

## **An Ecological Risk Assessment Framework for Effects of Onsite Wastewater Treatment Systems and Other Localized Sources of Nutrients on Aquatic Ecosystems**

**Rebecca A. Efroymson,<sup>1</sup> Daniel S. Jones,<sup>1,2</sup> and Arthur J. Gold<sup>3</sup>**

<sup>1</sup>Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; <sup>2</sup>Currently at Arcadis G&M, Inc., Oak Ridge, TN, USA; <sup>3</sup>Department of Natural Resources Science, Coastal Institute at Kingston, University of Rhode Island, Kingston, RI, USA

### **ABSTRACT**

An ecological risk assessment framework for onsite wastewater treatment systems and other localized sources of nutrients is presented, including problem formulation, characterization of exposure, characterization of effects, and risk characterization. The framework is most pertinent to the spatial scale of residential treatment systems located adjacent to small ponds, streams, or lagoons and some parts of shallow estuaries. Freshwater and estuarine ecosystems are distinguished based on differences in nutrient dynamics. Phosphorus exposure is the major determinant of phytoplankton production in most North American lakes. Nitrate can be directly toxic to aquatic biota such as amphibians. In shallow estuaries or lagoons, nitrogen is the primary stressor, which can be directly toxic to vegetation or can interact with biota to produce secondary stressors (limited light penetration, oxygen limitation, reduction in habitat, or reduction in forage vegetation or prey). Algal production, macrophyte production, fish community abundance and production, benthic community abundance and production, and amphibian community abundance and production are examples of risk assessment endpoint properties. Models and measurement methods for the characterization of exposure and effects are discussed, as well as sources and quantification of uncertainty. Example weight-of-evidence

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Address correspondence to Rebecca A. Efroymson, Environmental Sciences Division, Oak Ridge National Laboratory, P. O. Box 2008, Oak Ridge, TN 37831-6036, USA. E-mail: efroymsonra@oml.gov

tables are presented for failure scenarios involving traditional and emerging onsite wastewater system technologies.

**Key Words:** ecological risk assessment, nitrogen, nutrients, phosphorus, amphibian, macrophyte, eelgrass, seagrass, phytoplankton, wastewater, septic tank.

### INTRODUCTION

Ecological risk assessment is commonly used to predict the magnitude and likelihood of future hazards or to attribute causality to current or past stressors. The chemicals that are typically the subjects of ecological risk assessments include metals and organic toxicants, but risk assessments have occasionally focused on nutrients. For example, Borsuk (2004) quantified expert opinion to predict fish kills in the Neuse River Estuary, NC, USA, from nutrient pollution. King and Richardson (2003) developed an ecological risk assessment-based approach for developing water quality criteria. Valiela *et al.* (2000) provided a general risk framework for nitrogen loading to estuaries within a broader study of sustainable nitrogen loads to the Waquoit Bay, MA, USA, estuary. They sketched risk assessment considerations for nitrogen in estuaries, including the suggestion of potential assessment endpoints and measures of effect, the analysis of partitioning of nitrogen loading among various sources, the discussion of uncertainty of loading estimates, and the involvement of potential stakeholders. Numerous regional ecological risk assessments focused on multiple stressors that included nutrients (Walker *et al.* 2001; Cormier *et al.* 2000; Gentile *et al.* 2001). However, a detailed ecological risk assessment framework focused on nutrient releases into surface waters from individual onsite wastewater treatment (OWT) systems or other localized sources of nutrients has not previously been developed.

This article presents a framework for ecological risk assessment of nutrients from OWT. Most of the methods are also applicable to other localized sources of nutrients. The framework provides conceptual models and other aspects of problem formulation, measures of exposure and effects for nutrient impacts in freshwater and estuarine systems, and examples of weight-of-evidence processes for OWT failure scenarios. This ecological risk assessment framework was developed as a component of the "Integrated Risk Assessment for Individual Onsite Wastewater Treatment [OWT] Systems," within the National Decentralized Water Resources Capacity Development Project, supported by the U.S. Environmental Protection Agency (Jones *et al.* 2004). Four types of risk assessment frameworks, engineering, public health, ecological, and socioeconomic, were integrated, as many of these types of risks are dependent on each other (Jones *et al.* 2004). For example, the economics of water recreation is linked to the presence of water that is clean enough for drinking and swimming. Similarly, ecological risks depend on the failure rates of OWT systems as determined by engineering risk assessments.

This framework for ecological risk assessment of OWT systems and other localized sources of nutrients is presented in the typical format of risk assessments (USEPA 1998a): problem formulation, characterization of exposure, characterization of effects, and risk characterization.

## PROBLEM FORMULATION

### Assessment and Management Goals

Ecological risk assessments for OWT systems may be conducted for many purposes: (1) planning for or permitting of a new installation on a previously undeveloped site, (2) evaluation of the potential or observed effects of an existing OWT system, (3) assessment of incremental effects of additional treatment units in an existing development, (4) evaluation of potential retrofits for a currently failing OWT system, and (5) development of guidance for permitting. For example, Valiela *et al.* (2000) suggest that a management goal might be to retrofit all buildings in a watershed with septic systems that retain nitrogen at a particular efficiency. Charles *et al.* (2003) propose an OWT risk management model for determining the benefit of buffer zones in water catchments in Sydney, Australia. A typical risk management goal is to balance the risks of endangering public health and ecosystems and reducing local property values due to complete failure of an OWT system (*e.g.*, surface breakthrough) against the risk of increased installation and operating costs to the home owner and the risk of eliminating the opportunity for the community to develop the site in question.

Management goals may be categorized according to several attributes.

- **Incremental or total risk.** An ecological risk assessment may be intended to evaluate incremental risks associated with OWT alone, or the assessment may be intended to evaluate total risk from the OWT unit and other sources of nutrients.
- **Broad or narrow investigation.** In some assessments a broad range of ecological effects are of concern; others focus only on those that relate to existing water quality criteria.
- **Retrospective or prospective assessment.** The risk assessment may be concerned with impacts of past nutrient releases, concerned with prediction, or concerned with current conditions. The relative importance of measurement and modeling to estimate exposure and effects is determined partly by the temporal scope of assessment.
- **Results in terms of probability or magnitude.** The goal may be to determine if adverse effects are likely, or to determine the likely magnitude of the adverse effects.
- **Relative or stand-alone assessment.** The assessment may be intended to compare risks associated with different treatment technologies, numbers of units, operating conditions, and so on, or it may be non-comparative. In non-comparative assessments, the goal may be to identify any potential for risk, and therefore, it may be appropriate to use a high estimate of exposure and a high estimate of the effects that are potentially caused by that exposure. Comparative assessments use accurate estimates of exposures and effects, rather than conservative estimates, to avoid bias in the ranking of alternatives.
- **Explicit or unstated mitigation goal.** The risk assessment may be conducted with a restoration goal in mind, for example, "to extend and to maintain seagrass beds in water depths up to 1.7 m" (Sigua and Steward 2000).

### Types of OWT Systems

Many technologies can be combined to make up an OWT system. These systems can be classified based on their intended functions and vulnerabilities. For example, onsite wastewater soil adsorption systems (WSAS) can take many different forms (*e.g.*, beds, trenches, and mounds), can be located on sites with different conditions (*e.g.*, soil type, depth of unsaturated zone, and groundwater characteristics), and can be operated in a variety of ways (*e.g.*, gravityfed, cyclic dosing, and pressurized dosing). Source terms for nutrient transport models are characterized partly by the type of OWT system.

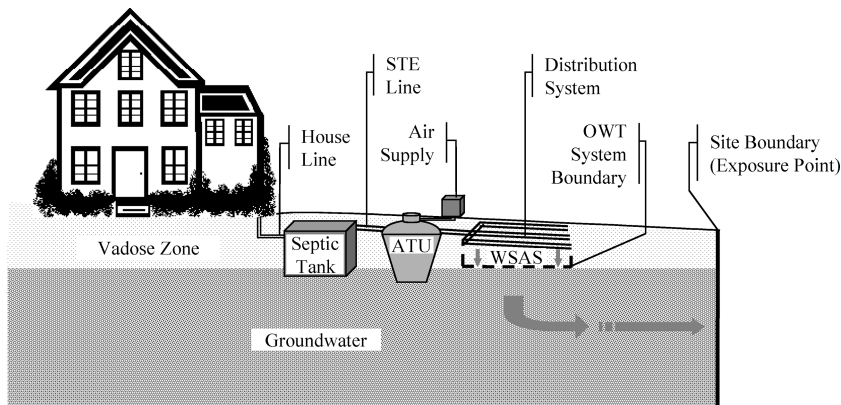
Modern systems include traditional septic systems and those systems that have more engineered unit operations. General categories of modern onsite wastewater treatment systems have varying effluent qualities: traditional, contemporary, and emerging systems (Jones *et al.* 2004). Traditional systems are assumed to have a septic tank, no tank-based advanced treatment, and gravity-fed WSAS. Contemporary systems are assumed to have a septic tank, an aerobic treatment unit, and a WSAS that requires less area for subsurface disposal, permitting retrofitting or advanced treatment in lots traditionally viewed as too small for adequate treatment. Emerging systems are assumed to have a septic tank, porous media biofilter, and disinfection unit, with drip irrigation. Example effluent characteristics for each system, including biochemical oxygen demand, total suspended solids, total nitrogen, total phosphorus, fecal coliforms, and viruses, are presented in Jones *et al.* (2004).

### Spatial and Temporal Bounds

This risk assessment framework was intended to address primarily the “micro-scale” of OWT systems, that is, an individual residential lot with a decentralized wastewater treatment system. Therefore, the framework is pertinent to residential treatment systems or other localized sources of nutrients located adjacent to ponds, streams, small lakes, lagoons, or shallow estuaries or smaller ditches of ponded water. The spatial bounds of the assessment are a particular surface water body, stream segment, or group of stream segments, consistent with recommendations in Suter *et al.* (2000), that is, areas in which wastes deposit, areas that are believed to be contaminated, and the extent of transport to the point where sufficient dilution volume ensures negligible risks. This ecological risk assessment framework assumes that surface water bodies are immediately adjacent to an effluent pipe or drain field, because its emphasis is on evaluating ecological exposures and effects rather than estimating chemical transport (Figure 1). Based on the localized scale of analysis, direct effects of nutrients on populations, communities, or ecological processes, rather than secondary effects from losses of forage or habitat or increases in predation, are emphasized in this article.<sup>1</sup> For example, this risk assessment framework addresses losses of abundance or production of the eelgrass (*Zostera marina*) population, but does not present relationships between eelgrass production or area and abundance of the many populations that depend on eelgrass.

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<sup>1</sup>An exception is indirect effects from nutrient-induced algal blooms that reduce light penetration, which are discussed here.



**Figure 1.** Schematic for a “Contemporary” Onsite Wastewater Treatment (OWT) system and the associated site boundary. STE refers to septic tank effluent, WSAS refers to wastewater soil absorption system, and ATU refers to aerobic treatment unit.

The time-scale of analysis is based on the lifetime of a treatment system, the lifespan of a particular species, regulatory requirements, or a decision by the risk manager. Particular events in time are evaluated, such as storm events that cause treatment failure and periods of high releases of nutrients that coincide with sensitive life stages of organisms. For example, high inputs of nutrients may occur in the spring, during thaws of frozen soils and fertilizer runoff. This time period coincides with periods of vertebrate breeding or larval development.

We focused our analyses on risk from individual systems rather than community systems. The use of multiple unit (*e.g.*, cluster) OWT systems is often stymied by the need for legal clarification on the right of entry, payments for maintenance and repair, and issues related to oversight and liability (USEPA 2003b; Lombardo 2004). Whereas the responsibility for maintenance, ownership, and liability for individual OWT systems is clearly centered on the contributing property owner, cluster treatment systems often require that the entire set of contributing properties agree to the creation of a “Responsible Management Entity” to handle maintenance and repair. Many communities and states are still developing approaches for cluster OWT systems, suggesting that individual OWT approaches are likely to remain in widespread use for a considerable time.

### Stressors

Nutrients in surface water may be viewed as ecological stressors if they are directly toxic to aquatic organisms or if they alter habitat or forage availability by increasing the biomass of an undesirable species or indirectly affecting oxygen concentrations, light penetration, or other aspects of habitat. Nutrient inputs to a surface water body have the greatest impact if background concentrations limit net primary production, net ecosystem production, or growth rates of phytoplankton populations.

Most nitrogen is expected to enter surface water as nitrate, because oxidation of ammonia, nitrite, and organic forms of nitrogen usually occurs in or near the

treatment unit, and nitrate is the most stable form of nitrogen in surface waters. Because of their chemical instability, nitrite, ammonia, and organic nitrogen are potentially significant stressors only when (1) they are released in large quantities from major point sources such as industrial effluents, livestock feed lots, slaughterhouses, or urban centers that do not have nitrification treatment of sewage (Rouse *et al.* 1999), (2) an OWT system is located in wet soils or forested watersheds (Valiela *et al.* 2000), or (3) the surface waters in which oxidized forms of nitrogen are released are anoxic or hypoxic. Reduced forms of nitrogen are not discussed in detail in this framework.

Nitrate is a potential stressor for organisms that are sensitive to the nutrient. Moreover, nitrogen is a potential stressor in nitrogen-limited areas, which are generally estuarine waters in temperate environments. Nitrogen is usually limiting to primary production in environments where the ratio of inorganic nitrogen to phosphorus is below the Redfield ratio of 16 moles of nitrogen to one mole of phosphorus (Redfield 1958; Howarth 1988). Phosphorus may also limit primary production in some marine ecosystems (Howarth 1988), including, for example, part of the Indian River Lagoon in Florida (Phlips *et al.* 2002), but this is not common. Three factors determine whether nitrogen or phosphorus is more limiting: (1) the ratio of nitrogen to phosphorus in surface water inputs; (2) the preferential reduction of nitrogen or phosphorus from the photic zone because of denitrification, preferential sedimentation of nitrogen in zooplankton fecal pellets, adsorption of phosphorus, or other biogeochemical processes; or (3) the extent to which nitrogen fixation balances other deficits in nitrogen availability (Howarth 1988).

Anthropogenic, bioavailable phosphorus (*e.g.*, soluble orthophosphate) is a potential stressor in most fresh waters in temperate environments. Most of the phosphorus released in septic tank effluent (about 85%) is in the form of soluble orthophosphate, with the rest as organic and inorganic phosphorus in suspended solids (Gold and Sims 2001). If the phosphorus travels a distance through soil before reaching a surface water body, a substantial fraction may precipitate with aluminum, iron, or calcium or sorb to clay particles. Effects of phosphorus are most commonly associated with OWT systems that suffer from hydraulic failure and border surface waters, whereas nitrate-nitrogen may travel long distances through groundwater (Gold and Sims 2001).

Furthermore, organic matter in treatment unit effluent may be a stressor for ecological receptors. This stressor is measured as carbonaceous biochemical oxygen demand (BOD). BOD is the amount of oxygen that would be consumed if all of the organic carbon in one liter of water were oxidized by microorganisms. BOD is a measure of the potential for an effluent to reduce the dissolved oxygen in a receiving surface water body.

Nutrients may act indirectly on some potential ecological receptors. Resulting algal blooms may cause light or oxygen limitations to macrophyte, fish or benthic communities. Light limitation, oxygen limitation, changes in populations that affect other populations (via predation, forage availability), or changes in communities that affect habitat are referred to as secondary stressors because they are not produced directly in septic tank effluent, but rather as a consequence of excess nutrients.

Other chemical stressors may originate from OWT systems but are not emphasized here. These could include pathogens, household products such as detergents

or paints, pharmaceuticals such as antibiotics, or undigested fat or sugar substitutes. For example, algal biomass and community structure were affected by amendments of an antibiotic, an anti-microbial agent, and a surfactant in recent laboratory studies (Wilson *et al.* 2003). Moreover, endocrine disruptors have been measured in effluents of municipal wastewater treatment plants (Servos *et al.* 2005), but not to our knowledge from onsite systems. More research is needed to determine if endocrine disruptors and other contaminants from OWT systems are significant stressors to aquatic populations and functions.

### **Background Levels of Stressors**

Exposures and risks are calculated with respect to background levels, which may be total nutrient loads in the absence of septic tank effluent or loads to reference locations that have no anthropogenic sources of nutrients. In many ecological risk assessments for anthropogenic chemicals, the source of concern is typically the major source of the chemical to surface water. However, risk assessments for OWT units are complicated by the multiple sources of nutrients that may lead to much greater nutrient exposure than the treatment unit of interest.

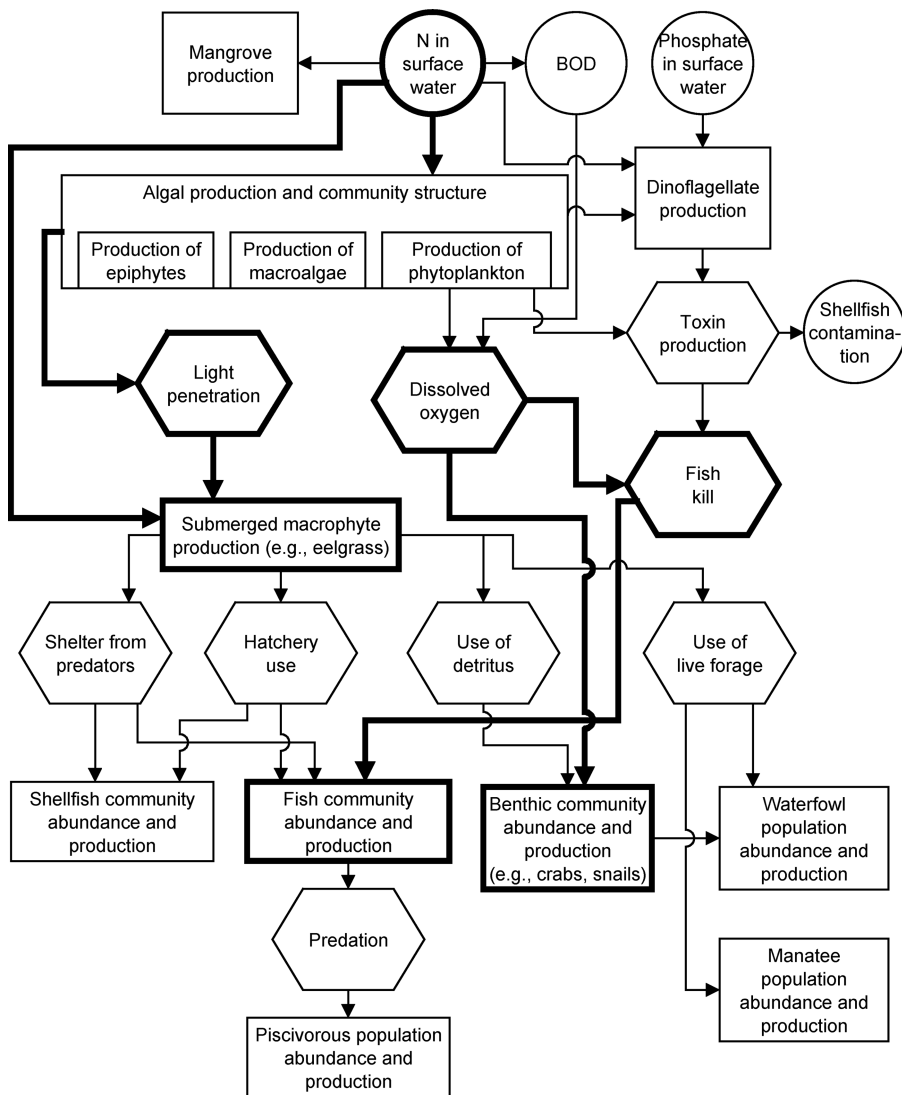
In addition to leachate from septic tanks, anthropogenically derived inputs of nitrogen include fertilizer application in agriculture and on lawns; livestock waste; effluents from industrial and wastewater treatment plants; atmospheric deposition of oxidized forms of nitrogen from the burning of fossil fuels, nitrogen fixation by leguminous crops, and urban storm water runoff. Nitrogen levels tend to be lower in summer than in other seasons because of the increased assimilation of the nutrient by plants during the growing season (Rouse *et al.* 1999). Background oxygen depletion events in estuaries that are associated with warm water and cloudy weather, as well as hypoxic events occurring at dawn during warm months, can be inappropriately attributed to eutrophication resulting from nitrogen enrichment (Valiela *et al.* 2000; D'Avanzo and Kremer 1994).

Anthropogenically derived inputs of phosphorus include fertilizer application, detergents, livestock waste, effluents from industrial and wastewater treatment plants, atmospheric deposition, and urban storm water runoff. The major sources of phosphorus inputs to streams are often geologic. Nutrients are also regenerated by algae and other organisms.

### **Conceptual Models**

The conceptual model describes and visually depicts the expected relationships among stressors, exposure pathways, and assessment endpoints (USEPA 1998a). The models and candidate ecological properties presented here are generic for assessments of wastewater treatment systems with an emphasis on local nutrient inputs from OWT. A conceptual model for a particular assessment would be tailored to a site with more specific ecological receptors and stressors after surveying that site and studying its ecology (*e.g.*, mangroves and manatees have very limited geographic range). This risk assessment framework distinguishes between two types of surface water ecosystems: (1) coastal lagoons (also called inland bays, salt ponds) and shallow estuaries and (2) freshwater lakes, streams, and ponds.

## Ecological Risk Assessment Framework for Wastewater Systems



**Figure 2.** Conceptual model for ecological risk assessment of wastewater treatment unit in a shallow estuary or lagoon. Potential assessment endpoint entities and properties are denoted by rectangles, circles are stressors, and hexagons are processes. Exposure pathways of focal assessment endpoint properties are indicated with bold lines.

### Estuary/Lagoon

A generic conceptual model for OWT unit effects in a shallow estuary or lagoon is depicted in Figure 2. These water bodies are usually less than 5 m and often less than 2 m in depth, and photosynthesis-supporting light can generally reach the bottom (Nixon *et al.* 2001). Lagoons, which are located behind barrier spits or islands, may not be well connected with the deeper coastal ocean. Nitrogen is the primary stressor,

which can be directly toxic or can interact with biota to produce secondary stressors (limited light penetration, oxygen limitation, reduction in habitat, or reduction in forage vegetation or prey). Organic matter (BOD) that is associated with wastewater is an additional stressor that can cause oxygen limitation.

In very shallow estuaries, most of the primary production is performed by seagrasses such as eelgrass, epiphytic algae, drift and attached macroalgae (sea weeds), and epibenthic microalgae (Nixon *et al.* 2001). Phytoplankton is generally less important. Nutrient levels are the key determinants of the structure of the primary producing community (Deegan *et al.* 2002).

The importance of seagrass beds lies in their use as habitats and temporary nurseries for fish and shellfish, sources of food for fish, food for waterfowl, detrital food for benthic invertebrates, food for manatees, and refuges from predation. Seagrasses require rather clear water, and they are found in sheltered lagoons just below the low-tide line, at maximum depths of usually only two or three meters. Seagrass reductions have been observed in numerous, nutrient-enriched shallow marine systems (Burkholder *et al.* 1992; Hauxwell *et al.* 2003; Short and Burdick 1996; Stevenson *et al.* 1993) and are therefore of concern in an ecological risk assessment for OWT systems.

Nitrate can act to increase the biomass of epiphytic algae and macroalgae, causing shading of seagrasses. The majority of the epiphyte community consists of algae, but the epiphytic complex consists of epiphytic macrophytes, microorganisms, macroalgae, metazoans, the extracellular excretions of these organisms, and mineral and organic particles sorbed on the organic matrix (Drake *et al.* 2003). At low biomass, this layer may prevent damage from ultraviolet radiation or repel potential herbivores (Drake *et al.* 2003), but at higher biomass, the epiphytic layer may shade seagrasses or possibly affect nutrient uptake and gas exchange and reduce photosynthesis (Drake *et al.* 2003; USEPA 2001)

In addition to shading, epiphytes directly compete for blue- and red-wavelength light with seagrass leaves (Drake *et al.* 2003). Shading of seagrasses by phytoplankton blooms is less common but occasionally observed (Nixon *et al.* 2001). Much research shows that nutrient enrichment stimulates the growth of epiphytes on seagrass leaves (Borum 1985; Neckles *et al.* 1993; Coleman and Burkholder 1994), except in cold regions (Nixon *et al.* 2001).

Eelgrass responds to inorganic nitrogen enrichment and shading through the elongation of leaves and a decrease in the allocation of biomass to below ground roots and rhizomes (Nixon *et al.* 2001). The lateral branching of rhizomes decreases, causing a decline in the density of shoots (Nixon *et al.* 2001). Hauxwell *et al.* (2003) also postulate that recruitment is diminished.

In addition to light-mediated effects, nitrate can have a direct toxic effect on eelgrass, particularly at warm temperatures (Burkholder *et al.* 1992; Touchette *et al.* 2003). The mechanism may involve uncontrolled nitrate uptake, which can lead to internal phosphorus limitation, carbon limitation, or other nutrient imbalances (Burkholder *et al.* 1992). Nitrogen amendment can also stimulate growth of mangrove forest trees in nitrogen-limited areas (Feller *et al.* 2003).

Phosphorus-limited lagoons such as part of the Indian River lagoon are not reflected in the generic conceptual model in Figure 2, but should be included in a site-specific model if phosphorus-mediated effects are possible. Mesocosms in Rhode

Island showed that even nitrate-limited systems could display secondary limitation associated with phosphorus; that is, phytoplankton blooms were larger in mesocosms treated with nitrate and phosphorus than in those treated with nitrate alone (Taylor *et al.* 1995).

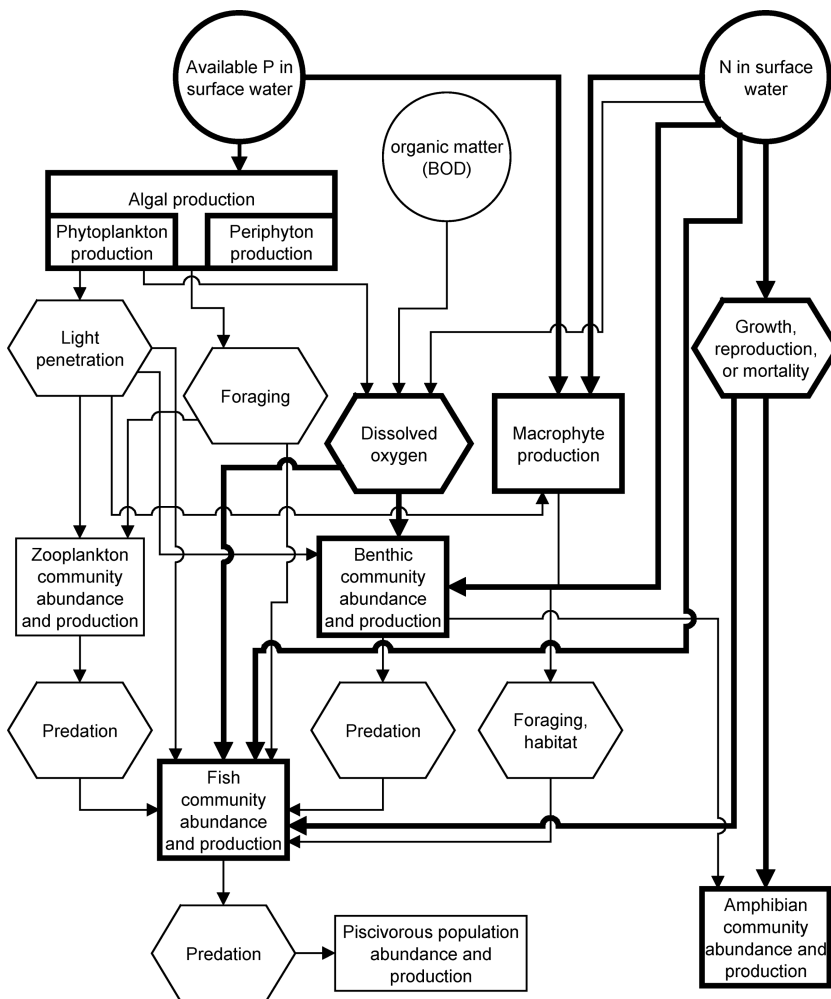
Losses of seagrass may lead to impacts on higher trophic-level organisms, but these effects are beyond the scope of this framework. For example, shifts from eelgrass communities to macroalgal communities that were associated with high nutrient inputs resulted in decreases in abundance, biomass, and diversity of fish (Hughes *et al.* 2002; Deegan *et al.* 2002). Macroalgae grow in dense mats on estuary sediments that reduce oxygen levels and alter the benthic community (Deegan *et al.* 2002). Moreover, declines of migrant Canada geese (*Branta canadensis*) and common goldeneye (*Bucephala clangula*) have been associated with the collapse of eelgrass beds, because of the vegetation and invertebrate prey reduction, respectively (Seymour *et al.* 2002).

Furthermore, algal blooms that are associated with high nutrient levels can deplete oxygen, especially at the benthic boundary layer. For example, decapod (*e.g.*, crab) abundance and biomass were reduced, apparently as a result of hypoxia (Deegan *et al.* 2002). Benthic and pelagic invertebrates and fish may be affected. Dinoflagellates such as *Pfiesteria* are protists that prey on fish and are implicated in major fish kills in estuaries and coastal waters in the mid-Atlantic and southeastern United States (Burkholder and Glasgow 1997). Nitrogen and phosphorus can directly stimulate growth of dinoflagellates or their algal prey. *Pfiesteria* outbreaks are observed in poorly flushed eutrophic estuaries that are impacted by human sewage, among other sources (Glasgow and Burkholder 2000).

### Fresh water

A generic conceptual model for OWT unit effects in a lake or stream is depicted in Figure 3. Phosphorus exposure is the major determinant of phytoplankton production in most North American lakes. The nutrient may also be limiting in streams, but high water flows and flood events may overwhelm the effects of nutrients (Biggs 2000). Lake eutrophication leading to increased phytoplankton biomass may result in increased hypolimnetic oxygen deficit, decreased water clarity and changes in species composition (Interlandi and Kilham 2001). Phytoplankton diversity is rarely selected as an assessment endpoint (usually not relevant to management goals, see later discussion), but it is notable that species diversity and productivity are often inversely related (Interlandi and Kilham 2001). Periphyton biomass in lakes and streams is sometimes related to nutrient concentrations, but at other sites no relationship is evident (because of nutrient gradients/boundary layer within benthic mats and that in many cases their substrate is also a nutrient source) (Bourassa and Cattaneo 1998).

The relationship between aquatic macrophytes and phytoplankton is not straightforward, but it appears that epiphytes and filamentous algae increase in the presence of high nutrient loads and compete with macrophytes by shading them (Phillips *et al.* 1978). In addition, macrophytes can secrete allelopathic chemicals that reduce the growth of cyanobacteria or other algae (Scheffer *et al.* 1997; Phillips *et al.* 1978).



**Figure 3.** Conceptual model for ecological risk assessment of wastewater treatment unit in a freshwater lake or stream. Potential assessment endpoint entities and properties are denoted by rectangles, circles are stressors, and hexagons are processes. Exposure pathways of focal assessment endpoint properties are indicated with bold lines.

Zooplankton and fish biomass may be partly controlled by phytoplankton biomass, nutrient ratios in phytoplankton, and palatability of phytoplankton. Zooplankton densities would be expected to increase in the presence of diets that are not limited by phosphorus content (Brett *et al.* 2000). Cyanobacteria are less palatable to zooplankton than other algal species (Scheffer *et al.* 1997). Models exist that relate fish yield to phytoplankton production or standing crop (Oglesby 1977), but these are not described in the characterization of effects because indirect effects are beyond the scope of this risk assessment framework.

## Ecological Risk Assessment Framework for Wastewater Systems

The toxicity of nitrate to amphibians has been observed in several studies, and the low volumes of water in ditches or vernal ponds increase the likelihood of exposures to toxic concentrations of nitrate. Direct toxicity to fish is also observed, usually at concentrations higher than those to which amphibians are sensitive. Indirectly, nitrate-resistant adult fish may increase the predation pressure on amphibian eggs and tadpoles if the fish do not experience toxicity.

### Assessment Endpoints

The U.S. Environmental Protection Agency (USEPA 1998a) recommends three criteria for selecting assessment endpoints: ecological relevance, susceptibility, and relevance to management goals. Suter *et al.* (2000) amend these with three additional criteria: appropriate scale, unambiguous operational definition, and practical considerations, such as availability of effects data or standard toxicity tests.

The rectangular boxes in the generic conceptual models in Figures 2 and 3 are options for assessment endpoint entities and properties (USEPA 1998a) for ecological risk assessments for OWT. In this ecological risk assessment framework, particular properties and pathways are selected for emphasis because they are direct effects of nutrients, light limitation or oxygen limitation. Endpoint entities that do not appear on these figures may be selected, for example, potential endocrine disruption in reptiles (Pelley 2003).

The assessment endpoints that are emphasized in this risk assessment framework are presented in Table 1. In many ecological risk assessment precedents, the entity that is of concern for plants, fish and invertebrates is the community (except for threatened or endangered species), whereas populations of terrestrial vertebrates are deemed to merit protection. For this reason, the population is listed as the endpoint entity only for amphibians (Table 1). Risks to individual organisms are typically only assessed in cases of threatened or endangered species.

The ecological relevance of most of these candidate endpoints is described above. For example, the seagrass community is emphasized because of its role as a unique habitat and breeding ground for many species and because it has been shown to be susceptible to nitrogen in many shallow marine systems. Amphibians are included because of their particular susceptibility to nitrate. As shown in Figures 2 and 3, particular pathways are emphasized as the focal assessment endpoint properties for this ecological risk assessment framework.

The direction of change that is of concern is stated when defining the assessment endpoint. For example, usually a decrease in production of seagrass is of concern, rather than an increase in production. However, some of these directional designations may vary from assessment to assessment. For example, the loss of macrophytes in freshwater systems may have negative ecological consequences for species relying on that habitat type. However, if the ecological risk assessment feeds into a socio-economic analysis, an increase in macrophyte production may be of greater concern, because this vegetation may interfere with use of water for fisheries, recreation, industry, agriculture, and drinking (Carpenter *et al.* 1998; Jones *et al.* 2004).

The level of effect on each property that is of concern is specified in an ecological risk assessment. For example, the risk assessment may be designed to answer the question of whether the preponderance of the evidence shows that the abundance

**Table 1.** Potential focal assessment endpoint entities, properties, and measures for ecological risk assessment of onsite wastewater treatment.

Surface water category	Entity <sup>1</sup>	Property <sup>1</sup>	Measure of exposure <sup>1</sup>	Measure of effect <sup>1</sup>
Estuary/lagoon	Seagrass population	Decrease in production	Concentration of nitrate	Macrophyte biomass density Algal biomass density Light penetration
	Benthic invertebrate community	Decrease in abundance or production	Concentration of dissolved oxygen	Benthic invertebrate biomass
	Fish community	Decrease in abundance or production	Concentration of dissolved oxygen	Fish abundance
Fresh water	Phytoplankton community <sup>2</sup>	Increase in production	Concentration of available phosphorus	Algal biomass density Chlorophyll <i>a</i>
	Macrophyte community	Change in production	Concentration of available phosphorus	Macrophyte biomass density
	Fish community	Decrease in abundance or production	Concentration of nitrate Concentration of dissolved oxygen	Fish abundance
	Benthic invertebrate community	Decrease in abundance or production	Concentration of nitrate Concentration of dissolved oxygen	Benthic invertebrate biomass
	Amphibian populations	Decrease in abundance and production		Concentration of nitrate

<sup>1</sup>Assessment endpoint entities, properties, and measures are not intended to be an exhaustive list. Entities and properties are the focus of the characterization of exposure and the characterization of effects in this ecological risk assessment framework.

<sup>2</sup>Particular populations of the phytoplankton community may be additional endpoint entities, such as cyanobacteria.

of a population is decreased by more than 20%, that a population becomes extinct, or that significant reductions are expected in 40% of species in a community. Levels of effect are not specified in this framework, because they are management decisions. Because nitrogen loading rate predictions in estuaries have about 30% uncertainty associated with them (Valiela *et al.* 2000), it would not be appropriate to choose a level of effect lower than that for these exposures. Moreover, the user of this framework should be aware that many of the toxicity data (*e.g.*, for amphibians) are presented as LC50s (concentrations causing mortality to 50% of individuals), and the actual concentration of concern for the population may be a lower concentration that affects fewer than 50% of individuals of a population.

### Measures of Exposure and Measures of Effect

Measures of exposure and measures of effects are the numerical outputs of environmental sampling, analysis, testing and modeling. Factors that may be considered in selecting measures of effect include: correspondence to an assessment endpoint, quantifiable relationship to an assessment endpoint, availability of existing data, simplicity of measurement, cost, appropriate scale, relationship to exposure pathway, relationship to mode of action of stressor, specificity to a particular causation factor, low variability, broad applicability, and availability of standard test method (Suter *et al.* 2000; Valiela *et al.* 2000). Measures of exposure and effects in Table 1 are only examples that represent a "first guess" about the types of exposure-response relationships that would be available. Not all of these measures are useful in all environments. For example, nitrate concentration may not be a useful measure of seagrass production where the concentration is not observed to increase in water with increases in nitrogen loading (Nixon *et al.* 2001).

## CHARACTERIZATION OF EXPOSURE

The characterization of exposure phase of assessment describes measurements or model results in terms that are useful for estimating effects (USEPA 1998a; Suter *et al.* 2000). That is, if the average annual input of phosphorus is known, it may need to be converted to the average annual concentration of phosphorus in the water body if the exposure-response relationship is based on this latter unit.

Measurements of most forms of nutrients and dissolved oxygen are easy, and if sufficient measurements are taken to characterize spatial and temporal variability, measurement is clearly more accurate than modeling for a risk assessment of current nutrient releases. (Note that measurements do not generally allow the investigator to estimate the incremental exposure associated with OWT systems; concentrations incorporate all sources.)

Prospective risk assessments require modeling of concentrations of nutrients in surface water at the exposure point. Retrospective risk assessments require modeling if historical measurements are not available. In some risk assessments, for which treatment units are located at a distance from a surface water body, modeling would include estimates of nutrient runoff, leaching to groundwater, and possible attenuation in groundwater. Dynamic modeling of nutrient transport is recommended if septic tanks fail, causing acute exposure, or if responses in exposure-response

models are acute or based on particular organism life stages or growing seasons. If dynamic models are used, field-verified models are most reliable.

The output of the characterization of exposure is not usually a single nutrient concentration or loading rate. Exposure is best characterized by a distribution of nutrient concentrations, loading rates, or other measures or exposure through time or space.

As stated previously, this ecological risk assessment framework assumes that surface water bodies are at the edge of a pipe or drain field; thus, transport through soil is not discussed. As the nutrient enters the water body, dilution is not instantaneous, but it can be rapid if the point of exposure is a small ditch or vernal pond. Water quality simulation models take loading rates or concentrations at points of entry in the water body and descriptions of mixing and reaction kinetics in a stream reach or other water body segment, and estimate pollutant concentration in a particular water body segment. The models may be steady-state or time-varying. Water quality models are most often deterministic but occasionally stochastic (Viessman and Hammer 1985). Simplifying assumptions are often made, such as steady stream flow, first-order decay of organic matter, and no influence of biota on nutrient concentrations.

Nitrogen loading (mass or mols per unit volume or unit area per day or year entering a water body) is a common exposure parameter for exposure-response models in lagoons and shallow estuaries. Short and Burdick (1996) provide an empirical relationship for estimating nitrogen loading ( $\text{kg}/\text{km}^2/\text{yr}$ ) from the number of houses in watersheds of Waquoit Bay, Massachusetts. Analogous relationships could be derived from measurements in other estuaries or by extrapolating Waquoit Bay results to similar developments.

Rough approximations of nutrient inputs to water bodies can be made if one knows the nutrient-loading rate to a treatment system, the fraction of the nutrient that can be found at different distances from it, the wastewater generated per capita per day, the number of people per household and the density of houses (Gold and Sims 2001). About 60–95% of phosphorus from effluents typically is found in soils within a few meters of the drainfield (Gold and Sims 2001). As stated earlier, very little phosphorus travels from OWT systems to surface water bodies, although exceptions can occur if the water is near the drainfield (Gold and Sims 2001). In a study of 18 samples of groundwater adjacent to a lake in the Puget Sound watershed, only 4 showed the likely transport of more than 1% of the phosphorus released in septic tank effluent 9 to 50 m to the lake (Gilliom and Patmont 1983). Similarly, phosphorus from septic tank effluents that is found in shallow groundwater decreases logarithmically with distance (Reneau 1979).

Even if nutrient inputs are known, a water quality model may be used to estimate concentrations in particular parts of lakes, lagoons, or streams. Gilliom and Patmont (1983) estimate that phosphorus in septic system effluent usually is diluted about 1000 times before entering lake waters, though it is unclear if that relationship still holds.

Jones and Bachmann (1976) calculate phosphorus concentrations in lakes based on the steady state solution of an equation by Vollenweider (1969) that requires basic information about nutrient inputs, flushing rates, and basin morphometry. Vollenweider's mass balance model approach to estimating nutrient concentrations in lakes is also summarized in USEPA (2000b).

## Ecological Risk Assessment Framework for Wastewater Systems

Most exposure-response relationships for ecological receptors in surface water require concentrations of nutrients as measures of exposure; ecotoxicologists tend to measure or model concentrations rather than loading rates. However, nutrient loading rates to surface water bodies are not always easily converted to concentrations. Nixon *et al.* (2001) note that in phytoplankton-based mesocosms such as those at the University of Rhode Island's Marine Ecological Research Laboratory, there is a good relationship between rate of nitrogen input and concentration of nitrate (Nixon *et al.* 2001). However, they find that inorganic nitrogen amendments in lagoon mesocosms that may contain seagrasses, epiphytic algae, macroalgae and benthic microflora decline rapidly in the summer. Moreover, Valiela and Cole (2002) demonstrate that wetlands that border lagoons can intercept (denitrify and bury) land-derived nitrogen. Thus, caution is appropriate when normalizing volumetric nitrogen loading for residence time to yield an expected or potential concentration.

Although low oxygen levels, BOD, and light attenuation are effects of nutrient-enhanced production, we treat them as exposure parameters here because they are not assessment endpoint properties, but rather, they determine assessment endpoint properties. Dissolved oxygen is typically measured. Clearly, BOD from wastewater effluent is one factor that may lower dissolved oxygen concentrations in surface water. In addition, temperature, reaeration, and rates of photosynthesis and decomposition of nutrient-stimulated phytoplankton and periphyton are predictors of dissolved oxygen concentrations. Viessman and Hammer (1985) provide an example of the formulation of a water quality model to predict dissolved oxygen at a downstream location, given BOD of waste discharged at an upstream location. Also, Nürnberg (1996) provides a regression of areal hypolimnetic oxygen depletion rates of North American lakes versus total phosphorus, and Kelly (2001) presents Boynton and Kemp's (2000) regression of rates of dissolved oxygen decline in the Chesapeake Bay against rates of total chlorophyll *a* deposition.

Chlorophyll *a* is treated as a measure of exposure in one model involving seagrasses in estuaries. Although chlorophyll *a* is best measured, concentrations may be modeled based on nutrient concentrations. For example, between 1  $\mu\text{M}$  and 20  $\mu\text{M}$  dissolved inorganic nitrogen in shallow estuaries, chlorophyll *a* tends to increase at slightly less than 1  $\mu\text{g/L}$  with every 1  $\mu\text{M}$  increase in dissolved inorganic nitrogen or approximately about 0.75  $\mu\text{g}$  chlorophyll per  $\mu\text{M}$  dissolved inorganic nitrogen (USEPA 2001, Figure 3-2b). Also, Nixon (2001) develops a relationship between mean chlorophyll *a* and nitrogen input from an experiment in which nitrogen, phosphorus, and silicon were added in molar ratio of 12:1:1 at Marine Ecosystems Research Laboratory in Rhode Island.

If non-anthropogenic background concentrations of nutrients or other environmental parameters are unknown, the USEPA suggests plotting concentrations in all lakes, streams, or estuaries and selecting the 25th percentile concentration, or plotting concentrations in reference lakes, streams, or estuaries, and selecting the 75th percentile to represent reference concentrations (USEPA 2000b,c, 2001).

For the purpose of retrospective risk assessments of a particular nutrient source, such as OWT systems, nutrient loading may be apportioned to multiple sources. For example, Valiela *et al.* (2000) attribute nitrogen loading to atmospheric deposition on six land cover types, fertilizer use on four land cover types, and wastewater disposal from septic systems in the Green Pond watershed, MA, USA.

## CHARACTERIZATION OF EFFECTS

Exposure-response relationships may be available or derived from field observations, laboratory or mesocosm tests with site-specific media, or relationships from published studies. These latter relationships may focus on exposure measures, ecological receptors and locations that are somewhat different from those of concern in a particular assessment, but they may be the only relationships available for retrospective or prospective assessments for which field observations or surface water samples are not available.

Exposure-response models may be (1) empirical models derived from measurements at one or more sites, (2) mechanistic models, or (3) thresholds (exposure levels above which effects occur at a defined level). In this risk assessment framework, only one mechanistic model was identified to characterize effects (see later discussion of macrophyte-phosphorus dynamics in fresh water (Phillips *et al.* 1978).

In choosing among exposure-response models, several principles apply. (1) The use of more than one type of evidence or model leads to increased confidence in the result and aids in the characterization of uncertainty. (2) Models derived from data collected in ecosystems that are similar to the ecosystem of concern (*e.g.*, oligotrophic versus eutrophic conditions; epiphyte versus macroalgae versus phytoplankton dominance; nitrogen versus phosphorus-limited conditions) and with species that are related to assessment endpoint entities are recommended. (3) If a site-specific model is not available, general models are usually preferable to site-specific models for other types of sites. (4) Laboratory or mesocosm-derived values are more reliable if they have been verified in the field. (5) Models that use available measures of exposure are most useful. (6) The most direct estimate (involving a model with few parameters) usually has the lowest uncertainty. (7) Thresholds are useful for evaluating if there is an effect, but not for quantifying its magnitude. (8) When field observations are used, it may not be possible to attribute causation, if multiple stressors are present or if multiple sources of one stressor are present.

The following paragraphs describe water quality criteria and exposure-response relationships for various biota in freshwater and estuarine/lagoon environments. Thresholds for effects and other exposure-response relationships for the focal assessment endpoint entities are presented later. In toxicology jargon, thresholds may be called Lowest Observed Adverse Effects Levels (LOAELs) or Lowest Observed Adverse Effects Concentrations (LOAECs). No specific level of protection is assumed. For example, because the level-of-effect component of the assessment endpoint is selected by a regulatory agency or other risk manager, this framework does not arbitrarily present concentrations of nitrate that are associated with a specific percentage loss of a seagrass bed. Examples of many different levels of effect are provided.

### Water Quality Criteria for Nutrients

Water quality criteria for nutrients that were derived to protect ecological receptors are candidates for use in the characterization of effects. As with any threshold or other effects level, the relevance to the particular site and ecological receptor of interest should be determined prior to using these values.

In the 1986 USEPA document *Quality Criteria for Water*, no water quality criterion for nitrate was recommended. However, criteria for ambient dissolved oxygen

## Ecological Risk Assessment Framework for Wastewater Systems

concentrations were 6.5 mg/L for the protection of larval stages of coldwater fish and invertebrates (9.5 mg/L for those embedded in sediments) and 6.0 mg/L for the protection of larval stages of warm water organisms (USEPA 1986).

More recently the USEPA (1998b) developed a *National Strategy for the Development of Regional Nutrient Criteria*, which describes a two-phase process for the development of water quality standards for nutrients. First, "nutrient criteria guidance" for nitrogen, phosphorus, and related parameters such as chlorophyll *a*, Secchi disk depth, and algal biomass have been developed. These are numerical ranges that vary based on the type of water body (streams and rivers, coastal waters and estuaries, lakes and reservoirs, and wetlands) and region of the country. Second, states and tribes are developing nutrient water quality criteria for water bodies in numerous ecoregions. Many of these criteria are based on ecological effects.

### Estuary/Lagoon

#### Seagrass

Seagrass biomass and cover may be measured directly in ecological risk assessments of current conditions. Exposure-response relationships for seagrasses are presented in Table 2. These range from thresholds to continuous relationships. Measures of exposure include nutrients, many of the secondary stressors that result from nutrients (light attenuation, chlorophyll *a*, epiphyte load), and direct measures of seagrass. Among nutrients, nitrogen loading rates and nitrate concentrations are emphasized, but phosphate is also considered. A wide range of exposure metrics are used, including the number of houses in a watershed (Table 2).

Many of the relationships in Table 2 involve single nutrients, although it is clear that combinations of nutrients or other factors can alter effects thresholds for seagrasses. For example, Stevenson *et al.* (1993) provide dissolved inorganic nitrate concentrations that are associated with regrowth of four species of submerged aquatic plants. In addition, Stevenson *et al.* (1993) acknowledge that their thresholds may not apply if one of the factors changes independently of others.

Measures of effect in Table 2 include photosynthetic rates, growth, and cover of seagrasses. Aerial photography and remote sensing can be used to measure seagrass habitat loss directly, but these methods are not sensitive to small changes in biomass density and cannot be used to attribute causation to particular nutrients or sources unless ground measurements are taken.

The original references cited in Table 2 indicate if the relationships are valid for the range of exposure concentrations observed or predicted at a site of concern. For example, seagrass biomass is somewhat predictable at given levels of nitrogen input, but above certain levels of nitrogen, factors other than depth, water residence time and nitrogen input are necessary to predict the dominant plant type in very shallow marine systems (Nixon *et al.* 2001).

#### Benthic invertebrate and fish communities

As stated earlier in the description of the conceptual model for shallow estuaries, effects on benthic and water column populations are possible due to trophic level interactions caused by changing vegetation (*e.g.*, relative dominance of seagrass and algae). However, these effects are unlikely to occur as a result of releases of nutrients

**Table 2.** Exposure-response relationships and effects thresholds for seagrasses.

Measure of exposure or indicator of effect <sup>1</sup> (X)	Effect (Y)	Type of model	Value or relationship <sup>2</sup>	Reference
Number of houses in watershed	% sediment area covered with eelgrass	Empirical, Waquoit Bay, MA	$\text{Log}(Y) = 1.666 - 0.0004(X)$	Short and Burdick (1996)
N loading (kg/km <sup>2</sup> /yr)	% sediment area covered with eelgrass	Empirical, Waquoit Bay, MA	$\text{Log}(Y) = 1.648 - 0.000044(X)$	Short and Burdick (1996)
N loading (kg/ha/yr)	Eelgrass loss	Threshold, loss of 80–96% of bed area in 1990s, Waquoit Bay, MA	30	Hauxwell <i>et al.</i> (2003)
N loading (kg/ha/yr)	Total disappearance of eelgrass	Threshold, Waquoit Bay, MA	60	Hauxwell <i>et al.</i> (2003)
N loading (kg/ha/yr)	% seagrass production/total production	Empirical, numerous estuaries	$Y = 145.653(X^{-0.550})$	Valiela and Cole (2002)
N loading (kg/ha/yr)	% seagrass area lost (10–30 yr)	Empirical, numerous estuaries	$Y = 0.693(x) + 14.211$	Valiela and Cole (2002)
N loading (kg/ha/yr)	Seagrass cover, production, extent of meadows	Threshold, numerous estuaries	20–100	Valiela and Cole (2002)
N loading (kg/ha/yr)	Seagrass cover, production, extent of meadows	Threshold, Cape Cod	20–30	Valiela and Cole (2002)
Nitrate-N loading ( $\mu\text{M}/\text{d}$ )	Eelgrass growth and survival	Experimental threshold, North Carolina mesocosm	3.5	Burkholder <i>et al.</i> (1992)
Total N concentration	Uninhibited eelgrass growth	Threshold (maximum), Chesapeake Bay	10 mmol/m <sup>3</sup>	References cited in Nixon <i>et al.</i> (2001)
Total N concentration	Uninhibited eelgrass growth	Threshold (maximum), coastal Denmark	70 mmol/m <sup>3</sup>	References cited in Nixon <i>et al.</i> (2001)
Input of total N	Decline of seagrass bed	Threshold, mesocosms, Rhode Island	2 mmol N/m <sup>2</sup> /d	Nixon <i>et al.</i> (2001)

(Continued on next page)

Dissolved inorganic N concentration	Regrowth of submerged aquatic vegetation ( <i>Ruppia maritima</i> , <i>Potamogeton perfoliatus</i> , <i>Potamogeton pectinatus</i> )	Threshold, estuarine gradient, Chesapeake Bay	<10 $\mu\text{M}$ (or N:P ratio > 100 or $\sim 1$ )	Stevenson <i>et al.</i> (1993)
Insolation at surface of canopy compared to water surface	Decline of seagrasses or submerged aquatic macrophytes	Threshold	11% (5–20%)	EPA 2001
Dissolved inorganic P concentration	Regrowth of submerged aquatic vegetation ( <i>Ruppia maritima</i> , <i>Potamogeton perfoliatus</i> , <i>Potamogeton pectinatus</i> )	Threshold, estuarine gradient, Chesapeake Bay	<0.35 $\mu\text{M}$ (or N:P ratio > 100 or $\sim 1$ )	Stevenson <i>et al.</i> (1993)
Total suspended solids	Regrowth of submerged aquatic vegetation ( <i>Ruppia maritima</i> , <i>Potamogeton perfoliatus</i> , <i>Potamogeton pectinatus</i> )	Threshold, estuarine gradient, Chesapeake Bay	<20 mg/L	Stevenson <i>et al.</i> (1993)
Chlorophyll <i>a</i>	Regrowth of submerged aquatic vegetation ( <i>Ruppia maritima</i> , <i>Potamogeton perfoliatus</i> , <i>Potamogeton pectinatus</i> )	Threshold, estuarine gradient, Chesapeake Bay	<15 $\mu\text{g/L}$	Stevenson <i>et al.</i> (1993)
Epiphyte biomass ( $\mu\text{g C/cm}^2$ )	photosynthetically available radiation-based photosynthesis of eelgrass and turtle grass, normalized to maximum rate	Empirical, Monterey Bay and Bahamas	$Y = -0.0025(x) + 1$	Drake <i>et al.</i> (2003)

(Continued on next page)

**Table 2.** Exposure-response relationships and effects thresholds for seagrasses. (*Continued*)

Measure of exposure or indicator of effect <sup>1</sup> (X)	Effect (Y)	Type of model	Value or relationship <sup>2</sup>	Reference
Epiphyte biomass ( $\mu\text{g C}/\text{cm}^2$ )	Spectral photosynthesis of eelgrass and turtle grass, normalized to maximum rate	Empirical, Monterey Bay and Bahamas	$Y = -0.0055(x) + 1$	Drake <i>et al.</i> (2003)
Seagrass leaf elongation	Unlikely bed survival	Environmental indicator	$> 1 \text{ cm/d}$	Nixon <i>et al.</i> (2001)
Density of seagrass	Unlikely bed survival	Environmental indicator	$< 100\text{--}150 \text{ shoots/m}^2$	Nixon <i>et al.</i> (2001)
Seagrass shoot to root ratio at midsummer	Unlikely bed survival	Environmental indicator	$> 1 \text{ or } 2$	Nixon <i>et al.</i> (2001)

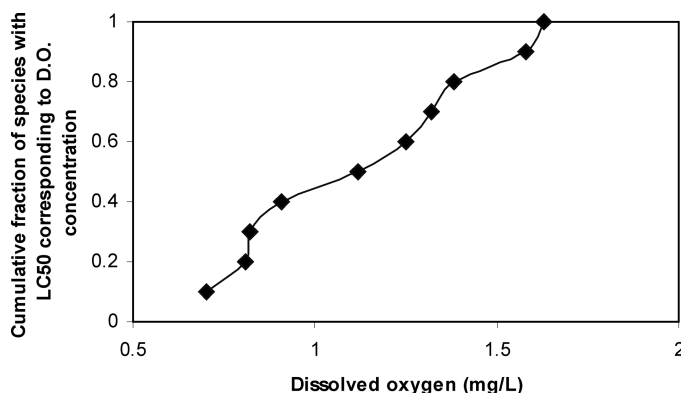
<sup>1</sup>Many of these measures of exposure represent effects of nutrients that serve as stressors for secondary effects. Others represent indicators of effects.

<sup>2</sup>The reader is cautioned to consult each study to determine the applicability of relationships before using them for a particular risk assessment.

from one or a few treatment units, so these exposure-response relationships are not presented here. Measurements of benthic invertebrate abundance and species richness may not allow the attribution of a probable cause.

Mortality due to low oxygen levels is a direct effect. Hypoxia is generally defined as critically reduced oxygenation of biological tissue caused by a water column oxygen concentration of less than 2 mg/L (Kelly 2001). Kelly proposes a hypoxic threshold value of total nitrogen of 80  $\mu$ M, based on several mesocosm and field studies and reprinted in USEPA (2001). However, because this value relates to hypoxia rather than to dissolved oxygen concentrations that cause specific, ecological effects, Kelly's value is of limited use in ecological risk assessment. Rosenberg *et al.* (1991) recommend an exposure limit of 1.4 mg/L oxygen for several days to weeks for coastal benthic communities. Standards for U.S. states are sometimes higher (*e.g.*, 5 to 6 mg/L), but are not necessarily intended to protect the most sensitive species (NRC 2000).

Numerous data are available on the effects of low oxygen on various species. Many of these data are summarized in USEPA (2000a) and are not repeated here. However, because many of these data are expressed as consistent results of standard toxicity test methods, these values are used to illustrate the utility of a species sensitivity distribution. A fraction or percentile of the distribution of concentrations associated with effects in various species can be used to identify a concentration to which that fraction of the community would be affected. For example, half of saltwater fish populations would be expected to have LC50s at 1.12 mg/L or lower dissolved oxygen (Figure 4). An untested species may be assumed to be a random draw from the distribution of tested species, or the distribution may represent the proportion of species in a fish community that is likely to be affected by a particular concentration of dissolved oxygen. For distributions of toxic levels of most nutrients, the X-axis would be expected to have a greater range of values than this curve related to toxicity of dissolved oxygen (Figure 4). The uses and forms of species sensitivity distributions are described in the many chapters of Postuma *et al.* (2002).



**Figure 4.** Species sensitivity distribution of LC50s for saltwater fish exposed to low concentrations of dissolved oxygen.

## Fresh Water

### Phytoplankton

Chlorophyll is typically the most direct measure of phytoplankton biomass, and several broad relationships between phosphorus concentrations in lakes and chlorophyll *a* are available for risk assessments (Table 3). Most regressions in Table 3 are linear, but van Nieuwenhuysse and Jones (1996) provide a few references to suggest that the total phosphorus-chlorophyll relationship for lakes may be curvilinear across broad ranges of phosphorus concentrations. They provide relationships between total phosphorus and chlorophyll in streams, with one model including stream catchment area. The relationships for streams tend not to be as tight as those for lakes because of the importance of flow generally, and flooding intervals, specifically (Biggs 2000). The relationship between total phosphorus and primary productivity in Wetzel (1983) is also nonlinear because of the self-shading effects of dense algal populations. In some systems the predictions of chlorophyll may be affected by grazing pressure (*e.g.*, high number of filter-feeding bivalves), high turbidity, and nitrogen limitation (Nixon *et al.* 2001).

Algal species dominance is more difficult to predict than total production. Wetzel (1983) provides a table of minimum phosphorus requirements per unit cell volume of algal genera that are common to lakes of progressively increasing productivity: *Asterionella*, *Fragilaria*, *Tavellaria*, *Scenedesmus*, *Oscillatoria*, and *Microcystis*. Blooms of cyanobacteria, a subset of the phytoplankton community, would be expected to be assessment endpoint entities in many risk assessments for onsite wastewater treatment. In an analysis of 17 world lakes, Smith (1983) showed that water bodies having an epilimnetic total nitrogen to total phosphorus ratio greater than 29, by weight, usually had low proportions of cyanobacteria. Scheffer *et al.* (1997) did not find a significant relationship between abundance of cyanobacteria and either the total nitrogen-to-phosphorus ratio or the concentration of phosphorus in 55 Dutch lakes, but did find a significant relationship with Secchi disk depth (transparency) and another multivariate shade indicator.

Eutrophication is not identified as an assessment endpoint entity in this risk assessment framework, but the process may be of interest to a risk manager. A risk assessment goal may be to determine whether or not the lake has transitioned to a higher trophic state in the past decade, that is, from oligotrophic to mesotrophic or mesotrophic to eutrophic. Various investigators provide classification schemes relating nutrient concentrations to trophic designations. For example, Wetzel (1983, Table 13–14) modifies a scheme from Vollenweider (1979) that includes ranges of total phosphorus concentrations, total nitrogen concentrations, chlorophyll *a* concentrations of phytoplankton, chlorophyll *a* peak concentrations, and Secchi disk depth.

### Periphyton

Periphyton biomass is not always related to nutrient concentration, and Bourassa and Cattaneo (1998) review several of the studies that observed significant relationships and those that did not. In one study, more than half of the periphyton biomass in 13 rivers in southern Ontario and western Quebec was explained by total

**Table 3.** Exposure-response relationships for phytoplankton in fresh water.

Measure of exposure (X)	Effect (Y)	Type of model	Value or relationship	Reference
Mean annual concentration, total P (mg/m <sup>3</sup> )	Mean annual chlorophyll <i>a</i> (mg/m <sup>3</sup> )	Empirical, lakes in Experimental Lakes Area, Ontario	$Y = 0.987 X - 6.520$	Schindler (1977)
Input of P, normalized for mean depth, water residence time, P sedimentation	Mean annual chlorophyll <i>a</i> (mg/m <sup>3</sup> )	Empirical, several oligotrophic lakes, mesotrophic lakes, eutrophic lakes	See paper; graph reprinted in Nixon <i>et al.</i> (2001)	Vollenweider (1976)
Total P concentration	Summer mean chlorophyll <i>a</i> (mg/m <sup>3</sup> )	Empirical, 143 lakes	$\text{Log } Y = 1.46 \text{ log } X - 1.09$	Jones and Bachmann (1976)
Total P concentration at spring overturn (mg/m <sup>3</sup> )	Summer mean chlorophyll <i>a</i> (mg/m <sup>3</sup> )	Empirical, 19 lakes in southern Ontario and 27 other North American lakes	$\text{Log } Y = 1.449 \text{ log } X - 1.136$	Dillon and Rigler (1974)
Summer mean total P concentration (mg/m <sup>3</sup> )	Summer mean chlorophyll <i>a</i> (mg/m <sup>3</sup> )	Empirical, 292 stream samples, worldwide	$\text{Log } Y = -1.65 + 1.99 \text{ log } X - 0.28 (\text{log } X)^2$	Nieuwenhuys and Jones (1996)
Summer mean total P concentration (mg/m <sup>3</sup> ); stream catchment area	Summer mean chlorophyll <i>a</i> (mg/m <sup>3</sup> )	Empirical, 292 stream samples, worldwide	$\text{Log } Y = -1.92 + 1.96 \text{ log } X_1 - 0.30 (\text{log } X_1)^2 + 0.12 + 0.12 \text{ log } X_2$	Nieuwenhuys and Jones (1996)
Predicted total P concentration (mg/m <sup>3</sup> )	Annual primary productivity (g C/m <sup>2</sup> /yr)	Empirical, Laurentian Great Lakes, other American Lakes, European Lakes	See Wetzel (1983), Figure 13-10	Wetzel (1983), from Vollenweider (1979)

phosphorus concentration (Chételat *et al.* 1999). In another investigation, almost half of the variation in mean monthly chlorophyll *a* in 25 New Zealand rivers was explained with a combination of dissolved nutrient data and days of accrual, to account for flood frequency (Biggs 2000). Bourassa and Cattaneo (1998) observed that in the range of 5 to 60  $\mu\text{g/L}$  of phosphorus in 12 Laurentian streams in Quebec, grazer biomass and mean grazer size explain a majority of the variability in periphyton, with current velocity and depth also being significant, but phosphorus not being significant. As with all assessment endpoints, available studies should be examined to determine which exposure-response relationship to use.

### Aquatic macrophytes

Macrophyte abundance and production may be measured or predicted based on complex processes that include nutrient availability, light penetration, and additional biotic factors. Bachmann *et al.* (2002) found that submersed macrophytes were absent in Florida lakes at phosphorus concentrations above 0.166 mg/L, but below that concentration, they might be present or absent. Some investigators have hypothesized that macrophytes decline when they are shaded by epiphytes and filamentous algae that are stimulated by high nutrient loads (Phillips *et al.* 1978), although Bachmann *et al.* did not observe this relationship. The pondweed *Potamogeton pectinatus* remained in a Netherlands lake at phosphorus levels above 0.6 mg/L (van den Berg *et al.* 1999). Charophytes (macroalgae) were observed to disappear from this lake at phosphorus concentrations above 0.3 mg/L, and recolonization required concentrations below 0.1 mg/L phosphorus (van den Berg *et al.* 1999).

Below Bachmann *et al.*'s high nutrient threshold, there was no relationship between nutrients and densities of submerged macrophytes. The lack of a relationship may be explained by the fact that macrophytes can obtain their nutrients from sediments in addition to the water column (Bachmann *et al.* 2002). Furthermore, Scheffer *et al.* (1993) found alternative equilibria in shallow lakes at similar nutrient levels, that is a clear state dominated by aquatic macrophytes or a turbid state with high algal biomass. Macrophytes tend to have more of a predictable effect on nutrient concentrations (because of uptake) than nutrients have on macrophytes (Bachmann *et al.* 2002).

Light penetration is also a factor in determining macrophyte biomass, but light thresholds may not be useful for indicating macrophyte dominance. Lakes in Florida showed a decrease in the biomass of macrophytes below water color values of about 150 Pt-Co (platinum-cobalt) units, but phytoplankton abundance also decreased at that color. The average Secchi disk depth in macrophyte-dominated lakes was greater than the depth in algal-dominated lakes, but there was a broad range of overlap between the two groups of lakes (Bachmann *et al.* 2002). For a single lake in the Netherlands, van den Berg *et al.* (1999) found a threshold Secchi depth (0.4 m) below which charophytes are not observed, but a slight negative correlation of *Potamogeton pectinatus* with Secchi depth.

High flows are a factor that is pertinent to macrophytes in streams. Wade *et al.* (2001) present a dynamic, mechanistic model for the Kennet River in southern England that represents the phosphorus cycle of reservoirs (including total and soluble reactive phosphorus) and in-stream processes that control the transfer of

phosphorus between those reservoirs. Water flow, suspension and deposition of suspended sediment, and growth of epiphytes and macrophytes are modeled. This site-specific model is a useful illustration that (1) exposure-response relationships can be represented by mechanistic models and (2) the processes that regulate macrophyte growth in fresh water are complex and not necessarily amenable to simple thresholds or regressions.

### Fish community

Fish community abundance and species richness may be measured. Or toxicity values from laboratory tests of nitrate and dissolved oxygen may be applied. Nitrate is generally the least toxic of the three forms of nitrogen (ammonia, nitrate and nitrite) to fish and amphibians (Rouse *et al.* 1999). Hecnar (1995) notes that LC50s for fish in several studies range from 800 to 12000 mg/L nitrate (180 to 2700 mg/L nitrate-N), high values that are consistent with the USEPA's 1986 decision not to set a water quality criterion for nitrate (see earlier discussion). However, significant mortality of eggs and/or fry of salmonid species have been recorded at 5 mg/L (steelhead trout, *Salmo gairdneri*), 10 mg/L (rainbow trout, *Salmo gairdneri*; cutthroat trout, *Salmo clarki*), and 20 mg/L (chinook salmon, *Oncorhynchus tshawytscha*) nitrate (1.1, 2.3, and 4.5 mg/L nitrate-N) in waters of low hardness (Kincheloe *et al.* 1979). Thus, if the fish community is an assessment endpoint entity, then existing effects data should be reviewed for toxicity to fish of different species and life stages.

A species sensitivity distribution of toxicity values for hypoxia would be analogous to the distribution in Figure 4, derived from USEPA (2000a). Effects of low oxygen in fresh water would be expected to be similar to those in salt water and to display similarly low variability.

### Amphibian populations

Amphibian abundance may be measured, or effects may be predicted using toxicity test data. Effects levels for toxicity of nitrate to amphibians are presented in Table 4. One or more of these effects levels may be chosen for use in a risk assessment (depending on the amphibian species of concern), or similar effects levels may be plotted in a species sensitivity distribution analogous to that in Figure 4, and the sensitivity of an untested species may be assumed to be a random variate in that distribution. This plot would facilitate comparisons with distributions of nitrate concentrations from the characterization of exposure. However, nitrate tolerance of amphibians such as the common frog (*Rana temporaria*) may vary based on the level of adaptation in a particular region (Johansson *et al.* 2001).

Effects on reproduction, growth, and survival are assumed to relate to the endpoint properties of abundance and production. Deformities are usually related to reproduction as well, and may be considered important by themselves. Therefore, tests that focus on these effects are more pertinent to and useful in the risk assessment than tests of behavior.

It is notable that ammonium nitrate was used as the source of nitrate in some tests, and toxicity of the ammonium ion may be partly responsible for the toxicity. Johansson *et al.* (2001) showed greater mortality of common frogs exposed to ammonium nitrate than of those exposed to the same concentration of sodium nitrate.

**Table 4.** Effects levels for toxicity of nitrate-N to amphibians.

Species	Common name	Stage	Chemical	Toxicity test endpoint	Concentration (mg/L)	Reference
<i>Bufo americanus</i>	American toad	Tadpole	Ammonium nitrate <sup>1</sup>	96-hr LC50	13.6, 39.3	Hecnar (1995)
<i>Pseudacris triseriata</i>	Chorus frog	Tadpole	Ammonium nitrate	96-hr LC50	17.0	Hecnar (1995)
<i>Rana pipiens</i>	Leopard frog	Tadpole	Ammonium nitrate	96-hr LC50	22.6	Hecnar (1995)
<i>Rana clamitans</i>	Green frog	Tadpole	Ammonium nitrate	96-hr LC50	32.4	Hecnar (1995)
<i>Pseudacris triseriata</i>	Chorus frog	Tadpole	Ammonium nitrate	Chronic: Development, behavior or mortality	2.5–10	Hecnar (1995)
<i>Rana pipiens</i>	Leopard frog	Tadpole	Ammonium nitrate	Development, behavior or mortality	2.5–10	Hecnar (1995)
<i>Rana clamitans</i>	Green frog	Tadpole	Ammonium nitrate	Development, behavior or mortality	2.5–10	Hecnar (1995)
<i>Bufo bufo</i>	Common toad	Tadpole	Ammonium nitrate	96 hr LC50	385	Xu and Oldham (1997)
<i>Bufo bufo</i>	Common toad	Tadpole	Ammonium nitrate	168 hr LC50	338	Xu and Oldham (1997)
<i>Bufo bufo</i>	Common toad	Tadpole/ meta-morph	Ammonium nitrate	30-d Subchronic, 21% lethality and 17% failure to resorb tails during metamorphosis	23	Xu and Oldham (1997)
<i>Bufo bufo</i>	Common toad	Tadpole	Sodium nitrate	Growth	9	Baker and Waights (1993)
<i>Bufo bufo</i>	Common toad	Tadpole	Sodium nitrate	Mortality	22.6	Baker and Waights (1993)
<i>Litoria caerulea</i>	Tree frog	Tadpole	Sodium nitrate	Development	9	Baker and Waights (1994)

<i>Litoria caerulea</i>	Tree frog	Tadpole	Sodium nitrate	Mortality	9	Baker and Waights (1994)
<i>Ambystoma gracile</i>	Northwestern salamander	Larva	Potassium nitrate	15-d, LOAEC (13% lethality)	12.5	Marco <i>et al.</i> (1999)
<i>Rana pretiosa</i>	Oregon spotted frog	Larva	Potassium nitrate	15-d, LOAEC (39% lethality)	12.5	Marco <i>et al.</i> (1999)
<i>Triturus helveticus</i>	Palmate newt	Larva	Ammonium nitrate	Mortality, Rapid metamorphosis	11.3	Watt and Jarvis (1997)
<i>Pseudacris regilla</i>	Pacific tree frog	Embryo	Sodium nitrate	10-d LC50	578.0	Schuytema and Nebeker (1999)
<i>Xenopus laevis</i>	African clawed frog	Embryo	Sodium nitrate	4, 5-d LC 50	871.6, 438.4	Schuytema and Nebeker (1999)
<i>Pseudacris regilla</i>	Pacific tree frog	Embryo	Sodium nitrate	10-d, LOAEC, length, weight	111.0	Schuytema and Nebeker (1999)
<i>Xenopus laevis</i>	African clawed frog	Embryo	Sodium nitrate	5-d, LOAEC, length	111.0	Schuytema and Nebeker (1999)
<i>Xenopus laevis</i>	African clawed frog	Embryo	Sodium nitrate	5-d, LOAEC, weight	56.7	Schuytema and Nebeker (1999)
<i>Xenopus laevis</i>	African clawed frog	Embryo	Sodium nitrate	5-d, LOAEC, deformity	230.4	Schuytema and Nebeker (1999)
<i>Xenopus laevis</i>	African clawed frog	Embryo	Sodium nitrate	4-d, LOAEC, frog embryo	>470.4	Schuytema and Nebeker (1999)
<i>Rana sylvatica</i>	Wood frog	Egg	Sodium nitrate	teratogenesis assay, weight, length	>9	Laposata and Dunson (1998)
<i>Ambystoma jeffersonianum</i>	Jefferson salamander	Egg	Sodium nitrate	Hatching success, deformity	>9	Laposata and Dunson (1998)

(Continued on next page)

**Table 4.** Effects levels for toxicity of nitrate-N to amphibians (*Continued*).

Species	Common name	Stage	Chemical	Toxicity test endpoint	Concentration (mg/L)	Reference
<i>Ambystoma maculatum</i>	Spotted salamander	Egg	Sodium nitrate	Hatching success, deformity	>9	Laposata and Dunson (1998)
<i>Bufo americanus</i>	American toad	Egg	Sodium nitrate	Hatching success, deformity	>9	Laposata and Dunson (1998)
<i>Rana cascadae</i>	Cascades frog	larva	Sodium nitrate, pH 5, pH 7	21-d mortality, stat signif	>4.5	Hatch and Blaustein (2000)
<i>Rana cascadae</i>	Cascades frog	larva	Sodium nitrate, pH 5	21-d mortality, 31%, not stat signif	1	Hatch and Blaustein (2000)
<i>Rana cascadae</i>	Cascades frog	larva	Sodium nitrate, pH 5, UV-B	21-d mortality, 50%, 25% mortality	1, 4.5	Hatch and Blaustein (2000)
<i>Xenopus laevis</i>	African clawed frog	tadpole	Sodium nitrate	mortality	>65.9	Sullivan and Spence (2003)

<sup>1</sup>Ammonia could be a contributor to toxicity in studies where ammonium nitrate was used. Table from Rouse *et al.* (1999) used with permission from *Environmental Health Perspectives* and amended with additional data.

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Therefore, thresholds derived from bioassays of ammonium nitrate should be used with caution.

Although beyond the scope of this framework, a risk assessment for larger-scale wastewater treatment at a distance from surface water bodies would consider several additional factors:

- (1) Potential effects of nitrate in soil on amphibians. In one study amphibian mortality was observed on recently fertilized fields (Schneeweiss and Schneeweiss 1997). Another investigation measured effects of ammonium nitrate on *Rana temporaria* (Oldham *et al.* 1997).
- (2) Nitrite as a stressor. Nitrite has been exhibited to impede tadpole development at levels as low as 3.5 mg/L (Marco and Blaustein 1999).
- (3) Potential indirect effects of nitrate on amphibians. Amphibian insect prey and some predators (fish) are sensitive to similar levels of nitrate as amphibians (Rouse *et al.* 1999). Also, effects of nitrate on tadpoles may be mediated by effects on their algal forage (Xu and Oldham 1997). Thus, amphibians may be impacted indirectly by aquatic community dynamics, and the direction of the expected effect would depend on the relative sensitivity of amphibians, prey, and predators to nitrate.

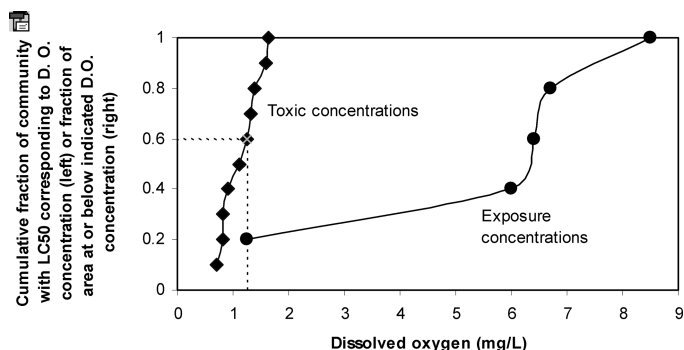
## RISK CHARACTERIZATION

### Screening-Level Risk Assessment

In some ecological risk assessments, the goal may be to eliminate sources, nutrients, or ecological receptors that have no potential for risk. This screening-level ecological risk assessment may consist of comparisons of nutrient concentrations or loading rates to reference concentrations or rates, as well as comparisons to conservative estimates of thresholds for ecological risk (*e.g.*, low estimates of effective concentrations of nutrients; high estimates of effective concentrations of dissolved oxygen). The screening-level risk assessment may be the goal of the entire undertaking, or it may be the first phase in a tiered risk assessment, to help focus the assessment on potential problems.

### Implementing Exposure-Response Models

If sufficient data are available, the risk characterization consists of a comparison of distributions of nutrient exposure concentrations or loadings and those of probable effects for each assessment endpoint. Exposure and effects characterizations are performed with equivalent spatial and temporal dimensions. Figure 5 presents an example of a graph in support of a risk characterization for saltwater fish exposed to potentially low dissolved oxygen levels. The example adds exposure concentrations to the species sensitivity distribution in Figure 4 and reinterprets the Y axis as the fraction of the community affected (rather than fraction of species affected) because exposure concentrations measured in the example represent five different spatial fractions (each 20%) of the fish community. (These five samples might represent five depths or five distances from a nutrient source.) Measurements at four of five



**Figure 5.** Species sensitivity distribution of LC50s for saltwater fish exposed to low concentrations of dissolved oxygen, compared to concentrations measured at five locations, each representing 20% of the lagoon area, at the sediment-water interface of a hypothetical lagoon.

locations are well above the median lethal dose for any portion of the community, but the dissolved oxygen level at one location (20% of the area) is likely to be lethal for a large fraction of fish species in the community. At the location with 1.25 mg/L dissolved oxygen, LC50s would be exceeded for about 60% of the fish community. Impacts on growth are likely to occur at higher concentrations of oxygen than those concentrations that are associated with mortality. For example, the USEPA water quality criteria for dissolved oxygen are 6 mg/L and higher (see earlier discussion).

Many of the relationships presented in the characterization of effects section are not direct measures of the assessment endpoint property. Therefore, extrapolations are necessary from chlorophyll *a* to algal biomass and from lethal concentrations of nutrients or concentrations that affect growth to the spatial range of a population that may be affected. For example, if the effluent from one treatment system flows into a ditch where tadpoles are located, would death of all tadpoles in that ditch significantly affect the local population of frogs?

### Weight of Evidence

If multiple measures of exposure exist, or if multiple, empirical, exposure-response models are available, all of these may be used to obtain distinct estimates of risks to the assessment endpoints. One example is the use of effects models for seagrass based on total nitrogen loading and those based on nitrate concentration in lagoons. In addition, biological surveys at the site may be used or performed.

Lines of evidence may be weighted differentially, based on the inherent quality of the models employed and on the quantity and quality of data used to implement them. Ultimately, a determination is made about whether an adverse effect is likely for a particular endpoint property and nutrient combination. Or if the goal of the ecological risk assessment is to estimate the magnitude of effect, the estimates of magnitude that result from using different methods to characterize exposure or effects may be weighted, and the result may be a weighted average estimate of the magnitude of effects. Criteria that may be used to weigh evidence include:

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(1) the relevance of data to the assessment endpoint; (2) the strength of an exposure-response relationship; (3) congruence of the temporal and spatial scope of the evidence with the endpoint; and (4) quality of the data in terms of sampling protocols, expertise, quality assurance, and reasonableness of conclusions (Suter *et al.* 2000).

Two example applications of the weight-of-evidence approach to risk characterization for individual OWT systems are presented. The first example expands on the hypothetical exposure-response model presented in Figure 5. In this example an emerging OWT system is proposed for a new home being built at the edge of a small estuarine lagoon. The site is considered to be unsuitable for a traditional or contemporary OWT system, because the set-back from the lagoon is extremely small (*i.e.*, the porous media biofilter and UV disinfection units are approximately ten feet from the site boundary, at which point the terrain slopes steeply downward for a short distance to the edge of the lagoon). It is assumed that in a rare event, the OWT system fails suddenly and “catastrophically,” discharging untreated wastewater that flows onto the soil surface and then into the lagoon.

Conservative assumptions and a simple dilution/mixing model are used to predict dissolved oxygen concentrations at five locations in the lagoon. These modeled concentrations are then compared with the effects data for saltwater fish (Figure 5). The results of this comparison are then considered in the context of the exposure scenario. In an actual assessment, the risk characterization would include an extensive discussion of the uncertainties associated with the predicted risks and a concise summary of the evidence and conclusions in a weight-of-evidence table. Only the weight-of-evidence table is presented in this example (Table 5).

The second example is for a traditional OWT system with a small farm pond located at the edge of the site boundary. The system was installed when a new home was built on a lot that was previously part of a small farm, the owners of which still reside in an adjacent home and retain ownership of the pond. These off-site residents and their visitors have casually observed a substantial decrease in the number of frogs inhabiting the pond since the OWT system in question was installed. They are concerned that wastewater effluent is responsible for the apparent decline in frogs in the pond. Based on this concern, county officials inspect the OWT system and sample the pond for wastewater constituents (*e.g.*, pathogens and nitrate). The system appears to be functioning properly, and the substantial set-back from the pond (*i.e.*, 100 feet from the drainfield) leads to the conclusion that the OWT system is unlikely to be a significant source of contamination to the pond. The water samples confirm this assumption. Thus, the weight-of-evidence strongly suggests that amphibians inhabiting the pond are not at risk from the OWT system in question (Table 6).

Both of these examples address risks associated with a single OWT unit. Many risk assessments for OWT systems are expected to be studies of larger scale that involve multiple OWT units and that will potentially impact watershed management. Most of these risk assessments will be prospective, will favor modeling over measurement, and may include only one line of evidence, as in Table 5. For these risk assessments to be useful, it will be important to verify model results for developments whose OWT systems are constructed as assumed in the risk assessment.

**Table 5.** Example weight-of-evidence summary for risks to aquatic communities in a small estuarine lagoon exposed to untreated wastewater in run-off from a failing, emerging OWT system.

Evidence	Result	Level of confidence in effect or non-effect	Level of confidence in cause-effect relationship	Explanation
Biological "survey"	N/A	N/A	N/A	Biological surveys are not available, because this is a prospective assessment for a new installation ( <i>i.e.</i> , the OWT system in question has not actually failed yet).
Predicted dissolved oxygen (DO) concentrations in the lagoon	+	Medium	High	The failure scenario assumes that untreated wastewater "breaks through" to the soil surface and flows into the lagoon. The CBOD of the wastewater is assumed to be very high ( <i>e.g.</i> , ~300 mg/L). Surface water DO levels are predicted for five locations based on the "known" volume and flushing rate of the lagoon in question, both of which are relatively small. DO levels at one of the five locations ( <i>i.e.</i> , the one nearest the OWT system) are expected to be acutely toxic to almost half of the fish at that location ( <i>i.e.</i> , the LC50 is exceeded for 40% of the tested species; Figure 5.3). A similar exposure-response analysis for benthic invertebrates also indicates that acute toxicity is likely at the location closest to the OWT system.
Weight of evidence	+	Medium	High	Only one line of evidence is available, but it suggests that a "total" failure of the OWT system in question would pose unacceptable risks to fish and invertebrates in the lagoon. Substantial uncertainty is associated with the exposure scenario, for which conservative assumptions were used. However, effects data are very reliable and a "fish kill" of any size is considered to be unacceptable by the decision makers.

**Table 6.** Example weight-of-evidence summary for risks to amphibians inhabiting a small pond potentially exposed to nitrogen from a traditional OWT system.

Evidence	Result	Level of confidence in effect or non-effect	Level of confidence in cause-effect relationship	Explanation
Biological "survey"	+	Low	Low	Residents and visitors have seen and heard fewer frogs in the pond over the last several years. This apparent reduction in abundance coincides with the building of a new house with a traditional OWT system immediately up-gradient of the pond approximately five years earlier. However, a scientifically designed survey has not been performed, and amphibian populations are known to be in decline throughout the state in which the site is located.
Predicted nitrate concentrations in groundwater discharging into the pond	-	Low	High	Installation of a traditional OWT system with the drain field located 100 feet from the pond was approved by the county. This set-back distance is based on conservative loading and treatment assumptions for the protection of human health. It is assumed that the nitrate concentration for groundwater entering the pond will not exceed 10 mg/L ( <i>i.e.</i> , a regulatory threshold for water supply wells in the area). This concentration is below those reported to be toxic in most of the published studies that are relevant for this site. It is assumed that the groundwater will be diluted by a factor of ten when discharged into the pond.
Measured nitrate concentrations in the pond	-	High	Moderate	Water samples were collected from the side of the pond closest to the OWT system on two occasions. Measured concentrations were well below all those reported to be toxic in the published studies that were determined to be relevant for this site. There is only moderate confidence that the OWT system in question is the source of the measured nitrate concentrations, because the site is in a farming community ( <i>i.e.</i> , agricultural inputs of nitrogen cannot be ruled out).
Weight of evidence	-	High	High	The weight of evidence strongly suggests that the OWT system in question is not currently having an adverse effect on amphibians inhabiting the pond.

### **Integration of Ecological Risks from Multiple Stressors**

After risks from nutrient and more indirect (*e.g.*, trophic) stressors are characterized, these risks are integrated for each assessment endpoint property. The stressors produced by wastewater treatment could act together to exert effects. For example, de Solla *et al.* (2002) found that low hatching success of amphibians at agricultural sites in British Columbia may be due to a combination of ammonia, biological oxygen demand and possibly organophosphates. Hatch and Blaustein (2000) observed interactions between pH and nitrate in determining survival of larval Cascades frogs (*Rana cascadae*), interactions between UV-B and nitrate on the activity level of larvae, and interactions of the three stressors in determining survival in some experiments.

Because we do not present risks from trophic interactions, we do not provide detailed guidance regarding the summation of risks from multiple stressors here. However, guidance is available in Suter (1999). If effects or exposures are not easily added, a mechanistic model or regression incorporating multiple stressor variables is needed. Regressions that incorporate multiple stressors are available for a few ecological receptors in a few surface water bodies. For example, chlorophyll *a*, a surrogate for phytoplankton biomass, may be predicted in the South Indian River Lagoon, Florida, using a regression that involves three variables: orthophosphate, total nitrogen, and turbidity (Sigua *et al.* 2000).

### **Uncertainty and Variability**

In any ecological risk assessment, sources of variability and uncertainty in results are described, and wherever possible, quantified. Field biologists often know the approximate magnitude of uncertainty associated with their measurements, as well as the spatial and temporal variability. The error associated with a regression is usually expressed by the researchers who derived the equation, and the error may be greater if the relationship is extrapolated from one location to an untested environment, from a mesocosm to the field, or from one species to another. In a comparison of parametric and nonparametric approaches to assessing uncertainty, Collins *et al.* (2000) found that standard deviations of modeled nitrogen loading to the shallow Waquoit Bay estuary, MA, USA, were about 38% of the loading rates, with uncertainties quantified for only a subset of the contributing variables. Based on this, Valiela *et al.* (2000) suggest an assumption of about 30% variation in nitrogen loading values. Parametric methods were not recommended because of the highly skewed distribution of the population of nitrogen loading rates (Collins *et al.* 2000). Collins *et al.* (2000) note that loading calculations for a large watershed incorporate spatial and temporal variability that is not present in individual field measurements. Additional sources of uncertainty arise in exposure-response relationships for ecological assessment endpoints, and risk assessors are among the few scientists who commonly propagate errors from exposure and effects estimates.

Summaries of options for qualitative and quantitative uncertainty analysis are found in Warren-Hicks and Moore (1998) and Suter *et al.* (2000). Resampling methods such as Monte Carlo analysis can be time consuming, and numerous quantitative uncertainty analyses are unnecessary if the decision will not be based on the level of uncertainty.

## CONCLUSIONS

Many of the ecological effects of OWT systems are expected to derive from nutrient loads to surface water. Effects on seagrasses in lagoons most often result from changes in nitrogen loading or related secondary stressors (*e.g.*, limited light penetration, oxygen limitation), and effects on algae and vascular plants in freshwater most often result from changes in phosphorus loading. Toxicity of low concentrations of nitrate to amphibians in surface water has also been observed. Ecological risk assessment is a useful process to quantify the magnitude and probability of those impacts. Numerous models, data, measurement methods, and assessment components are available to support ecological risk assessments of OWT systems. These include conceptual models that link stressors to assessment endpoints, measurements and methods to determine exposure, exposure-response relationships, the site-specific measurements from which the models are derived, and weight-of-evidence procedures. Although the confidence in risk assessments may sometimes be limited by a lack of site-specific information, the assessor can make use of empirical relationships that have been developed elsewhere. Potential effects on higher trophic-level organisms might come from altered forage or habitat conditions, and these impacts would need to be considered in comprehensive, watershed-scale assessments for housing developments with multiple OWT systems or other nutrient releases. An addendum to this framework would be needed to address the cumulative and emergent effects of multiple OWT systems and off-site treatment systems.

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