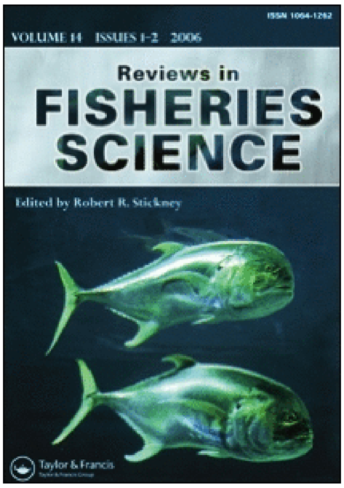

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Van T. La[a]; Steven J. Cooke[a]
[a] Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science, Carleton University, Ottawa, Ontario, Canada

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Advancing the Science and Practice of Fish Kill Investigations

VAN T. LA and STEVEN J. COOKE

Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science, Carleton University, Ottawa, Ontario, Canada

Occurrences of fish kills are increasing in aquatic ecosystems worldwide, and have been attributed to natural phenomena, as well as human modification and pollution of terrestrial and aquatic environments. Despite contemporary research activities, the science of fish kill investigations is still rudimentary and has advanced little since the 1960s. Here, we highlight the complexity of fish kills and provide a critical commentary on the key challenges that must be overcome in order to advance the science of fish kill investigation. Such challenges include recognizing the potential for carry-over effects, biotic factors, and multiple stressors when conducting fish kill investigations. We recommend an interdisciplinary approach that includes recent innovations in field physiology, functional genomics, and greater reliance on fish health professionals. We also recommend additional efforts to develop databases for tracking fish kills, as well as more attempts to publish fish kill studies in the peer-reviewed literature. The recommendations that we provide will advance our ability to identify fish kill causes, and consequently allow us to implement preventative measures to reduce the frequency and magnitude of fish kills worldwide.

Keywords fish kills, fisheries, fish mortality, conservation, management, aquatic systems

INTRODUCTION

Background and Context

Fish kills are a common phenomenon and are generally defined as localized mass die offs of fish that can occur in marine, estuarine, or freshwaters (Meyer and Barclay, 1990). A single fish kill event can number from several individuals to millions of dead fish. Frequent fish kills cause significant economic loss by reducing the population of recreationally and commercially valuable fish and as well as limit fish protein available for humans (Holmlund and Hammer, 1999). Other economic losses that result directly from fish kills include cleanup costs, and reduced tourism and recreation. Large fish kills also have a negative effect on regulation of food web dynamics and nutrient balance (see Holmlund and Hammer, 1999).

To highlight the severity of fish kills on the economy and the environment, we draw on three fish kill events in the Murray-Darling Basin, Australia that occurred from 2002–2004. These fish kills resulted in severe economic damage and significant loss of Murray cod (*Maccullochella peelii peelii*) (up to 3,000 individuals per event), a threatened keystone species, as well as the loss of other important recreational species such as trout cod (*Maccullochella macquariensis*) and silver perch (*Bidyanus biyanus*) (several thousand individuals per event) (Koehn, 2004). Immediate estimated costs associated with these fish kill events were 4–5.6 million AU dollars, not including the cost of prolonged fishery closures (Koehn, 2004). As a direct result of these events, the native population of fish in the Murray-Darling Basin are at 10% of their natural levels, and population recovery times per event are estimated to be up to 52 years (Koehn, 2004).

Unfortunately, fish kill events such as those in the Murray-Darling Basin are typical and occur worldwide. Most fish kill events are not isolated, and tend to recur often (Thronson and Quigg, 2008). For example, North Carolina reports approximately 30 fish kill events per year since 1996 (NCDWQ, 2000). Some fish kill events, especially those in smaller lakes and ponds, may remain unreported (Haslouer, 1979). For example, New South Wales reports an average of 34 fish kills per year, but actual numbers of fish kill events per year are estimated to exceed 60–80 (Koehn, 2004). The economic damage per fish kill compounded by the number of fish kills per year indicates

Address correspondence to Van T. La, Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario N1G 2W1, Canada. E-mail: vla@uoguelph.ca

Table 1 Examples of common proximate causes of fish kill events in freshwaters and estuaries

Cause	Definition[1]
Agricultural pollution	Pollution that pertains to pesticide, fertilizer and manure, silo and feedlot drainage, animal waste, etc.—can be direct or lead to other problems, such as hypoxia, as a result of biological oxygen demand
Acidification	Acidification by oxidation of sulphide minerals; can be delivered via precipitation (e.g., acid rain)
Biotoxin	Toxic algal and dinoflagellate blooms that are caused by *Karena brevis, Pfiestera,* etc.
Disease	Various bacteria, parasites, fungus, and viruses
Exhaustion	Physical exhaustion of fish typically leading to cardiac collapse (e.g., during challenging migration)
Extreme temperature changes	Rapid changes in temperature (e.g., cold shock)
Gas bubble trauma	Gas-supersaturation downstream from dams or other infrastructure or natural barriers
Industrial pollution	Pollution arising from various resource extraction, processing, and manufacturing activities (e.g., mining, food and kindred products, chemicals, metals, petroleum, and paper products)
Low dissolved oxygen (hypoxia or anoxia)	Low levels of oxygen in the water, usually associated with urban runoff, decay of organic material (i.e., biological oxygen demand), rainfall events, etc.
Municipal pollution	Pollution arising from refuse disposal, water system, swimming pools, power, and sewage systems
Transportation pollution	Pollution that pertains to rail, trucks, barge or boats, and pipeline ruptures
Unknown/undetermined	Fish kill events in which no cause can readily be determined

[1]Definitions were adapted from Thronson and Quigg (2008) and Haslouer (1979).

significant economic damage to fisheries, recreation, food web dynamics, and aquatic ecosystems. As a result, an understanding of the causes of fish kills and why they occur is fundamental in order to implement preventative measures to reduce their frequency and magnitude. Here, we provide what is not meant to be an exhaustive review of fish kills, but rather a critical commentary on the key challenges that need to be addressed in order to advance the science and practice of fish kill investigations.

The Causes of Fish Kills

Although fish kills can be of natural phenomena, human alteration and pollution of aquatic and terrestrial systems is increasing fish kill frequency and scale worldwide. Despite the fact that fish kills are common globally, few countries outside of North America have regional or national summaries of the frequency and magnitude of fish kills, and their potential causes. The only available summary reports that we are aware of outside of North America are a regional summary in New South Wales (Walsh et al., 2004; rivers), and a national summary in Ireland between 1983 and 1984 (Fahy, 1985). Of these fish kills, most were ultimately caused by anthropogenic activities, more specifically oxygen depletion from agricultural waste, ammonia toxicity, silage, and toxic sprays. Reported fish kills were also associated with high rainfall, when increased run-off into aquatic systems is most likely. In addition, irrigation practices likely concentrate toxic chemicals in the water, making them more harmful. Similar situations where fish kills have been attributed to anthropogenic activities outside of North America have been noted in Kenya (Ochumba, 1990), Norway (Leivestad and Muniz, 1976), and Brazil (Munoz et al., 1994).

In North America, regional summaries of fish kill causes in Missouri (see Czarnezki, 1983; freshwater), Kansas (Haslouer, 1979; Brunson, 1986; freshwater), Florida (Hoyer et al., 2009; freshwater), North Carolina (see NCDWQ, 2000; a combination of freshwater and estuarine), Texas (Thronson and Quigg, 2008; coastal waters), and a national summary for the United States between 1980 and 1989 (Lowe et al., 1991) reveal that only a small fraction of fish kills are caused by natural phenomena, which include extreme changes in seasonal temperatures such as winterkills and summerkills, disease, and parasites. These natural causes, however, can be modulated by human activities through global environmental alterations such as climate change. These regional summaries reveal that the majority of fish kills are caused by anthropogenic activities, which emanate from agricultural, industrial, municipal, and transportation-related activities. Specific causes include manure and pesticide application, chemical spills, hydropower operations, sewage, and eutrophication leading to harmful planktonic blooms (e.g., NCDWQ, 2000; Thronson and Quigg, 2008).

For additional context, we used a variety of search engines (Web of Science, Google Scholar) to locate all fish kill events that were summarized in both the peer-reviewed literature and technical reports for North American estuaries and freshwaters from 1890–2006 (excluding the summaries produced by Kansas, Missouri, Florida, and North Carolina to avoid regional bias) and identified 170 fish kill events. We determined that the major proximate causes of fish kills were agricultural pollution (19.5%), biotoxins (17.2%), and chemical pollution (7.1%) (see Table 1 for definitions). Minor causes were extreme changes in temperatures (5.9%), low dissolved oxygen (5.3%), gas bubble trauma (3.6%), disease (3.6%), exhaustion (2.4%), and acidification (1.2%). Overall, 66.9% of surveyed fish kills were ultimately caused by anthropogenic activities, while only 10.1% were caused by natural events. Although this search was not exhaustive or global in focus, it demonstrates that fish kills in North America are primarily caused by anthropogenic activities. This strongly suggests that with increasing human activities, we will expect to see more fish kills. Also remarkable is how 23% of North American fish kill causes were undetermined. Previous summaries such as those in New South Wales and Ireland

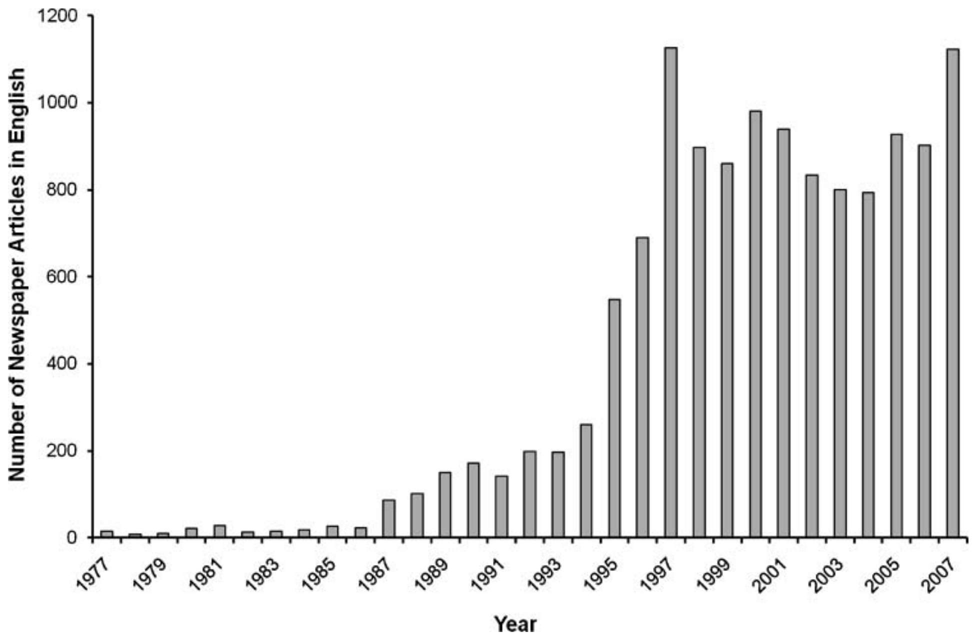

Figure 1 Number of newspaper articles printed in English using a power search with keyword "fish kills" on LexisNexis from 1977–2007.

indicate that anthropogenic activities are also a leading cause of fish kills in other regions of the world, demonstrating that the causes of fish kills in North America are not unique. During this exercise it became apparent how few fish kill case studies were available in peer-reviewed outlets and how sufficient scientific information was unavailable to support fish kill investigations.

Public Interest and Perception on Fish Kills

We attempted to determine trends and patterns in media reports of fish kills to establish public interest and perceptions on this topic. We used the LexisNexis search engine and searched for the term "fish kills" from 1977–2007 to find the number of fish kill articles in newspapers printed in English per year worldwide (Figure 1). Although we focused on newspaper articles printed in English, these articles are representative of over 350 major newspapers across North America, Europe, Australia, Asia, and Africa. In general, from 1977 through the mid 1980's there were relatively few fish kills reported in the media ($<$ 50/year). Between 1987 and through 1994, the number of reports rose steadily to over 200/year. By 1997, there were over 800 media reports on fish kills appearing annually through to 2007 when we terminated the literature search. Clearly, the frequency of fish kills that are discussed in the media has increased over the past 30 years, reflecting an increase in public scrutiny and demand for determining the causes of fish kills and reducing their occurrence. There is little evidence, however, that the science and practice of fish kill investigation has paralleled the frequency of occurrence of these events or public concern.

ADVANCING THE SCIENCE OF FISH KILL INVESTIGATIONS

There are several documents routinely used by practitioners when investigating fish kills (i.e., Hill, 1983; Meyer and Barclay, 1990; Southwick and Loftus, 2003) but none provide extensive commentary on the challenges associated with their evaluation. In contrast, two earlier papers highlighted the challenges associated with determining the cause of fish kills (i.e., Wood, 1960; Burdick, 1965), however, there has been little discussion and discourse in the primary literature regarding the science of fish kills investigation. Improving the protocol of fish kill investigations with greater reliance on science to improve policy, management, and decision making is crucial considering the increase in fish kill events in recent years. Given this background, here we provide a critical commentary on the key challenges that must be overcome in order to advance the science of fish kill investigation, and ultimately, implement its prevention. Many of the examples that we use are from North America as most publications are from this region. However, we acknowledge that similar challenges likely occur worldwide and thus our recommendations should extend to other jurisdictions. Throughout we provide potential solutions for addressing these challenges.

Need for a Standard Definition of What Constitutes a "Fish Kill"

Currently, the definition of what constitutes a "fish kill" varies among practitioners and jurisdictions. For example, North Carolina defines a fish kill as an event that results in a minimum of 25 dead fish (NCDWQ, 2000), where Rhode Island does not have a minimum requirement (Rhode Island Department of Environmental Management, 2009). One of the more common definitions for "fish kill" is a "sudden and unexpected mass mortality of wild or cultured fish" (Lugg, 2000). Clearly, the phrase "mass mortality" is subjective. In order to consistently identify and respond to fish kills, a standard definition is required. Such a definition is important for compiling statistics and also in determining when to begin a formal investigation.

Here, we proffer a standard definition that can be used on a nationwide or global scale. We suggest that an event should be defined as a fish kill if the mortality event is (1) not part of the fishes' natural life cycle (e.g., mass mortality following spawning activity in semelparous fish); (2) if a minimum of 25 dead fish are found in one square kilometre (lentic) or river kilometre (lotic) and within a 48-hr period, and (3) if mortality was not caused by predation, including by humans (i.e., harvest).

We proffer the number 25 dead fish because it is a historically unobserved and statistically unlikely number compared to the number of dead fish that die of natural causes(i.e., old age) that one may expect to readily find during a 48-hr period (Schneider, 1998). Also, the minimum number of dead fish required to constitute a fish kill by various jurisdictions range from five dead fish (Australia; Commonwealth of Australia, 2007) to 25 dead fish (North Carolina; NCDWQ, 2000). We suggest that the number of dead fish in the upper limit of this range should be used in order to accommodate larger and more speciose water bodies found worldwide. We also suggest that the Food and Agriculture Organization of the United Nations, the World Organization of Animal Health, or the Association of World Fisheries Societies should take a leadership role in mandating a clear, standard fish kill definition with quantitative context and timeframe such as the one proposed here.

Need for Standard Protocols for Fish Kill Investigation

A major problem with current fish kill investigations is that there is no globally accepted standard protocol. Hill (1983), Meyer and Barclay (1990), and to a lesser extent, Southwick and Loftus (2003), provide a general framework for fish kill investigations. These protocols, particularly the ones in the Hill (1983) and Meyer and Barclay (1990) documents, are focused on "freshwater". Moreover, Meyer and Barclay (1990) is an out of print technical report and it is consequently difficult to obtain. A major concern is that most protocols cannot be easily adapted outside of the developed world. For example, Meyer and Barclay (1990) encourage practitioners to rely heavily upon analytical health laboratories with expertise in fish pathology. Although this is prudent, many jurisdictions do not have such facilities or capacity. The Food and Agricultural Organization of the United Nations, the World Organization of Animal Health, or the Association of World Fisheries Societies could take a leadership role in developing a standard protocol for investigations that could work in both developed and developing countries where there is a broad range of technological capacity.

The most advanced protocol that we were able to obtain is a national investigation and reporting protocol by the Commonwealth of Australia (2007), which includes sections on each phase of fish kill investigation and communication for all water body types. This detailed document sets out standard minimum requirements for each stage of management of a fish kill incident and includes useful flowcharts, information on sampling methods, the roles and responsibilities of officers assigned to fish kills investigation, and resources for investigating officers. We encourage other nations to follow Australia's example and create national standard protocols for fish kill investigation.

There is still a need, however, to adopt a standard fish kill investigation protocol that can be used on a global basis. Essentially, there could be a core investigative protocol that would apply broadly to all fish kill investigations with more specialized and detailed investigative options for when the capacity exists. We have modified a generalized fish kill investigation plan (adopted from Meyer and Barclay, 1990) to consider the current status and challenges for investigating fish kills (Table 2). This plan highlights opportunities for improvement across the common investigative phases and can aid in developing a standard protocol that can be used globally. Overall, standard investigative approaches would facilitate compilation and analysis of different fish kill reports enabling the detection of potential global trends.

Need for Clear and Simple Fish Kill Reporting Mechanisms

There is a need for a formal fish kills reporting strategy that is easily accessible and convenient for public use. Often it is a citizen, not a government official, that initially detects a fish kill, and as a result, there is a time-lag between fish kill notification and scientific investigation. A quick and efficient fish kill reporting strategy from public citizens to government officials is crucial to promptly direct investigators to the fish kill area to initiate investigations before valuable samples begin to degrade. However, formal fish kill forms are often tedious. For example, the government of Rhode Island has formal fish kill report forms that are available online, where each fish kill reporting citizen is expected to print the form, fill it out, and then submit it via mail or fax (State of Rhode Island, 2010). A similar form is also used in New South Wales, Australia (NSW Government, 2010). Such forms are often tedious, and require detailed information such as the specific species, condition, and size of the fish affected. We suspect that most citizens may not be willing to send such reports because it is too troublesome or time-consuming. In addition, mail and fax are inconvenient methods of sending information between parties in many regions worldwide compared to the internet and telephone.

We propose that future fish kill reporting strategies should use simple web-based submission procedures or government-paid central hotlines (e.g., Florida has a hotline; Florida Fish and Wildlife Conservation Commission, 2010a). Ideally, people should be able to report fish fills to existing central

Table 2 Generalized investigation steps in fish kill investigations (adopted from Meyer and Barclay, 1990), current status and challenges associated with each investigative phase, and opportunities for improvement

Generalized Investigation Steps	Current Status and Challenges	Opportunities for Improvement
Fish kill reported/suspected	• Reporting by fax, mail, or telephone by public • Can be tedious and time-consuming • Time lag between fish kill notification and scientific investigation	• Reporting by web-based submission or phone call using fish kill hotlines • Reduce time lag between fish kill notification and scientific investigation
Investigator designated	• Often regional biologist with little expertise on fish kill investigation	• Create training programs for two sets of investigators (1) First responder: general knowledge of fish kills and ability to take samples (2) Specialist: specialized in fish kill research and has knowledge of all assessment methods and investigative tools
Site visit for reconnaissance	• Few visits per kill; often delayed or non-existent • No standard protocol	• Implement long-term monitoring programs • Require more site-visits per kill immediately after fish kill is reported/suspected • Create standard protocol
Collect chemical, physical, and biological samples	• Often delayed resulting in degraded samples • Few samples taken • No control to compare pre-fish kill to post-fish kill conditions	• Implement long-term monitoring programs • Take more samples, taking into account biological variation • Consider using reference sites • Adopt interdisciplinary approach
Fish kill count to evaluate resource damage and monetary value assessment	• Often underestimated • Scavengers remove carcasses • Economist value assessments on species for compensation costs	• Take into account scavengers and difficulties in detecting dead fish • Continue value assessments of species and number of fish killed for compensation • Conduct studies to understand counting biases
Laboratory analysis and database mining	• Samples often degraded or limited; difficult for pathological analysis	• Collection of more samples immediately after fish kill reported • Adopt interdisciplinary approach in data analysis
Collate and synthesize information	• Information prior to fish kill unavailable, difficult to determine causation • Most investigations try to identify a single cause, but in reality rarely reveal more than correlation	• Consider several factors as most fish kill causes are complex • Consider carry-over effects
Prepare a report	• Non-existent in some jurisdictions, while others compile annual regional reports	• Implement annual reporting on regional/national/global levels on fish kills • Publish case studies in peer-reviewed outlets
Regulatory or management action	• Some monitoring; most do not have fish kill management plans • Reactive approach to fish kills rather than attempting to predict and prevent events	• Implement long-term monitoring programs • Identify potential causes and structure management action in response to those causes • Adopt a proactive approach to fish kill prevention

government websites or hotlines given that using existing centralized systems is more economical and easily accessible to the public. Some jurisdictions have already established web-based submission fish kill notification reports (e.g., Florida; Florida Fish and Wildlife Conservation Commission, 2010a; West Virginia; Cacapon Institute, 2010). We encourage more jurisdictions to follow Florida and West Virginia's example. The majority of households have either a phone or internet connection and are more likely to be used because they are efficient, convenient, inexpensive, and will significantly decrease the time-lag between fish kill notification and fish kill investigation. We also propose that citizens that report fish kills should only be responsible for providing sufficient information for investigators to determine if immediate action is required rather than providing all specificities of each fish kill event, which could dissuade their participation.

Need for Standardized Electronic Databases to Track Fish Kill Events

Several jurisdictions regularly compile annual fish kill reports that are readily available to the public (Lowe et al., 1991).

For example, North Carolina began a fish kill annual reporting strategy in 1997 that identified fish kills across the state in a standardized manner (NCDWQ, 2000). Each report concisely identifies the cause of the fish kill, species affected, number of fish killed, and water body. These summary reports yield information of what areas are more prone to fish kills, their causes, and probability of reoccurrence. Ideally, a standardized database could be developed that would enable all fish kills (at least nationally) to be tracked. Such a database would require use of rigorous and well-defined scientific data standards and protocols to enable sharing and analysis. We suggest that a fish kill database should include the following searchable raw data fields:

Reporter
Affiliation
Begin date of fish kill
End date of fish kill
Begin date of fish kill investigation
End date of fish kill investigation
Species affected
Number of species affected
Number of samples taken
Estimated time of sampling after death
Minimum size of fish affected
Maximum size of fish affected
GPS location (UTM coordinates)
Water body type (e.g., freshwater lake, river, estuary, coastal marine)
Water body size (km^2 or river km)
Proximate cause(s)
Ultimate cause(s)
Budget of investigation
Textual description of fish kill reported

Regionally or globally, such a database could be used to identify broad-scale trends. For example, knowing how many species are affected by a fish kill will allow investigators to determine if the majority of fish kills are caused by species-specific stressors (e.g., viruses) or more general stressors (e.g., pollution), which can then be analyzed by region or water body type or size. Such analyses and results would inform management strategies, and subsequently become an integral component of fish kill monitoring (Hale and Hollister, 2009).

Need to Recognize Multi-Factor Stressors

Most investigations attempt to identify a single causal factor for a fish kill event (Lowe et al., 1991; Glasgow et al., 2001; Thronson and Quigg, 2008). Stressors resulting in fish kill events can be additive, multiplicative, synergistic, and antagonistic (Folt et al., 1999). In biological systems, it is becoming increasingly clear that most stressors do not act alone (Jacobson et al., 2003) and this can have unexpected ecological consequences (Christensen et al., 2006). For example, Robinson and Deano (1985) revealed that the synergistic effects of acidity and aluminum were likely responsible for fish kills in Louisiana dating back to 1911. More recently, Kangur et al. (2005) reported that a fish kill in a lake in Estonia was the result of synergistic effects of cyanobacterial bloom, low water level, and high temperatures. Determining how different stressors interact is often difficult and poorly understood.

Water temperature can have a strong influence on organismal biology, and combined with global climate change, water temperatures may increasingly be implicated in fish kills alongside other stressors (Roessig et al., 2004). Fish may be able to cope with a range of stressors if temperatures are within their optimal range. However, as water temperatures approach or exceed their optimal temperatures, stressors that are normally benign can become lethal (Pörtner and Farrell, 2008). Given the realities of global climate change, it is likely that water temperature will be an additional stressor for many fish kills (Roessig et al., 2004).

Need to Acknowledge the Potential for Long-Term Carry-Over Effects That Can Complicate Fish Kill Investigations

It is a common belief that the occurrence of a fish kill must be associated with an immediate stressor (Lugg, 2000). Fish kills, however, can also be the result of a delayed reaction or carry-over effect to a stressor that had occurred several weeks or months prior to the fish kill event. Essentially, a previous stressor can modulate the response of an individual to subsequent stressors, even if separated by time and/or changes in life-history stage. Carry-over effects are just beginning to be understood in an ecological context (Norris, 2005), with little research on understanding the extent to which carry-over effects occur in fish. Fish kill investigations will benefit by implementing long-term monitoring of fish populations and the environment, and establishing good communication with the local community to obtain more accurate historical information. In addition, fish kill investigations will profit by taking into account potential stressors or events that occurred several months before attempting to establish fish kill causation.

A classic example of fish kill mortality due to delayed carry-over effects occurred in the Greater Yellowstone ecosystem (Bozek and Young, 1994). In 1990, Bozek and Young (1994) observed a fish kill in a burned watershed. To determine fish kill causation, they performed a rigorous study that compared burned watersheds and unburned watersheds, which involved external fish pathological analysis and the collection of suspended sediments and discharge data. This research indicated that fish died of asphyxiation due to increased suspended sediments as a carry-over effect from a large wildfire that had occurred two years prior to the fish kill event (Bozek and Young, 1994). Wildfires, aside from obvious loss of vegetation, can cause indirect changes to the ecosystem such as greater nutrient availability, soil modification and changes in water chemistry and hydrology, which can have effects several years after the fire has taken place (Minshall et al., 1997). Such events that have long-term consequences demonstrate that fish kills can be caused by unclear carry-over effects and that fish kill investigators need to consider the potential that a temporally separate event may contribute to a fish kill.

Need to Recognize That "Causation" Is Rarely Demonstrated but Should Be the Target of Any Fish Kill Investigation

It is important to note that in many of the studies that have investigated fish kills, the factors associated with fish kills are usually discussed as if they represent a causal mechanism(s). In reality, at best we are able to detect correlations or associations that may indicate a causal link, but they do not prove that such a link exists (Wright, 1921; Shipley, 2000).

To determine causation, it would be necessary to perform large-scale experiments and artificially manipulate various factors, which is inherently difficult in ecology (Scheiner and Gurevitch, 1993). When investigating fish kills, often *a posteriori*, it is almost impossible to enact such a study design (such as the before-after-control-impact approach; Underwood, 1992) due to the lack of appropriate data on reference sites and usually an absence of proper replicates. As such, investigators have often had to rely on less than optimal strategies for attempting to infer causal relationships (see Suter et al., 2002) which include use of graphical methods of model construction (e.g., Stow and Borsuk, 2003).

An example of a series of fish kills that has stimulated field and laboratory studies to identify the cause of mortality is the mass en route mortality of adult migratory sockeye salmon (*Oncorhynchus nerka*) during their homeward migration in the Fraser River, BC. The extensive fish kills documented over several years exceeded 90% of some populations (Cooke et al., 2004) placing the stocks at risk of extinction. Early efforts to identify the "cause(s)" of the mortality were focused on understanding the effects of different environmental conditions (e.g., water temperature, flow, disease, turbidity) on salmonids and looking for temporal associations between these factors and the extent of mortality (Cooke et al., 2004). Based on these initial "paper" exercises, several experiments were designed and executed to test hypotheses related to the causes of mortality—it was believed that elevated water temperature was the cause. Laboratory experiments were used that involved capturing migrating fish, artificially exposing them to different temperatures in a laboratory, re-releasing the fish, and then monitoring survival (Crossin et al., 2008). This work revealed that both acute and chronic exposure to elevated water temperature resulted in mortality, but in different ways. For example, chronic exposure to high temperatures resulted in accelerated energy use, disease development, and senescence. The acute exposure to elevated water temperature resulted in collapse of aerobic scope while actively migrating (Farrell et al., 2008). Not only did the researchers establish a causal link, they also identified the mechanism by which water temperature would lead to mortality. Similar controlled experiments have been conducted to determine the cause of massive annual fish kills in the Salton Sea, California (i.e., osmoregulatory failure at cool temperatures; Sardella et al., 2009) and estuarine and coastal fish kills on the eastern seaboard of the United States (toxic dinoflagellates; Burkholder et al., 1995). Once associations are observed in the field, greater reliance on laboratory experiment would be useful for improving ability to ascribe causation. Moving towards identifying causal factors would help to reduce the uncertainty associated with fish kill cases when dealing within the regulatory and judicial realms.

Need to Understand How Biotic Factors Modulate Fish Kills

It is currently unknown which individuals in a population/community are likely to suffer mortality despite the fact that biotic characteristics (e.g., fish size, sex, reproductive state, energy stores, health, condition) may influence the extent of a fish kill. Essentially, there is immense inter-individual variation in a range of phenotypic characteristics and this variation is correlated with the fitness of individual fish (Bennett, 1987). When exposed to different stressors, such variation could modulate responses and mortality rates. For example, a recent synthesis (i.e., Hanson et al., 2008) revealed that although rarely considered, inter-sexual variation can influence responses to a range of stressors and presumably influence fish kill sensitivity. Similarly, the reproductive status can also influence fish response to different stressors given the energetic burden and endocrine changes associated with reproduction. Although some fish kill investigations include evaluations of fish condition using general proxies such as condition factor, few include evaluations of energy density. Energetic condition seems like a logical metric and potential modulator of fish mortality. It is important for fish kill investigators to consider baseline variation in biotic characteristics and how it could influence fish kill occurrence and severity. There is also opportunity, however, for large-scale experimentation to better understand how inter-individual variation in organismal condition influences responses to different stressors.

Previous fish kill investigations have used the number of species affected in a fish kill as a cue to what may have caused the fish kill initially. For example, if one species is affected and not others, then the cause is likely a species-specific cause such as a virus or infection, where one species is much more susceptible (Munoz et al., 1994). A complete understanding on how stressors affect fish of different phenotypes will also provide valuable information to help determine not only potential causes but also aid in determining appropriate course of action. For example, if fish kills that focus on female fish are very frequent, then it may be more a priority to determine the cause of those fish kills given that females are essential for population growth and sustainability. As a result, we can infer potential causes based on phenotypic variation of the dead fish and also implement appropriate population recovery strategies depending on which fish in the population are most affected.

Need for Interdisciplinary Approaches to Fish Kill Investigation

Interdisciplinary research tends to be motivated by the need to address a complex problem which requires methods and knowledge from a number of disparate disciplines (Rhoten and Parker, 2004). Interdisciplinary approaches to research have been touted as being particularly relevant for addressing some of the most urgent environmental and conservation problems (Steele and Stier, 2000). Fish kills are an excellent example of a complex environmental problem that would benefit from more interdisciplinary perspectives. Most fish kill investigations currently combine information on biology, chemistry, pathology, and environmental conditions. However, there are other disciplines that also have the potential to yield innovative approaches to investigating fish kills and identifying their causal basis.

Specifically, we regard functional genomics as a promising tool for the investigation of fish kills given that functional genomics research captures the interplay of genetic and environmental factors on the fate and condition of fish (Feder and Mitchell-Olds, 2003). Functional genomic tools such as microarrays enable researchers to profile the expression of thousands of genes at once, enabling the assessment of response to environmental stressors on a genome-wide scale (Klaper and Thomas, 2004). Fish kill investigations would benefit from identifying the gene clusters and physiological pathways that are up-regulated or down-regulated in fish that die compared to fish that survive, or control fish from a reference system. Genomic approaches are particularly useful to identify potential causes that were not being considered given the thousands of genes that can be evaluated. In addition, there have been numerous advances in field physiology (Costa and Sinervo, 2004) which enable researchers to use portable diagnostic tools in the field to assess fish health and condition (Cooke et al., 2008), providing new and timely information to aid in fish kill investigations. Comprehensive interdisciplinary investigations of fish kills are uncommon (but see work on harmful algae; e.g., Glibert et al., 2002), but such an approach is needed to truly identify and understand the complexity of fish kills. We acknowledge that functional genomics is an expensive tool and may not be feasible to do on a large scale and for every fish kill event. We, however, recommend it as a valuable tool that should be used especially in areas where there are high frequencies of fish kills where causes cannot be determined.

Need for More Assistance from Aquatic Health Specialists and Veterinarians with Expertise on Wild Fish

In the last decade or so, there has been a significant increase in the number of trained veterinarians and fish health specialists with expertise in aquatic wildlife including fish (Buttitta, 1998; Kuehn, 2002), as well as the production of related educational and training resources (e.g., Stoskopf, 1993). These highly trained professionals possess a wealth of knowledge on the biology, physiology, and epidemiology of fish (Hartman et al., 2006), knowledge that is extremely useful for the investigation of fish kills. We acknowledge the history and role of fish pathologist and animal health specialists in fish kill investigations (see Abt and Bullock, 1996), however, it is our belief that the expertise of these professionals is underutilized in the investigation of fish kills. In addition, when involved, veterinarians and fish health specialists are often acting in the role of pathologist and rarely are involved with the field aspects of a fish kill investigation. Because veterinarians have traditionally had little training in aquatic medicine (Kuehn, 2002), it is possible that they are simply not consulted because fish biologists are unaware that such capacity now exists. Many natural resource agencies now employ both fish health specialists and veterinarians with expertise in aquatic animal medicine. We suggest that these professionals should be involved in fish kill investigations (including the field site visits) and that mechanisms be set up such that these consultations are inherent with any fish kill investigation.

Need for Use of Ecological and Environmental Observatories to Better Understand and Monitor Fish Kill Events

In recent years there has been growing interest in the use of large-scale ecological and environmental observatories to monitor and understand biotic and abiotic processes (Keller et al., 2008). Such systems typically rely on an expansive network of sensors that record information on water chemistry (Glasgow et al., 2004; Johnson et al., 2007), oceanography (Glasgow et al., 2004), geology (Favali and Beranzoli, 2006), and biology (Welch et al., 2003; Cooke et al., 2005). The general premise of these observatories is that they provide scientists with new opportunities to study multiple, interrelated processes over time scales ranging from seconds to decades. To date, these systems have been deployed in a range of environments including coastal shelf regions (Welch et al., 2003), whole lakes (Cooke et al., 2005; O'Connor et al., In Press), and estuaries (Welch et al., 2003). Given that fish kills are not always predictable and the inherent difficulties of studying an event after it has occurred, the collective network of ecological and environmental observatories provides a unique opportunity to study fish kills. Theoretically, fish kills could be monitored and studied in real time; however, the most likely approach would be to reconstruct conditions after they had occurred. Moreover, if there are a suite of characteristics associated with the fish kill event(s), it may be possible to use ecological and environmental networks to predict the timing and location of future fish kills.

As an example, O'Connor et al. (In Press) used a whole-lake ecological observatory in eastern Ontario equipped with an under-ice acoustic telemetry array to monitor largemouth bass behaviour and subsequent mortality during a massive winterkill event. Essentially, the researchers were able to monitor a winterkill in real time bringing novel insight into the anatomy of a winterkill. Although we were unable to find any published examples (aside from the forthcoming paper by O'Connor et al.) of where fish kills have been monitored or studied using a large-scale ecological or environmental observatory, this technology has been recognized as a critical need for early warning systems and rapid response to harmful algal bloom events (Glasgow et al., 2004) which can promote fish kills.

Need for Scientific Studies Designed to Improve Understanding of How to Investigate Fish Kills

To date, there have only been three studies (Labay and Buzan, 1999; Ryon et al., 2000; Patterson et al., 2007) that have evaluated different approaches for investigating fish kills or attempted to address some of the inherent challenges in fish kill research. These studies tended to focus on the ability of investigators to locate dead fish. For example, Ryon et al. (2000) simulated a fish kill in a small stream. In general, the surveys of tagged dead fish were effective. However, there was some evidence of substantial carcass removal by scavengers leading the authors to conclude that fish kill investigations in streams may underestimate actual mortality. Labay and Buzan (1999) revealed that within a kill event, smaller individuals and less abundant species are underestimated compared to larger individuals and more abundant species. In a study of fish kills among upriver migrating sockeye salmon, Patterson et al. (2007) revealed that the paucity of carcass observations was attributed to a variety of factors (such as turbidity and water temperature) that influence the visibility of salmon carcasses in large rivers. As such, they conclude that fisheries managers should not always expect to see large numbers of dead salmon in years of high en route mortality.

Unfortunately, the studies described above represent the only science that we are aware of that were conducted explicitly to improve the investigation of fish kills. In fact, many fish kill investigative techniques rely on knowledge gained from the use of rotenone for planned fish kills or fish population monitoring in inland waters, something that does not likely apply to all systems, and may be influenced by the fact that the mortality is driven by a toxicant. We encourage more experimental work of this nature to determine the optimal sampling strategies and investigative techniques for studying fish kills.

Need for More Peer-Reviewed Fish Kill Case Studies and Scientific Papers

Although fish kills are a significant concern to human and ecosystem health, it is a severely understudied topic within peer-reviewed literature. A likely reason is that often fish kill investigations are localized and fail to generate conclusive results, which are thus deemed unworthy of publication. We encourage fish kill investigators to prepare their work for submission to a peer-reviewed outlet in order to advance the science and practice of fish kill investigations. Several journals in fisheries, aquatic, and environmental science accept "case study" papers which detail localized problems such as a fish kill.

Need for Improved Training Opportunities for Fisheries Practitioners on Investigation of Fish Kills

To ensure that fish kills are investigated by individuals with appropriate expertise, it is necessary to have two levels of "training" directed at investigators with different levels of specialization. Initial investigators at a fish kill site are often general biologists who may not have fish kill expertise. These initial investigators should have adequate training to determine the type of response needed and be able to collect initial field samples prior to the arrival of the second wave of more specialized investigators (herein called the "specialists"). These specialists should have expert training specific to fish kill investigations, have a network of other experts (e.g., veterinarians, pathologists, water chemists) with whom they can consult, and be sufficiently familiar with what each discipline can bring to an investigation. Training courses for specialists should therefore emphasize new developments in fish kill investigation science and practice, whereas courses for initial investigators should focus on initial sample collection and problem diagnostics (i.e., when to call in the specialist).

Need to Improve Communication with the Public When Fish Kills Occur

One of the themes that emerged from our anecdotal examination of media reports of fish kills was public concern about human health, particularly with respect to swimming in fish kill waters or in eating fish captured from systems where fish kills occurred. Another common theme in the media accounts was frustration with a perceived lack of government response. There seems to be an assumption that there is a "quick test" that will yield a definitive answer on the cause of the fish kill. We interpret the above patterns as a general failure with the public to understand the complexity of fish kills and the challenges associated with their investigation. It is also symptomatic of a bigger failure of fish kill investigators to provide rapid, clear and credible information to the public when a fish kill occurs. One approach would be to inform the public of the complexity of fish kill investigations which could be achieved through websites and electronic pamphlets that are written for a general rather than a scientific audience. Another approach can include more media coverage on fish kill investigation efforts. We recognize that it is difficult to provide rapid answers to fish kills; however, it is also important to ensure that any public concerns are either allayed or addressed. Human dimensions studies focused on understanding perceptions of the public regarding fish kills and their investigation would be useful for helping to inform how regulatory agencies respond to fish kill events. Any attempts to improve the communication between fish kill investigators, regulators, and the general public will serve towards generating public support for fish kill investigations.

CONCLUSIONS

The increase of fish kills are a growing concern for the public, government officials, and scientists. Increasing fish kill events indicate that aquatic systems are at risk (Whitfield and Elliot, 2002). In order to reduce fish kills, immediate action is required. Many of our recommendations (e.g., creating and managing global databases, recruiting specialists, integrating genomics, creating large-scale observatories) may be considered expensive and possibly not practical for some practitioners. However, if we examine the economic cost of implementing these strategies compared to the loss of fisheries profit (lost production) or potential cleanup costs due to fish kills each year, the economic cost is very minimal.

To highlight the economic advantage of implementing our recommendations, we draw on the United States as an example. Based on Environmental Protection Agency data from 1977–1987, it has been estimated 141 million fish die per year nationally because of fish kills (Pimentel et al., 1993). In the 1980s, the monetary value of a fish was estimated to be $1.70 (AFS, 1982 as cited in Pimentel et al., 1993). As a result, the total economic loss due to fish kills in the 1980s amounted to approximately 240 million dollars per year in the United States. This estimate, however, is considered a low approximation because many fish are washed away and removed by scavengers before they can be counted (Pimentel et al., 1993).

Economic loss at the present time is likely to be much more because of 1) inflation and subsequent increased value of fish and 2) the increase in frequency and magnitude of fish kills. For example, in the 1980s, the number of dead fish in North Carolina was estimated to be approximately 2.5 million for an average of 15 fish kill events per year (Lowe et al., 1991), while fish kills reported in 2008 resulted in over 7.5 million dead fish for 61 fish kill events (NCDWQ, 2008). Similar dramatic increases in fish kill events and mortality has also been reported in other states (e.g., Florida from the 1990s to 2000s; Florida Fish and Wildlife Conservation Commission, 2010b). Also, a survey in New South Wales revealed that there is a large increase in reported fish kills from the 1980s (16.6 kills per year; 1980–1989 data) to the 1990s (35.7 kills per year; 1990–1996 data) (Lugg, 2000), indicating that the increase in fish kill events and the subsequent economic losses of millions of dollars per year are not unique to the United States and are a likely occurrence worldwide. Combating these losses by implementing monitoring and research programs as we have described herein is a very minimal investment compared to the economic losses due to fish kills.

In addition to the economic impact of fish kills, it is important to evaluate the damage caused by fish kills to aquatic ecosystems, and what it will mean for fisheries industry and the environment in the future. As fish are killed by various stressors, there may be a significant shift in ecosystem balance and a change in reproduction dynamics and sustainability of fish populations. As fish populations decline, it is likely that profits will decline as well. Currently, the Australian government net profit on fisheries is 2.2 billion dollars per year (Government of Australia, 2010). If fish populations continue to decline as a result of fish kills, this industry will likely collapse since many commercial fish have long recovery periods (e.g., Murray cod can take up to 52 years to recover after a fish kill event; Koehn, 2004).

We therefore emphasize that there is a crucial need to act upon the ever-increasing frequency of fish kills, and that current investigation methods are not providing a clear picture of what is occurring in our aquatic ecosystems. The objective of this critical commentary was to identify key challenges in the science and practice of fish kill investigation. In doing so, we have generated a framework of what needs to be done to elevate the science and practice of fish kill investigations which will assist with the prevention of anthropogenically-induced fish kills.

We have emphasized the need for a global definition of fish kills, as well as the critical need for a standardized protocol, electronic databases, and simple reporting strategies for fish kill investigations. We recognize that this cannot and should not be done by every jurisdiction on their own and require some level of national or ideally global coordination. As such, we suggest that the Association of World Fisheries Societies, the World Organization of Animal Health and/or the Food and Agriculture Organization of the United Nations are appropriately positioned to facilitate the development and mandating of such fundamental standard methods and definitions. We have also recommended that fish kill science adopt an interdisciplinary approach, something essential to address such complex problems. This requires more assistance from aquatic specialists and veterinarians, and embracing new approaches including the use of field physiology techniques, genomics approaches, and large-scale ecological and environmental observatories. We have also described how carry-over effects and biotic factors must be considered in fish kill investigations. There is also a need to move towards documenting "real" causation through the use of mechanistic experimental approaches to compliment *in situ* fish kill investigations. Accompanying these changes, should be improvements in training of fish kill investigators. Finally, given that all natural resource issues require an understanding of the human dimension, we encourage greater attempts to use social science to understand how to best address public concern regarding fish kills. Failure to adopt these strategies will retard our ability to identify the proximate and ultimate causes of fish kills, resulting in an overall increase in anthropogenic fish kills and further loss of our aquatic systems.

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