens from January 2005 through January 2007 with comparisons to historical impingement data. Historical impingement mortality data from 1980-1985 assessed effects on the aquatic community of Chickamauga Reservoir for operational monitoring discharge permit requirements. An additional impingement study was conducted during December 2001 through February 2002, to compare peak numbers of fish impinged to historical impingement monitoring. No significant impacts were observed to the aquatic community in either of these studies and both datasets were similar in the numbers and species impinged.

Per an agreement reached in September 2001 with TDEC, Division of Water Pollution Control, TVA performs Reservoir Fish Assemblage Index (RFAI) (Hickman and Brown 2002) sampling annually to demonstrate that SQN operation is not impacting the balanced indigenous population in Chickamauga Reservoir. The primary reason for gathering these data is to support the continuation of a Section 316(a) thermal variance for SQN. However, the RFAI monitoring also gives an indication of the overall adverse environmental impact of plant operations to the reservoir fish assemblage and benthic community, including impacts from the plant's cooling water intake.

Plant Description

SQN is located on the west shore of Chickamauga Reservoir at Tennessee River Kilometer (TRK) 779.7 (TRM 484.5) (Figure 1). Construction began in 1970 and commercial operation for Unit 1 began in 1981 and Unit 2 in 1982. The two units (pressurized water reactors) have a total nameplate rating of 2,441 megawatts (MW). Natural draft cooling towers enable SQN to operate in an open or helper mode. In open mode operation, with both units at maximum power, total water demand is 72.45 m³/s (2,558 cfs). CCW is drawn from Chickamauga Reservoir into the intake channel through an opening approximately 165 m (541 ft) long and 3 m (9.8 ft) high near the bottom of a skimmer wall situated near the river channel. This allows SQN to withdraw cooler water from the lower portion of the water column. From the intake channel, water passes through six, 3 m wide traveling screens to the intake pumps. Mesh openings on screens are 0.95 cm² (3/8 in²). Both units were near full load during January 2005 through January 2007 (Figure 2). Average daily generation for the two combined was 2,373 MW; Unit 1 averaged 1,186 MW and Unit 2 averaged 1,187 MW. Six intake pumps were usually in operation, resulting in an average daily intake flow of 71.8 m³/s (2,536 cfs). Velocity at the traveling screens averaged 37 cm/sec (1.2 fps).

Methods

Impingement sampling began on January 25, 2005, and weekly samples were collected through January 15, 2007. To simplify comparisons in this report, data from January 25, 2005 through January 23, 2006 will be referred to as Year-One, and from January 30, 2006 through January 15, 2007, as Year-Two. To collect each sample, intake screens were rotated and washed on a prearranged schedule by the plant assistant unit operator to remove all fish and debris. After 24 hours, screens were again rotated and washed with Aquatic Monitoring and Management (AM&M) crew on site. Fish and debris were collected in a catch basket constructed of 9.5 mm (3/8 in) mesh located at the end of the sluice pipe where the monitoring crew removed and processed the sample. Fish were sorted from debris, identified, separated into 25 mm (1 in) length classes, enumerated, and weighed. Data were recorded by one member of the AM&M crew and checked and verified (signed) by the other for quality control. Quality Assurance/Quality Control procedures for impingement sampling (TVA 2004) were followed to ensure samples were comparable with historical impingement mortality data.

Moribund/Dead Fish

Fish collected from a 24-hour screen wash were usually all dead when processed. Incidental numbers of fish which appeared to have been dead for more than 24 hours (i.e., exhibiting pale gills, cloudy eyes, fungus, or partial decomposition) were not included in the sample. Also, during winter, threadfin shad occasionally suffer die-offs or stress from cold-shock and are impinged after death or in a moribund state (Griffith and Tomljanovich 1975, Griffith 1978). If these die-off incidents were observed, they were documented to specify that either all, or a portion of impinged threadfin shad collected during the sample period were impinged due to cold-shock and may not have been impinged otherwise. Any fish collected alive were returned to the reservoir after processing.

Data Analysis

Impingement data from weekly 24-hour impingement samples were extrapolated to provide estimates of total fish impinged by week and total for each year of study. In rare situations when less than a 24-hour sample was possible, data were normalized to 24 hours. Historical data collected during 1981-1984 were averaged over a 52-week period, while data collected during 1985 were from January through July only. During 2001-2002, impingement data were collected from December through February and therefore represent only the winter period.

To facilitate the implementation of and compliance with the EPA regulations for Section 316(b) of the CWA (Federal Register Vol. 69, No. 131; July 9, 2004), prior to its suspension by EPA, fish lost to impingement were evaluated by extrapolating the losses to equivalent reductions of adult fish, or of biomass production available to predators in the case of forage species. In conformance with methods utilized by EPA in its Technical Development Documents in support of the Phase II Rule (EPA 2004), EPRI (Formerly known as the Electric Power Research Institute) has identified two models for extrapolating losses of fish eggs, larvae, and juveniles at intake structures to numbers or production of older fish (Barnhouse 2004). The Equivalent Adult (EA) model quantifies entrainment and impingement losses in terms of the number of fish that would have survived to a given future age. The Production Foregone (PF) model applies to forage fish species to quantify the loss from entrainment and impingement in terms of potential forage available for consumption by predators. These models require site-specific data

on the distribution and abundance of fish populations vulnerable to entrainment and impingement. TVA also used these models to determine the "biological liability" of the CCW intake structure based on the EPA guidance developed under the suspended rule.

Results and Discussion

Impingement sampling at SQN from January 2005 to January 2007 resulted in collection of 2,889 fish (22 species) during Year-One and 5,766 fish (21 species) during Year-Two (Table 1). Threadfin shad were predominate (91%) in the samples, followed by bluegill (3%), freshwater drum (2%), and channel and blue catfish (1% each) (Table 2). All other species contributed less than 1% of the total number collected. Annual estimates of number impinged and corresponding biomass are compared by species and year in Table 2. Rate of impingement was highest during November and December during Year-One (2005-2006), while peak impingement occurred during August, October, and November during Year-Two (2006-2007) (Table 3, Figure 3). Estimated annual impingement was calculated by extrapolating impingement rates from weekly samples. An estimated 20,223 fish were impinged during Year-One and 40,362 during Year-Two; of these, the majority was threadfin shad (Table 2). Estimated impingement during Year-Two was more than double the impingement estimate during Year-One due to collection of greater than two times more threadfin shad during Year-Two.

With the exception of samples collected during 1980-1982, annual historical impingement estimates for SQN were similar to those calculated during this study (Table 4, Figure 4). Although estimated impingement was much higher from 1980-1982, threadfin shad accounted for the majority of fish impinged in these samples as well as in samples collected during 1983-1985. The 2001-2002 data represented samples collected only in the winter when peak numbers are typically impinged at SQN (Kay and Baxter 2002). Impingement estimates for all species, except threadfin shad, were low and consistent with the 1980-1985 historical data and with data collected during the current study. Threadfin shad was the dominant species collected during 2001-2002, comprising 97% of the total number collected and 74% of the total weight (Table 5). Gizzard shad, freshwater drum, and sunfish comprised a notable proportion of historic impingement samples following threadfin shad (Tables 5 and 6). This was similar to the dominant species collected during this study.

Threadfin and/or gizzard shad typically comprise over 90% of fish impinged on cooling-water intake screens of thermal power stations in the Southeastern U. S. (EPRI 2005). They also comprise an average of 35%-56% of total fish biomass where they occur (Jenkins 1967). Threadfin shad have a high fecundity rate, move in large schools, and are intolerant to cold temperatures, often resulting in high mortality rates in winter. These traits are probably major contributing factors to the annual and seasonal fluctuation in numbers of fish impinged at SQN. A recent study by Fost (2006) indicated that cold-stressed threadfin and gizzard shad can be classified as either impaired or moribund. Impaired shad could recover if environmental conditions improved and would therefore not die if not impinged. Moribund fish on the other hand, are assumed to not be able to recover and die regardless of impingement. Fost's data indicated that threadfin shad began to exhibit reduced or impaired swimming performance at 7.5°C (45.5°F).

Plotted weekly ambient water temperatures for SQN (Figure 5) appear to be negatively correlated with peak shad impingement as previously reported by numerous studies

(EPRI 2005, Griffith and Tomljanovich 1975, Griffith 1978; McLean et al., 1980). No die-offs of threadfin shad were observed at SQN during the two years of monitoring by AMM crews or were reported by power plant personnel.

Application of the EA and PF models to the total numbers estimated impinged resulted in reduced numbers of fish which would have been expected to survive to either harvestable (EA) size/age or to provide forage (PF) (Table 4). This reduced number is considered the "biological liability" resulting from plant CCW impingement mortality based on the guidance developed for the now suspended 316(b) regulations. The numbers of fish representing SQN's biological liability for Year-One and Year-Two were 1,868 and 821, respectively.

As part of TVA's Vital Signs Monitoring Program resident fish communities were sampled in Chickamauga Reservoir upstream TRK 789.4 (TRM 490.5) and downstream TRK 775.7 (TRM 482.0) of SQN since 1999 (Baxter and Simmons 2007). Resulting data were analyzed using a multi-metric RFAI to rate the overall health and condition of the fish community at these sampling locations. Fish communities at both sites upstream and downstream from SQN have averaged a rating of "Good" during 1999-2006, indicating that SQN is not adversely impacting the resident fish community (Baxter and Simmons 2007).

Summary and Conclusions

Fish impingement rates at SQN during 2005-2007 were much lower than during 1980-1981, but were similar to historical data collected from 1982-1985. Threadfin shad has been the dominant species impinged during all years sampled and comprised 91% of fish impinged during this study. Biological liability after EA and PF reduction was low. Low impingement rates at SQN and "Good" RFAI scores for sites just upstream and downstream of SQN indicated that the SQN CCW intake is not adversely impacting the Chickamauga Reservoir fish community.

References

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Table 1. List of Fish Species by Family, Scientific, and Common Name Including Numbers Collected in Impingement Samples During 2005-2007 at TVA's Sequoyah Nuclear Plant.

Family	Scientific Name	Common Name	Total Number Impinged	
			Year-One	Year-Two
Clupeidae	<i>Alosa pseudoharengus</i>	Alewife	10	4
	<i>Dorosoma cepedianum</i>	Gizzard shad	17	25
	<i>Alosa chrysochloris</i>	Skipjack herring	10	10
	<i>Dorosoma petenense</i>	Threadfin shad	2,529	5,373
Cyprinidae	<i>Pimephales notatus</i>	Bluntnose minnow	0	2
	<i>Pimephales vigilax</i>	Bullhead minnow	1	3
	<i>Moxostoma spp.</i>	Unidentified redhorse	0	1
	<i>Notropis atherinoides</i>	Emerald shiner	1	0
Ictaluridae	<i>Ictalurus furcatus</i>	Blue catfish	25	40
	<i>Ictalurus punctatus</i>	Channel catfish	50	32
	<i>Pylodictis olivaris</i>	Flathead catfish	3	11
	<i>Ameiurus natalis</i>	Yellow bullhead	1	0
Atherinidae	<i>Labidesthes spp.</i>	Unidentified silverside	0	1
Moronidae	<i>Morone saxatilis</i>	Striped bass	4	0
	<i>Morone chrysops</i>	White bass	2	4
	<i>Morone mississippiensis</i>	Yellow bass	24	10
Centrarchidae	<i>Lepomis spp.</i>	Unidentified sunfish	0	1
	<i>Lepomis macrochirus</i>	Bluegill	122	120
	<i>Lepomis auritus</i>	Redbreast sunfish	2	1
	<i>Lepomis microlophus</i>	Redear sunfish	1	0
	<i>Micropterus salmoides</i>	Largemouth bass	5	5
	<i>Micropterus punctulatus</i>	Spotted bass	1	13
	<i>Pomoxis nigromaculatus</i>	Black crappie	0	47
	<i>Pomoxis annularis</i>	White crappie	3	3
Poeciliidae	<i>Gambusia affinis</i>	Western mosquitofish	1	0
Percidae	<i>Sander canadense</i>	Sauger	1	0
Sciaenidae	<i>Aplodinotus grunniens</i>	Freshwater drum	76	60
Total Number of Fish			2,889	5,766
Total Number of Species			22	21

Table 2. Estimated Annual Numbers, Biomass, and Percent Composition of Fish Impinged by Species at Sequoyah Nuclear Plant During 2005-2007.

Species	Estimated Number			Estimated Biomass (g)			Percent Composition by Number
	Year-One	Year-Two	Average	Year-One	Year-Two	Average	
Threadfin shad	17,703	37,611	27,657	59,612	70,539	65,076	91
Bluegill	854	840	847	6,636	5,054	5,845	3
Freshwater drum	532	420	476	63,686	28,385	46,036	2
Channel catfish	350	224	287	78,309	25,683	51,996	1
Blue catfish	175	280	228	67,998	70,021	69,010	1
Black crappie	0	329	165	0	385	193	1
Gizzard shad	119	175	147	6,902	2,506	4,704	T
Yellow bass	168	70	119	6,545	2,779	4,662	T
Skipjack herring	70	70	70	9,982	14,770	12,376	T
Alewife	70	28	49	560	791	676	T
Flathead catfish	21	77	49	6,391	67,326	36,859	T
Spotted bass	7	91	49	700	217	459	T
Largemouth bass	35	35	35	231	91	161	T
White bass	14	28	21	3,857	5,117	4,487	T
White crappie	21	21	21	91	42	67	T
Bullhead minnow	7	21	14	35	49	42	T
Striped bass	28	0	14	140	0	70	T
Redbreast sunfish	14	7	11	2,065	987	1,526	T
Bluntnose minnow	0	14	7	0	14	7	T
Unidentified redhorse	0	7	4	0	3,605	1,803	T
Emerald shiner	7	0	4	7	0	4	T
Yellow bullhead	7	0	4	35	0	18	T
Unidentified silverside	0	7	4	0	21	11	T
Redear sunfish	7	0	4	70	0	35	T
Unidentified sunfish	0	7	4	0	28	14	T
Western mosquitofish	7	0	4	7	0	4	T
Sauger	7	0	4	3,010	0	1,505	T
TOTAL	20,223	40,362	30,293	316,869	298,410	307,640	

Table 3. Numbers of Fish Impinged at Sequoyah Nuclear Plant by Month and Percent of Annual Total During Year-One, Year-Two, and for Both Years Combined.

Month	Total Number of Fish Impinged Year-One	Percent of Annual Total	Total Number of Fish Impinged Year-Two	Percent of Annual Total	Years One and Two Combined	Percent of Two-Year Total
Jan	295	10	570	10	865	10
Feb	9	0	179	3	188	2
Mar	46	2	86	1	132	2
Apr	68	2	30	1	98	1
May	4	0	13	0	17	0
Jun	5	0	5	0	10	0
Jul	41	1	173	3	214	2
Aug	62	2	751	13	813	9
Sep	193	7	242	4	435	5
Oct	262	9	1,515	26	1,777	21
Nov	358	12	1920	33	2,278	26
Dec	1,546	54	282	5	1,828	21
Total	2,889		5,766		8,655	

Table 4. Total Numbers of Fish Estimated Impinged by Year at Sequoyah Nuclear Plant and Numbers Following Application of Equivalent Adult and Production Foregone Models During 2005-2007.

	1980-1981	1981-1982	1982-1983	1983-1984	1984-1985	2005-2006	2006-2007
Extrapolated Annual Number Impinged	94,528	81,158	20,685	41,076	27,195	20,223	40,362
Number after EA and PF Reduction	4,851	5,843	2,256	4,162	2,761	1,868	821

Table 5. Percent Composition (By Number and Weight and After EA and PF Models Applied) of Major Species of Fish Impinged at Sequoyah Nuclear Plant Between December 18, 2001 and February 25, 2002.

Species Composition	Percent by Number	Percent by Weight
Threadfin shad	96.98	74.09
Bluegill	0.80	0.64
Freshwater drum	0.77	14.68
Gizzard shad	0.43	1.33
Alewife	0.23	0.82
Channel catfish	0.28	1.33
Striped bass	0.24	0.46
Mosquitofish	0.13	0.01
Logperch	0.03	0.08
Fathead catfish	0.02	4.68
Bluntnose minnow	0.02	0.03
Redear sunfish	0.02	0.02
Redbreast sunfish	0.01	0.73
Largemouth bass	0.01	0.27
White crappie	0.01	0.83

Table 6. Percent Composition (By Number and After EA and PF Models Applied) of Major Species of Fish Impinged at TVA's Sequoyah Nuclear Plant During 1980-1985 and 2005-2007.

Species Composition	1980-1981		1981-1982		1982-1983		1983-1984		1984-1985		2005-2006		2006-2007	
	% by Number	% after PA and EF	% by Number	% after PA and EF	% by Number	% after PA and EF	% by Number	% after PA and EF	% by Number	% after PA and EF	% by Number	% after PA and EF	% by Number	% after PA and EF
Threadfin shad	83	63	72	46	49	25	70	44	65	42	87	59	93	77
Lepomis	8	16	4	7	8	12	9	14	6	12	4	9	2	5
Gizzard shad	4	3	9	6	22	11	2	1	8	5	1	0	0	0
Skipjack herring	0	0	0	0	1	1	3	2	4	3	0	0	0	0
Ictalurids	0	0	0	0	2	7	1	5	1	4	3	15	2	10
Freshwater drum	2	3	8	14	12	19	9	15	6	9	3	6	1	2
Spotted bass	0	0	1	2	0	0	0	0	0	0	0	0	-	1
White crappie	-	3	0	0	1	2	0	0	0	0	0	0	1	2
Yellow perch	-	3	-	6	1	6	-	4	-	3	0	0	0	0
Yellow/White bass	-	3	3	11	2	6	-	4	-	3	1	6	-	2
Bullhead minnow	0	0	0	0	0	0	0	0	2	1	0	0	0	0
Total	97	94	97	92	98	91	95	94	93	96	99	95	99	99

Dash denotes not a major species during that year.

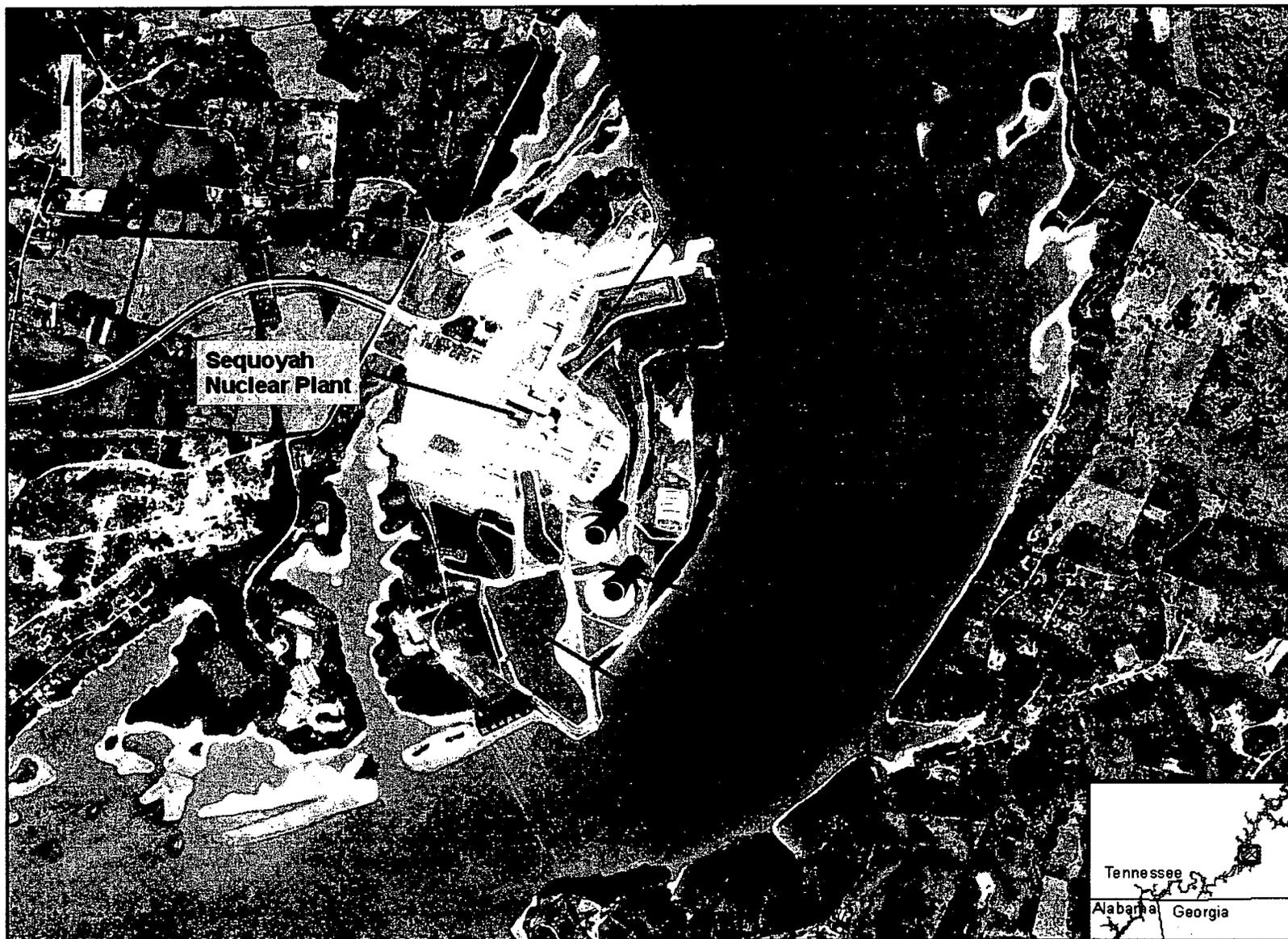


Figure 1. Aerial photograph of Sequoyah Nuclear Plant including CCW intake structure, skimmer wall, intake basin, and diffuser cooling pond.

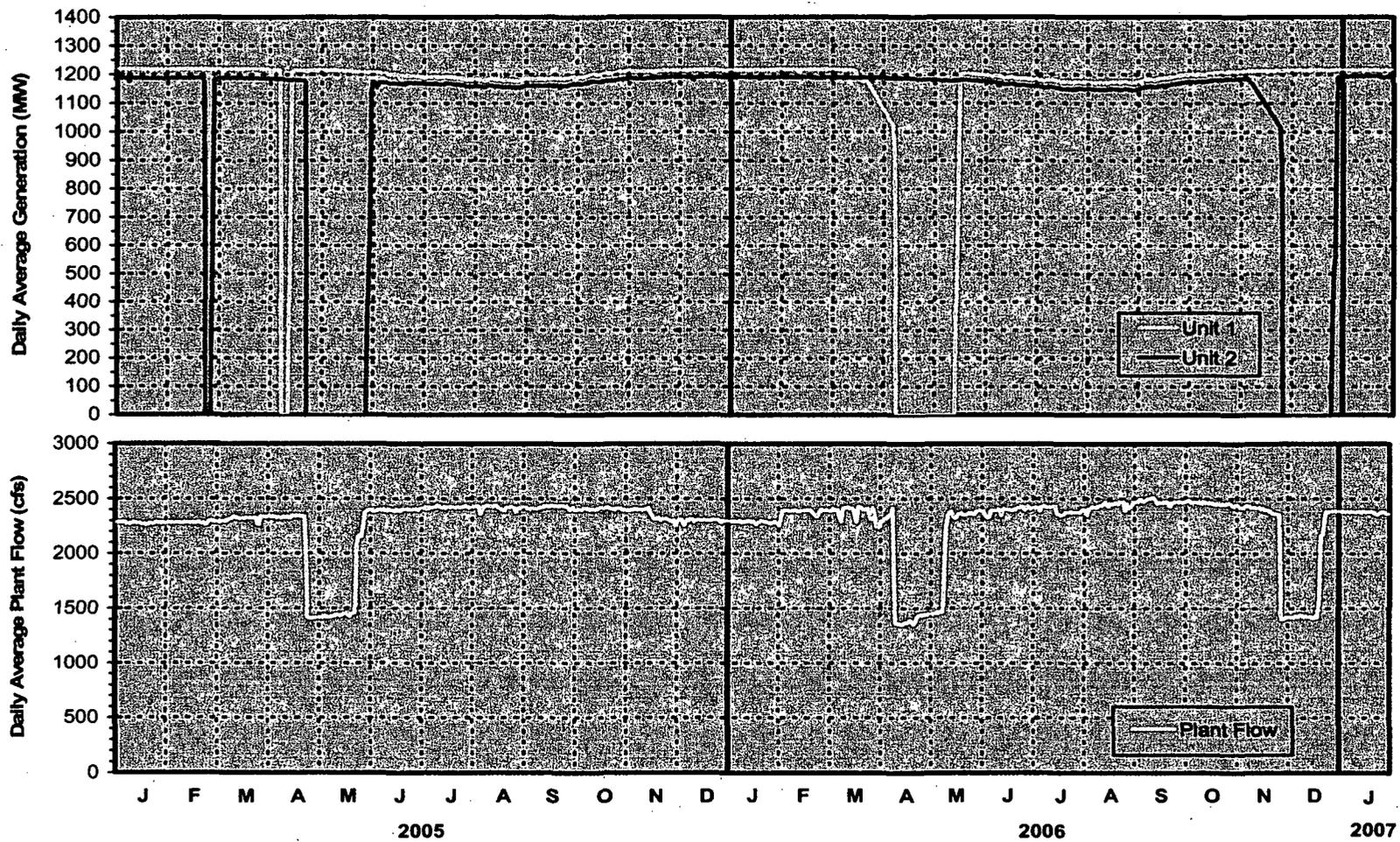


Figure 2. Average daily generation (MW) and intake flow (cfs) at Sequoyah Nuclear Plant during January 2005 through January 2007.

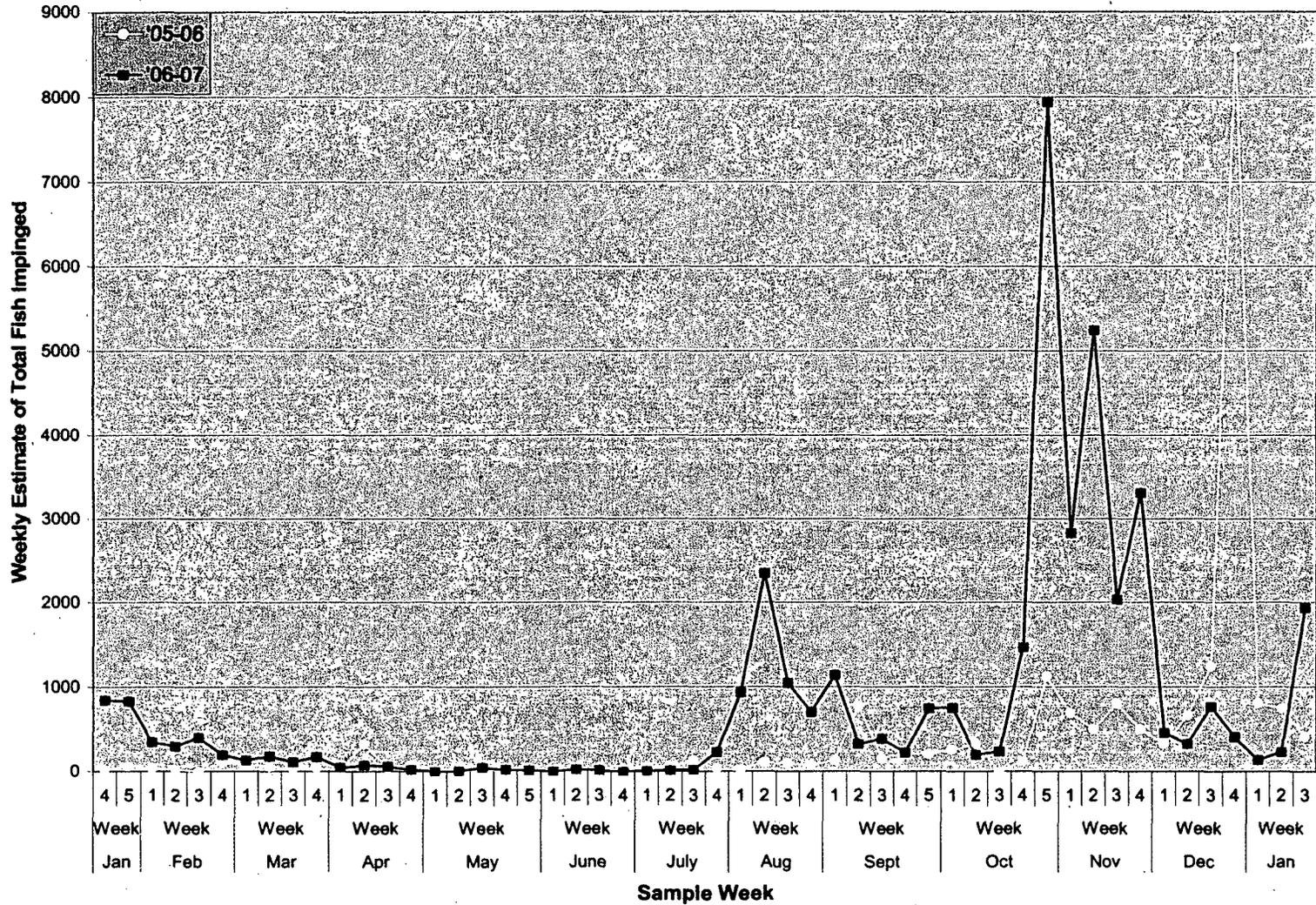


Figure 3. Estimated weekly fish impingement at Sequoyah Nuclear Plant during 2005-2007.

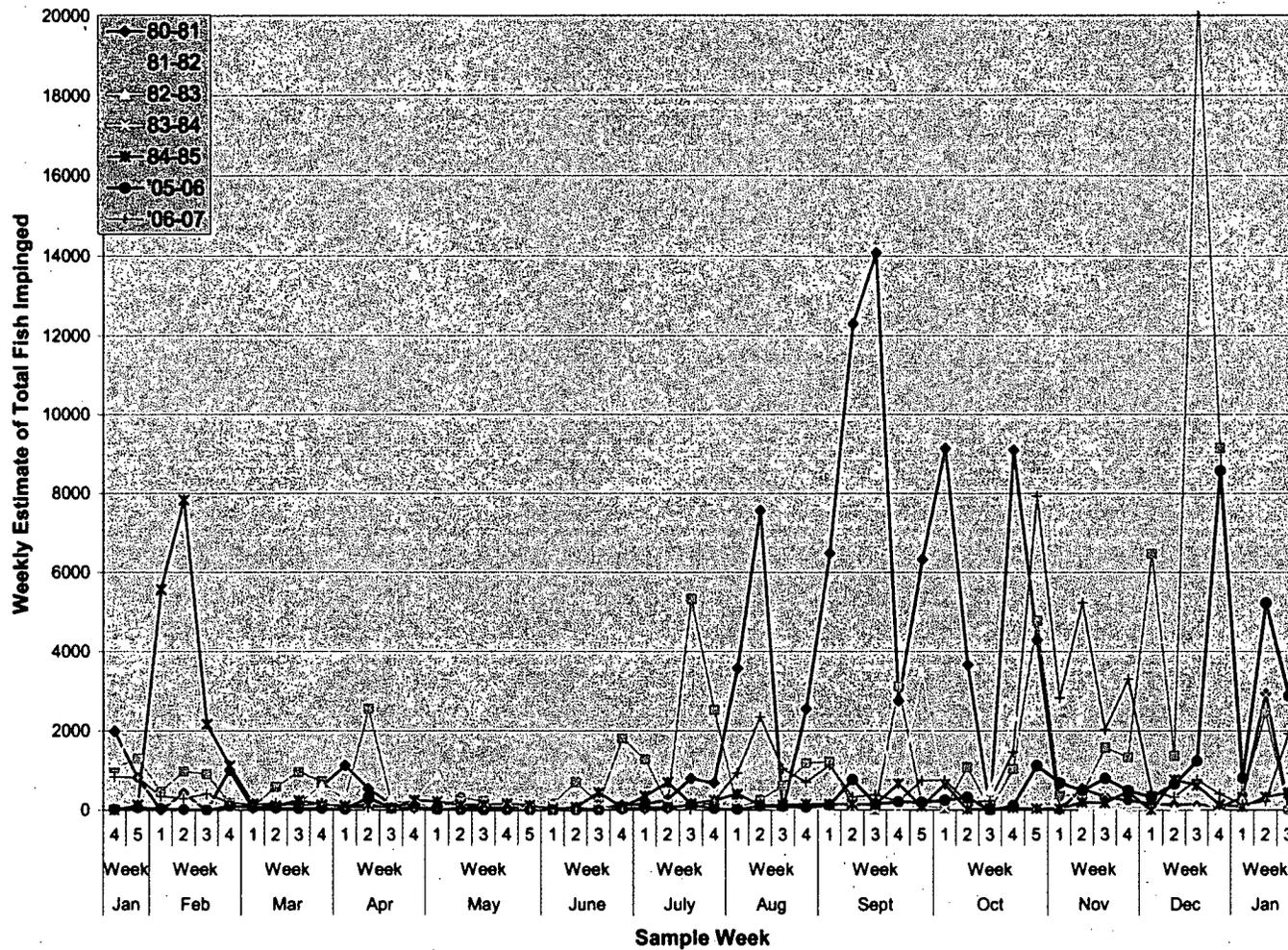


Figure 4. Comparison of estimated weekly fish impingement at Sequoyah Nuclear Plant during historical and recent monitoring periods.

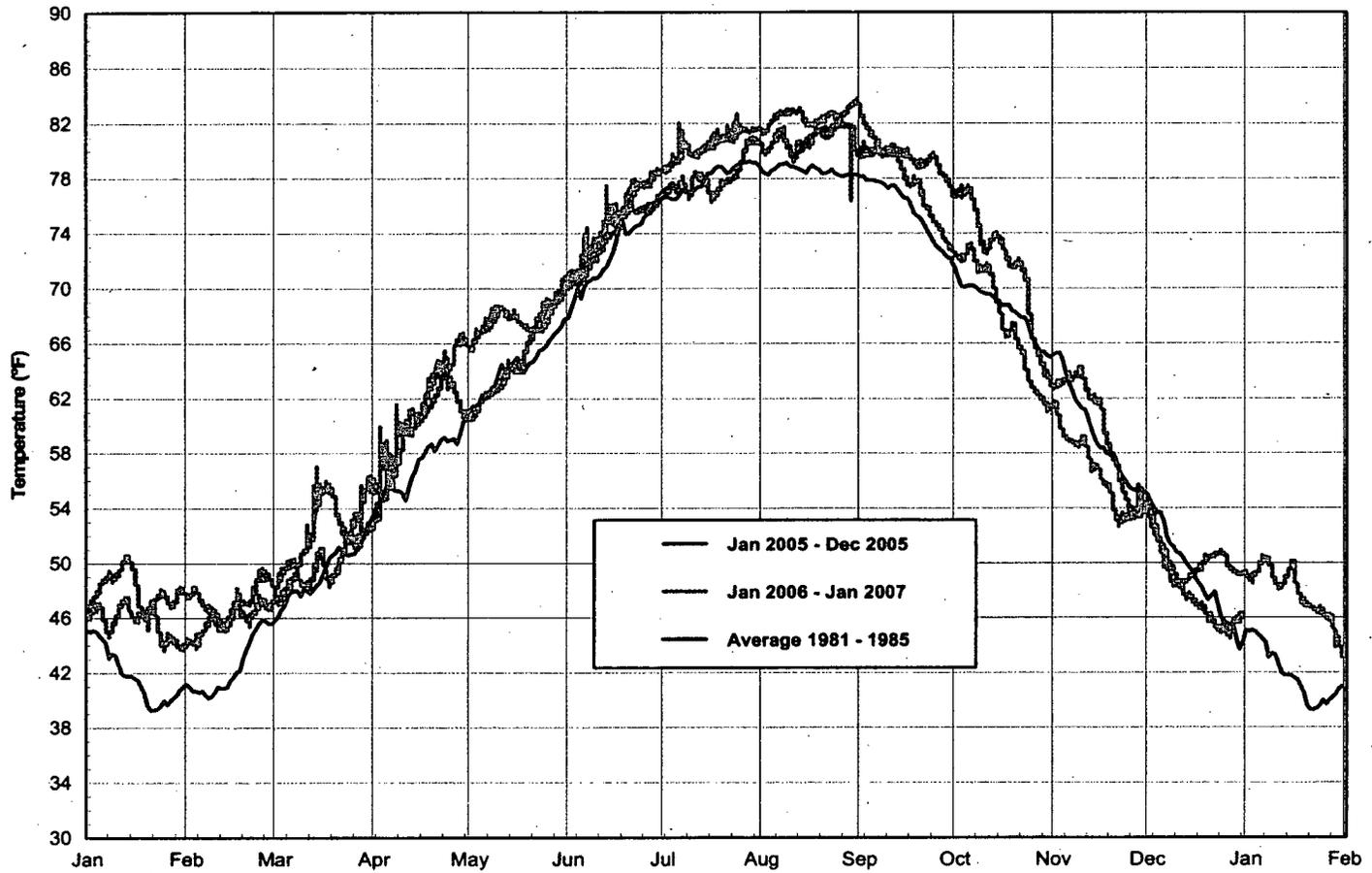


Figure 5. Ambient daily (24-hr avg) water temperature at Sequoyah Nuclear Plant intake during historical (1981-1985) and recent (2005-2007) impingement monitoring.

The Role of Temperature and Nutritional Status in Impingement of Clupeid Fish Species



The Role of Temperature and Nutritional Status in Impingement of Clupeid Fish Species

1014020

Final Report, March 2008

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PRODUCT DESCRIPTION

Episodic impingement of high numbers of juvenile and adult clupeid fish species such as gizzard and threadfin shad, menhaden, and herring is a common occurrence, particularly during winter at many power plant cooling water intake structures (CWIS). In fact, annual impingement estimates are frequently dominated by the large numbers of clupeids associated with these episodes. Minimizing the number of fish impinged at CWIS is important for both environmental protection and operational reasons. This report presents the results of investigations of two environmental factors, cold shock and nutritional state, that are known to contribute to the impingement of clupeids. These results can be used to help predict when impingement events are likely to occur and to assess the relative contribution of project operations and natural causes to fish impingement.

Results and Findings

A review of the literature on mass mortalities of clupeid species, particularly gizzard and threadfin shad, revealed that such events are common, especially in larger freshwater lakes, rivers, and reservoirs. However, research to date into the causes of this mortality as well as the general physiological responses of clupeids to potential environmental stressors is limited. The principal reasons for such die-offs often vary among species. Laboratory studies confirm that cold temperatures and cold shock resulting from a rapid decline in temperature can reduce swimming endurance in gizzard and threadfin shad and render them more susceptible to impingement. The results of these studies will be useful for identifying the environmental conditions under which one might expect the cause of impingement to be largely of natural origin. For example, when thermal regimes at a CWIS are similar to those that resulted in loss of equilibrium in laboratory experiments, i.e., $< 2\text{ }^{\circ}\text{C}$ for gizzard shad and $< 5\text{ }^{\circ}\text{C}$ for threadfin shad, we would expect that the bulk of impinged fish were not killed directly by impingement. This study also identified physiological indicators of susceptibility to impingement such as hematocrit and condition factor whose measurement could potentially be used to predict or explain episodic impingement events. The use of multiple indicators of stress helps to explain confounding stressors that may be present in natural ecosystems. Using physiological and performance-level indicators to assess impingement susceptibility appears promising, but further studies are needed to evaluate the relative importance of cold shock and nutritional status on impingement.

Challenges and Objectives

Under the Clean Water Act (CWA) §316(b), the applicant for a National Pollutant Discharge Elimination System (NPDES) permit must demonstrate that the location, design, construction, and capacity of its cooling water intake structure represents Best Technology Available (BTA) for minimizing adverse environmental impact. As of preparation of this report, the U.S. Environmental Protection Agency (EPA) is re-writing, per a 2007 U.S. Appeals Court finding, the Rule to implement CWA §316(b) for existing power plants (Phase II Rule). Many studies

have demonstrated a relationship between the incidence of natural mortality for several fish species (particularly clupeids) and increased power plant impingement. EPA has recognized the need to evaluate naturally moribund fish and shellfish entering CWIS. In the now remanded Phase II Rule, EPA noted that estimates of impingement mortality should be based on the impingement and harm of healthy fish, not the incidental capture of moribund and dead fish. The revised EPA Phase II Rule may retain the requirements in the previous Rule; and the key challenge will be to demonstrate technical, defensible criteria for the identification of impinged fish that were already dead or dying when they entered the intake. The development of these criteria is the subject of this report and future EPRI research.

Applications, Values, and Use

This report is planned as a technical support document providing information and ideas EPRI members can use when discussing impingement compliance options with permitting agencies in areas where there are occurrences of high episodic natural mortality of fish.

EPRI Perspective

This report provides information to EPRI members to support their CWA §316(b) compliance efforts. Most notably, the report supports the documentation of the natural occurrence of dead and moribund fish, thereby reducing estimates of annual impingement mortality that can be attributed to processes and structures associated with a power plant's CWIS.

Approach

This issue was initially addressed by reviewing and summarizing the technical literature on natural mortality events exhibited by clupeids for the purpose of examining the relationship between naturally stressed and moribund fish and impingement at CWIS, as well as for designing laboratory studies to investigate key relationships. The project team then conducted laboratory studies on the responses of two common freshwater shad species (gizzard and threadfin) to rapid reductions in water temperatures and their potential for recovery from cold shock. Additional laboratory studies investigated the relationship between various physiological indicators of stress and the susceptibility of impingement by these species.

Keywords

Clean Water Act §316(b)
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Fisheries
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Cover photo of threadfin shad collected during an episodic event at a power plant on the Ohio River.

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1

INTRODUCTION

As a result of concerns in the late 1960s and early 1970s over the potential effects of fish entrainment and impingement losses at electric generating facilities, Congress included §316(b) as part of the amendments to the Federal Water Pollution Control Act of 1972 (commonly referred to as the “Clean Water Act”). Under the Clean Water Act §316(b), an applicant for a National Pollutant Discharge Elimination System (NPDES) permit must demonstrate that the location, design, construction and capacity of its cooling water intake structure (CWIS) represents Best Technology Available (BTA) for minimizing adverse environmental impact.

In 1995, the U.S. Environmental Protection Agency (USEPA) began a three-phased process to develop the rules related to §316(b).¹ The final Phase I Rule, for new facilities, was published on 18 December 2001 (66 FR 65255) and was amended on 19 June 2003 (68 FR 36749). The final Phase II Rule, for existing electric generating facilities was published on 9 July 2004 (69 FR 41575). The Phase II Rule applies to existing facilities whose construction commenced prior to 17 January 2002 and that have cooling water intake structures with a design capacity greater than or equal to 50 million gallons per day (MGD), and use 25 % or more of the water withdrawn for cooling purposes. The Phase III rule, for smaller (<50 MGD) power plants and certain industrial facilities, was published 16 June 2006 (71 FR 35005).

USEPA’s regulations establishing requirements for cooling water intake structures at Phase II existing facilities were challenged by industry and environmental stakeholders. On judicial review, the Second Circuit decision (*Riverkeeper, Inc. v. EPA*, 475 F.3d 83, (2d Cir., 25 January 2007)) found some provisions illegal and remanded several provisions of the Phase II rule on various grounds. The provisions found illegal included the option to use restoration and cost-benefit analyses. The key provisions remanded to EPA include:

- EPA's determination of the BTA under §316(b);
- the rule's performance standard ranges;
- the cost-cost compliance alternative; and
- the Technology Installation and Operation Plan provision

In response to the decision, EPA suspended the Phase II rule on 9 July 2007 (72 FR 37107). In lieu of the suspended Phase II Rule EPA required that permitting authorities develop case-by-case, best professional judgment (BPJ) controls for existing facility cooling water intake structures that reflect the best technology available for minimizing adverse environmental

¹ *Cronin v. Browner*, No. 93 Civ. 0314 (AGS)(S.D.N.Y.), Order of 21 November 2000.

impact. CWA provision 40 CFR 125.90(b) directs permitting authorities to establish §316(b) requirements on a BPJ basis for existing facilities not subject to categorical §316(b) regulations.

Though remanded, it is anticipated that some features of the Phase II Rule related to technology-based performance standards will be retained when USEPA revises the rule in the future². The suspended Phase II Rule had established performance standards for cooling water intake structures that would have required substantial reductions in impingement mortality and entrainment relative to a Calculation Baseline. The Calculation Baseline is the impingement mortality and entrainment that would hypothetically occur if the facility had a shoreline, near-surface intake, traveling screen with a standard 3/8 inch mesh with its face oriented parallel to the shoreline, but no other measures to reduce impingement mortality and entrainment. Among other requirements, the remanded Phase II Rule had required a reduction of impingement mortality by 80 to 95% from the Calculation Baseline for all Phase II in-scope power plants in the U.S.

The USEPA has recognized the need to evaluate naturally moribund fish and shellfish entering cooling water intake systems (USEPA 2006). For example, as part of the Verification Monitoring Plan for compliance alternatives in §125.94(a)(2), (3), (4), or (5), an applicant proposal was to be submitted outlining how naturally moribund fish and shellfish entering the CWIS will be identified and used to meet performance standards in §125.94(b). Although the Verification Monitoring Plan is part of the remanded Phase II rule, it is reasonable to expect that permitting authorities will take into account the numbers of naturally dead and moribund fish entering the CWIS when evaluating the need for controls to minimize adverse impacts to fish populations. In a letter regarding calculation baseline estimates at the Muscatine Power Plant (Iowa), EPA Region VII stated that "... moribund fish should not be counted in the impingement calculation baseline. Sampling of impingement should count all fish, but moribund fish should not count toward the calculation baseline" (USEPA 2006).

Many studies have demonstrated a relationship between increased power plant impingement and the incidence of natural mortality for several fish species, particularly for clupeid species (Griffith and Tomljanovich 1975; Loar et al. 1978; McLean et al. 1979; McLean et al. 1980; McLean et al. 1981; McLean et al. 1985; LaJeone and Monzingo 2000). However, field evaluations of the condition of fish (e.g., living, dead, moribund, recoverable) prior to impingement can be difficult and are rare. EPRI recently

completed³ a 2-yr survey of impingement at 15 power plants on the Ohio River. Of the 112 seasonally-combined events (8 seasons at 13 plants and 4 seasons at 2 plants), there were 16 seasonally-combined impingement events of more than 10,000 fish that occurred at 7 of the 15 plants. These 16 seasonally combined events included large numbers of gizzard shad (*Dorosoma cepedianum*; 13 seasonally-combined events), threadfin shad (*Dorosoma petenense*; 3), freshwater drum (*Aplodinotus grunniens*; 2), and skipjack herring (*Alosa chrysochloris*; 1). During these high seasonally-combined impingement events, 25% (average) of the impinged fish were alive at the time of sample collection. In four of the seasonally-combined events living fish

² USEPA began a revised rule-making effort in the fall of 2007 and tentatively plan on releasing a draft revised Phase II Rule in late 2008 or early 2009. Also of note is that the 2nd Circuit Court decision of 2007 has been appealed to the U.S. Supreme Court for review.

³ Ohio River Ecological Research Program: Impingement Mortality Characterization Study at 15 Phase II Generating Stations. EPRI Draft Report, January 2008. Final report planned for June 2008.

comprised less than 1% of the impingement counts for the predominant species. Nearly all of the fresh dead fish (i.e., those that had recently died or perhaps moribund) were captured during fall and winter samples (Figure 1-1). Studies at the Muscatine Plant (Iowa) found that more than 95% of fish entrapped on barrier nets in December (357 total fish) and February (961 total fish) were either moribund or dead (HDR LMS 2006).

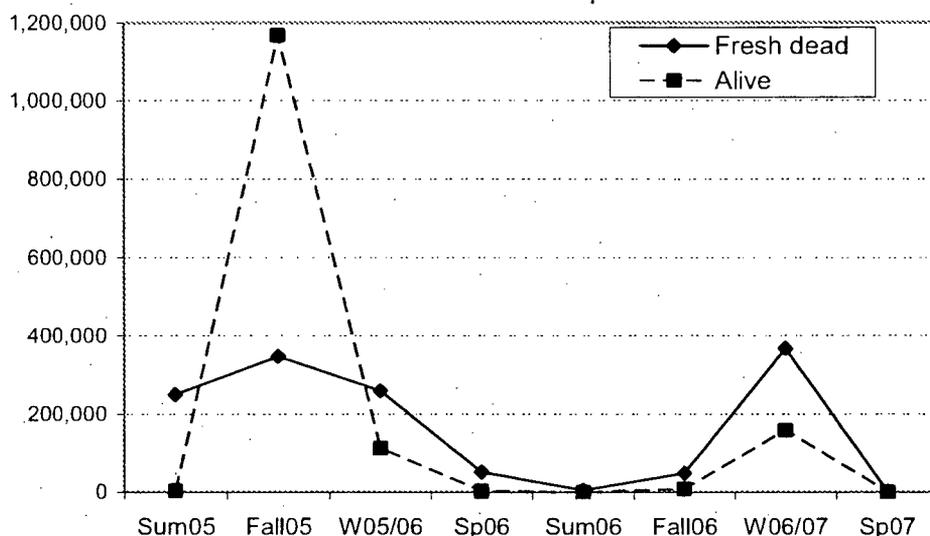


Figure 1-1

Total number of impinged fish (alive and fresh dead) per season at 15 intake structures on the Ohio River (NOTE: the impingement data for fall 2005 included almost 1.1 million live but moribund threadfin shad collected at one power plant in a single day of sampling).

Laboratory studies designed to better understand the factors that contribute to fish impingement are necessary to assign project responsibility and adjust fish protection technology performance accordingly. For example, information on the behavior and physiological state of cold-stressed fish prior to impingement may help industry, regulatory, and resource agencies determine the proportions of impinged fish that were already dead or dying when they entered the CWIS. Further, if used as a monitoring tool, behavioral or physiological indicators may be valuable for predicting the environmental conditions and/or fish population dynamics under which large impingement episodes will occur. This knowledge would enable utilities to adjust the operations of their power plants to reduce the loss of debilitated, but recoverable, fish as well as prevent blockage of cooling water flow.

Cold shock and starvation have been proposed as primary causes of winter mortality for many species including gizzard shad. White et al. (1986) conducted an extensive study on the physiological and biochemical responses of cold-shocked gizzard shad relative to susceptibility to impingement. They found that even though lipid reserves are relatively high in gizzard shad going into winter, they have trouble mobilizing this energy reserve when temperatures are very cold and thus go in to a starvation mode even though they contain high fat reserves. This results in a quick utilization of liver and muscle glycogens, which in turn results in other tissues being utilized for energy. In severe cases, liver function declines and failure of other physiological functions often follows. For example, cell membranes begun to lose their ability to transport

materials properly which can result in poor energy delivery to the brain and subsequent brain dysfunction. Loss of brain function results first in disorientation and eventually a comatose condition like that seen during winter mortality events. White et al. (1986) concluded that poor over-winter survival of gizzard shad in Sandusky Bay (Ohio) is a result of enzymatic acclimation occurring too late in the season causing eventual physiological failure.

The purpose of this report is to present the results of two efforts by funded by EPRI to better understand the condition of fish that become impinged at CWIS and the environmental conditions associated with impingement events. The first study, presented in Chapter 2, was a literature review of natural mortality events in clupeid fishes (i.e., shad, menhaden, and herring species). By understanding the conditions under which natural events occur, resource managers and project operators can more accurately assess the contribution of natural causes to impingement at CWIS.

The second study, presented in Chapters 3 and 4, was a series of laboratory experiments with gizzard shad and threadfin shad under different cold shock thermal regimes and feeding history designed to better understand the relationships among cold shock, nutritional status, and susceptibility to impingement. These studies were intended to further the understanding of these relationships as reported by White et al. (1986) and others.

This report is planned as a technical support document providing information and ideas that EPRI members can use when discussing impingement compliance options with permitting agencies in areas where there are occurrences of high episodic natural mortality. The information presented in this report will help establish guidelines for identifying the time of year and temperature dynamics that are likely to result in high incidences of naturally moribund and dead fish in CWIS impingement samples.

As part of its corporate objectives to provide scientifically sound information for development of cost-effective environmental policies and regulations as well as information for cost-effective and scientifically sound compliance efforts, EPRI has supported a variety of studies that evaluated scientific methodologies and summarized potential environmental effects of cooling water withdrawals. These studies have done much to advance the current state-of-the-art for addressing issues related to §316(b). In addition to this document, other EPRI reports that provide information relevant to §316(b)-related compliance sampling include:

Fish Protection at Cooling Water Intake Structures: A Technical Reference Manual (EPRI Report 1014934, 2007)

Effects of Fluctuating Temperatures on Fish Health and Survival (EPRI Report 1012545, 2007)

Latent Impingement Mortality Assessment of the Geiger Multi-Disc™ Screening System at the Potomac River Generating Station (EPRI Report 1013065, 2007)

Technical Resource Document for Modified Ristroph Traveling Screens: Design and Construction Technology Plan and Technology Installation and Operation Plan (EPRI Report 1013308, 2006)

Laboratory Evaluation of Modified Ristroph Traveling Screens for Protecting Fish at Cooling Water Intakes (EPRI Report 1003238, 2006)

Design Considerations and Specifications for Fish Barrier Net Deployment at Cooling Water Intake Structures (EPRI Report 1013309, 2006)

Field Evaluation of Wedgewire Screens for Protecting Early Life Stages of Fish at Cooling Water Intake Structures: Chesapeake Bay Studies (EPRI Report 1002542, 2006)

Field Evaluation of Wedgewire Screens for Protecting Early Life Stages of Fish at Cooling Water Intakes (EPRI Report 1010112, 2005)

Impingement and Entrainment Survival Studies Technical Support Document (EPRI Report 1011278, 2005)

Entrainment Abundance Monitoring Technical Support Document (EPRI Report 1011280, 2005)

Impingement Abundance Monitoring Technical Support Document (EPRI Report 1008470, 2004)

Parameter Development for Equivalent Adult and Production Foregone Models (EPRI Report 1008832, 2005)

Extrapolating Impingement and Entrainment Losses to Equivalent Adults and Production Foregone (EPRI Report 1008471, 2004)

Impacts of Volumetric Flow Rate of Water Intakes on Fish Populations and Communities (EPRI Report 1005178, 2003)

Evaluating the Effects of Power Plants on Aquatic Communities: Summary of Impingement Survival Studies (EPRI Report 1007821, 2003)

Evaluating the Effects of Power Plants on Aquatic Communities: Guidelines for Selection of Assessment Methods (EPRI Report 1005176, 2002)

Evaluating the Effects of Power Plant Operations on Aquatic Communities: An Ecological Risk Assessment Framework for §316(b) Determinations (EPRI Report 1005337, 2002)

Technical Evaluation of the Utility of Intake Approach Velocity as an Indicator of Potential Adverse Environmental Impact under Clean Water Act Section 316 (EPRI Report 1000731, 2001)

Review of Entrainment Survival Studies: 1970-2000 (EPRI Report 1000757, 2000)

Taken together these documents provide utility managers, regulators, and interested parties technically sound guidance for the §316(b) determination process. It is EPRI's intent that these

documents be accepted as objective resources by a diversity of users involved in the regulatory process, including scientists, engineers, managers, and lawyers working for the utility industry, regulatory and resource management agencies, academic and private consultants, and environmental advocates.

2

NATURAL MORTALITY EVENTS IN CLUPEID FISHES: A LITERATURE REVIEW

Introduction

The family Clupeidae includes a wide diversity of prolific species, including blueback herring (*Alosa aestivalis*), alewife (*Alosa pseudoharengus*), American shad (*Alosa sapidissima*), gizzard shad and threadfin shad. Many clupeid species have been introduced into lakes and reservoirs as a forage base for recreationally important game species. Under optimal environmental conditions and low predator pressures, clupeid populations can expand quickly. For example, in the mid-1950s, about 1,000 threadfin shad were introduced into Havasu Reservoir, Colorado, and within one year the population numbered in the millions and had spread downstream of the reservoir (Moyle 2002). Large populations without controls can quickly exceed carrying capacity for the water body, resulting in mass mortality from starvation and disease. Studies have shown correlations between clupeid density and juvenile mortality for species like gizzard shad (Stock 1971; Kampa 1984; Buynak et al. 1992; Welker et al. 1994). Owing to their large numbers, clupeids often comprise a large proportion of the fish that are impinged at CWIS (Loar et al. 1978).

Many introduced clupeids have narrow thermal and water quality tolerance ranges, causing mass mortality during harsh periods. Sudden and drastic changes in temperature cause behavioral and physiological changes in many clupeid species. A rapid drop in temperature can cause loss of swimming and schooling abilities and a decrease in feeding (Griffith and Tomljanovich 1975). At temperatures near their lower tolerance limits, clupeids experience loss of equilibrium, erratic swimming, movement to the surface, and lack of response to external stimuli (Griffith 1978). These behavioral changes not only make clupeids vulnerable to predation, but they can become more susceptible to power plant impingement. Rapid decreases in water temperature can occur naturally, or as a result of plant operations. The winter shutdown of industrial facilities that produce warmwater discharges can cause debilitating or lethal cold shock among clupeids that congregated near these warmwater discharges during winter (Burton et al. 1979).

This chapter presents the results of a literature search and review to address the relationship between the occurrences of naturally stressed and moribund fish and impingement at CWIS. This literature review focused on five members of the herring family (Clupeidae) – threadfin shad, gizzard shad, alewife, Atlantic menhaden (*Brevoortia tyrannus*), and Gulf menhaden (*Brevoortia patronus*). Each of these five species have a documented history of large scale die-offs, represent a significant component of impingement at cooling water intake structures, and are found over a broad geographic range encompassing fresh, brackish and marine waters. Specific objectives of the review were to identify and summarize available information for the species listed above related to each of the following areas:

- Susceptibility of each species to die-offs;
- Seasonality of such die-offs;
- Contributing environmental conditions and other causal factors (i.e., stressors);
- Physiological processes and indicators of stressor exposure;
- Relationship to cooling water intake impingement; and,
- Recorded occurrences of large-scale die-offs, including species and geographic locations.

The search for information was conducted in five phases. First, literature contained within library holdings at ASA Analysis & Communication, Inc. relevant to §316(b) issues were identified and accessed. Second, a thorough search of the Internet was conducted for relevant information. Third, a broad-based search was conducted through the Dialog® system. This search focused on three databases: Biosis Previews, National Technical Information Service (NTIS), and the Electric Power Database. A broad search of these databases yielded over 1,100 relevant titles. The full record, including the abstract, was printed and used to identify the most useful and relevant literature. Fourth, the reference lists in all of the literature identified in phases 1–3 of the search were reviewed to identify additional materials. Finally, individuals with prior research experience in areas related to fish impingement and §316(b) issues were contacted to obtain additional, often unpublished, reports and papers.

Seventy three relevant reports and published reference materials were identified and retrieved as part of this effort. An annotated listing of these materials is provided in Appendix A and is summarized by species below.

Threadfin shad

Threadfin shad is one of the most important forage species in many water bodies, especially in Southeastern lakes and reservoirs (Schael et al. 1995). In these water bodies, this species often provides an important source of food for largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), and striped bass (*Morone saxatilis*). As a result of its importance as a forage species for many recreationally important fish species, threadfin shad have been introduced over wide geographic areas of the country. However, threadfin shad are a short-lived, fragile fish prone to frequent die-offs when conditions are sub-optimal (Higginbotham 1988). For example, threadfin shad are known to suffer mass mortality when water temperatures fall below 5-6°C. In addition, this species is sensitive to dissolved oxygen depletion during summer months and can exhibit large die-offs after spawning as a result of cumulative physiological stress. The reduced physiological condition of threadfin shad during summer months is believed to have increased impingement at the Comanche Peak Steam Electric Station in Texas from 1993 through 1994 (TUEC 1994). During the period from late July to late August when water temperatures were at their highest, 81 % of the annual impingement of threadfin shad occurred at this station.

While it appears that threadfin shad can be susceptible to a wide variety of stressors, temperature appears to be the primary contributor to most large-scale mortality events. Griffith (1978) found that threadfin shad started dying at 9°C and that none of his study fish survived at 4°C. In

addition, threadfin shad mortality can be high when they are exposed to water temperatures at 9°C for several months (Strawn 1965). A sudden drop in temperature can not only cause detrimental behavioral changes and decreased feeding, but can also cause loss of equilibrium and death (Griffith 1978). Loss of equilibrium due to cold shock can cause hemorrhaging and fungal infections (Colby 1973). In fact, because of their sensitivity to low water temperatures, threadfin shad survival in some water bodies may require access to warm water discharges from power plants. For example, during a 1983 survey of the upper Mississippi River threadfin shad were only collected near the Portage Des Sioux power plant, and survival of threadfin shad in Montrose Lake, Missouri, is believed to be dependent on the warm water discharges from a steam generating plant (Pflieger 1997).

Low temperatures also appear to be a primary factor affecting impingement rates at many cooling water intakes. This is a common occurrence for many Southern power plants, as threadfin shad impingement typically increases when water temperatures fall below 10°C (Loar et al. 1978). A study of 32 Southeastern United States power plants found that threadfin shad accounted for more than 90 % of all fish impinged, with peak impingement of this species occurring in winter (Loar et al. 1978). Increased threadfin shad impingement occurred at the following power plants when water temperatures were below 15°C: Green River, Kentucky; Allen, North Carolina; Marshall, North Carolina; Riverbend, North Carolina; Arkansas One, Arkansas; Oconee, South Carolina; Wateree, South Carolina; and Eagle Mountain, Texas. Impingement of large numbers of threadfin shad at Kingston Station, Tennessee, coincides with threadfin shad die-offs in the reservoir on which this steam electric power plant is located (McGee et al. 1977; McLean et al. 1985). The highest densities of impinged threadfin shad coincided with a sudden drop in water temperature (Figure 2-1). Impingement of threadfin shad at Kingston Station increased to 5,000 shad per day in December when temperatures dropped to 7°C. As water temperatures continued to decrease to 4°C, 42,000 threadfin shad were impinged on 8 December (McLean et al. 1980).

In years with mass mortality during severe winters, all age and size classes are affected and a majority of the threadfin shad population was eliminated (McLean et al. 1985). However, this highly fecund and fast-growing species has the ability to rebound quickly in the years following a significant die-off. Fish which hatch in spring are capable of spawning that same summer, enabling a population to quickly rebound following mass mortality. For example, an estimated 95 % of the threadfin shad population was removed from Watts Bar Reservoir during the winters of 1976-1977 and 1977-1978 as a result of impingement mortality and winter kill. However, the threadfin shad population had rebounded by autumn of each year following the die-offs (McLean et al. 1980).

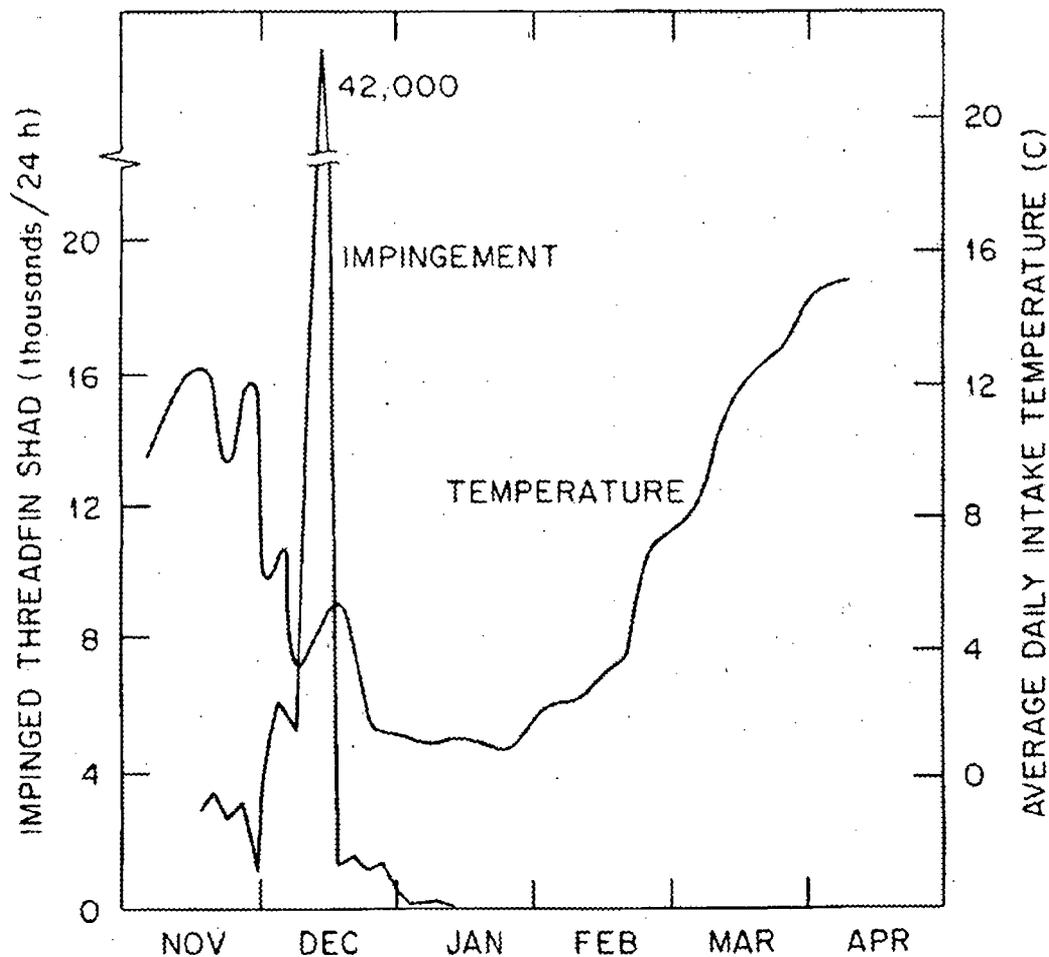


Figure 2-1
Impingement of threadfin shad at Kingston Steam Plant and water temperatures at the intake canal from November 1976 through April 1977. (From: McGee et al. 1977).

The following reports provide details of documented mass mortality events of threadfin shad:

Watts Barr Reservoir, Tennessee 1976-1977 – Mass threadfin shad mortality due to a severe winter (McLean et al. 1985)

Pee Dee River, North Carolina May 2002 (NCDWQ 2002)

Sacramento - San Joaquin Delta, California – Mass die-off during winters when temperatures drop to 6-8°C (Moyle 2002)

Clear Lake, California – Extirpated during severe winter of 1990-1991 (Moyle 2002)

White River Basin, Missouri – Occasional massive winter mortality (Pflieger 1997)

Lake Texoma, OK 2001 – Severe winter kill in mid-February 2001 (OK Department of Wildlife Conservation 2001)

Bull Shoals Reservoir, Arkansas 1983-1984– Severe winter kill (Arkansas Game and Fish Commission 1995)

Norfolk Lake, Arkansas 1996 – Severe winter kill (Arkansas Game and Fish Commission 1997)

Smith Mountain Lake, Virginia 2002-2003 – Nearly complete die-off of population due to severe winter (Virginia Department of Game and Inland Fisheries 2004)

Gizzard Shad

Gizzard shad are native to most Southeastern states, but have since been introduced throughout much of the country (Cooper 1983; Kirtland 1844, cited in HDR LMS 2006). Introduced as a forage base for game species, gizzard shad, unfortunately, often outgrow their predators and quickly overpopulate a system. In many systems, gizzard shad are viewed as a nuisance fish as a result of an overpopulation of large adult fish. In addition, gizzard shad can compete with juvenile predators and other planktivorous fishes leading to declines in sport and native fish communities (Johnson et al. 1988; Michaletz 1997).

Overpopulations of gizzard shad combined with severe winters and low dissolved oxygen often lead to mass mortalities. Typically, mass gizzard shad mortalities tend to occur in the northern part of their range as a result of severe winters. For example, Kirtland (1844, cited in HDR LMS 2006) reported heavy winter kills of gizzard shad in the Ohio River, and White (1986) described the winter kills as being density dependent. Gizzard shad larvae and juvenile survival has been correlated with water temperature in several midwest reservoirs; early cohorts not only grew slower as a result of lower temperatures, but also suffered higher mortalities than later age classes (Michaletz 1997). Winter die-offs are often more severe for younger age classes, as they typically deplete energy reserves more rapidly than do larger gizzard shad (Shuter and Post 1990). In addition, gizzard shad which are spawned later in the year have less time to build-up fat reserves (White et al. 1986). For example, during severe winters in Sandusky Bay, Lake Erie 100 % mortality has been recorded for young-of-the-year (YOY) gizzard shad in the 40-85 mm size range and 99.9 % mortality for YOY between 90-140 mm (White et al. 1986). In other studies on Lake Erie, gizzard shad populations exhibited a shift in size range of YOY fish from mid-autumn through early spring. Because growth does not occur during this period, this shift in mean length and length range has been attributed to size selective mortality over the winter (Carrots 1976). Similarly, high gizzard shad impingement counts at the Muscatine Plant in Iowa were initially dominated by YOY fish; later in the winter, the mean size of impinged shad increased (HDR LMS 2006). Gizzard shad often dominate fish biomass in many bodies of water and can consume all of the food resources that might otherwise be available to other fish species. For example, higher gizzard shad levels can yield reduced growth in bluegills (Michaletz 1998). This reduced bluegill growth can lead to reduced growth in largemouth bass which preferentially feed on bluegills.

Periods of higher impingement rates for gizzard shad often correspond with periods of large winter die-offs; further, the size range of impinged gizzard shad also tends to overlap with size ranges of those fish subject to winter mortality. For example, gizzard shad impinged from 1979 through 1984 at Eastlake, Avon Lake, and Edgewater generating facilities located in Lake Erie's Central Basin comprised mainly YOY fish in the 40-125 mm size range, similar to the size of fish which exhibited natural mortality (White et al. 1986).

Gizzard shad are most susceptible to winter die-offs in the northern part of their range, as they are not physiologically adapted for survival during extended cold periods. Gizzard shad begin showing signs of disorientation when water temperatures are around 6 or 7°C (Cox and Coutant 1975). Gizzard shad rely on stored lipid reserves during the winter months, as feeding stops when water temperature declines to around 11°C, but level of activity remains unchanged (White et al. 1986). In addition, when water temperatures drop below 8°C, gizzard shad are unable to mobilize fat reserves and begin utilizing liver, muscle glycogens, and other tissues as sources of energy even though lipids remain. As the liver is metabolized, liver function begins to fail causing jaundice. In cold water, gizzard shad lose cell function and are unable to diffuse waste and materials across cell membranes. After several weeks of these stressful conditions, gizzard shad begin to lose brain function which results in loss of equilibrium, erratic swimming, and finally ends in a comatose state and death (White et al. 1986).

The following reports provide details of documented mass mortality events for gizzard shad:

Several East Tennessee Reservoirs Spring 1983 – Large gizzard shad die-offs due to cumulative stresses and low lipid reserves (Adams et al. 1985)

Sandusky Bay, Lake Erie – Mortality of 5 million gizzard shad per acre (White et al. 1986)

Ohio River near Cincinnati, Ohio 1844 – Large winter kill (Kirtland 1844)

Buckeye Lake, Ohio 1928 and 1940 – Winter die-off after cold snap; gizzard shad struggling at surface (Trautman 1928; Trautman 1940)

Lewis and Clark Lake, South Dakota – 100 % mortality of age 0+ age class after 103 days of ice cover (Walburg 1964)

Western Basin Lake Erie 1955 – winter kill after cold snap; erratic swimming behavior (Bodola 1955)

Huron River, Ohio 1982 – Die-off of millions of yearling gizzard shad after cold snap in weather (Cleveland Plain Dealer 1982)

Acton Lake, Ohio – Complete mortality of age 0+ age class (Hlohowskyj 1983)

Elephant Butte Lake, New Mexico (Jester and Jensen 1972) and Presque Isle Bay, PA (Neumann et al. 1977) – large winter kill

Nebraska Lakes – Winds breaking down stratification causing deep waters to cool rapidly from 4°C to 0°C which caused mortality in gizzard shad (Heidinger 1983)

Alewife

Alewife have been introduced both purposely and accidentally into many northern lakes, such as the Great Lakes, and serve as a forage base for native and introduced salmonids and walleye. Although the alewife has lower thermal tolerances than other clupeids, such as threadfin shad, seasonal die-offs are common in land-locked populations. In Lake Ontario and Lake Michigan, large seasonal alewife die-offs occurred in the 1960s and 1970s following severe winters (O’Gorman and Schneider 1986; Flath and Diana 1985).

As a result of introductions of Pacific salmon and the revitalized lake trout (*Salvelinus namaycush*) and walleye (*Stizostedion vitreum*) stocks, the alewife is a vital link in the food chain of the Great Lakes. In the 1960’s, Lake Michigan alewife experienced an average yearly mortality rate of 68 % which was attributed to winterkill and spawning stresses (Brown 1968). These large die-offs in Lake Michigan are thought to be an indirect result of competition leading to a reduction in fat reserves (Brown 1972). Annual die-offs in Lake Michigan correlated with the time of year in which energy reserves are lowest, an indication of insufficient feeding due to environmental stresses, competition, or a reduced plankton population (Flath and Diana 1985). As a result of population declines in several of the Great Lakes, large alewife die-offs are no longer a common occurrence; poor recruitment following the severe winters of 1976-1982 are believed to be the primary cause of Lake Michigan alewife declines (Eck and Wells 1987).

Sudden exposure to warmer temperatures in littoral areas may also cause spring and early summer die-offs in alewife populations (McCauley and Binkowski 1982). Alewife may succumb to warm inshore waters after prolonged exposure to cold temperatures during harsh winters which deplete fat reserves (Colby 1973). The large die-offs in Lake Michigan in June and early July 1967 are believed to be a result of fish encountering warm littoral water as they moved inshore from deep cold water. This theory is supported by the fact that fish appeared robust, many contained rapidly digestible zooplankton, and all size classes of male and female alewife were affected (Brown 1968). Studies have indicated the upper lethal temperature for alewife is 25°C (McCauley and Binkowski 1982). Seasonal percent lipids stored by alewife from Lake Michigan are typically at their lowest (3-5 %) in late spring and early summer (Flath and Diana 1985).

A severe winter in 1992-1993 in Lake Ontario was believed to have severely stressed the alewife population, causing a winter kill; many alewives remained in the littoral areas after spawning instead of moving to deeper water, which increased their vulnerability to impingement (Ross et al. 1996). A sound deterrent system used at James A. Fitzpatrick Nuclear Power Plant during this period exhibited decreased effectiveness when water temperatures were below 13°C, as a result of a diminished response of fish (Ross et al. 1996). Although severe winters can greatly reduce alewife populations, high fecundity and high early life stage survival allow alewife populations to quickly recover in 1 to 2 years (Brown 1972; Kohler and Ney 1981).

The following reports provide details of documented mass mortality events for alewife:

Lake Michigan June and July 1967 – Large alewife die-off, possible temperature shock or algal toxicity (Stanley and Colby 1971; Brown 1968)

Lake Michigan Early 1980s – Large decline in alewife population (Eck and Wells 1987)

Lake Ontario, New York Spring 1993 – Highest mortality in 10 years (Schneider and Schaner 1994)

Lake Ontario, New York – Alewife winter kill (O’Gorman and Schneider 1986; Bergstedt and O’Gorman 1989)

Lake Michigan and Lake Ontario – Alewife mass mortality mainly in spring (Pritchard 1929; Graham 1956; Smith 1968)

Lake Michigan 1960s – 68 % average yearly mortality (Brown 1968)

Claytor Lake, Virginia 1977-1978 – Large alewife die-off associated with severe winter (Kohler and Ney 1981)

Lake Wononskopomuc, Connecticut – Alewife die-off (Warshaw 1972)

Atlantic & Gulf Menhaden

Both species of menhaden are found in marine and brackish waters along the Atlantic and Gulf coasts. Atlantic menhaden are found from Western Nova Scotia to Florida, while the Gulf menhaden occurs from Cape Sable, Florida, to Veracruz, Mexico. Both species serve as important forage for a variety of larger aquatic predators and also, as adults, support important commercial fisheries in certain regions. While most examples of significant mortality events have been reported for Atlantic menhaden, it is reasonable to expect similar events in the closely related Gulf menhaden.

Menhaden mass mortalities appear to be less influenced by temperature stresses and more commonly caused by disease and overcrowding. For example, the interactions of large populations of menhaden with predatory fish can promote large fish kills, as predators like bluefish and striped bass pursue schools of menhaden into small coves. These overcrowded menhaden schools quickly deplete dissolved oxygen concentrations in the small embayments, leading to anoxic conditions and large menhaden kills (ASMFC 2001). For example, a school of Atlantic menhaden near Core Banks, North Carolina in 1997 was estimated to have a biomass of 60,000 million tons, with fish 9 m deep in the water column. This large concentration of fish is believed to have led to oxygen depletion and a large kill (Smith 1999). Oviatt et al. (1972) reported that dissolved oxygen concentrations within small schools of Atlantic menhaden were depleted by 12 % compared to the concentrations in water outside of the school.

In addition to the effects of temporary anoxia, Atlantic menhaden mortalities have been reported in numerous estuaries along the East Coast as a result of ulcerative mycosis disease and toxic dinoflagellates (Ahrenholz et al. 1987; Noga et al. 1991; Burkholder et al. 1992; Faisal and Hargis 1992). Sudden decreases in water temperature as a result of winter shutdown at large

power plants can cause Atlantic menhaden mortalities. For example, a temperature decrease from 15 to 5°C caused all menhaden to die within 36 hr in laboratory studies (Burton et al. 1979).

Details of Atlantic menhaden mass mortalities are provided in the following reports:

Pamlico River, North Carolina May 2002 – Increasing water temperature and changes in dissolved oxygen may have caused fish kill (NCDWQ 2002)

Alligator Creek, North Carolina April 2002 – Shallow creek, no explanation for large kill (NCDWQ 2002)

Neuse River, North Carolina July 2002 – High water temperatures and low dissolved oxygen (NCDWQ 2002)

New York Harbor – Annual die-off of millions of menhaden (Westman and Nigrelli 1955)

Chesapeake Bay – Annual die-off caused by virus (spinning disease) (Stephens et al. 1980)

Southern Maine 1980s & 1990s – Menhaden kills due to oxygen depletion in coves (Vaughan 1990; Conniff 1992)

East Coast Estuaries – Kills caused by toxic dinoflagellates (Ahrenholz et al. 1987; Sindermann 1988; Noga et al. 1991; Burkholder et al. 1992; Faisal and Hargis 1992)

Core Banks, North Carolina 1997 – School induced low dissolved oxygen concentrations (Smith 1999)

Oyster Creek Nuclear Generating Station, NJ 1972, 1973, 1974, 1975 – Menhaden kills likely a result of cold shock (Coutant 1977)

Discussion

Based on the summarized literature, reports of mass mortalities of clupeids are quite common, especially in larger freshwater lakes, rivers and reservoirs, and brackish and marine embayments. However, to date, studies of the causes of this mortality as well as the general physiological responses of clupeids to potential environmental stressors have been limited. This lack of published research often leaves fisheries managers guessing at the causes of mass mortality, how to prevent such occurrences, and how to predict large clupeid die-offs.

Perhaps the most extensive research on the topic was conducted by White et al. (1986). These studies provide detailed information on the physiological response of gizzard shad to thermal stress and provide clues to link mass mortalities to cold stress, but it is unclear whether such information is relevant to other clupeids. The authors found that the amount of stored lipids appears to play a role in determining winter kill of several clupeid species, but is not an effective means of determining cold stress mortality in gizzard shad, as gizzard shad are unable to utilize stored fat reserves below 8°C. At these low temperatures, gizzard shad begin metabolizing liver tissue and lose cellular function, which eventually leads to decreased liver and brain function.

Necropsies of gizzard shad that died in cold stress-related mass mortality events revealed loss or breakdown of liver function, enlarged gallbladder, scale base hemorrhaging, jaundiced internal organs and eyes, and progressive darkening of bile.

Unfortunately, details on menhaden, alewife, and threadfin shad mass mortality are not as well documented. Alewife mass mortality was a common occurrence in the Great Lakes, Lake Huron, Lake Michigan, Lake Erie, and Lake Ontario. These die-offs seemed to be linked to severe winters, but since the introduction of predatory salmonids, mass mortality has not been as common an occurrence. Alewife populations in several of the Great Lakes have been significantly reduced by poor recruitment following winterkill, predation by Pacific salmon and lake trout, or by competition with other planktivores and invasive dreissenid mussels, and coincidentally die-offs have not been as noticeable. These observations suggest that mass winter mortality may be a density-dependent process. Research indicates the mass alewife mortalities which occurred in 1983-1984 were a result of poor condition in the alewife population, as temperatures were not as severe as previous winters (Bergstedt and O'Gorman 1989). In direct contrast, alewife collected prior to the severe winter of 1981-1982 were in good condition and, as a result, winter mortality was not severe (O'Gorman 1986). In contrast to gizzard shad energetics, fat reserves have been reported to play an important role in alewife survival during harsh winters and may be an effective tool for predicting mass die-off (Brown 1972; Colby 1973; Bergstedt and O'Gorman 1989).

Based on documented occurrences, mass mortality of menhaden appears most likely to result from either a sudden change in water quality or disease. Menhaden often travel in large schools which have the ability to quickly degrade oxygen concentrations when confined in small areas. Theories of mass menhaden mortality include large schools being chased into small confined embayments by predators such as bluefish and striped bass. The respiration of several hundred thousand menhaden in a small area could quickly consume available dissolved oxygen, leading to asphyxiation. Other sources of mass menhaden mortality include ulcerative mycosis disease, toxic dinoflagellates, and thermal shock as a result of power plant shutdown (Coutant 1977; Smith 1999).

Although predicting mass mortalities is often difficult and problematic, identifying symptoms of cold shock in fishes is well documented. Fish exposed to temperatures at or near lower tolerances exhibit a short period of increased swimming and hyperactivity, followed by decreased movements, a decrease in response, and finally loss of equilibrium, which is shortly followed by death (Coutant 1977). Cold stress in clupeids also leads to vulnerability to predation and power plant impingement. Several Southeastern power plants have documented high threadfin shad impingement coinciding with a substantial drop in temperature and mass natural mortality (Griffith and Tomljanovich 1975; Loar et al. 1978; McLean et al. 1985). Laboratory studies suggest the uncoordinated swimming of cold-stressed threadfin shad prevents escape from power plant intake structures (Griffith and Tomljanovich 1975).

3

LABORATORY STUDIES ON CRITICAL THERMAL LIMITS

Introduction

As noted in Chapter 2, high power plant impingement rates among clupeids have often coincided with observations of cold-stress-related reductions in swimming capabilities and mass mortalities in the nearby river and reservoir. Recognizing that naturally cold-stressed and moribund fish may contribute to high impingement counts, it is important to quantify the effects of low temperatures on clupeid behavior, physiology, and mortality. That is, to better understand the relationship between natural environmental conditions and impingement events we need a better understanding of the thermal tolerances of clupeids.

One traditional approach to quantifying temperature tolerance (both minimum and maximum) of fishes is the critical thermal methodology (CTM). The critical minimum temperature (CTMin) is defined as the pre-death lower thermal point at which locomotion becomes disorganized and a fish loses the ability to escape from conditions which may ultimately lead to its death. This method usually involves exposing fish to a constant linear decrease in temperature until loss of equilibrium (LOE) or another endpoint is reached. The CTMin is typically defined as the median temperature at which individuals in a group of fish began to exhibit LOE. CTMin is species-specific and is a function of acclimation temperature (Beitinger et al. 2000; Brett 1956; Elliot 1981), acclimation time (Doudoroff 1942), and rate of temperature decline (Gunter and Hildebrand 1951). Fish acclimated to higher temperatures typically have a higher CTMin and, conversely, fish acclimated to low temperature may have a lower CTMin. The effect of rate of temperature decline on CTMin is not as straightforward. It is generally accepted that, if the rate of decline is fast, there is little time for acclimation and the CTMin will be higher than at slower rates of decline where some acclimation occurs along the way. However, recent work with critical maximum temperatures suggest that slower rates of temperature increase can result in lowered CTMax because of a longer exposure time to temperatures above some threshold where thermal stress occurs (EPRI 2007). A similar relationship might also exist for CTMin.

A range of temperatures causing general distress, loss of equilibrium, and mortality have been reported for gizzard and threadfin shad (Griffith and Tomljanovich 1975; Cox and Coutant 1976; Neumann et al. 1977; Griffith 1978; Heidinger 1983; McLean et al. 1985). The variability in methods and reported responses makes it difficult to assess the contribution of environmental conditions to impingement at cooling water intake structures. The primary objective of this study was to determine the cold tolerance of gizzard and threadfin shad from a Tennessee reservoir during either gradual or immediate cold shock. A secondary objective that evolved during the study was to determine the ability of these species to recover after LOE.

Methods

Fish Collection and Care

Gizzard shad were collected in March 2006 and threadfin shad in September 2006 by electrofishing on the Clinch River, Tennessee. Live shad were transported to Oak Ridge National Laboratory in 151-L barrels filled with ambient river water, equipped with aerators, and treated with 400 g of sodium chloride. Shad were then held at 24°C for 3 to 5 d in 889-L circular tanks. Each tank was equipped with an aerator, and a constant 0.6 L/min flow through was maintained. Shad were acclimated to feeding on frozen brine shrimp and laboratory conditions during this period. Following the 3-5 d acclimation, test fish were transferred to a 530-L rectangular tank, receiving 0.25 L/min of flow.

Gradual Cold Shock and Subsequent Recovery

Test groups of 22 gizzard shad (mean total length = 143 mm, weight = 24 g) or 20 threadfin shad (mean total length = 128 mm, weight = 17 g) were placed in a 530-L rectangular tank and acclimated for one week at $15 \pm 0.2^\circ\text{C}$ prior to testing. Each group was then subjected to a cold shock at a declining rate of 0.5°C/hr until LOE. Portable chillers paired with temperature controllers were used to regulate exposure temperatures within $\pm 0.2^\circ\text{C}$. As tank temperature dropped, the time and temperature at which LOE occurred was recorded. Individuals within a test group exhibited LOE at different temperatures; we considered the CTMin to be the median temperature at which fish in a group lost equilibrium. Half of the fish were randomly assigned a holding period of 30 min in the cold shock tank after losing equilibrium before being placed in a recovery tank. The other half of the test group was transferred immediately after LOE to one of 12 recovery aquaria (30.5 cm^3) within a larger tank, which was the same size as the cold shock tank. The larger tank was filled to a depth of 17.8 cm to serve as a water bath and maintained at the same temperature as the cold shock tank. The 12 recovery aquaria were filled to a depth of 17.8 cm and equipped with a water supply and aerator. Individual fish were placed into an aquarium and water was dripped into the aquarium at $\sim 25 \text{ mL/min}$ to create a warming rate of about 1.0°C/hr . The initial aquarium temperature, time of recovery, aquarium temperature at recovery, weight, and length were recorded for each fish. If individuals regained equilibrium for more than 15 min, recovery was noted.

Instantaneous Cold Shock

A test group of 20 gizzard shad (mean total length = 143 mm, weight = 24 g) were placed in a 530-L rectangular tank and acclimated for one week at $15 \pm 0.2^\circ\text{C}$ prior to testing. Ten fish were plunged into a rectangular tank maintained at 4°C and another 10 fish into a tank maintained at 6°C . The tanks remained at these temperatures for the first 24 hr after which the tanks were allowed to warm at room temperature for the next 4 d. Time and temperature at which LOE occurred and recovery from LOE were recorded during the experiment if either occurred.

Results—Gizzard shad

Critical Thermal Minimum Determination and Recovery

Activity levels decreased as temperatures approached 5°C, and fish became totally lethargic by 4°C. Below 4°C there was little response to vibration in the water and capture by netting. The median LOE temperature for gizzard shad exposed to cold shock at 0.5°C/hr was 1.7°C and ranged from 1.0 to 2.7°C (Figure 3-1). All gizzard shad recovered as water warmed within recovery aquaria. On average, recovery occurred at 2.6°C, 0.8°C above the average LOE temperature.

Instantaneous Cold Shock

Gizzard shad plunged into the 6°C water bath did not lose equilibrium or die during the 5 d of testing. The 10 fish plunged into the 4°C water bath all experienced LOE during the 5 d period (Figure 3-2). Within the first 15 min of being transferred from the holding tank to the 4°C water bath, 8 of 10 had lost equilibrium. The remaining two fish experienced LOE during the 24-48 hr period. Two fish died on the third day of testing and one fish on the fourth day. The fact that the water warmed to 24°C (9°C higher than the acclimation temperature) during the recovery period may have contributed to the three mortalities. The seven remaining fish recovered (i.e., regained equilibrium) and survived the 5 d of testing.

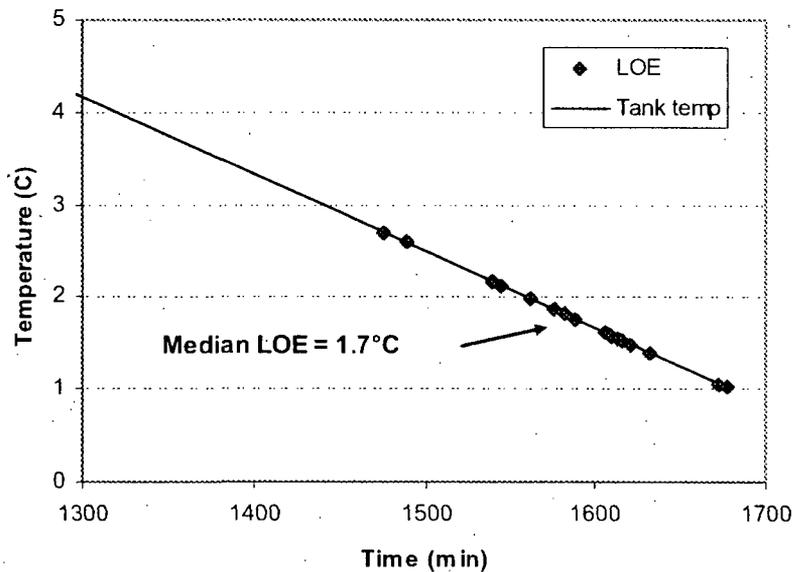


Figure 3-1
Time and temperature of LOE of 22 gizzard shad exposed to cold shock at a rate of 0.5°C/hr and acclimation temperature of 15°C.

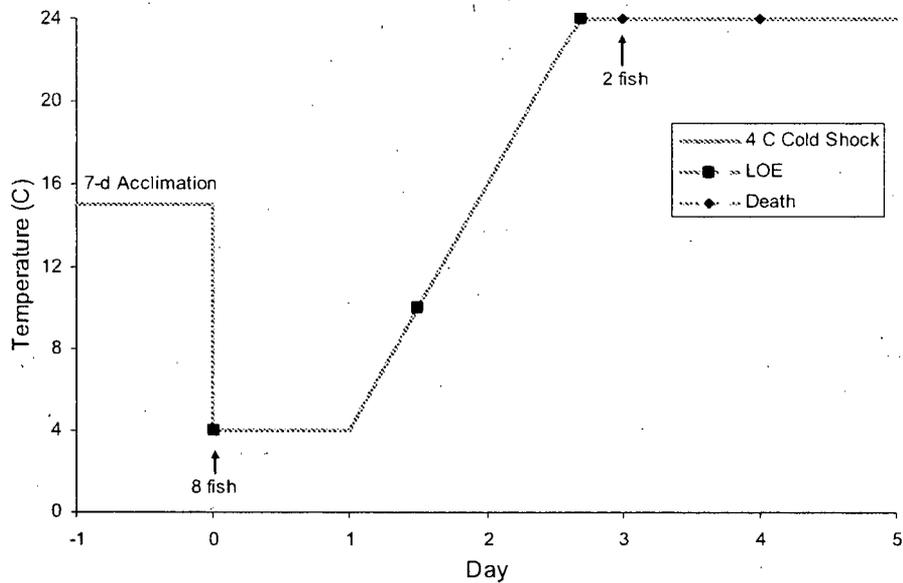


Figure 3-2
 Time of LOE and death for 10 gizzard shad acclimated to 15°C then plunged into 4°C water bath for 24 hr and then warmed at room temperature over a 5-d period.

Results—Threadfin shad

Critical Thermal Minimum Determination and Recovery

Several anecdotal signs of distress were observed in threadfin shad during the cold shock treatment. Individuals began to swim out of sequence rather than in a school, often swimming into the side of the tank. Although the general activity level of these fish appeared to increase as temperatures decreased, there was little direct response to vibration and netting at 8.5°C. The median LOE temperature for threadfin shad exposed to cold shock at 0.5°C/hr was 4.8°C and ranged from 4.6 to 6.4°C (Figure 3-3). All threadfin shad recovered as water was warmed within the recovery aquaria. On average, recovery occurred at 7.5°C, 2.5°C above the average LOE temperature.

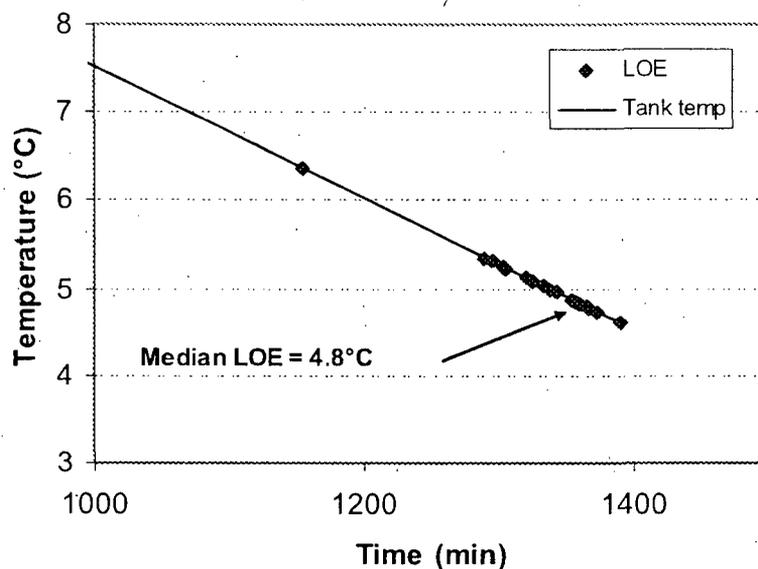


Figure 3-3
Time and temperature of LOE of 20 threadfin shad exposed to cold shock at a rate of 0.5°C/hr and acclimation temperature of 15°C.

Discussion

The CTMin value we determined for gizzard shad is consistent with those reported by others, with one exception. We found the CTMin for gizzard shad to be 1.7°C. Neumann et al. (1977) reported survival at temperatures below 1°C for a short period, and Heidinger (1983) suggested that mortality occurs in gizzard shad at temperatures between 0 and 4°C. Cox and Coutant (1976) performed acute cold shock testing with gizzard shad acclimated at 15°C and reported a CTMin of 6°C whereas we observed no LOE at that temperature when applying similar methods. The CTMin values for gizzard shad exposed to gradual cold shock and instantaneous cold shock varied. Gizzard shad exposed to acute cold shock experienced LOE at 4°C whereas LOE did not begin to occur until 2.7°C under gradual cold shock. Some individuals did not lose equilibrium until 1°C. Obviously, the rate of temperature change affects the CTMin of gizzard shad.

We found the CTMin for threadfin shad to be 4.8°C. Griffith (1978) found CTMin values for threadfin shad between 4 and 6°C with mortality of the least tolerant at low temperatures as high as 9°C and 100% mortality by 4°C. Similarly, McLean et al. (1985) reported impingement of threadfin shad increased significantly when water temperatures dropped below 7°C. Threadfin shad were not exposed to instantaneous drops in temperature, therefore the relationship is still unclear for this species. However, Griffith (1978) reported a CTMin similar to ours for threadfin shad using a rate of change of 1.0°C/hr compared to our 0.5°C/hr.

Signs of behavioral distress during cold shock prior to LOE or death in threadfin shad have been reported by other investigators. Griffith (1978) found that threadfin shad exposed to acute temperature declines began showing signs of behavioral distress as much as 5°C higher than

lethal temperature, and he observed a lack of response to movement and vibration at 6-7°C above lethal temperature. Griffith and Tomljanovich (1975) reported moribund threadfin shad exposed to 1-4°C temperature declines in 4 hr swam individually rather than in schools prior to LOE. These observations are consistent with the anecdotal signs of distress in gizzard and threadfin shad we observed.

Various acclimation temperatures were not tested to determine how the CTMin was affected for either species, although we know that CTMin generally declines with acclimation temperature to a point. Cox and Coutant (1976) reported that the timing of equilibrium loss for gizzard shad was a function of exposure and acclimation temperature. However, the variation in CTMin was less than 1°C at the three acclimation temperatures of 15, 17.5, and 20°C tested by Cox and Coutant (1976).

Threadfin and gizzard shad that experienced LOE under the gradual cold shock recovered when exposed to warmer water. We found 100 % survival regardless of the CTMin temperature for any given individual. However, we did not monitor long term survival, and we declared recovery if equilibrium was regained for greater than 15 min. Griffith (1978) also reported threadfin shad were capable of recovery if placed into water 3.0°C above an individual's CTMin but survival was not 100 %; fish with the lowest temperature tolerance had greater survival. The recovery ability of gizzard shad after reaching their CTMin has not been previously reported for comparison. However, gizzard shad are the more temperature tolerant species and would be expected to recover under colder conditions than threadfin shad.

In summary, the CTMin of gizzard shad appears to be between 1.5 and 4°C depending on the acclimation temperature and possibly other factors. Rate of temperature decline is an important factor when determining the CTMin for gizzard shad. Acute temperature drops yielded CTMin values several degrees warmer than those resulting from gradual cold stress. This is important considering that many natural die-offs are the result of strong cold fronts chilling water bodies quickly. Rates of change greater than 1.0°C/hr appear necessary to affect the CTMin of gizzard or threadfin shad. If water temperatures drop below 6°C, threadfin shad may begin to lose equilibrium, regardless of the rate of temperature decline. If temperatures drop below 3°C, gizzard shad may also become susceptible. These values can be used to make general rules about assigning the relative role of natural mortality during winter impingement events. Power plant managers should monitor water temperatures in the vicinity of water intakes for these critical limits. If critically low ambient water temperatures are imminent, the impingement of threadfin and gizzard shad might be reduced by altering plant operations.

This study has shown the importance of differentiating between moribund, impaired, and unimpaired fish relative to susceptibility to impingement. By definition, it is assumed that moribund fish would not recover and would die regardless of impingement, whereas impaired fish (such as those stressed by cold water temperatures in our experiment) would not necessarily die due to natural causes, but their condition may lead to death via impingement. Similarly, impaired shad are more susceptible than healthy shad to natural predation. As an analogy to natural predation, power plants could be considered selective predators that remove weak individuals from the population that would have been removed by natural predators. Several studies have related cold shock to increased predation rates in fish (Coutant et al. 1974; Coutant et al. 1976; Wolters and Coutant 1976). Best professional judgment of the permitting authorities (or future USEPA regulations for Phase II facilities) will apparently allow the estimation of

impingement losses to account for moribund fish (EPA 2006). However, further scientific evidence would be useful to clarify the natural environmental fate of shad impaired by cold shock.

4

ASSESSING COLD SHOCK EFFECTS THROUGH PERFORMANCE AND PHYSIOLOGICAL RESPONSE

Introduction

In the NPDES permitting process for cooling water intake structures, best professional judgment of the permitting authorities (or future USEPA regulations for Phase II facilities) may allow the estimation of impingement losses to be corrected for moribund fish. Presently, moribund fish entering the intake are identified by observational or visual criteria. However, observation of general fish behavior as an indicator of prior impairment may be misleading; cold-stressed shad may increase their activity level, even though swimming is impaired and susceptibility to impingement is increased. Quantification of other parameters, such as the fish's physiological state or particular components of its behavior, may be useful techniques for evaluating the influence of natural environmental conditions on impingement.

Impingement likely increases when shad are subjected to temperatures that affect their physiological function and performance. Increased susceptibility to impingement occurs at some point above the LOE temperature for both gizzard shad and threadfin shad, and may be an indicator of natural mortality in the water body induced by cold shock. The premise for the studies reported in this chapter is that the level of acute and chronic cold stress prior to LOE can be quantified using physiological-bioindicators, and that these stress responses are related to moribundity.

The use of plasma cortisol and chloride to quantify sub-lethal stress responses in fish is well established (Barton et al. 2002, Strange and Schreck 1978). White et al. (1986) used plasma cortisol and chloride to quantify stress response to cold shock in gizzard shad. Reduced ration has also been shown to affect natural mortality in gizzard shad (Adams et al. 1985). Lipids are typically stored during periods of high food availability (summer and fall) and utilized during periods of low food availability or non-feeding periods (winter and early spring) (Adams 1999). The influence of feeding at cold temperatures and duration of starvation on susceptibility to impingement has not been investigated. Bodola (1966) reported that gizzard shad discontinued feeding at 11°C. Both gizzard and threadfin shad are lethargic during cold periods, but the energy demand to maintain physiological homeostasis continues, which requires the utilization of energy reserves if feeding has ceased. The physiological condition of fish, quantified using various bioindicators of nutrition, could reveal the role of ration in natural mortality. Hematocrit, triglycerides, and total protein have been used as general indicators of nutrition and starvation in fish (Adams et al. 1985, 1992; Barton et al. 2002). The condition factor (K), an index that relates weight and length, reflects energy storage and metabolism due to starvation (Dutil et al. 2003).

Swimming performance or endurance is a useful behavioral measurement for relating physiological condition to impingement. Griffith and Tomljanovich (1975) used swimming performance to determine the ability of cold-shocked threadfin shad to avoid impingement and found high impingement mortality below 8°C. Martinez et al. (2004) demonstrated that starved Atlantic cod (*Gadus morhua*) exhibited a reduced swimming endurance compared to cod that had been fed. However, the combined effects of reduced ration and cold shock on swimming performance have not been investigated.

The challenge for environmental managers and regulators is to determine whether fish impinged on intake screens would have died anyway because of natural environmental conditions. Bioassessment techniques can be used to reveal the effects of suboptimal environmental conditions on swimming performance and physiological state. When coupled with onsite observations of water temperatures, wind speed and direction, and fish condition, this performance and physiological response data may help explain the causes of impingement events. To assist in assessing the causes of impingement, a bioassessment tool or simplified procedure is required that can quantify the stress condition of gizzard and threadfin shad as they become vulnerable to impingement.

In this regard, the primary objectives of this study were to: 1) identify the critical points where cold shock and reduced ration affect the ability of gizzard and threadfin shad to escape impingement and 2) identify physiological and performance indicators that may indicate increased susceptibility of gizzard and threadfin shad to impingement.

Methods

Fish Collection and Care

Gizzard and threadfin shad were collected by electrofishing from August to October 2005 on the Clinch River, Tennessee. Water temperatures ranged from 20-28°C. Live shad were transported to Oak Ridge National Laboratory in 151-L barrels equipped with aerators and treated with 400 g of sodium chloride. Shad were then held at 24°C for 3 to 5 days in 889-L circular tanks. Each tank was equipped with an aerator and a constant 0.6 L/min through-flow was maintained. Shad were acclimated to feeding on frozen brine shrimp and laboratory conditions during this period.

General Methods

Following the 3-5 day acclimation, test fish were transferred to 530-L rectangular tanks receiving 0.25 L/min of flow in groups of either 34 gizzard shad (mean total length = 153 mm, weight = 30 g) or 45 threadfin shad (mean length = 134 mm, weight = 17 g) per tank. The number of individuals in each test group exceeded the number required for testing to allow for mortality during acclimation. Portable refrigeration units paired with temperature controllers were used to regulate exposure temperatures within $\pm 0.2^\circ\text{C}$. Each group was acclimated for one week at $15 \pm 0.2^\circ\text{C}$ prior to testing in one of two general treatments, either cold shock alone or a combination of reduced ration with cold shock. The protocol used for the two experiments is illustrated in Figure 4-1. A single test group for each experiment was repeated for each species.

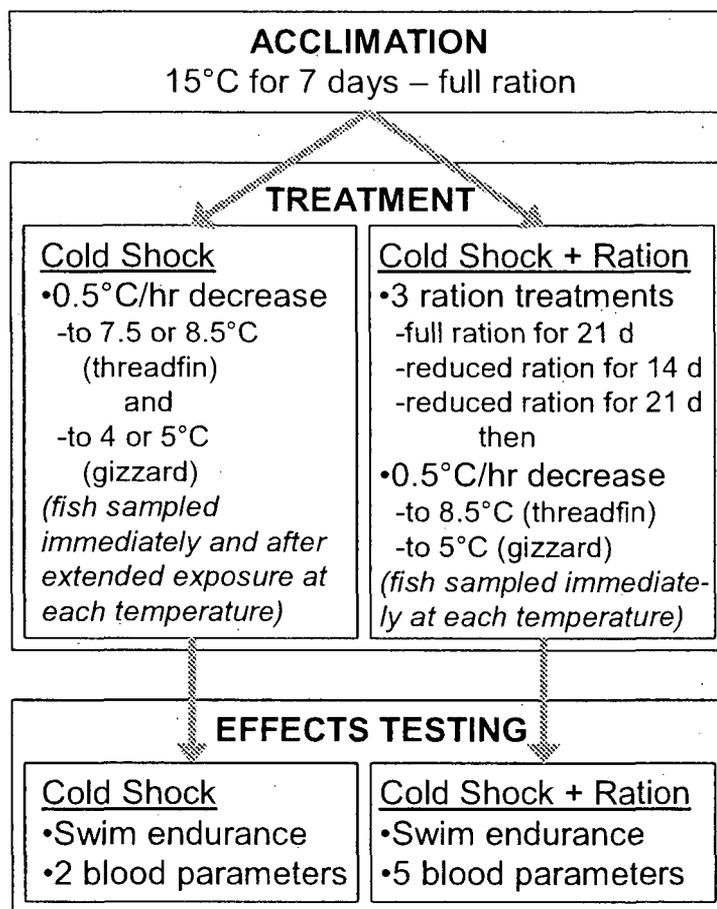


Figure 4-1
Summary of the protocol used during cold shock and reduced ration experiments.

At the time of testing, 18 gizzard or 24 threadfin shad were removed from their exposure tanks for blood collection. Shad were quickly removed from the exposure tanks with small dip nets to minimize handling stress and immediately anesthetized with tricaine methanesulfonate (MS-222). Three shad were removed at a time and bled within 2 to 4 min of being placed in MS-222. Fish were bled using 21G1 Vacutainer™ needles paired with 13 X 75 mm heparinized Kendall™ collection tubes. Total length (mm) and weight (g) were measured for each individual. Within a test, blood samples were randomly pooled, due to the low volume of serum derived from individuals, to form 6 pooled groups of 3 gizzard or 4 threadfin shad for each treatment. Threadfin shad were smaller than gizzard shad so more individuals were needed per pooled group. Hematocrit was measured for each pooled sample. Plasma was separated from whole blood by centrifugation, transferred to 1.5-mL cryotubes, and stored in liquid nitrogen until analysis. Plasma cortisol concentration was determined via Coat-A-Count® solid-phase 125I-cortisol radioimmunoassay. Plasma chloride was determined using a spectrophotometric assay by Pointe Scientific™.

For swimming performance tests, 10 gizzard or threadfin shad were removed from treatment tanks (the same tanks from which the fish to be bled were taken) and placed into the corral area

of the swimming performance (test) channel (Figure 4-2). The dimensions of the test channel and methods of the test allowed 5 fish to be tested simultaneously. The test channel was maintained at the target temperature using portable refrigeration units. The flow (~0.15 m/s) in the test channel was produced by a ¾ horsepower centrifugal pump. Water was pumped from the corral zone and introduced to the upper end of the test channel through a series of increasing-diameter pipes and a 0.32-cm mesh screen to even the flow distribution within the test zone of the channel. The performance test channel was 10.8 cm wide X 122 cm long. Water depth was held at 14.6 cm. The power to the pump was surged 3-4 times to allow the fish to orient upstream and gain swimming balance prior to initiating full flow velocity.

Each individual was observed during a maximum period of 1 hr to determine if impingement occurred at the rear screen for > 15 s. Impinged fish were removed immediately, and total swim time (≤ 1 hr), total length (mm), and weight (g) of each individual were recorded. Condition factor ($\text{weight} / (\text{length}^3) * 1000$) was calculated for individuals.

Statistical analyses on all data were performed using SAS, version 9.1, and SPSS, version 14. A value of $P < 0.05$ was considered significant for all tests and simultaneous confidence was held at $P = 0.05$ for all *post hoc* tests. Correlations between variables were investigated using Pearson correlation coefficients. Differences between test groups in swimming performance and physiological indicators were analyzed with analysis of variance (ANOVA). The Shapiro-Wilk statistic was used to test the assumption of normally distributed errors. If data were not normal, a natural log transformation of the dependent variable or ranked data was used in the ANOVA. Homogeneity of variance between treatments was assessed with Levene's test. If significant differences in mean values were indicated by the ANOVA F test, paired means were evaluated using the least significant difference (LSD) test. Dunnett's mean separation test for unequal group variances was used when heterogeneous group variances exceeded a 3-fold difference between any treatment pair (van Belle 2002).

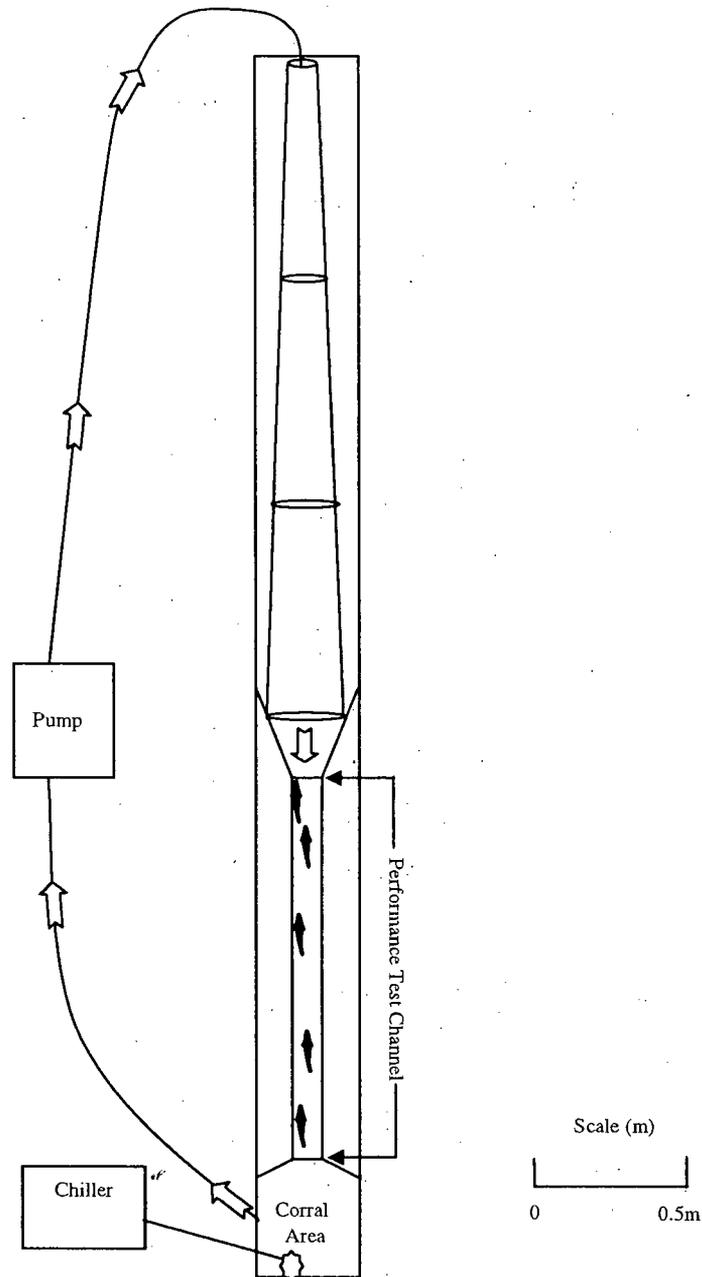


Figure 4-2
Schematic of the swimming performance channel (top view).

Experiment 1. Effects of Cold Shock on Swimming Performance and Physiological Condition

The CTMin results reported in Chapter 3 were used to determine the cold shock treatment temperatures for the swimming performance and physiological state experiments (Fost 2006). Cold shock was induced by decreasing temperature at $0.5^{\circ}\text{C}/\text{hr}$ starting at the acclimation temperature of 15°C and concluding at one of two target temperatures, either 4 or 5°C . We also

tested gizzard shad that were held for an additional 6 hr after reaching the target temperatures to determine the effect of extended or prolonged exposure at those temperatures. We expected that extended exposure at a stressful temperature would either increase the level of thermal stress and be apparent in the bioindicators or, alternatively, provide some level of acclimation resulting in a less stressful response. Figure 4-3 illustrates the four different thermal scenarios tested for each species (plus 15°C controls).

We repeated one treatment for each species to evaluate experiment repeatability and determine if any changes occurred over the period of time fish collections were being made. The 5°C gizzard shad test group was repeated 4 weeks after the initial tests, and results were compared to the initial test group of the same thermal regime.

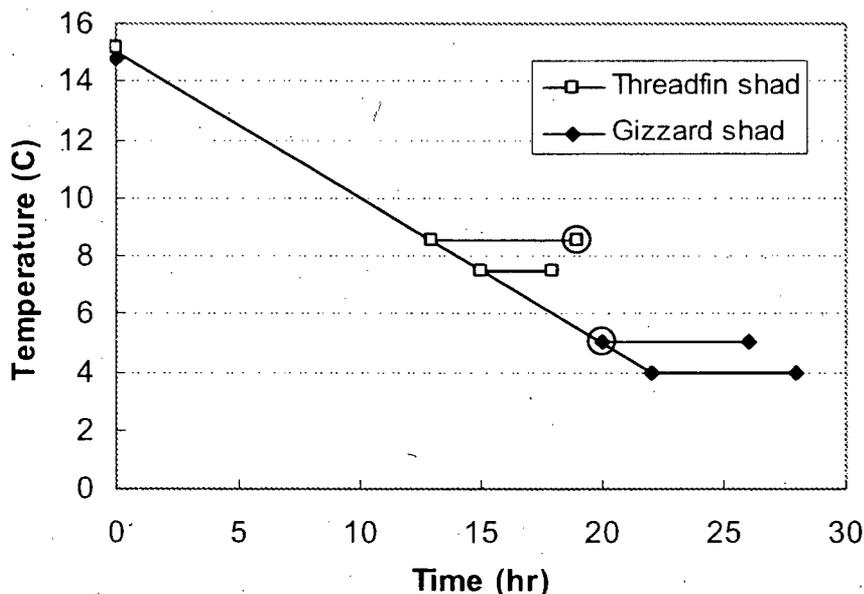


Figure 4-3
Thermal scenarios tested (lines) with points of sampling for both threadfin shad (n=24 at each square) and gizzard shad (n=18 gizzard shad at each diamond). Repeated trials are indicated by open circles.

Cold shock experimentation with threadfin shad was the same as that for gizzard shad except that target temperatures were 7.5° and 8.5°C (Figure 4-3). Two groups of threadfin shad were held for extended exposure at 8.5°C (6 hr) and 7.5°C (3 hr) after the initial temperature decline. We repeated the 8.5°C extended treatment 3 weeks after the initial test and the results were averaged with the initial treatment used for comparison. Controls for both species were sampled at the acclimation temperature of 15°C.

Fish were monitored for abnormal behavior or signs of distress during the cold exposure. Plasma cortisol (ng/mL) and chloride (mEq/L) levels in each pooled group were measured to determine acute stress response to the test temperatures. Swim tests were also performed on the same pool of fish but not the same fish to determine if swimming performance was related to physiological condition. Condition factor was calculated for each individual to determine if swimming performance was also correlated with condition.

Experiment 2. Effects of Combined Cold Shock and Reduced Ration on Swimming Performance and Physiological Condition

Each test group was fed a reduced ration of 0.5 % of their mass in frozen brine shrimp for 14 and 21 days. Following these 14 and 21 d feeding periods, the control groups were fed 5 % of their mass in frozen brine shrimp for 14 days. Gizzard shad were cold shocked to a temperature of 5°C and threadfin shad to a temperature of 8.5°C at a rate of 0.5°C/hr from 15°C. The entire 21-day reduced ration group was repeated 24 hr after the initial for each species and the results were averaged with the initial treatments for comparison. The test groups were observed for changes in swimming activity during the reduced ration period.

Following the reduced ration period and cold shock treatment, blood was withdrawn, and total length (mm) and weight (g) were measured for each individual. We measured plasma cortisol (ng/mL), chloride (mEq/L), total protein (mg/dL), triglycerides (mg/dL), and hematocrit (%) level in each pooled group. Total protein and triglycerides were determined using a centrifugal fast analyzer (Cobas brand). A separate group of fish from the same treatment tank was tested for swimming endurance.

In March 2006 we collected additional fish from the field to determine if the nutritional status of laboratory shad after 14 or 21 days of reduced ration was similar to that found in fish collected from the reservoir in late winter.

Results

Effects of Cold Shock on Swimming Performance and Physiological Condition

Gizzard Shad—

- As in the experiments in Chapter 3, we observed signs of distress (abnormal behavior) during the gizzard shad cold shock treatments. Activity levels decreased as temperatures approached 5°C and fish became totally lethargic (but upright) by 4°C. There was little startle response to vibration in the water and netting at 4°C.
- In swimming performance tests, cold-shocked gizzard shad had significantly lower mean swimming times for all treatment groups than the control ($P=0.005$; Figure 4-4). Mean swimming time was less in gizzard shad cold shocked to 4°C than to 5°C. Swimming performance of gizzard shad in extended test groups (referred to henceforth as '4°C Ext' and '5°C Ext') was not different statistically from fish sampled immediately upon reaching the temperature, but the pattern of decreased endurance with increased exposure to cold was consistent with the overall trend.
- Mean condition factor was not different among test groups and there was no correlation between condition factor and mean swimming performance for gizzard shad.

- Cold shock affected cortisol and chloride ($P < 0.001$) levels in gizzard shad. Mean plasma cortisol and chloride were significantly higher in all cold shocked groups compared to controls (Figure 4-4). Mean plasma cortisol was highest for the two treatments at 4°C (the lowest temperature tested), but mean plasma chloride did not differ among cold shock treatments.
- There was not a significant correlation between swimming performance and either cortisol ($R^2 = 0.62$; $p = 0.11$) or chloride ($R^2 = 0.56$; $p = 0.14$; Figure 4-5).

Threadfin Shad—

- As in the experiments in Chapter 3, we observed signs of distress in threadfin shad during the cold shock treatments at temperatures 2-3°C above the LOE. Individuals began to swim out of sequence rather than in a school, often swimming into the side of the tank. The activity level of these fish appeared to increase as temperatures decreased. There was little response to vibration and netting at 8.5°C and below.
- Cold shock had a significant effect on swimming performance of threadfin shad ($P < 0.01$). As with gizzard shad, the results show a clear trend of decreasing swim endurance with increasing exposure to cold (Figure 4-4).
- Mean condition factor did not differ among test groups, and, like gizzard shad, there was no correlation between condition factor and mean swimming performance for threadfin shad.
- Mean plasma cortisol was significantly lower in three of the four threadfin shad test groups compared to the control ($P < 0.001$; Figure 4-4). Plasma cortisol levels for the 7.5°C Ext test group were significantly lower than those sampled immediately (Figure 4-4).
- We found no correlation between swimming performance and cortisol ($R^2 = 0.28$; $p = 0.27$) or chloride ($R^2 = 0.0004$; $p = 0.975$; Figure 4-5). Mean plasma chloride was significantly different from the control for only two treatments, the 8.5°C Ext and the 7.5°C Ext ($P < 0.005$; Figure 4-4). The 7.5°C test group had the lowest mean chloride among the test groups (Figure 4-4).

Repeated Treatments for both Species—The gizzard shad repeated treatment did not differ from the initial group in swimming performance or mean chloride but did differ in mean cortisol (Table 4-1). The threadfin shad repeated treatment had a significantly longer mean swimming performance and lower mean cortisol compared to the initial group but no difference in mean chloride (Table 4-1).

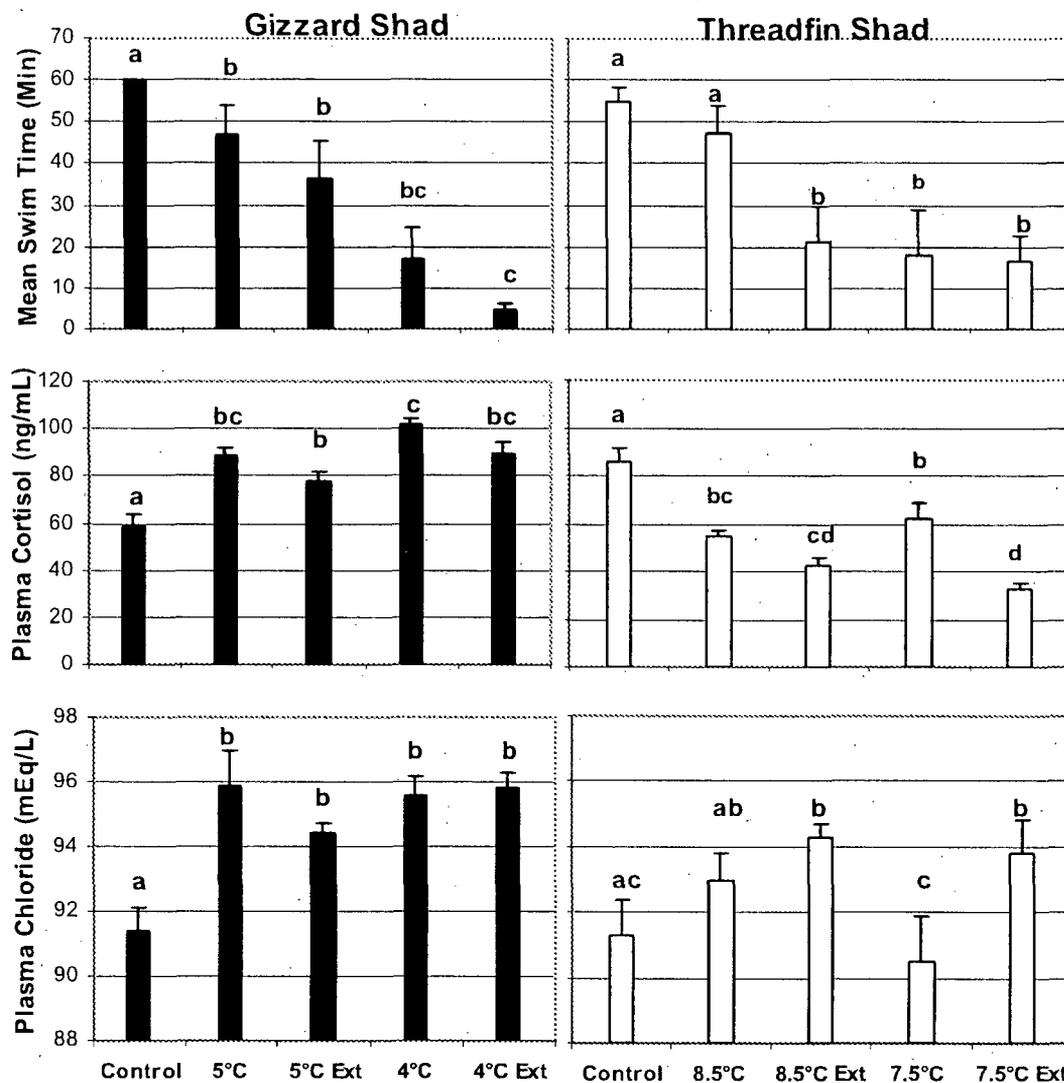


Figure 4-4
 Mean (+1 SE) swimming time, plasma cortisol, and plasma chloride of gizzard and threadfin shad exposed to cold shock treatment beginning at 15°C and declining at a rate of 0.5°C/hr to the test temperature. Gizzard shad were tested at 15°C (control), 5°C, after 6 hr at 5°C (5°C Ext), 4°C, and after 6 hr at 4°C (4°C Ext). Threadfin shad were tested at 15°C (control), 8.5°C, 8.5°C + 6 hr (8.5°C Ext), 7.5°C, and 7.5°C + 3 hr (7.5°C Ext). Treatments that are statistically different (P < 0.05) have different letters.

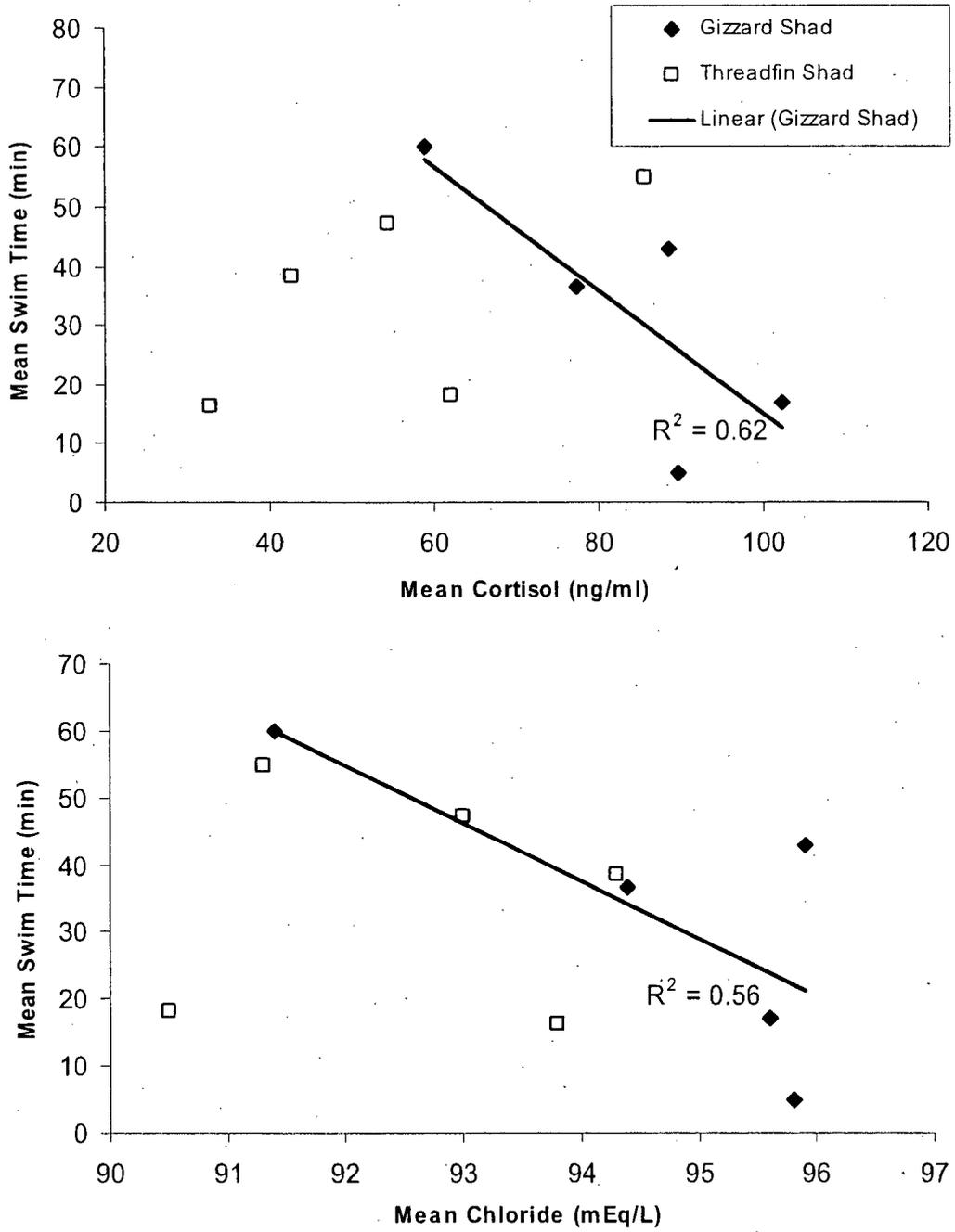


Figure 4-5
Linear correlations of mean cortisol and mean chloride to mean swim time of gizzard and threadfin shad.

Table 4-1

A comparison of several stress indicators (means) for original and repeated test groups of gizzard and threadfin shad. The 5°C test group (cold shock) and the 21 d test group (reduced ration and cold shock) were repeated with gizzard shad. The 8.5°C Ext test group (cold shock) and the 21 d test group (reduced ration and cold shock) were repeated with threadfin shad. Significant differences ($P < 0.05$) between means are indicated by asterisks.

Dependent Variables (test group repeated)	Original Treatment	Repeat Treatment	P value
Cold Shock			
Gizzard Shad (5°C test group)			
Swim Time (min)	42.33	43.60	0.9382
Cortisol (mEq/L)	88.60	69.52	<0.0001*
Chloride (ng/ml)	95.92	93.90	0.0871
Threadfin Shad (8.5°C Ext test group)			
Swim Time	22.32	54.56	0.0049*
Cortisol	42.63	21.64	0.0001*
Chloride	94.28	94.25	0.9568
Reduced Ration + Cold Shock			
Gizzard Shad (21 d test group)			
Swim Time	60.00	37.85	0.0250*
Cortisol	93.95	71.08	<0.0001*
Chloride	96.50	96.23	0.8498
Total Protein (mg/Dl)	95.83	97.50	0.4605
Triglycerides (mg/Dl)	83.42	81.42	0.5877
Hematocrit (%)	21.33	21.00	0.3960
Condition Factor	8.64	8.35	0.8743
Threadfin Shad (21 d test group)			
Swim Time	51.28	55.40	0.3722
Cortisol	44.54	50.51	0.0400*
Chloride	93.42	91.44	0.1453
Total Protein	70.00	72.50	0.1670
Triglycerides	27.00	45.08	0.0008*
Hematocrit	15.67	16.00	0.2891
Condition Factor	7.24	7.21	0.1248

Effects of Combined Cold Shock and Reduced Ration on Swimming Performance and Physiological Condition

Gizzard Shad—

- Gizzard shad generally remained active during the treatment periods (14 or 21 d) of reduced ration.

- Groups fed a reduced ration did not have significantly different mean swimming performance after cold shock than fish fed a full ration ($P=0.69$; Figure 4-6). A treatment effect of reduced ration was observed for both cortisol and chloride ($P<0.001$).
- Mean cortisol for the 14-d reduced ration group was higher than both the 21-d reduced ration group and control (Figure 4-6, Table 4-2).
- Mean plasma chloride values for both reduced ration groups were lower than the control.
- Treatment effects were also observed in total protein ($P<0.01$), triglycerides ($P<0.0001$), and condition factor ($P<0.01$; Figure 4-7).
- Mean plasma total protein was significantly higher in the 21-d reduced-ration group than the 14-d reduced-ration group.
- Mean plasma triglycerides decreased between 14 and 21 d of reduced ration.
- Mean condition factor was lower in the 21-d group than control.
- Gizzard shad collected in March of 2006 had lower mean condition ($K=7.4$) than fall-collected fish held in the laboratory for 21-d of reduced ration ($K=8.1$).

Threadfin Shad—

- Threadfin shad schooled and remained active during the reduced-ration test period.
- Groups fed a reduced ration did not have significantly different swimming performance compared to controls ($P=0.61$; Figure 4-6).
- Treatment effects were not found with cortisol ($P=0.60$) or chloride ($P=0.08$; Figure 4-6).
- Treatment effects were observed for total protein ($P=0.0001$), triglycerides ($P<0.0001$), hematocrit ($P<0.0001$), and condition factor ($P<0.01$; Figure 4-7).
- Total protein was significantly lower in the reduced ration groups, but there was no difference between the two reduced ration groups.
- Triglycerides were significantly higher in the 14-d reduced-ration group than controls and 21-d reduced-ration group.
- Hematocrit was significantly lower in both reduced ration groups than the control with those held longest (21-d group) having the lowest hematocrit.
- Condition factor was lower in the 21-d reduced-ration group than the 14-d group or control, which were not different.
- As with gizzard shad, threadfin shad collected in March of 2006 had lower mean condition ($K=6.7$) than fall-collected fish after 21-d of reduced-ration ($K=6.9$).

Repeated Treatments for both Species—Mean swimming performance and mean cortisol were significantly lower in the repeated 21-d reduced ration group of gizzard shad compared to the initial 21-day reduced ration group (Table 4-1). No other differences were found between the repeated and initial group of gizzard shad. Higher mean cortisol and lower mean triglycerides were the only differences between the repeated and initial 21-d reduced ration groups of threadfin shad.

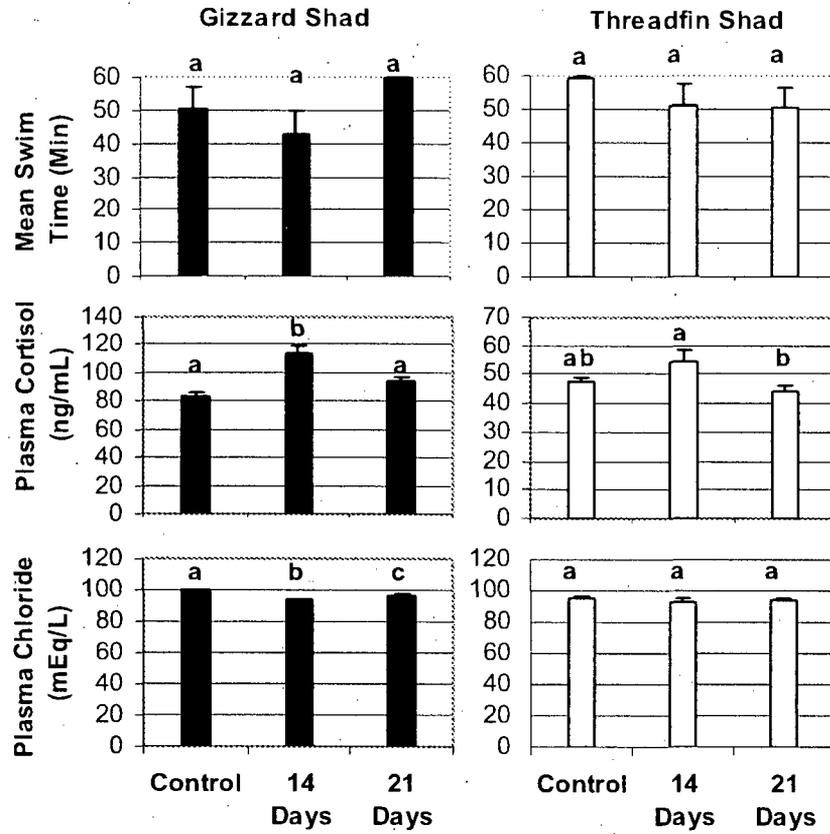


Figure 4-6
 Mean (+1 SE) swimming time, plasma cortisol, and plasma chloride of gizzard and threadfin shad exposed to cold shock after one of three protocols: 14 d of full ration, 14 d of reduced ration, or 21 d of reduced ration. Treatments that are statistically different ($P < 0.05$) have different letters.

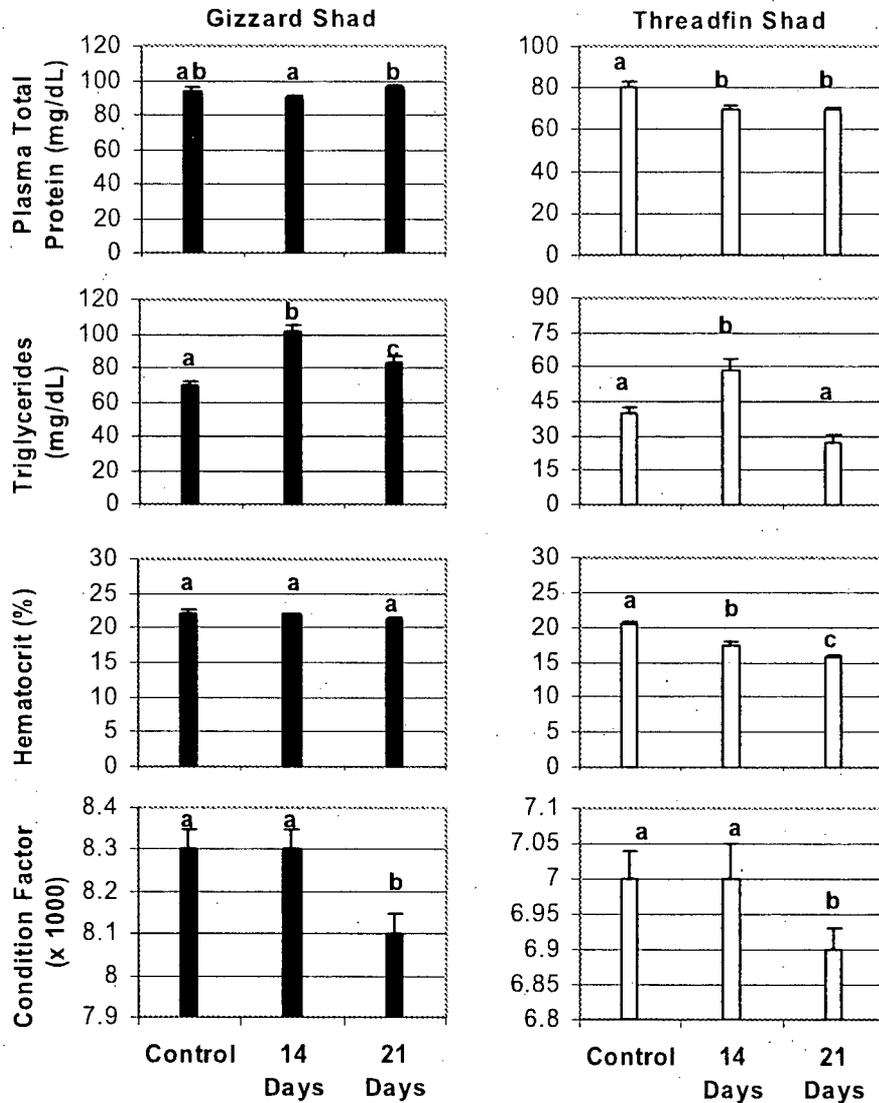


Figure 4-7
 Mean (+1 SE) condition factor, hematocrit, plasma total protein, and plasma triglycerides of gizzard and threadfin shad exposed to cold shock after one of three ration treatments: 14 d of full ration, 14 d of reduced ration, or 21 d of reduced ration. Treatments that are statistically different ($P < 0.05$) have different letters.

Discussion

The responses of gizzard and threadfin shad to cold shock alone and to a combination of starvation and cold shock were measured to gain insight into various factors that may contribute to impingement of these species at CWIS. Swimming endurance, cortisol, and chloride levels of gizzard shad and threadfin shad all responded to cold shock treatment, though not always as expected.

Swimming endurance of gizzard shad acclimated to 15°C was reduced at water temperatures of 4 to 5°C, suggesting that susceptibility to impingement likely increases at these temperatures and below. For threadfin shad, we did not find a statistically significant decrease in endurance at 8.5°C, but that appears to be the point below which we are likely to see effects. Significant effects were observed at a test exposure temperature of 7.5°C. Similarly, Griffith and Tomljanovich (1975) showed that the ability of threadfin shad to resist impingement was severely impaired at temperatures below 8°C, but at higher temperatures impingement was slightly or not at all impaired. The temperature at which threadfin shad were affected was warmer than for gizzard shad, making them more susceptible to cold-stress-related impingement when the two occur in the same water body. It is worth noting that most of the shad used in the control trials were able to sustain swimming for the maximum period of 60 min at a velocity of 0.5 ft/s, which is the velocity often used as design criteria for cooling water intake screens.

Cortisol was expected to increase as a response to cold-shock-induced stress, which has been observed in similar studies. Hyvarinen et al. (2004) found increasing cortisol levels when brown trout were cold shocked in an ice bath. In this study, we found the expected response for gizzard shad, but for threadfin shad, we found the opposite response. This may be due to a reduced ability of threadfin shad to mount a stress response under abnormally cold temperatures, as was reported by Strange (1980) for channel catfish. Davis (2004) also showed that fish held at colder temperatures had delayed responses in cortisol (and chloride) in comparison to those fish held at warmer temperatures. Alternatively, the lack of a statistically significant response by threadfin shad could be the result of the control group experiencing unknown stressors such as reduced water quality or handling effects that also elevated cortisol levels. The cortisol levels in the control groups for both species were higher than resting values reported for most fish species (0-50 ng/mL; Davis 2004); however, no published research involving shad cortisol levels was found for comparison. Because the control group had abnormally higher levels of cortisol compared to reported resting levels for other species, we suspect that the presence of an unknown stressor is the most likely explanation for the threadfin shad outcome.

Chloride levels in fish typically decline in response to a stressor. For example, Miles et al. (1974) observed decreases in plasma chloride in muskellunge (*Esox masquinongy*) resulting from capture and handling. However, we found in this experiment that levels usually increased with cold shock. Baseline chloride values were lower in all groups than those typically reported for unstressed fish (100-130 mEq/L; Barton et al. 2002). As with cortisol, there may have been an unaccounted for stressor that affected the fish while held in the laboratory. Additionally, the acclimation period of one week at 15°C may not have been sufficient to stabilize osmoregulatory function in this species, or perhaps laboratory confinement was more stressful for these species than those species tested by other investigators.

One objective of this study was to find indicators of susceptibility to impingement. We compared the bioindicator responses to swimming endurance and found cortisol and chloride in gizzard shad to be negatively correlated with endurance, which suggests that either of these could be potential indicators of susceptibility to impingement. In most species, chloride changes are the inverse of cortisol (Davis 2004), but in this case chloride response tracked that of cortisol. Johansen et al. (1994) found that when cortisol levels of rainbow trout increased above resting levels due to a stressor (toxicant), swimming performance decreased.

Prolonged exposure at cold temperatures seemed to worsen the effect on swimming endurance for both species. With extended exposure of 3-6 hr at a test temperature, we found no evidence of additional stress in cortisol and chloride measures. To the contrary, based on cortisol in gizzard shad and both cortisol and chloride in threadfin shad, there appeared to be acclimation or recovery from stress. Strange (1980) showed that channel catfish became acclimated to stress after several days and cortisol subsequently declined. Similarly, Strange and Schreck (1978) showed that cortisol levels in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) began to decrease 3.5 hr after a stressor was presented and removed.

Compromised nutritional status as a result of several weeks or months of reduced food availability has been correlated with impingement of fish in late winter and early spring (Adams et al. 1985). Previous studies on a variety of fish species indicated that swimming performance, total protein, triglycerides, hematocrit, and condition factor would decline as the duration of the starvation increased. Martinez et al. (2004) demonstrated that starved Atlantic cod (*Gadus morhua*) had reduced swimming performance compared to cod that were fed. McMillan and Houlihan (1991) reported rainbow trout having reduced serum total protein after several days of fasting. Ruane et al. (2002) compared triglyceride levels in common carp (*Cyprinus carpio*) fed different rations and found a direct relationship between triglyceride and ration. Adams et al. (1985) reported lower hematocrit and condition factor levels in stressed gizzard shad compared to unstressed shad. In our study, gizzard and threadfin shad showed little response in swimming endurance or in the short-term stress indicators (cortisol and chloride) after 14 and 21 d of reduced ration followed by cold shock. The treatments did result in lowered condition factor and lowered blood hematocrit levels. We did not find a relationship between swimming endurance and any of the physiological indicators that was common between the two species. Either the lack of feeding had no effect on the stress response or the period of starvation was not long enough to cause an observed affect. Since these species typically experience periods of low food availability in winter, possible adaptation to periods of reduced feeding could occur, therefore helping to explain, in part, the lack of a clear response in the lab to reduced ration.

To better understand the implications of reduced ration under natural conditions, we collected gizzard and threadfin shad from the Clinch River in March 2006 after a winter period when feeding was greatly reduced. These fish had significantly lower condition factors, (7.4 for gizzard and 6.7 for threadfin shad) than those we had collected during the summer and held for 21 d under reduced ration (8.1 for gizzard and 6.9 for threadfin shad). Therefore, even though the reduced ration period of 21 d in the laboratory resulted in poorer condition compared to controls, condition of fish in the lab did not quite approximate that of shad collected from the reservoir in late winter. If the condition of fish in the lab had been similar to that of shad collected in the field in late winter, greater declines in nutritional status indicators and swimming performance may have occurred in those fish subjected to cold shock treatment. Changes in hematocrit and condition factor were significant in threadfin shad but not gizzard shad after 21 d of reduced ration. This differential response between species is possibly due to gizzard shad storing proportionately greater amounts of lipids than threadfin shad (Adams 1999).

Hematocrit and condition factor are relatively simple indicators that could be used in the field to rapidly determine susceptibility to impingement. These are the types of rapid assessment indicators that might be used to detect impairment of fish prior to impingement. The applicability of these measures and other easily and rapidly applied indicators of susceptibility could be

further assessed by investigating physiology and swimming performance of shad whose condition replicates the condition of shad collected from the water body in late winter. Such laboratory studies could define the relationships between environmental stresses (cold shock or starvation) and responses of individual fish (e.g., hematocrit and condition) and subsequent changes in swimming performance and susceptibility to impingement. Once the relationships are established, field studies could be designed to predict the potential for large impingement events based on measurements of environmental or physiological state.

The overall results of this study indicate that rapid declines in temperature and cold temperatures, particularly those slightly above the temperatures of LOE, would render gizzard and threadfin shad more susceptible to impingement. Potential indicators of susceptibility to impingement have been identified in this study (e.g., hematocrit and condition factor) and could be performed on fish in the vicinity of intake structures as a preliminary assessment of the applicability of this assessment technique. The use of multiple indicators of stress helps detect and account for confounding stressors which may be present in natural ecosystems.

Using physiological and performance-level indicators to assess impingement susceptibility appears promising, but further studies are necessary to evaluate the relative importance of varying cold shock regimes and nutritional status to impingement susceptibility. More testing is needed for both species on the effects of:

- rate of temperature decline relative to the acclimation temperature,
- lower acclimation temperatures relative to the cold shock test temperatures, and
- a longer acclimation period prior to testing.

Further research addressing the role and importance of nutritional status on impingement susceptibility should include:

- longer starvation periods using fish collected in late winter,
- combining several different cold shock temperatures with the reduced ration treatment, and
- analysis of the physiological recovery of fish held under reduced ration and cold shocked.

This type of information would clarify the relationship between physiological indicators and susceptibility to impingement and increase capability for predicting and assessing impingement mortality.

5

SUMMARY

Summary of Results

The literature on mass mortalities of clupeid species reveals that such events are common, especially in larger freshwater lakes, rivers and reservoirs, and brackish and marine embayments. The principal reasons for such die-offs often vary among species. Research into the causes of this mortality as well as the general physiological responses of clupeids to potential environmental stressors is limited.

More reports on gizzard shad die-offs were found than for the other clupeid species, though this does not necessarily mean this species is affected more often or is more susceptible. Accompanying studies often provided detailed information on the physiological response of gizzard shad to thermal stress and provided clues to link mass mortalities to cold stress. Researchers found that the amount of stored lipids appears to play a role in determining winter kill of several clupeid species, but that parameter in itself is not an effective means of determining cold stress mortality in gizzard shad. Alewife die-offs seemed to be linked to severe winters, but there also appears to be a density-related factor. For example, greater mortality has been observed in a less-severe winter when population density was high than in a severe winter with lower alewife densities. Threadfin shad are native to the southern United States, but have been introduced as a forage base to many higher latitude states where they commonly suffer winter mortality. The inability to survive the winter in some locales has been used as a benefit by fisheries biologists trying to better control managed fish populations. Although mortalities are often severe following adverse conditions, this species has the ability to quickly repopulate a water body (McLean et al. 1980). Mass menhaden mortality appears most likely a result of a sudden change in water quality or disease.

An underlying hypothesis of the laboratory experiments presented in this report is that fish that become impinged represent a portion of the population that is in some way compromised or weaker than individuals that are not impinged. Our studies were not designed to identify the survivors or the fit individuals, but to better understand the lack of fitness of those that are most susceptible to impingement.

In studies on lower critical thermal limits, we found that threadfin shad acclimated at 15°C began to exhibit loss of equilibrium at 6.0°C, with a CTMin (median LOE) of 4.8°C. The first gizzard shad to lose equilibrium occurred at a lower temperature, 2.7°C, with a CTMin of 1.8°C. Shad acclimated to temperatures <15°C would probably have slightly lower CTMins, and exposure to a different rate of temperature change would also have an effect on the CTMin. To investigate the response to the most extreme rate of temperature change, we plunged gizzard shad

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acclimated at 15°C directly into water at either 4 or 6°C. No fish lost equilibrium at 6°C and all fish eventually lost equilibrium when plunged into 4°C water.

An interesting finding in our study related to the ability of gizzard shad to recover from cold shock. For fish that lost equilibrium during a gradual drop in temperature, apparent recovery occurred within 15 min of being placed into water that was no more than 2°C higher than the temperature at which they experienced LOE. It is worth noting that the length of time that fish were in a state of disequilibrium prior to being exposed to warmer temperatures during the experiment was short (on the order of a few minutes). A longer exposure to debilitating temperatures before warming is more likely in the field, which would likely reduce the rate of recovery. For those that lost equilibrium during the plunge experiments, 80 % eventually recovered when the water temperature was slowly raised over the next several days. For those fish that did not recover, mortality did not happen immediately, but occurred 3-4 d after the initial plunge.

The results of the CTMin and recovery experiments suggest that determining when a gizzard or threadfin shad becomes moribund is not straightforward, because under some circumstances fish that appear debilitated might just be temporarily impaired and might recover. These distinctions are important when trying to evaluate the relative contributions of natural causes and CWIS to fish impingement and mortality.

We also reported here on studies of the responses of gizzard and threadfin shad to cold shock alone and to a combination of starvation and cold shock to gain insight into factors that contribute to impingement of these species at CWIS. We expected that shad acclimated at 15°C would experience effects on swimming endurance as temperature fell below 8°C (for threadfin shad) and below 5°C (for gizzard shad). These values are about 3°C above the CTMin values for these species that we determined in separate experiments, and about 1-2°C above the point at which we observed apparent recovery from LOE in static water tanks. In an effort to simulate late winter nutritional status we also subjected some fish to 2-3 weeks of near starvation, but found that this did not produce an additional negative effect on swimming endurance. Subsequent field sampling suggests that a longer period of starvation is needed to simulate late-winter field conditions.

We evaluated two biochemical stress indicators for a response to cold shock alone and found that both blood serum cortisol and chloride responded to cold shock, but the responses were not consistent enough to conclude that these indicators would be useful for evaluating cold shock or predicting impingement susceptibility. The results are not easily interpreted because of the interacting effects of stress accumulation, acclimation, and recovery as well as a potential inability to mount a stress response as the organism's physiological systems become compromised due to continual stress exposure.

We examined the interaction between nutritional status and stress response by evaluating additional bioindicators – hematocrit, total protein, and triglycerides. For both shad species, hematocrit responded negatively with increased starvation, and triglycerides increased after 14 d starvation and then decreased after 7 more days. There was no relationship for either parameter with swimming endurance.

The overall results of this study confirm that cold temperatures and cold shock as a result of a rapid decline in temperature can render gizzard and threadfin shad more susceptible to impingement. Potential indicators of susceptibility to impingement have been identified in this study (e.g., hematocrit and condition factor) and could be analyzed for fish in the vicinity of intake structures as a preliminary assessment of the applicability of this assessment technique. The use of multiple indicators of stress may help to explain confounding stressors that may be present in natural ecosystems.

The results of this study can also be useful for identifying the environmental conditions under which one might expect that the cause of impingement is largely of natural origin. For example, when thermal regimes at a CWIS are similar to those that resulted in loss of equilibrium in laboratory experiments, we would expect that the bulk of impinged fish were not directly killed by impingement. By comparing historic temperatures at a site to those tested in the laboratory it might be possible to designate entire seasons when impingement is likely a result of primarily natural causes. Both of these approaches were recently proposed by Muscatine Power and approved by EPA Region VII as a means to assign likely cause of death during winter impingement events (USEPA 2006)

Future research needs

Field evaluations of the condition of fish (e.g., living, dead, moribund, recoverable) prior to impingement can be difficult, but power industry, regulatory agency, and resource agency personnel need to estimate the proportions of impinged fish that are already dead or dying when they become impinged. Laboratory studies of fish response to cold stress can help explain episodic impingement observations at operating power plants.

Using physiological and performance-level indicators to assess impingement susceptibility appears promising, but further studies are needed to evaluate the relative importance of cold shock and nutritional status on impingement. Future studies should be designed to address the following questions:

- Is there a difference between that fraction of a population, including a particular size and/or age groups, that gets impinged and the rest of the population?
- Are there bioindicators that can be used to predict when impingement is eminent or likely? Can they be readily measured at a reasonable cost?
- Similarly, can lab-based empirical results be used to predict which environmental conditions are likely to result in an impingement event?
- Are there indicators that can be used to evaluate the contribution of natural causes to impingement on intake screens?

Future testing on cold shock should include analyses of:

- rates of temperature decline,
- acclimation temperatures,
- acclimation durations, and

Summary

- various combinations of the above.

Further research addressing the role and importance of nutritional status on impingement susceptibility should include:

- laboratory experiments with longer starvation periods using fish collected in late winter,
- combining different cold shock temperatures with the reduced ration treatment, and
- analysis of nutritional bioindicators in fish collected from the field during different seasons and at different nutritional levels.

There are other indicators than those used in our study that might be good predictors of susceptibility to impingement. Future work on other bioindicators should focus on the following classes of indicators:

- **Performance:** This class includes tests that relate to a fish's ability to escape or avoid impingement and could include experiments that test swimming endurance (as presented in this report) and high speed video analysis of startle response.
- **Physiological condition:** In this report we used cortisol and chloride as indicators of exposure to a stressor. There are other indicators that could be used as indicators of organ dysfunction, osmoregulation, and carbohydrate and protein metabolism. In addition, there are new genomic and proteomic techniques that evaluate the suite of proteins manufactured by an organism in response to stress and how that suite changes in response to different stressors.
- **Nutritional/bioenergetic:** There are several indicators of nutritional status or general condition that might have a predictable relationship to impingement susceptibility or swimming performance (e.g., condition factor, liver-somatic index, visceral-somatic index, spleno-somatic index, serum triglycerides and cholesterol, and body lipids and phospholipids).

Such studies could clarify the relationships between physiological indicators and susceptibility to impingement, and thereby increase our ability to predict and assess impingement mortality. Laboratory data would help in the design of adequate field tests of these techniques, providing preliminary information on the numbers of fish to sample and the sampling frequency needed to determine background states and rates of change. Compared to our controlled experiments, field studies of the causes of increased susceptibility to impingement would need to account for complicating factors that might confound predictions from the laboratory-based responses. For example, water temperature changes in nature are rarely as uniform as those tested in these experiments, but rather exhibit decreases and increases in an irregular pattern. The presence of thermal refugia in the river or reservoir may protect fish from debilitating temperature decreases, resulting in fewer moribund or impaired fish than would be predicted from these studies. Thus, while it is recognized that measuring many of these physiological indicators as early warning signs of imminent episodic impingement events may not be practical at most power plants, the findings would provide possible explanations for the occurrences of such events.

Lastly, an alternative approach to finding bioindicators of susceptibility to impingement prior to impingement is one that identifies post-mortem indicators of health status prior to impingement (e.g., death or moribund). Candidate indicators could be those that indicate the cause of death or

those that indicate time of death. For example, quantifying how gill color and eye opacity change after death under different environmental conditions could be used to establish a time of death relative to time of impingement.

6

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APPENDIX

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Factors affecting abundance, growth, and survival of age-0 gizzard shad. Michaletz, P. H. 1997. **Transactions of the American Fisheries Society.** 126:84-100. Growth of YOY gizzard shad was positively correlated by temperature and to a lesser degree food availability. Early cohorts suffered higher mortalities than late cohorts.

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Dynamics of alewives in Lake Ontario following mass mortality. O'Gorman, R. and C. P. Schneider. 1986. *Transactions of the American Fisheries Society*. 115(1): 1-14. Alewife in Lake Ontario quickly recovered following the massive die-off in the winter of 1976-1977. Ability of the alewife population to quickly recover has been attributed to the longevity of survivors, high survival of yearlings, and the production of large year classes.

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Smith, S.E. 1968. Species succession and fishery exploitation in the Great Lakes. *Journal of the Fisheries Research Board of Canada* 25:667-693.

Effects of temperature on electrolyte balance and osmoregulation in the alewife (*Alosa pseudoharengus*) in fresh and sea water. Stanley, J. G. and P. J. Colby. 1971. *Transactions of the American Fisheries Society*. 4:624-638. Study researched the effects of cold temperature on electrolyte balance and osmoregulation in alewife. Cold stress caused ionoregulatory failure in the alewife studies. Large winter die-offs in Great Lakes may be a result of a failure to osmoregulate.

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Effects of alewives on zooplankton of Lake Wononskopomuc, Connecticut. Warshaw, S.J. 1972. *Limnology and Oceanography* 17:816-825. Details effect of alewife predation on zooplankton community in Lake Wononskopomuc, CT. Plankton community shifted to smaller forms eight years after alewife introduction, but reversed back to larger species after large alewife winter die-off.

Growth and survival of larval fishes: roles of competition and zooplankton abundance. Welker, M.T., C.L. Pierce, and D.H. Wahl. 1994. *Transactions of the American Fisheries Society* 123:703-717. Study assessed growth and survival of gizzard shad and bluegill at different population and food densities. Gizzard shad survival and growth were negatively correlated with gizzard shad density and positively correlated with macrozooplankton prey. Bluegill growth was positively correlated with prey availability.

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