

## Final Report

### Development of a Conceptual Model for Non-Indigenous Species for the Mid-Atlantic States

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Cherry Point, Washington State. April Markiewicz photograph

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## 1. Introduction and scope

The goal of this project was to develop a conceptual model and the risk assessment process for non-indigenous or invasive species with application to the Mid-Atlantic States. The broader goal was to have a risk assessment conceptual model that would be applicable with modification to other areas of the United States. These goals have been met.

During the course of this study a generalized conceptual model was published (Landis 2004) that outlined the basic considerations for invasive species risk assessment with consideration for spatial relationships. The effect of spatial arrangement was further investigated by Dienes, Chen and Landis (in press, *Risk Analysis*), which demonstrated via modeling the influence of geography on the invasion process. The research won the best paper award for the 2004 Society for Risk Analysis annual meeting.

The research outlined above laid the foundation for the construction of a generalized conceptual model for the estimation of risk due to invasive species. The generalized conceptual model incorporates the relative risk model approach of source-stressor-habitat-impact (Landis and Wieggers 2005). In order to track the multiple scales of interaction the hierarchical patch dynamics framework of Wu and David (2002) have been employed. To date this approach has been used in four distinct risk assessments.

Our test case was a risk assessment for the European Green Crab risk at the Cherry Point coastal region along the Washington Coast. As an addendum to this work the risk due to the macroalgae *Sargassum* was also calculated. The lessons learned in this process were next applied to calculating risks due to the Nun Moth (Mid Atlantic watersheds) and Asian oyster (Chesapeake Bay) as our test cases for the East Coast. The risk assessment for the European Green Crab has been conditionally accepted for publication in *Human and Ecological Risk Assessment*.

This final report summarizes the generalized conceptual model and the risk assessments on the European Green Crab, Asian Oyster and European Green Crab. The distributions for the uncertainty analyses for these studies are found in Appendix A. A summary of the assessment for *Sargassum* is included as Appendix B.

### 1.1 Introduction and background to invasive species

In recent years, non-indigenous or invasive species have become an increasing concern both ecologically and economically. Greater than 120,000 non-indigenous species of plants, animals and microbes have invaded the United States, United Kingdom, Australia, India, South Africa and Brazil (Pimentel 2001). While these introductions include economically valued species such as corn (*Zea mays* L.), domestic chicken (*Gallus* spp.) and cattle (*Bos taurus*), anywhere from 10-30 percent of the introduced species are pests and cause major environmental impacts (Pimentel 2001; Williamson and Fitter 1996). The United States alone suffers losses of approximately 58.3 billion dollars per year as a result of damage and mitigation costs due to accidentally as well as intentionally introduced pests (Pimentel 2001). As global trade increases, the United States and other countries will continue to chance accidental introduction of invasive species (Ricciardi and Rasmussen 1998) and the possibility of impacts will continue.

The study of invasive species has been approached largely on a case-by-case basis and field studies often have been too idiosyncratic to be used to derive general hypothesis of invasive species establishment (Vermeij 1996). Laboratory and garden studies are hampered by problems of scale, replication and control (Wardle 2001, Doak *et al.* 1998). Hypothesized mechanisms for invasive species establishment and spread abound and most may be separated into two general categories: 1) attributes of the non-indigenous species and 2) attributes of the community into which the invasive species has arrived. Studies considering the former are typically searches for lists of common traits among the various species of invasive species, and exceptions to these lists are common (Mack *et al.* 2000).

First, one of the strongest predictors of plant introductions is if the species has established in another location (Kolar and Lodge 2001). In fish invasions of the Great Lakes the factors that determined establishment were relatively faster growth, toleration of a wider range of temperature and salinity and had a past history of invasiveness (Kolar and Lodge 2002). Quickly spreading fish has the features of slower relative growth rate, tolerance of a wide temperature range, and poor survival at higher temperature ranges. Examining past patterns of invasion or home range characteristics can also prove predictive for a variety of species (Kolar 2004). The second category of hypotheses includes vacant niche, enemy escape, disturbance, and species richness or diversity (Mack *et al.* 2000, Shea and Chesson 2002). However, we may not yet know enough about ecosystem functioning to relate these types of observations to the larger questions of prediction, policy and management (National Science and Technology Council 1999).

Scientists have recognized the need for a way to determine which species will be introduced and more importantly, cause impacts, in order to allocate resources for prevention, detection, management and control efforts (Grosholz and Ruiz 1996; Ricciardi and Rasmussen 1998). Nevertheless, only a small number of attempts have been made to assess the risk of terrestrial invasive species introduction and impacts and even less for aquatic species. A few studies have been conducted and methodologies suggested to determine the risk of invasive species introductions via ballast water, a major transport vector of aquatic introduced species (Hayes 1998, Hines *et al.* 1999). The focus of these studies, however, was ballast water management in which the goal was to determine the risk of transport and survival of organisms within the ballast water and subsequent introduction. The investigators did not specifically consider the risk of impacts, which are the responses of the valued entities to inherent alterations caused by an invasive species.

While it is possible to only assess the risk of introduction, in which the endpoint is the introduction to a new environment, the investigator implicitly assumes the establishment of any invasive species in a new region is an undesired event (Hewitt and Hayes 2002). Another way of defining risk is the likelihood of impacts following introduction. This acknowledges that the undesired impacts must occur for the particular introduction event to be a cause for concern. The Aquatic Nuisance Species Task Force (ANSTF 1996) used this definition of risk to develop the Generic Non-indigenous Aquatic Organisms Risk Analysis Review Process. While the review process identifies important considerations in evaluating risk of introduction and impacts, it is only a "skeleton" process, designed to accommodate a variety of approaches from very subjective to quantitative and thus, lacks detailed standardized methodology.

Ecological risk assessment, using the Relative Risk Model (RRM) methodology (Landis and Wieggers 1997; Wieggers *et al.* 1998, Landis and Wieggers 2005), is currently one approach used to predict the risks of impacts at a regional scale. The RRM quantitatively

ranks sources of stressors and habitats by using Geographic Information Systems (GIS) to analyze spatial datasets to determine risk at the regional level. This approach has proven very useful in many circumstances including the multi-stressor risk assessments for the Fjord of Port Valdez, Alaska (Wieggers *et al.* 1998), Cherry Point, Washington (Hart Hayes and Landis 2004) and several others. These risk assessments, however, have only been used for chemicals and other abiotic stressors and have not yet considered organisms, specifically invasive species, as stressors. This study is the first of its kind to use the ecological risk assessment method with the Relative Risk Model to determine the risk of introduction and associated impacts by a non-indigenous species.

The objective of this program is to adapt current risk assessment and Relative Risk Model methodology to the issue of invasive species and apply the methodology to predict the probability of risk of introduction and effects of an invasives. Three case studies are presented.

The first is the introduction of the European Green Crab to the Cherry Point, Washington region. Risk is calculated for two source scenarios: 1) current conditions, as well as 2) possible future conditions in which El Nino may influence passive current dispersal. The second scenario is for the Asian Oyster being intentionally introduced to the Chesapeake Bay. The final case study is the risk due to an accidental introduction of the Nun moth, a European species, to the forests of the Mid-Atlantic States.

## **2. Ecological Risk Assessment for the European Green Crab Risk Assessment at Cherry Point, Washington**

### **2.1 Introduction**

The European green crab (*Carcinus maenas*) serves as the initial species for this study. *C. maenas* is often referred to as an invasive species by environmental managers (WANSPC 1998). For the purposes of this study, an invasive species is an organism introduced to an area beyond its historic range that is able to survive, grow and sustain itself through reproduction long enough to cause impacts to another biological entity.

*C. maenas* was chosen to serve as the model species since it possesses many of the general attributes of invasive aquatic species, including 1) wide distribution in original range, 2) wide environmental tolerance, 3) rapid growth, 4) early sexual maturity, 5) high reproductive capacity, 6) broad diet, 7) gregariousness, 8) natural mechanisms of rapid dispersal, and 9) commensal with human activity (Ricciardi and Rasmussen 1998). *C. maenas* is also thought to have caused undesirable impacts following introduction into a new region (Cohen *et al.* 1995). Furthermore, it has not yet been introduced to the study area but is considered a species of concern by environmental managers (WANSPC 1998).

### **2.2 Risk Assessment Approach**

#### **2.2.1 Problem Formulation**

##### **European green crab (*Carcinus maenas*)**

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*C. maenas* is a non-swimming crab in the family Portunidae (Crothers 1967) that originates from the Atlantic coast of Europe, ranging from Norway and the British Isles south to Mauritania (Grosholz and Ruiz 1995). As a result of anthropogenic transport, *C. maenas* has extended its range to include Australia, South Africa as well as the east and west coasts of the United States (Cohen *et al.* 1995; Crothers 1968; Le Roux 1990). Following introduction in 1817 to the western Atlantic, *C. maenas* has become established from New Jersey to Nova Scotia (Cohen *et al.* 1995). Between 1989 -1990, *C. maenas* was introduced to the west coast in San Francisco Bay, California (Cohen *et al.* 1995; Grosholz *et al.* 2000). Since that time, *C. maenas* has spread to several small estuaries on the coasts of California, Oregon and Washington (Cohen *et al.* 1995; Grosholz *et al.* 2000) and in 1999- 2000, individuals were found in several sites on the west coast of Vancouver Island, British Columbia (Jamieson *et al.* 2002). The rapid spread of *C. maenas* to Oregon, Washington and Canada is thought to be the result of larval dispersal associated with

abnormal currents during the 1997/1998 El Nino event (Hunt 2001; Jamieson *et al.* 2002; Behrens Yamada and Hunt 2000).

The larval life cycle of *C. maenas* includes one protozoal stage, four zoeal stages and one megalopal stage (Crothers 1967). The solely planktonic protozoal and zoeal stages are typically found in the offshore water column while the megalopal stage can be planktonic and benthic, seeking refuge in vegetation, and exhibiting onshore movement in order to settle to the benthic habitat (Crothers 1967, Quieroga *et al.* 1997). Following settlement, the juvenile molts approximately 11 times and develops into an adult growing to a maximum size of 86mm carapace width (Crothers 1967).

*C. maenas* can tolerate salinities ranging from 4-33 ppt (Crothers 1967) with the exception that higher salinities (>20 ppt) are required for development from the egg to the megalopal stage (Anger *et al.* 1998). *C. maenas* is also temperature tolerant, inhabiting areas with seasonal temperatures ranging from 22 degrees Celsius in the summer to -1 degrees Celsius in the winter (Cohen *et al.* 1995). A minimum temperature of 7 degrees Celsius, however, is required for feeding and growth of juveniles and adults (Berrill 1982; Ropes 1968) and therefore extended periods of cold temperatures (< 7 degrees Celsius) coupled with low salinity can be detrimental to a population (Berrill 1982).

*C. maenas* can live in a variety of benthic habitats including hard and soft substrata such as sand, mud, and gravel-cobble (Crothers 1967; Cohen *et al.* 1995; Jamieson *et al.* 1998; Klein Breteler 1976; Ruiz *et al.* 1999) but most crabs, especially juveniles, prefer complex refuge including mussel beds, macroalgae, eelgrass and other vegetation (Cohen *et al.* 1995; Crothers 1967; Hedvall *et al.* 1998; Moksnes 2002; Ropes 1968). McDonald (2001) noted that in Washington, most *C. maenas* are captured in native vegetation or meadows of the non-indigenous cordgrass, *Spartina alterniflora* and may be due to competition with the red rock crab (*Cancer productus*).

Other competitors of *C. maenas* both in its native as well as non-native ranges include other crabs (Crothers 1967, Hunt 2001, Cohen *et al.* 1995). In fact, juvenile and adult *C. maenas* have been shown experimentally to fiercely compete with early benthic phase Dungeness crab (*Cancer magister*) for food and shelter (McDonald 2001). *C. maenas* also competes for shelter with other shore crabs such as *Hemigrapsus oregonensis* (Cohen *et al.* 1995, Jensen *et al.* 2002), which exhibits competitive dominance over *C. maenas*.

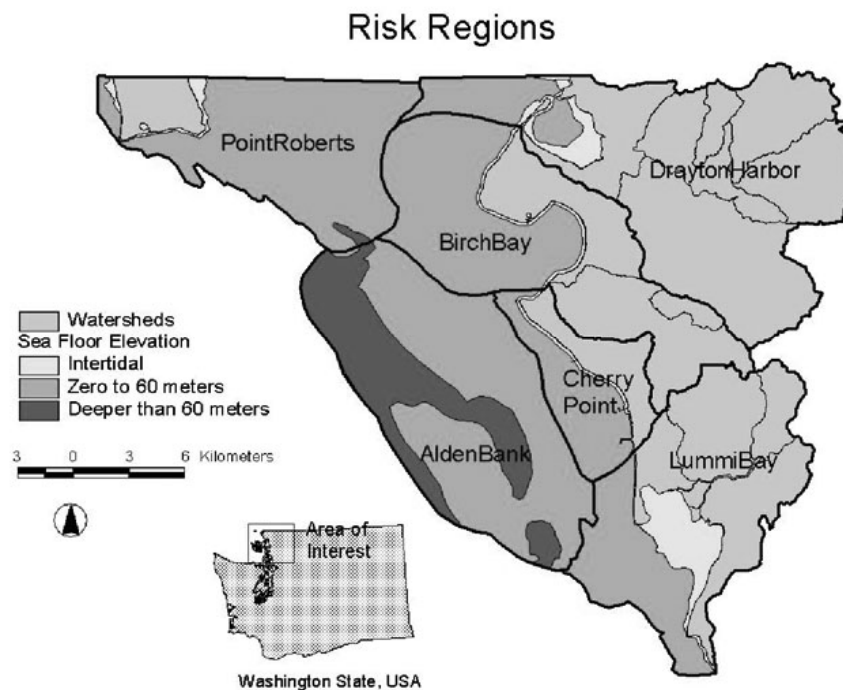
Predators are numerous in both the native and non-native ranges of *C. maenas*. A variety of species, including shrimp and cannibalistic juveniles eat the planktonic and newly settled larvae (Moksnes 2002; Le Calvez 1987, Cohen *et al.* 1995). As adults, *C. maenas* are preyed upon by fish, birds, otters, seals and larger conspecific crabs (Hunt 2001, Cohen *et al.* 1995, Lohrer and Whitlatch 2002). In Oregon, Hunt (2001) notes that predators of *C. maenas* include Western gulls (*Larus occidentalis*), Glaucous-winged gulls (*Larus glaucescens*) and the crabs, Dungeness and red rock crabs.

*C. maenas* are well known as voracious predators. They prey upon organisms from at least 104 families and 158 genera in five plant and protist and 14 animal phyla (Cohen *et al.* 1995; Crothers 1968). While prey preferences vary between regions, *C. maenas* prefer mollusks (Grosholz and Ruiz 1996; Dare *et al.* 1983) and have been shown to eat 10-25mm mussels per day (Dare *et al.* 1983). This excessive predation of *C. maenas* upon mollusks is thought to be the cause of a dramatic decline in the clam, *Mya arenaria* in New England and southeast Canada (Cohen *et al.* 1995) and a change in the shell morphology of

dogwhelks in North Wales (Hughes and Elner 1979). Walton *et al.* (2002) suggests that *C. maenas* is likely to cause substantial ecological changes upon molluscan populations.

### Description of Study Area – Cherry Point Region, Washington State

The Cherry Point region of Washington State extends from Point Roberts in the north to Lummi Bay in the south (Figure 2.1). This moderately developed area is the site of two major refineries, British Petroleum and Tosco Ferndale Oil Refinery and an Aluminum plant, Intalco (EVS 1999). Washington Department of Natural Resources (WDNR) manages the near shore environment at Cherry Point. In keeping with its management goal of preserving natural resources, WDNR recently designated the Cherry Point region a candidate natural reserve, which prioritizes natural resources over economic management decisions. This region has also served as the study area for a previous risk assessment of abiotic stressors (Hart Hayes and Landis 2004).



**Figure 2.1:** Overview of the Study area. The six sub-regions of Cherry Point, WA originally identified by Hart Hayes and Landis (2004).

Hart Hayes and Landis (2004) previously divided the study area into six sub-regions (Figure 2.1) using watershed and bathymetric boundaries available in GIS datasets. We used the same sub-regions for this analysis as well because, with the exception of Alden Bank, each sub-region possesses distinct shoreline features (e.g. open shoreline and bays, both natural and modified). These features influence the habitat characteristics within each sub-region, which in turn may affect the survival of the *C. maenas*. The sub-regions include:

1. Birch Bay sub-region – a bay consisting of mostly open shoreline, exposed to moderate to long fetch. Receives some wind waves and currents (Dethier 1990).
2. Cherry Point sub-region – open shoreline, again exposed to moderate to long fetch, having wind waves and currents (Dethier 1990). Water depths reach 70m just offshore, making the sub-region suitable for large vessel traffic.
3. Drayton Harbor sub-region – a bay partly enclosed by the Semiahmoo spit. Exhibits minimal wave action or currents (Dethier 1990).
4. Lummi Bay sub-region – a bay partly enclosed by a bar (Sandy Point). Minimal wave action and currents possible (Dethier 1990).
5. Point Roberts sub-region – shoreline extends along a peninsula immediately south of the U.S/ Canadian border. Much more exposed shoreline on the western side of the peninsula. Receives some wind waves and currents.
6. Alden Bank – a shallow bank 7.3 km offshore from Cherry Point sub-region. Completely submerged with a depth of 5m (Landis *et al.* 2000).

### **Description of Habitats**

Hart Hayes and Landis (2004) identified 10 habitat types present in the Cherry Point study area. The habitats are 1) gravel-cobble intertidal, 2) sandy intertidal, 3) mudflats, 4) eelgrass, 5) macro-algae, 6) soft bottom nearshore subtidal, 7) water column, 8) streams, 9) wetlands and 10) forest. The stream, wetland and forest habitats were excluded from this analysis as they represented the terrestrial component of the Cherry Point study area which does not apply in this aquatic-based risk assessment.

### **Description of Assessment Endpoints**

Assessment endpoints are those entities that represent economic, ecological or cultural values of the stakeholders within the region. In the previous Cherry Point risk assessment for abiotic stressors, six organisms were selected as biological endpoints by the Cherry Point Technical Working group, a representative assembly of local stakeholders. The six endpoints selected were the 1) Coho salmon (*Onchorhynchus kisutch* (Walbaum)), 2) juvenile Dungeness crab (*Cancer magister*), 3) English sole (*Parophrys vetulus*), 4) great blue heron (*Ardea herodias*), 5) common littleneck clam (*Protothaca staminea*), 6) surf smelt (*Hypomesus pretiosus*). Pacific herring (*Clupea harengus pallasii*) and eelgrass (*Zostera marina*) were later added to the list.

Coho salmon are an anadromous fish which spend their adult lives in the northern Pacific Ocean. From July through August, they return to the coastal and Puget Sound areas of Washington State to spawn in the rivers from August to February (Laufle *et al.* 1986). The juveniles then return to the ocean in July and August of the following year. Coho salmon are prey for many large mammals but are also predators of many organisms, especially decapod crustacean larvae (Laufle *et al.* 1986; WDFW 2003). These fish are valued in both commercial and recreational fisheries in the study area.

The Dungeness crab is commonly found in the coastal and inland waters of the Pacific Ocean but only the juvenile stages primarily utilize the nearshore habitat. Following larval settlement in April and May, the juveniles live in shallow coastal and estuarine waters, preferring sandy mud, eelgrass, and bivalve shell habitats (McDonald 2001; Pauley *et al.* 1986). Upon reaching 25mm carapace width, the juveniles move to subtidal areas where they mature to adults (Gunderson *et al.* 1990). These crabs are considered a valued species as they are a very important commercial and recreational fishery in Washington State (Pauley *et al.* 1986).



Juvenile English sole represent one of the major commercial groundfish species on the Pacific coast (Toole *et al.* 1987). They are abundant in the shallow inland coastal waters of Washington and British Columbia (Gunderson *et al.* 1990; Toole *et al.* 1987). They prefer to live in sand, mud, or eelgrass habitats and feed primarily upon copepods, cumaceans, amphipods and polychaetes (Gunderson *et al.* 1990; Toole *et al.* 1987). The juveniles typically emigrate from the nearshore habitats when they reach 75 to 80mm in size (Gunderson *et al.* 1990; Toole *et al.* 1987).

Great blue herons are year-round inhabitants of beaches in the Strait of Georgia. They are typically found foraging for small fish among eelgrass and kelp beds with the highest abundances from June through August (Butler 1995). These birds are important culturally and aesthetically.

Common littleneck clams are a widespread bivalve species ranging from the Aleutian Islands in Alaska to Baja California, Mexico. These clams inhabit beaches with coarse sand or gravel, stones or shells (Chew *et al.* 1987). Due to their wide distribution and high abundance along the eastern Pacific coast, the littleneck clam is both a commercial and recreational species, with 95 percent of the commercial catch of littleneck clams coming from Washington (Chew *et al.* 1987).

Surf smelt are a schooling forage fish present in the eastern Pacific, from Alaska to California. They utilize the nearshore habitat particularly during spawning when they deposit their eggs upon coarse sand and fine gravel substrates in the upper intertidal zone. Surf smelt are known to spawn in the Birch Bay/ Cherry Point region year round (WDFW 2003b, 2003d). This species is fished commercially and yields approximately 100,000 pounds (45454.5 kg) annually in Washington State.

Another eastern Pacific fish species that utilize nearshore beaches for spawning is the Pacific herring. From April through June, the herring spawn on vegetation and substrates in intertidal and shallow subtidal waters (EVS 1999; WDFW 2003c). Euphasids, copepods and amphipods are the main prey of herring (WDFW 2003b). The Pacific herring represent an important commercial sport bait fish industry and the Cherry Point herring stock is the largest in the state.

Eelgrass is a species of seagrass abundant throughout the protected estuaries of the Pacific Northwest region of the U.S. It can be found growing in sand and mud substrates ranging from 1.8m above MLLW to 30m in depth (Phillips 1984). Eelgrass performs several ecological functions including nutrient recycling, high primary production, and habitat stabilization (Phillips 1984). Eelgrass beds also provide shelter and food for juvenile stages of several finfish and shellfish species.

### **Identification and Description of Potential Sources of *C. maenas***

Several sources or transport vectors have been responsible for the accidental introduction of marine non-indigenous species, including *C. maenas*. Based on literature concerning current and historical European green crab transport vectors, we identified six possible sources of introduction of *C. maenas* to evaluate in this study. Five are classified as anthropogenic and one is a non-anthropogenic or natural source.

1. *Aquaculture shipments*: This vector has aided in the accidental introduction of several nonindigenous species in Washington, including the oyster drill (*Ocenebra japonica*)

and cordgrass (*Spartina alterniflora*) (WANSPC 1998). All life stages, especially *C. maenas* juveniles, could be transported with the aquaculture products. Currently, the Washington Administrative Code (WAC) 220-77-040 applies to aquaculture and prohibits the importation of unauthorized aquaculture shipments. Non-compliance presents the greatest risk associated with this vector.

2. *Live seafood shipments*: Non-indigenous species may be transported in packing materials, such as seaweed, used for shipping live seafood. Juvenile *C. maenas* are commonly found in New England rockweed (*Fucus* spp.) and kelp (*Ascophyllum nodosum*), which are used in shipments of live bait worms and Atlantic lobsters (Cohen *et al.* 1995). Introduction occurs when the packing materials and containers are disposed of improperly (WANSPC 1998). Washington Department of Fish and Wildlife (WDFW) officials are responsible for inspecting shipments and holding areas for edible shellfish.

3. *Ballast water*: Vessels can transport large amounts of ballast water, up to 113,000 tonnes, which is later discharged at ports while loading cargo. Organisms from a variety of taxonomic groups can survive transport within the ballast water tanks (Hines *et al.* 1999) suggesting European green crab larvae and juveniles may be transported in ballast water as well. According to WAC 220-77-090, WDFW requires that all vessels which are subject to chapter 77.120 RCW must report ballast water management information at least twenty-four hours prior to entering Washington waters. Also, as of July 1, 2004, vessels that have not adequately exchanged their ballast water must treat their ballast prior to discharge into Washington waters. This regulation, however, does not apply in this study as the analysis was conducted prior to the new regulations. Like the aquaculture shipments, non-compliance is an issue with this source as well.

4. *Research Release*- Institutions, such as public and private research laboratories and schools, may possess non-indigenous species as research subjects. If strict protocols do not exist or are not followed, accidental escape and introduction can occur. Any life stage of *C. maenas* may potentially be released. All institutions must obtain a permit to use invasive species and controls are required for effluent release (WANSPC 1998). Additionally, a WDFW official must regularly inspect and approve the research facilities (Russel Rogers, WDFW, pers. comm., 14 Jan. 2004).

5. *Educational Release*: Additional educational facilities, such as aquariums, may also possess and display invasive species for teaching purposes. The educational facilities, much like research institutions, chance accidental release of any life stage of *C. maenas* if proper precautions are not followed (WANSPC 1998). Educational facilities are also required to follow WDFW regulations (Russel Rogers, WDFW, pers. comm., 14 Jan. 2004).

6. *Passive current dispersal associated with El Nino Southern Oscillation (ENSO) events*: This source (hereafter referred to as passive current dispersal) is a form of secondary transport in which the organism has already been introduced to a region and then spreads to the area of concern primarily via larval dispersal. Following an El Nino event, the eastern Pacific current regime changes and the normal spring transition is delayed, resulting in increased and extended northward flowing currents (Davidson Current), less upwelling, less offshore movement and increased sea surface temperatures (Jamieson *et al.* 2002; Lynn *et al.* 1995; Sorte *et al.* 2001). These phenomena may allow for successful northward larval transport and survival. This type of extended dispersal for *C. maenas* larvae is plausible as it has been observed for other types of crab larvae in the eastern Pacific during El Nino events (Sorte *et al.* 2001).

## Identification of Exposure Factors

A regional risk assessment of invasive species must consider that the exposure to the stressor becomes the probability of a successful biological invasion event (Landis 2004). For the purposes of this study, exposure is further defined as the probability of *C. maenas* introduction, survival, growth and possibly reproduction. Several factors that may influence *C. maenas* survival and growth include physical parameters such as temperature and salinity, biological parameters including resource competition, predation and availability of suitable refuge as well as disturbances, either anthropogenic or natural. It is important to recognize that once the invasive species population is established in the habitat, however, it will increase and fluctuate due to a number of reasons (Landis 2004). The pattern of habitat patches can also influence the probability of a successful invasion (Deines *et al*, in press).

An additional consideration is that the various life stages of the organism may have different requirements and should be considered separately. For instance, we considered the factors influencing exposure of the larvae, a planktonic life stage, and the exposure factors for benthic juvenile and adult life stages independently.

## Identification of Effects and Impacts

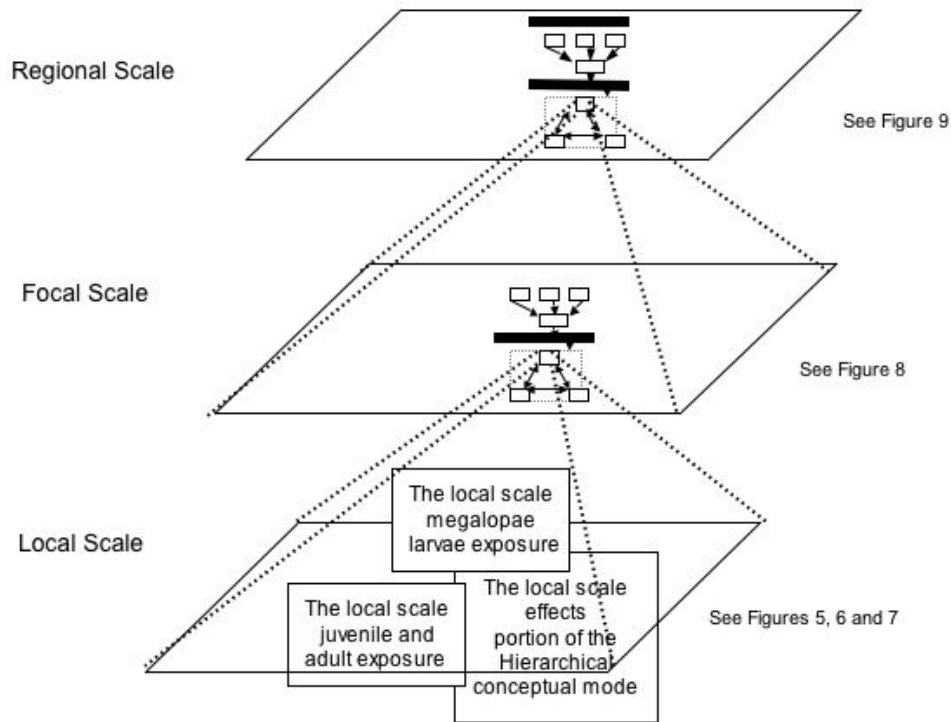
Following introduction and survival within a new environment, invasive species can directly and indirectly impact the valued characteristics of the receiving environment through a broad range of mechanisms, or effects (Landis 2004). Some of the potential effects, or inherent potential alterations, that *C. maenas* may cause are classified into two categories: potentially beneficial and potentially undesirable, based upon the goals associated with the assessment endpoints. The potentially beneficial effect of *C. maenas* was identified as increased prey availability for an assessment endpoint. Potentially undesirable effects of *C. maenas* include predation, resource competition and physical habitat alteration. Impacts to the endpoint such as the replacement of the species or a change in biodiversity, population dynamics, age structure or community composition may occur as a result of the above effects. The processes that govern these impacts are fundamentally ecological and evolutionary and are therefore, contingent, probabilistic and dynamic (Landis 2004).

### 2.2.2 Conceptual Model Development

Once the risk components were identified they were integrated into a conceptual model, which is a representation of the predicted relationships among the stressor, the exposure scenarios and assessment endpoint responses. We created a conceptual model that not only allows for the exploration of each potential pathway leading to impacts but also illustrates the invasion process and addresses the concept of scale and the exposure factors that influence invasion at each scale.

In designing the conceptual model, we used the hierarchical patch dynamics paradigm (HPDP) (Wu and Loucks 1995; Wu and David 2002) as a framework. The HPDP incorporates three levels or scales: local, focal and regional (Figure 2.2). The focal scale is defined as the scale at which the phenomenon or process under study characteristically operates (Wu and Loucks 1995; Wu and David 2002) and is the primary scale in which the analyses are conducted in this study. In addition to the focal scale, Wu and Loucks (1995) and Wu and David (2002) recommend considering the two scales adjacent to the focal scale: the local and regional scales. Regional, or higher, scales are characterized by slower and larger entities (overall context and constraints) whereas lower, local scales are defined by faster and smaller entities (mechanisms and initial conditions) (Wu and David

2002). It is important to note that the relationship between the scales is relatively symmetric and does not specifically imply a top-down or bottom-up control.



**Figure 2.2** A schematic diagram of the hierarchical conceptual model for Cherry Point, Washington illustrating the integration of the local, focal and regional scales. This model not only addresses the concept of scale but allows for exploration of each potential pathway leading to impacts and illustrates the invasion process and the factors that influence invasion. *C. maenas* patch interactions at the focal scale include the immediate Cherry Point region, and at the regional scale include and the patches along the entire North American Pacific coast.

The diagram for the conceptual model in this study is similar to that of Wu and David (2002). In this model, each scale, beginning with the local scale, has an increasing spatial extent which in turn affects the types of factors influencing the invasion process. The local scale of the conceptual model is nested within the focal scale which is in turn integrated into the regional scale (Figure 2).

The local scale has the smallest spatial scale and provides the mechanistic processes for the overall model. At this scale, introduction of *C. maenas* occurs in discrete habitat patches within the Cherry Point area and effects are due to interactions of *C. maenas* with the endpoints within each patch. The factors influencing exposure are more localized with

respect to habitat patches such that small factors, such as a freshwater discharge pipe, may influence the localized temperature and salinity as well as possible contaminant load within a particular habitat patch.

The various distinct life stages must be considered separately. As a result, we developed detailed local scale exposure models for megalopae larvae (Figure 2.3) and juveniles and adults (Figure 2.4), as well as an effects model for both life stages (Figure 2.5). Only the megalopal larval life stage is considered based on the assumption that megalopae are the main larval life stage introduced by the sources. This is due to the possibility that the larvae will develop into megalopae during transport, depending on the duration of the journey. Furthermore, megalopae are more benthic (Crothers 1968) and are therefore assumed to remain in the sub-region to which they were transported.

All of the interactions occurring within each patch at the local scale were then integrated together to represent the focal scale (Figure 2.6). In our study, the focal scale is the spatial extent of the Cherry Point study region and includes the overall habitats and associated endpoint populations. The factors influencing exposure at this scale are generalized for the entire region (e.g. average temperature and salinity ranges within Cherry Point, interactions with endpoints at the population and metapopulation level, overall disturbances within Cherry Point) and the effects are considered at the population level within Cherry Point.

The other scale adjacent to the focal scale considered in the conceptual model is the regional scale (Figure 2.7), which has the largest spatial scale and provides a general context for the overall model. The spatial extent of the regional scale is that of the entire Pacific coast of the United States. Consequently, this scale considers all populations of *C. maenas* on the U.S. Pacific coast and the corridors between these populations, which are transport vectors, such as ballast water, and passive current dispersal. The factors influencing exposure are also of a much larger scale and include ocean regime and climate which in turn influence events such as the Pacific Decadal Oscillation (PDO) and El Niño/La Niña.

### **2.2.3 Analysis**

The third phase in an ecological risk assessment, the analysis phase, involves relating exposure and effects to each other (U.S. EPA 1998) and investigating each route to the impact. To analyze the risk of exposure and effects, we used the Relative Risk Model methodology developed by Landis and Wiegiers (1997) and Wiegiers *et al.* (1998). This methodology has been used numerous times to comparatively determine risk at a large scale (Hart Hayes and Landis 2004; Moraes *et al.* 2002; Obery and Landis 2002; Wiegiers *et al.* 1998). The RRM ranks risk components and filters each possible combination. In using the RRM, the following assumptions were considered (Landis and Wiegiers 1997; Wiegiers *et al.* 1998):

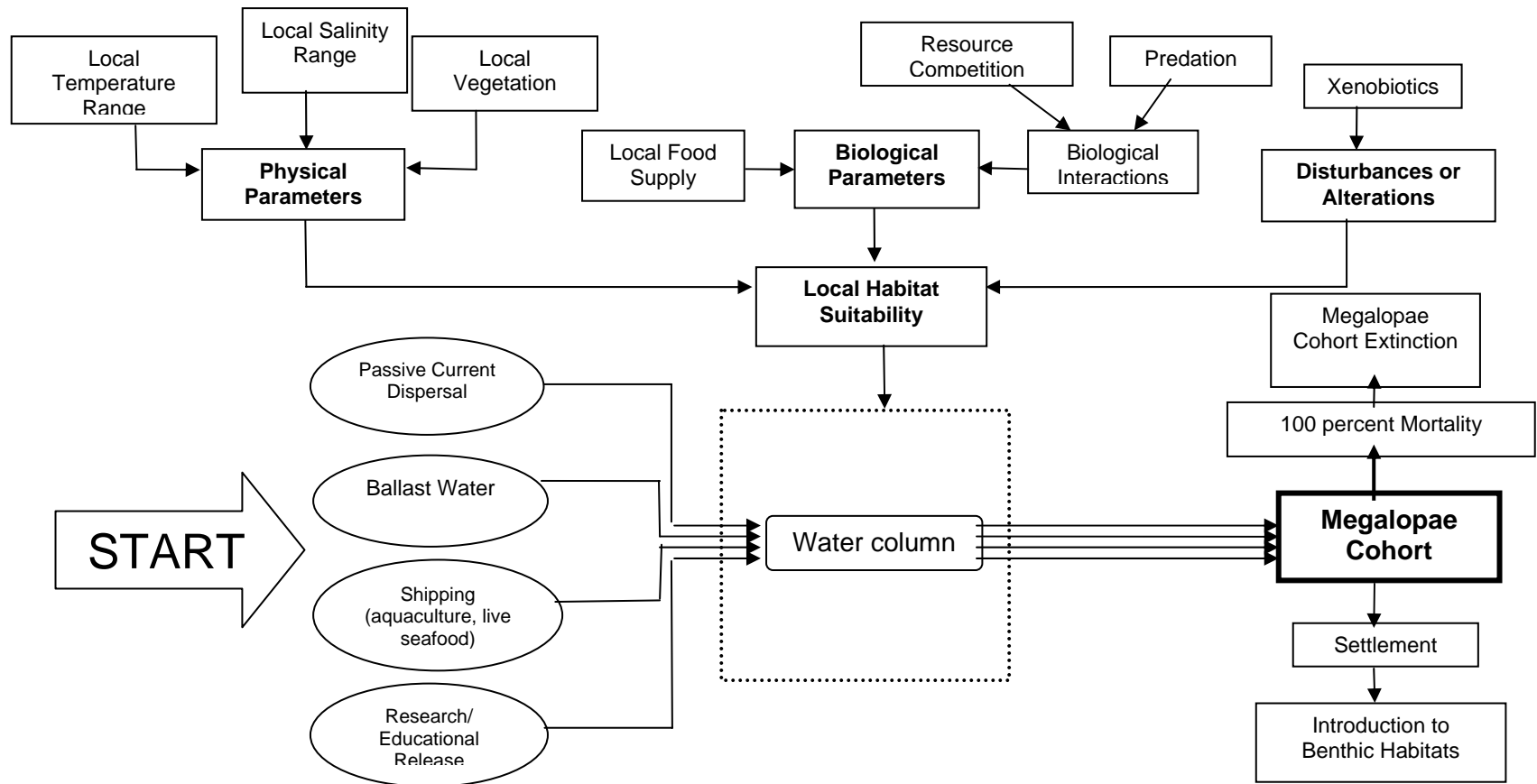
1. The type and density of assessment endpoints is related to the available habitat;
2. The sensitivity of receptors to stressors varies between habitats; and
3. The severity of effects in sub-regions of the Cherry Point region depends on relative exposures and the characteristics of the organisms present.

### **Development of Habitat and Source Ranks**

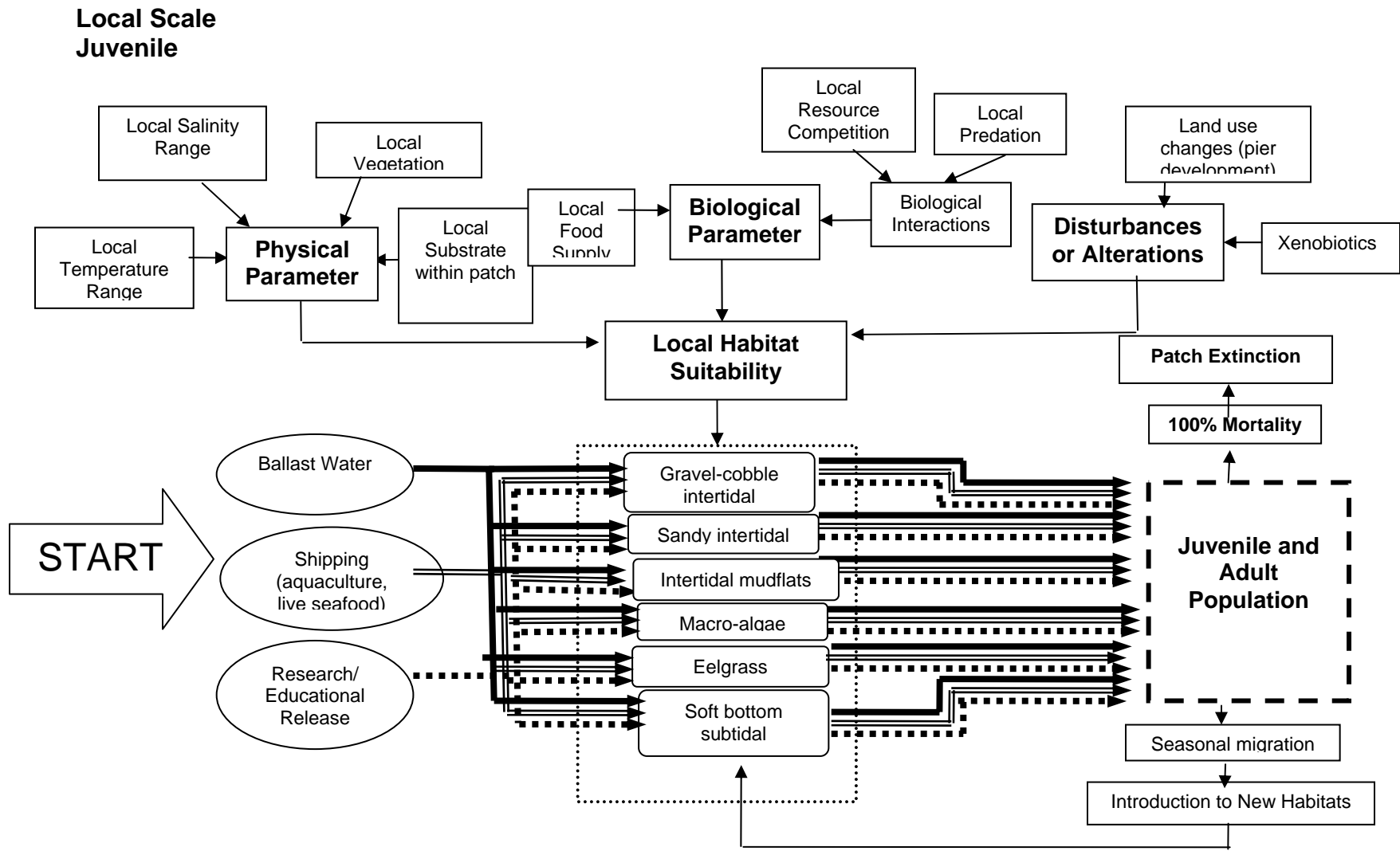
We incorporated the habitat ranking scheme that was previously determined in the Cherry Point risk assessment of abiotic stressors (Hart Hayes and Landis 2004) (Table 2.1).

These ranks were based on size of habitat (km<sup>2</sup>) and classified into categories using natural breaks in GIS datasets. The habitat ranks ranged from zero to six on a two-point scale, where zero represents lowest potential for exposure (no habitat present) and six represents highest potential for exposure (relatively largest amount of habitat present).

## Local scale megalopae larvae exposure



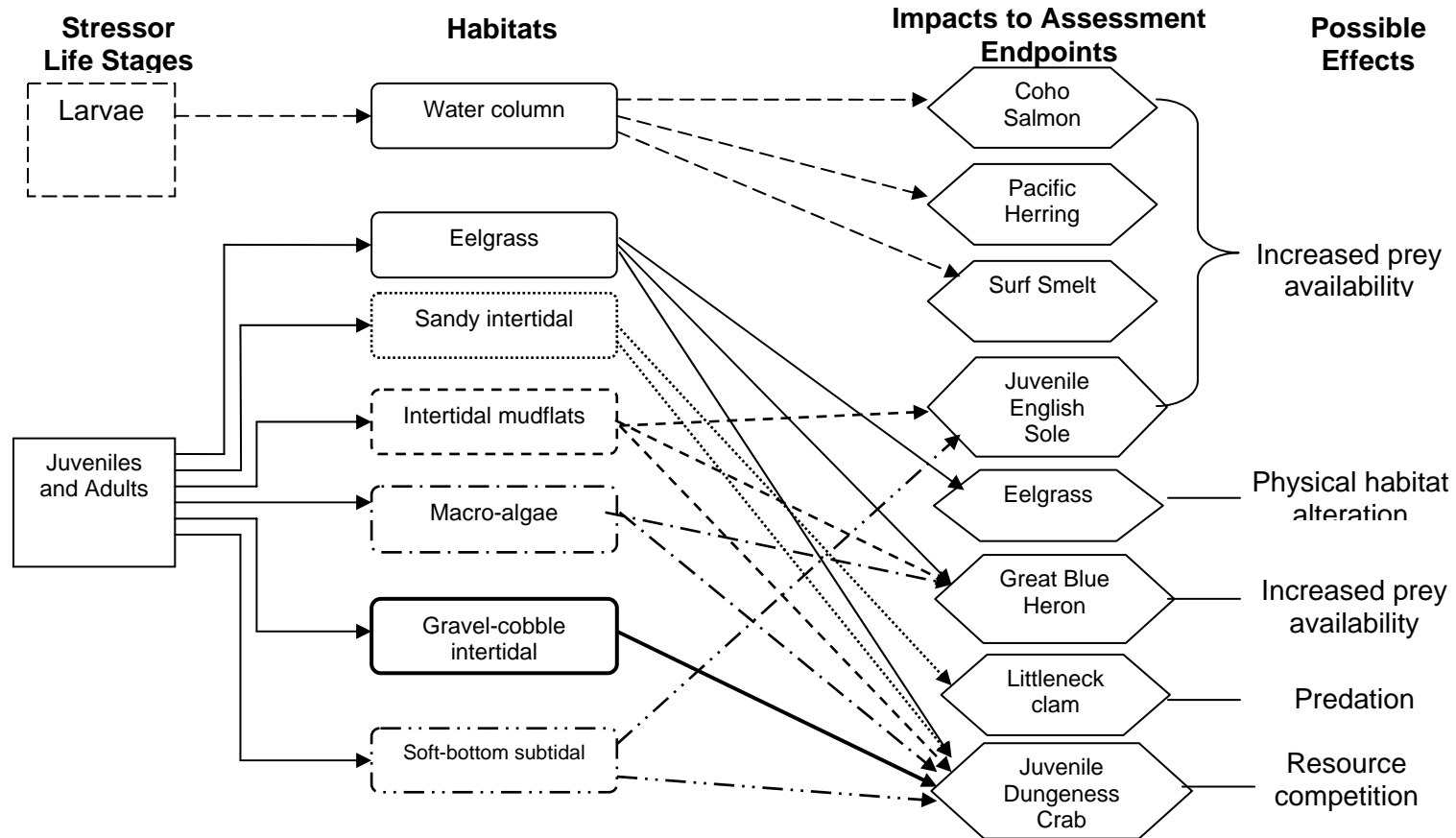
**Figure 2.3:** The local scale megalopae larvae exposure portion of the hierarchical conceptual model. All sources and habitats relevant to the megalopae life stage as well as the related local habitat suitability parameters are included. When evaluating each source-habitat pathway, proceed to the local scale juvenile and adult life stages conceptual model upon reaching the “Introduction to Benthic Habitats” box (Figure 2.4). For related effects, see the local scale effects conceptual model (Figure 2.5).



**Figure 2.4:** The local scale juvenile and adult life stages exposure portion of the hierarchical conceptual model. All sources and habitats relevant to both life stages as well as the related local habitat suitability parameters are included.

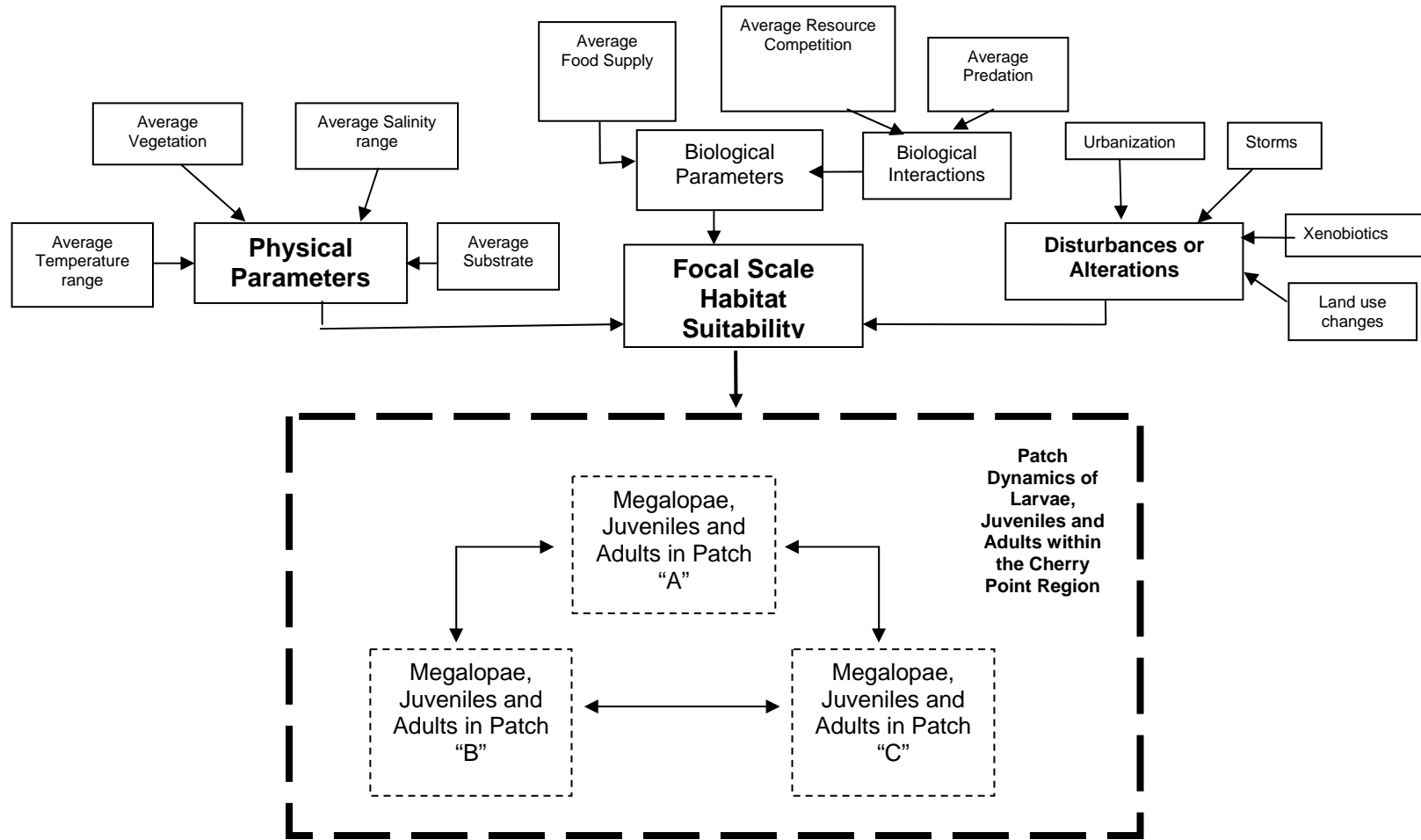


## Local Scale Effects

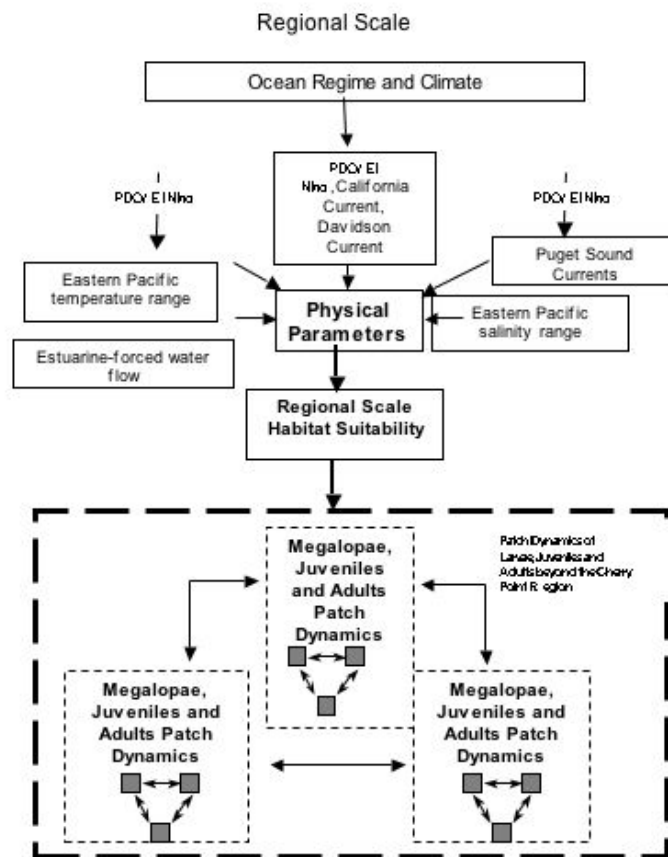


**Figure 2.5.:** The local scale effects portion of the hierarchical conceptual model. This model illustrates the potential effects pathways resulting from local patch overlap and subsequent interaction with any of the three *C. maenas* life stages.

## Focal Scale



**Figure 2.6.** The focal scale of the hierarchical conceptual model. This scale includes all *C. maenas* patches within the Cherry Point area and the interactions between the patches due to migration, transport vectors and larval dispersal, as indicated by arrows. Additionally, all relevant habitat suitability parameters at the focal scale are included.



**Figure 2.7:** The regional scale of the hierarchical conceptual model. This scale includes all *C. maenas* patches within the entire Pacific coast region of the United States and the interactions between the patches through transport vectors and larval dispersal, as indicated by arrows. The large scale parameters that influence regional scale habitat suitability are also included.

Sources were not present in more than one sub-region and consequently numerical ranking criteria relative to the sub-regions could not be developed in the same way as the habitat ranking scheme. Alternatively, the source ranks were based solely on presence/absence of each source with zero representing absence of source (low exposure potential) and six indicating presence of source (high exposure potential) (Table 2.2). To be consistent with the habitat ranking scheme, zero and six were again used as the minimum and maximum ranks possible for the sources. For example, we assigned a rank of six to the Cherry Point sub-region because in 2003 several vessels discharged ballast water at the Cherry Point and Ferndale piers, which are located within the sub-region (SERC 2003).

Since this study evaluates the risk of two source scenarios, current conditions and future conditions with El Nino-driven passive current dispersal, the ranking assignments for each scenario differed slightly. A rank of six was assigned to the source, passive current dispersal, for the second source scenario which represents future El Nino conditions. The passive current dispersal source rank remained a zero for current conditions, as 2003/2004 was not an El Nino year (Federov *et al.* 2003).

### **Development of Exposure and Effects Filters**

Filters are weighting factors used to determine the relationship between risk components: sources, habitats and impacts to assessment endpoints (Wiegiers *et al.* 1998). The exposure and effects filters were evaluated based on specific criteria and were then assigned values ranging from zero to one, which indicated whether the conceptual model pathway was complete from either source to habitat (exposure) or from habitat to endpoint (effects). A zero indicated an incomplete pathway while a one indicated a complete pathway. The exposure filters in this study were developed based on the following components, adapted from Wiegiers *et al.* (1998):

- A. Will the source release the stressor?
- B. Will the stressor then occur and persist in the habitat? (e.g. is the habitat suitable to allow for survival, growth and reproduction of the stressor?)

For each exposure filter component, we considered specific criteria and then assigned a numerical value to each of the components. Exposure filter component A, which considers whether or not the source will release the stressor, was dependent upon three criteria, or source characteristics:

1. Origin of the source
2. Application of treatment or precautionary methods
3. Interaction of the source with the aquatic environment

We created a decision tree (Figure 2.8) to aid in the assignment of the filter value related to exposure filter component A. A value of zero indicated the source would most likely not release the stressor while a one indicated release would occur.

Several criteria were essential in evaluating the habitat suitability for exposure filter component B. Suitable habitat is dependent upon physical and biological parameters, which were previously identified in the conceptual model. These parameters include the following:

1. temperature
2. salinity
3. food supply
4. potential predators/resource competitors
5. preferred habitat/refuge

It is important to note that disturbances were not considered as parameters in this analysis as there is not enough evidence available to determine the role of disturbances in invasion (Ruiz *et al.* 1999).

Again a decision tree (Figure 2.9) was designed to assign the filter values related to exposure filter component *B*. The values assigned were either 0, 0.5 or 1 with a zero indicating unsuitable habitat, 0.5 representing moderately suitable habitat and one representing highly suitable habitat.

Similar to the exposure filters, the effects filters were developed based on three components, partially adapted from previous risk assessments (Hart Hayes and Landis 2004; Wiegers *et al.* 1998). The three effects filter components included the following:

- A. Does the endpoint occur in and utilize the habitat?
- B. Is there seasonal overlap in habitat usage between the stressor and the endpoint?
- C. Are effects (either beneficial or undesirable) to the endpoint possible from interaction with the stressor?

To assign values for whether the endpoint utilized the habitat (effects filter component *A*), we incorporated a previously determined ranking scheme (Hart Hayes and Landis 2004) in which a zero indicated the endpoint did not use the habitat, a 0.5 indicated the endpoint used the habitat only marginally, while a one indicated the endpoint completely used the habitat. GIS and other published literature were used to determine the predicted distribution and abundance of the endpoints. All values assigned were the same as those in Hart Hayes and Landis (2004) except for herring and surf smelt, in which we changed any previous values of 0 and 0.5 to 1 because new data (WDFW 2003a) suggests that spawning occurred in all sub-regions and therefore, herring and surf smelt were present in all sub-regions.

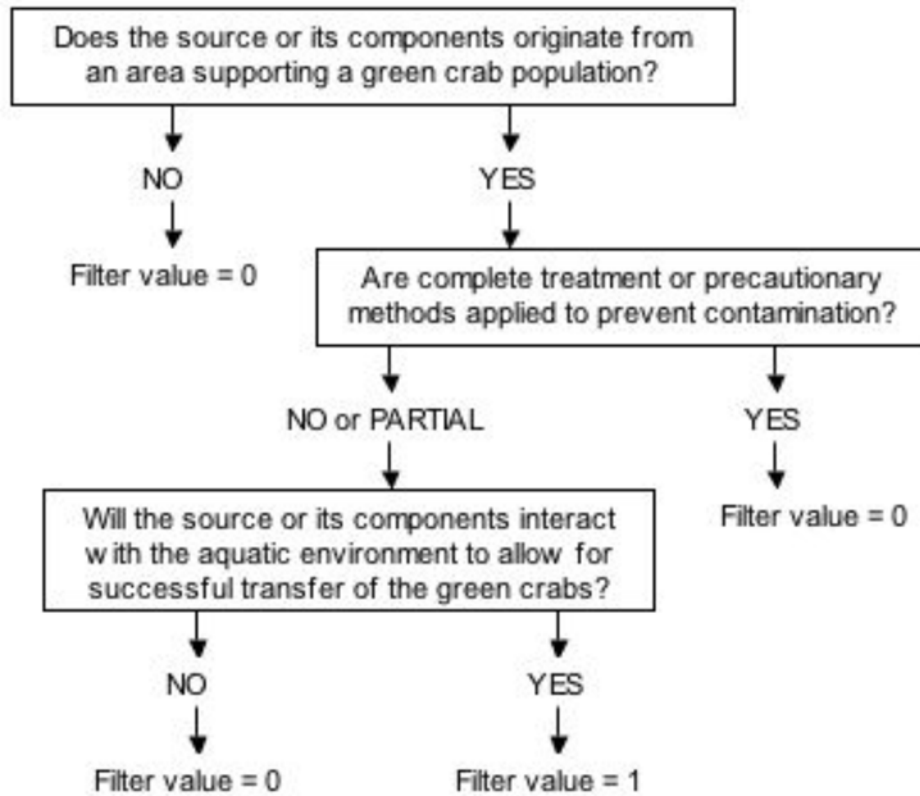
Seasonal overlap between the stressor and each endpoint (effects filter component *B*) was addressed by assigning a zero if overlap was not possible while a value of one was assigned if overlap was possible. All endpoints were assigned a value of one as seasonal habitat overlap was expected with *C. maenas*, indicated by the life history of each organism.

Finally, both beneficial and undesirable effects (effects filter component *C*) were considered by assigning a zero for no effects possible, a positive one if undesirable effects were possible and a negative one if beneficial effects were possible. For example, the juvenile Dungeness crab received a value of one because the literature indicated undesirable effects from *C. maenas* could occur (McDonald 2001). Conversely, since the Coho salmon preys upon crab larvae (Laufle *et al.* 1986; WDFW 2003b), it received a value of negative one, indicating beneficial effects were possible.

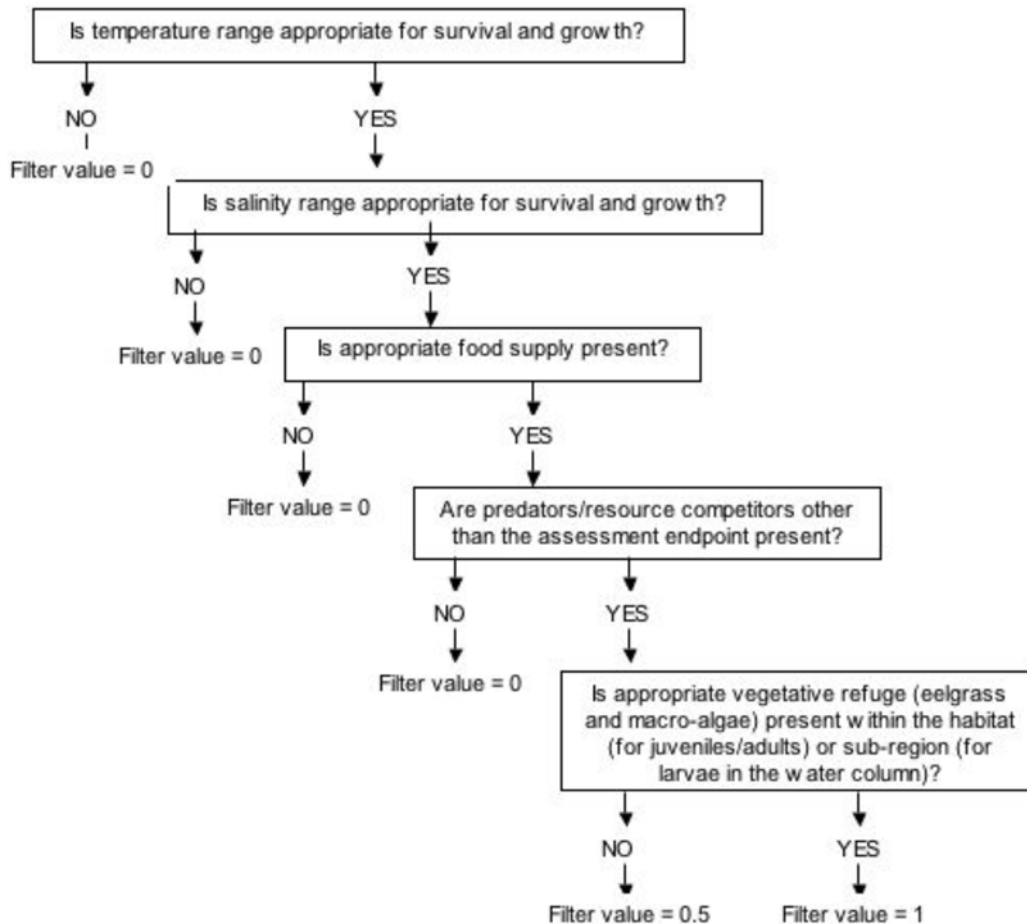
While the additional components for exposure and effects filters added more complexity to the model, the multiple components allowed for more in depth uncertainty analysis later in the study. The uncertainty within each component could now be addressed separately.

#### **2.2.4 Risk Characterization**

Risk characterization is the final phase of a risk assessment and is the process of integrating exposure and effects data to estimate the risk. As typical in using the RRM, all ranks were converted into a point system. To generate the exposure and effects filters for each pathway, the filter components of the exposure and effects filters were multiplied. Finally the source and habitats ranks were integrated with the exposure and effects filter products to



**Figure 2.8:** The decision tree used to determine the value for exposure filter component A (e.g. Will the source release *C. maenas*?).



**Figure 2.9:** The decision tree used to determine the value for exposure filter component *B* (e.g. less the habitat suitable enough to allow for survival and growth of *C. maenas*?). Generate the risk for each source-habitat-endpoint pathway. The risk scores were then summed to produce the following predictions: 1) risk in sub-regions, 2) risk in habitats, 3) risk to assessment endpoints and 4) contribution of each source to risk.

### Uncertainty Analysis

Following determination of the final risk scores in the risk characterization phase, the sources and amounts of uncertainty within each component of the RRM were identified and addressed using Monte Carlo analysis. This type of uncertainty analysis is a probabilistic approach to quantifying the change in model outputs as a function of model inputs. The inputs within the RRM are the ranks and filters and the outputs are the final risk scores.

Using methodology similar to that of Hart Hayes and Landis (2004), we classified the uncertainty for each filter component and rank as low, medium or high based on the amount of confidence within each assigned value. We then assigned discrete statistical

distributions to represent the uncertainty within the ranks and filter components with medium and high classifications according to specific criteria listed in Tables 3 and 4. The ranks and filter components with low uncertainty classifications were not assigned a distribution, but instead retained their original value. The uncertainty distributions are found in Appendix A.

All source ranks except for live seafood shipments, were assigned low uncertainty due to the occurrence of documentation indicating presence or absence of each source within the each sub-region. Live seafood shipments source ranks for all sub-regions except Alden Bank were assigned medium uncertainty, as there is no available documentation of establishments within the sub-region receiving live seafood shipments. In addition, while live seafood shipments are known to enter the U.S. from Canada (Mike Williams, WDFW, pers. comm. 15 Mar 2004) at the Blaine Port of Entry (Drayton Harbor sub-region), the final destinations of the shipments are often unknown.

Several of the habitat ranks received medium and high uncertainty classifications. We used the previous assignments of high uncertainty for eelgrass and macro-algae habitat ranks for the Alden Bank sub-region. High uncertainty was assigned because no vegetation data were available this far off shore, though vegetation was probably present (Hart Hayes and Landis 2004). We then assigned medium uncertainties to eelgrass and macro-algae habitats within all other sub-regions. The GIS data used to assign the ranks are rather outdated and consequently, the populations may have changed in abundance and distribution from the time the data was recorded.

We did not change the medium uncertainty classifications originally assigned by Hart Hayes and Landis (2004) for the soft bottom subtidal ranks in all sub-regions because no substrate data was available for the study area. In determining the area for this habitat on which ranks were based, Hart Hayes and Landis (2004) assumed that the subtidal region of the study area mostly consisted of soft bottom substrate, as opposed to vegetation or rocky substrate. This discrepancy between soft bottom subtidal and vegetated or rocky substrate is important as it may influence the suitability of the habitat.

We evaluated the uncertainty of each filter component value separately. Exposure filter component A for ballast water (Will the source release the stressor?) was given a value of one. However, much uncertainty remains concerning ballast water transport of crab larvae. For instance, the actual amount of crab larvae within the ballast tanks of the vessels using the pier at Cherry Point is unknown. This number is highly variable because the amount of larvae loaded by the ballast water alone is dependent upon factors including timing of larval production and location of nearest reproductive populations. Additionally, there is uncertainty concerning the actual ability of larvae to survive in less suitable conditions (no light, decreased food supply, damage during uptake). Therefore, we classified the uncertainty for *C. maenas* release by ballast water as high.

We had assigned a value of zero to exposure filter component A for live seafood shipments because most of the shipments coming into the U.S. are from British Columbia, which does not support a *C. maenas* population. However, trans-shipments carrying non-indigenous species have come through Canada and entered Blaine, which is the city within the Drayton Harbor sub-region (Mike Williams, WDFW, pers. comm. 15 Mar 2004). This filter component is also subject to the same uncertainties that applied to ballast water including timing and number of organisms being loaded as well as unknown ability to survive during transport under less suitable conditions (live seafood are packed moist, not in water).



Furthermore, it is uncertain whether the products within these shipments interact with the marine environment.

In addition to the exposure filter component *A* uncertainty assignments in Source Scenario 1 (current conditions), the exposure filter component *A* was assigned high uncertainty for El Nino-driven passive current dispersal in Source Scenario 2. This scenario evaluates the risk for future conditions, in which an El Nino year would occur. The high uncertainty classification was warranted because while El Nino years are relatively predictable, the intensities of the individual El Nino can vary (Federov *et al.* 2003). The conditions associated with a mild El Nino year may not be sufficient to allow for larval transport from the California, Oregon or Washington *C. maenas* populations. All other uncertainty assignments in source scenario 2 remained the same as those used in source scenario 1.

All exposure filter component *B* values, which describe habitat suitability in each sub-region, were classified as having high uncertainty. The high uncertainty classifications were due to a number of factors. Recent data concerning the abundance and distribution of possible resource competitors and predators other than the assessment endpoints were unavailable. Even if the data were available, much uncertainty remains concerning how the system functions and thus, it was difficult to accurately predict species interactions. Simberloff and Alexander (1994) noted that in addition to species interactions, biological stressors can reproduce and evolve over time, making exposure and effects difficult to quantify. This overall stochasticity of the system further added to the uncertainty surrounding habitat suitability.

We used similar uncertainty classifications for the effects filter component *A* (endpoint habitat utilization) as those used for the effects filters in the previous Cherry Point risk assessment (Hart Hayes and Landis 2004). Due to the lack of site specific data, the filters for the Coho salmon, English sole and littleneck clam were originally assigned medium uncertainty in each sub-region. All other filters were assigned low uncertainty because data regarding habitat utilization by the other endpoints were available. For this study, we increased the uncertainty for Pacific herring and surf smelt from low to medium because the data, which had been used in the previous risk assessment, was actually based on spawn presence and not presence of the juvenile or adult forms. Thus, we had to extrapolate from this benthic habitat utilization data to the water column, assuming that the number of fish present is relative to the amount of spawn within each sub-region.

All effects filter component *B* values, which consider seasonal overlap, were assigned low uncertainty, as all endpoint distributions would overlap seasonally with *C. maenas*. Conversely, the uncertainty was greater for the effects filter component *C* values (possible effects). The great blue heron, English sole, Pacific herring and surf smelt were all assigned a value of zero for the possibility of *C. maenas* causing effects. Potentially undesirable effects would be minimal if any and major beneficial effects would most likely not occur because *C. maenas* (larvae, juveniles or adults) are not a primary food source for these endpoints (Butler 1995; Gunderson *et al.* 1990; WDFW 2003b). Nevertheless, we assigned medium uncertainty to these values due to the possibility that these organism may switch to using *C. maenas* as a food source if it were present, though it would mostly be opportunistic feeding, resulting in low beneficial effects.

As for the other endpoints, we assigned high uncertainty to the eelgrass effects filter components *C* value of one. There is evidence of *C. maenas* damaging eelgrass indirectly

while searching for food (Davis *et al.* 1998), but whether or not this action was severe enough to warrant a value of one was questionable. Low uncertainty was assigned to the effects filter components for the Coho salmon, Dungeness crab and littleneck clam as there was sufficient data available to suggest undesirable effects (Dare *et al.* 1983; Davis *et al.* 1998; Grosholz and Ruiz 1996; McDonald 2001).

Once we had assigned all of the uncertainty classifications, we ran the Monte Carlo simulations using Crystal Ball® 2000 software as a macro in Microsoft® 2002 Excel. The simulations were run for 1,000 iterations and the output was generated in the form of statistical distributions representing the range of possible final risk estimates for each sub-region, source, habitat and endpoint. While the simulations were running, we noted that the tails and overall shape of the distributions were no longer changing after 1,000 iterations, indicating this number was sufficient to ensure accuracy in the results.

### **Sensitivity Analysis**

We then conducted a sensitivity analysis using the Crystal Ball® 2000 software as well. This type of analysis examines the sources of uncertainty, influenced by either the model sensitivity or parameter uncertainty (Goulet 1995; Warren-Hicks and Moore 1998). Model sensitivity is the influence of a parameter within a model and parameter uncertainty includes the range of possible parameter values. During sensitivity analysis, correlation coefficients are generated to rank model parameters according to their contribution to prediction uncertainty. Consequently, a high rank correlation indicates that the uncertainty within the model parameter has great importance in influencing the uncertainty within the model.

## **2.3 Results**

### **2.3.1 Risk Characterization**

#### Source Scenario 1

The risk characterization phase yielded overall final risk scores for each sub-region, source, habitat, and assessment endpoint, for both source scenarios. In the first source scenario, Cherry Point was the only sub-region at risk, having a final risk score of 42. The only source contributing to this risk was ballast water, which also had a risk score of 42. Within the Cherry Point sub-region, the greatest risk was to the eelgrass habitat while the water column habitat had the lowest risk (Figure 2.10a). The water column actually had negative risk, due to beneficial effects of *C. maenas* larvae to the Coho salmon. The juvenile Dungeness crab was the endpoint at greatest risk of undesirable effects (Figure 2.10b).

#### Source Scenario 2-EI Nino

The second source scenario, which includes passive current dispersal associated with EI Nino events, yielded much higher risk scores than Source Scenario 1. In the event that current dispersal occurred, Lummi Bay and Drayton Harbor were the sub-regions most at risk (Figure 11a). Alden Bank was the only sub-region with a negative overall risk, with beneficial effects to certain endpoints contributing to this negative value. Passive current dispersal was the source that contributed the most to the risk in comparison with all other sources (Figure 2.11b). The only other source contributing to risk was ballast water. For the overall Cherry Point study area, the eelgrass habitat was most at risk (Figure 2.11c). The juvenile Dungeness crab was the assessment endpoint at greatest risk (Figure 2.11d). Also, the only endpoint with a negative risk in the entire study area was the Coho salmon.

### 2.3.2 Uncertainty Analysis

The Monte Carlo analysis produced probability distributions for each sub-region, source, habitat and endpoint risk estimates. The distributions show all possible risk estimates and the probability of those estimates occurring as a result of the uncertainty within the model inputs, or parameters.

#### Source Scenario 1

##### Sub-regions

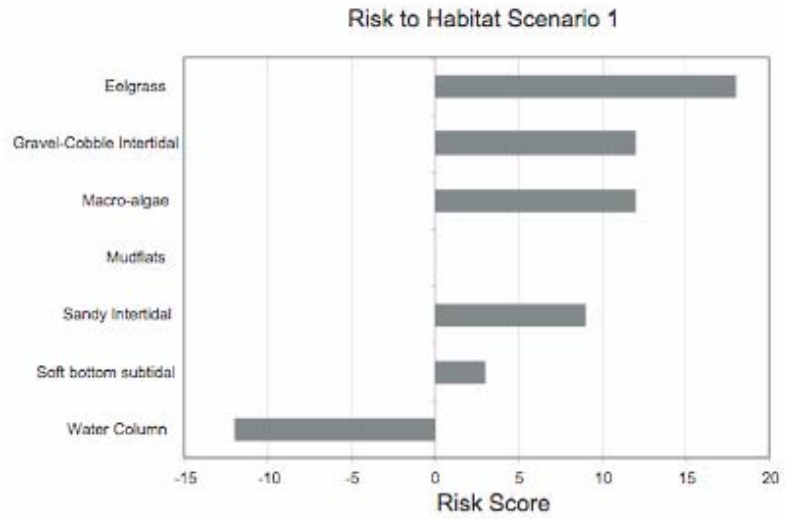
All sub-region uncertainty distributions had means similar to the estimates predicted by the Relative Risk Model (RRM). Birch Bay, Drayton Harbor, Lummi Bay and Point Roberts had wide ranges, due to extreme outliers in the uppermost 10 percent of the distributions. The distributions indicated, however, that there was an 80 to 85 percent probability of the risk estimate for each sub-region equaling zero. The Cherry Point sub-region distribution also had a wide range but was right-skewed with no more than a 36 percent probability of each risk estimate occurring. This reflects the uncertainty in the risk estimate predicted by the RRM. No range was possible for the Alden Bank sub-region since sources of *C. maenas* were considered absent.

##### Sources

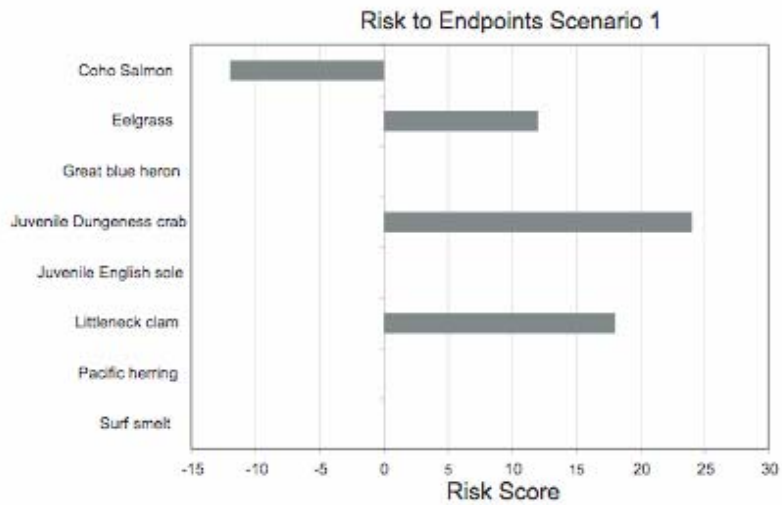
The uncertainty distributions for the following sources had small range widths: aquaculture shipments, passive current dispersal via El Nino, educational release and research release. These sources were considered absent for the first source scenario. The ballast water and live seafood shipment probability distributions were right-skewed, each having no more than a 43 percent chance of the risk estimate equaling zero with the probability for every other risk estimate occurring never exceeding 5 percent. The live seafood shipment distribution, however, had a much wider range, indicating more variability, and thus less confidence in the RRM risk estimate for live seafood shipments.

##### Habitats

The probability distributions for gravel-cobble intertidal, soft bottom subtidal and sandy intertidal habitats had means that were very similar to the value predicted by the RRM. The distributions were right-skewed but the range widths were relatively small, with no greater than a 27 percent chance of each risk estimate occurring for each habitat. The water column distribution had a mean similar to the predicted RRM value but was a slightly wider, left-skewed distribution, again with no more than a 27 percent chance of each risk estimate occurring. The macro-algae distribution had a wide range and was right-skewed, having no more than a 43 percent chance of the risk estimate equaling zero with the probability of all other risk estimates occurring never exceeding 20 percent. The mudflat habitat had a slightly smaller range, however, there was approximately an 80 percent probability of the risk estimate equaling zero. While the mean of the probability distribution for the eelgrass habitat was similar to the RRM estimate, the distribution was skewed and the range was the widest of all habitat distributions, suggesting the least confidence in this RRM estimate.

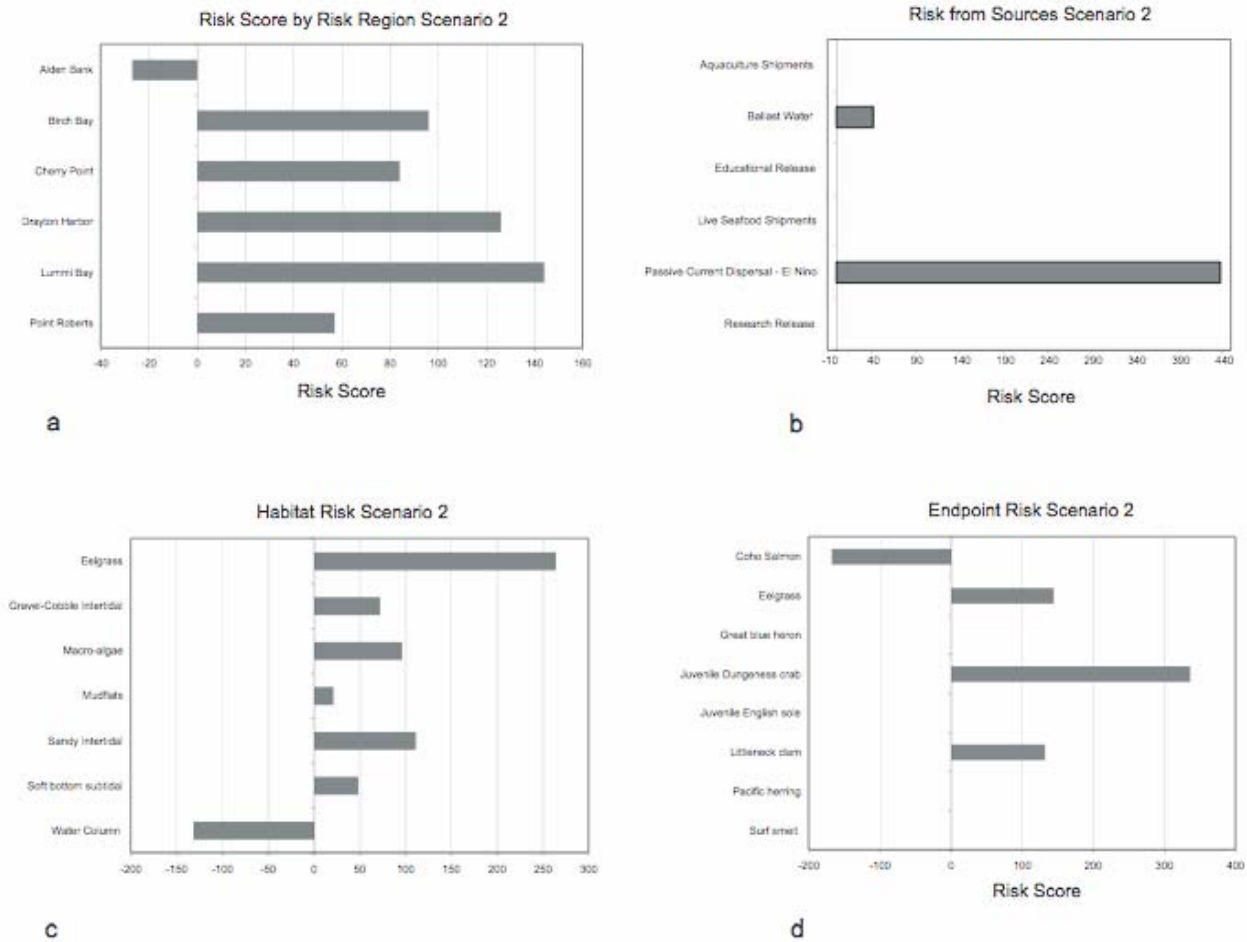


**a**



**b**

**Figure 2.10:** Relative risk to habitat (Figure 2.10a) and Assessment Endpoints (Figure 2.10b) for Source Scenario 1 without an El Nino event.



**Figure 2.11:** Relative risk to each assessment endpoint for Source Scenario 2.

## Assessment Endpoints

The two endpoints, Pacific herring and surf smelt, had distributions with means very similar to that predicted by the RRM estimates. Both distributions were left-skewed due to extreme outliers in the lowermost 10 percent of the distributions and had approximately an 85 percent chance of the risk estimate for each endpoint equaling zero. While having slightly wider ranges, the juvenile English sole and great blue heron distributions also had means similar to the predicted RRM values, were again left-skewed due to extreme outliers in the lowermost 10 percent of the distributions and had an 85 percent chance of the risk estimate for each endpoint equaling zero. Though the Coho salmon distribution was left-skewed with a slightly smaller range width, there was no more than a 27 percent chance of each risk estimate occurring. While the littleneck clam and eelgrass had right-skewed distributions the eelgrass distribution showed a 56 percent chance of the risk estimate equaling zero with the probability for every other risk estimate occurring never exceeding 14 percent. Conversely, the littleneck clam distribution revealed there was no greater than a 20 percent chance of each risk estimate occurring. The juvenile Dungeness crab uncertainty distribution had the widest range of all endpoints with no more than an 18 percent chance of each risk estimate occurring, indicating a large amount of variability and thus less confidence within the RRM estimate for this endpoint.

## Source Scenario 2 El Nino

### Sub-regions

The means for all sub-region probability distributions were slightly higher than the risk estimates predicted by the RRM. All sub-region distributions, except for Alden Bank, were right-skewed with fairly wide ranges, with Lummi Bay having the widest range. The distributions for the Birch Bay, Drayton Harbor, Lummi Bay and Point Roberts sub-regions revealed there was no more than a 35 percent chance of each risk estimate for each sub-region occurring. The Cherry Point sub-region showed there was no greater than a 14 percent probability of each risk estimate for this sub-region occurring. As mentioned above, the Alden Bank distribution was different from the others in that it was a left-skewed distribution with a smaller range, while having a 40 percent chance of the risk estimate equaling zero with the probability for every other risk estimate occurring never exceeding 10 percent.

### Sources

The probability distributions for aquaculture shipments, educational release and research release had small range widths as these sources were again considered absent in the source scenario. The ballast water and live seafood distributions were right-skewed with a mean similar to, but lower than, the RRM prediction. The ranges were relatively small and each distribution showed that there was no more than a 42 percent chance of the risk estimate equaling zero with the probability for every other risk estimate occurring never exceeding 10 percent. Passive current dispersal had an approximately normal probability distribution that was much wider than the other source distributions. In addition, the distribution showed that there was no greater than a 3 percent chance of each risk estimate occurring, which indicated a large amount of variability within the passive current dispersal RRM estimate.

### Habitats

The mudflat habitat had a wide, right-skewed distribution with no greater than a 27 percent probability of each possible risk estimate occurring. Both the macro-algae and soft-bottom subtidal habitats had normal distributions with means similar to, but lower than, the RRM estimate. The ranges were wide with no more than an 8 percent chance of each risk estimate occurring for these two habitats. The probability distribution for the water column had a mean similar to the predicted RRM value but was left-skewed with a wide range and showed no more than a 7 percent probability of each risk estimate occurring. The gravel-cobble and sandy

intertidal distributions were very wide and right-skewed, revealing no greater than an 8 percent probability of each risk estimate occurring for these two habitats. The means were also slightly lower than their respective RRM estimates. Of all habitat distributions, the eelgrass habitat distribution had the widest range. The distribution was also right-skewed with no more than a 6 percent chance of each risk estimate occurring. This variability within all habitat distributions suggests less confidence within the RRM values estimated for all habitats.

#### Assessment Endpoints

The means of the probability distributions for all eight endpoints were slightly lower than the predicted RRM estimates. Four endpoints, including the great blue heron, English sole, Pacific herring and surf smelt, had wide, left-skewed distributions which were the result of extreme outliers in the lower and more negative 10-20 percent of the distributions. These distributions showed an 80 percent probability of the risk estimate equaling zero. The eelgrass and littleneck clam distributions were right-skewed with a wide range. The eelgrass distribution, however, revealed that there was no greater than a 37 percent probability of each risk estimate occurring while the littleneck clam distribution showed that there was no greater than a mere 5 percent probability of each risk estimate occurring. The Coho salmon and Dungeness crab probability distributions were normal with a wide range. The Coho salmon distribution showed there was no greater than a 9 percent chance of each risk estimate occurring for this endpoint. The distribution range for the Dungeness crab was the widest of all endpoint probability distributions. Furthermore, the Dungeness crab distribution revealed there was no more than a 3 percent chance of each risk estimate occurring, indicating more variability within this RRM estimate.

#### 2.3.3 Sensitivity Analysis

The sensitivity analysis produced rank correlations for each sub-region, source, habitat, and endpoint risk estimate. The rank correlations indicate whether the uncertainty of any model parameters influences the uncertainty within the final risk estimates.

#### Source Scenario 1

##### Sub-regions

For the Cherry Point sub-region, the model parameter contributing the most uncertainty was the exposure filter component A (source characteristics) for ballast water, having a rank correlation value of 0.58. The model parameter, exposure filter component A for live seafood shipments, contributed the most uncertainty to the risk estimates for the following regions: Birch Bay, Drayton Harbor, Lummi Bay and Point Roberts. The rank correlations for live seafood shipments ranged from 0.58 to 0.89 for these sub-regions. It is important to note that several other parameters are listed as influencing the uncertainty as well. Alden Bank did not have an available source of *C. maenas* for this source scenario and thus, sensitivity analysis for this sub-region was not conducted.

##### Sources

The uncertainty within the exposure filter component A for ballast water in the Cherry Point sub-region most influenced the uncertainty within the ballast water risk estimate. The rank correlation for this model parameter was 0.64. Live seafood shipments did not have a dominant model parameter, suggesting several model parameters influenced the uncertainty within this estimate. All other sources were considered absent for this source scenario with low uncertainty and therefore sensitivity analyses were not conducted.

## Habitats

The live seafood shipments exposure filter component *A* was the model parameter with the most dominant effect on the uncertainty in the mudflat habitat risk estimate, having a rank correlation of 0.56. The other habitats including eelgrass, gravel-cobble intertidal, macroalgae, sandy intertidal, soft-bottom subtidal and water column did not have a dominant model parameter, indicating that multiple model parameters contribute to the uncertainty within the risk estimates.

## Assessment Endpoints

The uncertainty within the effects filter component *C*, which considers whether effects are possible, was highly correlated with the uncertainty in the risk estimates for the following endpoints: eelgrass, great blue heron, juvenile English sole, Pacific herring and surf smelt. The rank correlation was lowest for eelgrass (0.62) while it ranged from 0.86 to 0.90 for the other endpoints. The Coho salmon, littleneck clam and juvenile Dungeness crab did not have one model parameter that exhibited a dominant effect upon the uncertainty within the risk estimates, but instead, several model parameters contributed to the uncertainty.

## Source Scenario 2 El Nino Sub-regions

The uncertainty within the risk estimates for Birch Bay, Drayton Harbor and Lummi Bay was highly correlated with the parameter, passive current dispersal (exposure filter component *A*). The correlations for these three sub-regions were 0.57, 0.66 and 0.64, respectively. Figure 2.12a portrays the sensitivity chart for the Birch Bay sub-region. Cherry Point, Alden Bank and Point Roberts did not have one parameter exhibiting a dominant effect upon the uncertainty in the risk estimates, indicating that multiple model parameters contributed to the uncertainty within the risk estimates.

## Sources

Similar to the first source scenario, the exposure filter component *A* for ballast water in the Cherry Point sub-region most influenced the uncertainty within the ballast water. Additionally, the uncertainty within the risk estimates for live seafood shipments and passive current dispersal was influenced by more than one model parameter as there was no apparent dominant correlation for either source. All other sources were considered absent for this source scenario with low uncertainty and consequently no sensitivity analyses were conducted.

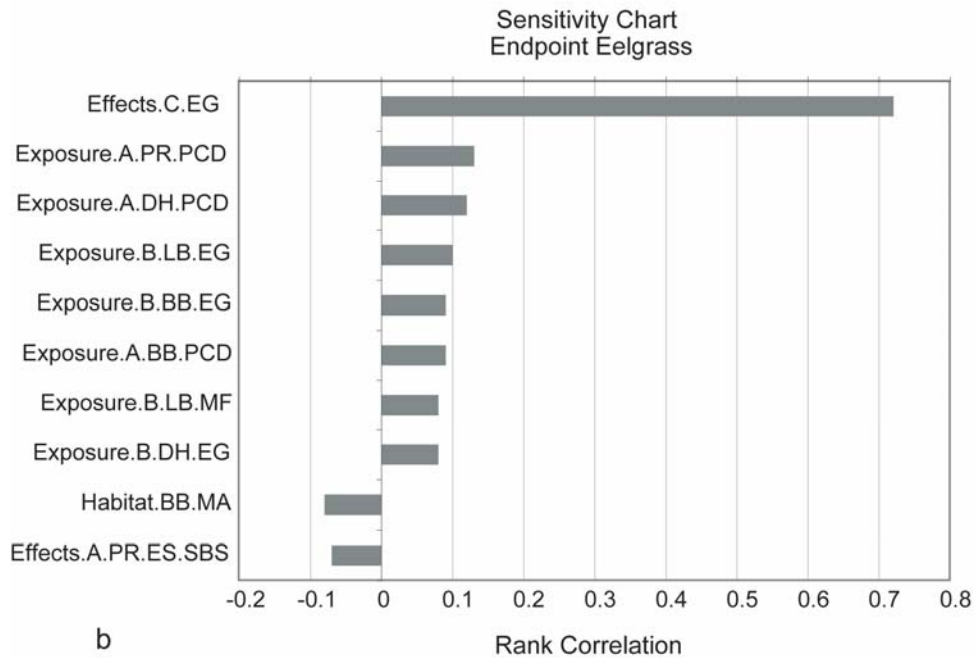
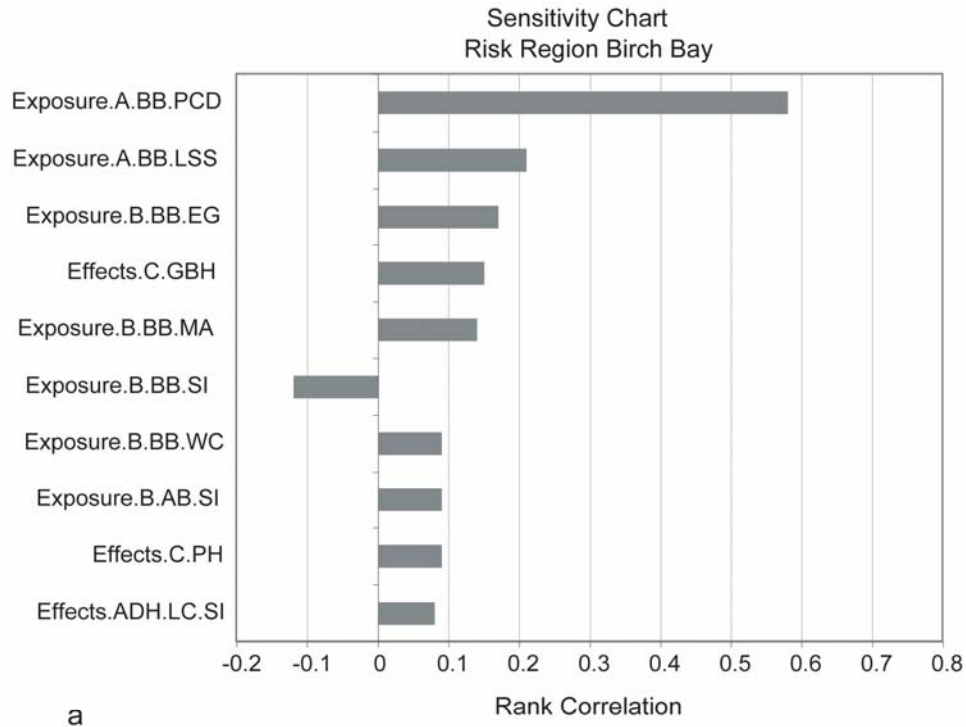
## Habitats

The uncertainty within each habitat risk estimate was affected by several model parameters. Therefore, the habitats did not have one dominant parameter contributing to the uncertainty and consequently all of the rank correlations were low.

## Assessment Endpoints

Similar to the first source scenario, the uncertainty within the effects filter component *C* was again highly correlated with the uncertainty in the risk estimates for eelgrass, great blue heron, juvenile English sole, Pacific herring and surf smelt. The importance of the effects filter for the endpoint eelgrass is portrayed in Figure 12b. The eelgrass rank correlation again was the lowest (0.74) while the correlations for the other endpoints were all above 0.90. Additionally, the Coho salmon, littleneck clam and juvenile Dungeness crab did not have one model parameter which contributed the most uncertainty to the risk estimates. Several model parameters all had similar lower correlation values and thus, contributed to the uncertainty more equally.





**Figure 2.12.** Sensitivity Chart as measured by rank correlation for the El Nino scenario. In Figure 2.12a the factor Exposure.A.BB.PCD is the exposure filter component A for passive current dispersal in Birch Bay. The most important factor in Figure 2.12b is Effects.C.EG, the effects component C for eelgrass.

## 2.4 Discussion

The issue of non-indigenous species has become a concern to environmental managers, and several investigators have expressed the need to determine which species will be introduced and cause impacts (Grosholz and Ruiz 1996; Ricciardi and Rasmussen 1998). Ecological risk assessment, using the RRM methodology, offers a way to assess the risk of impacts of a stressor in a quantitative manner. Though this process is robust enough to evaluate the risk of any type of stressor, either physical, chemical or biological, it had never before been used to assess the risk of invasive species. Using *C. maenas* as a model species, we demonstrated that the risk assessment and RRM methodology can be used to estimate the risk posed by the introduction and impacts of a non-indigenous species.

In adapting the RRM methodology for use in determining the risk of introduction and impacts by an invasive species, we modified the exposure and effects filters to better suit a biological entity. Since exposure for a biological stressor is the probability of a successful invasion event, the exposure filter was broken into two components, source characteristics and habitat suitability, which influence the introduction, survival, growth and reproduction of an organism. To account for temporal variability and the possibility of effects, the effects filter was divided into three components detailing the abundance of the endpoints in the habitat, seasonal overlap with *C. maenas* and the types of effects possible (beneficial vs. undesirable). These modifications allow for a more detailed analysis of risk and the associated uncertainty.

In the first source scenario representing current conditions, Cherry Point is the only sub-region that exhibited risk. This is primarily due to the only available source, ballast water, occurring in this sub-region. Within the sub-region, the habitat that is at the greatest risk of introduction and impacts is the eelgrass habitat, which in addition to being considered an assessment endpoint, is also utilized by the juvenile Dungeness crab (McDonald 2001; Pauley *et al.* 1986), another assessment endpoint. In contrast to the eelgrass habitat, the water column habitat actually exhibits negative risk, which is due to the beneficial effects of *C. maenas* larvae as prey for the Coho salmon utilizing this habitat. The results concerning potentially beneficial effects, however, should be interpreted with caution. Long-term undesirable impacts to other habitats and endpoints may eventually outweigh the more immediate, short-term beneficial effects.

The juvenile Dungeness crab is the endpoint that is most at risk in this scenario, which was expected since it is an abundant crab that has been shown in the laboratory to compete with *C. maenas* for resources (McDonald 2001). Additionally, the littleneck clam is moderately at risk, which was also predictable as bivalves are a primary prey item of *C. maenas* (Cohen *et al.* 1995; Dare *et al.* 1983; Grosholz and Ruiz 1996). Both the Dungeness crab and the littleneck clam represent important fisheries in the state of Washington (Pauley *et al.* 1986; Chew and Ma 1987) and consequently the predictions of substantial risk of impacts to these endpoints should be an area of major concern to environmental managers.

While these risk estimates are initially useful in determining which sub-regions, sources, habitats and endpoints should receive more immediate attention by environmental managers, uncertainty analysis was necessary to determine the amount of confidence in the risk estimates. Monte Carlo analysis offers a way to characterize the uncertainty within the risk estimates and also identify the model parameters, if any, that influence the uncertainty the most. Uncertainty analysis for the first source scenario revealed that the uncertainty in the model parameters caused much variability in the possible RRM estimates. For instance, while the Cherry Point sub-region was determined to be at a higher risk, many risk estimates were actually possible due to a large amount of uncertainty. Sensitivity analysis determined that the uncertainty

concerning whether ballast water will actually release *C. maenas* contributed most to this variability.

Unlike the sub-region risk estimates, the lack of confidence in the habitat and endpoint risk estimates was due to uncertainty concerning multiple model parameters, including exposure and effects components. As a result of the numerous sources of uncertainty within the model parameters, the RRM may have actually underestimated the risk to the juvenile Dungeness crab, littleneck clam and eelgrass. This underestimation of risk could result in lack of protection of these endpoints and discourage preventative measures that are actually necessary. The variability in the risk estimates for the remaining endpoints; great blue heron, juvenile English sole, Pacific herring and surf smelt, is mostly due to uncertainty concerning the beneficial effects of *C. maenas* to these endpoints. Further research concerning these effects may lead to a reduction in the risk estimate uncertainty and clarify whether or not beneficial effects are possible or if the endpoints remain unaffected.

When considering the possibility of El Nino-driven passive current dispersal as an additional source for the second source scenario, the risk to most sub-regions, habitats and endpoints increase dramatically. All sub-regions now are at risk, though Lummi Bay and Drayton Harbor are the two sub-regions with the greatest risk. This is most likely due to the presence of those habitats and endpoints having the greatest amount of risk in the Lummi Bay and Drayton Harbor sub-regions. Once again, the eelgrass habitat is the habitat with the highest risk while the water column habitat is again estimated to have negative risk as a result of potentially beneficial effects to the Coho salmon that utilize the habitat. The juvenile Dungeness crab is the endpoint most at risk overall while the littleneck clam and eelgrass again are predicted to be the endpoints with moderate risk. Environmental managers should focus on those habitats and endpoints having considerable risk of impacts when determining the appropriate preventative or mitigative measures to employ.

Similar to the first source scenario, there is much variability and therefore less confidence in the risk estimates for the second source scenario. The risk estimates for the Lummi Bay and Drayton Harbor sub-regions were possibly overestimated due to the extremely large amount of uncertainty concerning the possibility of *C. maenas* introduction from passive current dispersal. Once again, with the exception of the great blue heron, juvenile English sole, Pacific herring and surf smelt, there is an extreme amount of variability in the habitat and endpoint risk estimates. This variability is due to uncertainty within numerous model parameters, including source characteristics, habitat suitability exposure factors and effects factors.

Though ecological risk assessment and the RRM methodology alone are useful in predicting risk from an invasive species, uncertainty analysis is essential in determining the credibility of the risk estimates and providing a basis for efficient data collection or application of refined methods (U.S. EPA 1998). In the case of *C. maenas* and Cherry Point, Washington, the uncertainty of whether ballast water, passive current dispersal and live seafood shipments can actually release *C. maenas* into the habitats greatly affects the sub-region risk estimates. More research must be conducted to determine if the ballast water discharged in the Cherry Point sub-region actually contains viable larvae or post larvae of *C. maenas* or other decapods. Also more information is required concerning the viability of organisms in ballast water.

More information is also required concerning live seafood shipments within the Cherry Point study area as the uncertainty for the exposure of this source also greatly affects the sub-region risk estimates. A database of establishments receiving live seafood shipments and the origins of the shipments would be especially useful in determining whether these shipments could

potentially introduce *C. maenas* to the Cherry Point study area. Viability of decapods in seafood shipments in general is also an area in need of investigation.

Unlike the other two sources, passive current dispersal is a natural event in which the uncertainty of exposure is associated with the intensity of the El Nino event, which is highly unpredictable (Federov *et al.* 2003). The presence of appropriate currents within Puget Sound is also an important component necessary to transport *C. maenas* larvae to the region. Monitoring for larval populations along the west coast of the U.S. and Puget Sound during El Nino events is necessary to determine the possibility of successful larval transport of decapod larvae, specifically *C. maenas* larvae, to the Cherry Point study area.

While the dominant sources of uncertainty were identified for several of the risk estimates, the variability within most estimates was due to multiple sources of uncertainty. Much of this uncertainty can be attributed to lack of data concerning the abundance and distribution of assessment endpoints and other native species, as well as unknown interactions with *C. maenas* and unavailable source information. In addition, the natural stochasticity of the ecological system only serves to further complicate the uncertainty. Overall, much uncertainty still remains concerning the ecological system and the non-indigenous species in question.

Until the time when more of the uncertainty can be reduced through additional research efforts, ecological risk assessment and the RRM can serve as a guide for environmental managers to reduce the risk of introduction and impacts through preventative and possibly mitigation efforts. Government agencies such as the Washington Department of Fish and Wildlife can continue utilizing ballast water management strategies and monitoring for *C. maenas* in the Straits of Georgia and Juan de Fuca. Since passive current dispersal is a natural source, not under the control of humans, prevention may not be possible should this form of transport occur. If that is the case, efforts can be directed toward eradication strategies in sub-regions predicted to be most at risk as well as monitoring of endpoint populations predicted to be affected by *C. maenas*.

### **3. Ecological Risk Assessment for the Asian Oyster in Chesapeake Bay, Maryland**

#### **3.1 Introduction to the Asian Oyster (*Crassostrea ariakensis*)**

The Asian Oyster (*C. ariakensis*) is found in the coastlines of China, Southern Japan, Taiwan, the Philippines, Thailand, Vietnam, northern Borneo, Malaysia, Pakistan and India (Tschang and Tse-kong, 1956; Rao, 1987; Zhou and Allen, 2003). The population outside of the China and Southern Japan regions has not been genetically confirmed (Allen et al., 2002). Asian Oyster was accidentally introduced to Oregon in the 1970's with *Crassostrea gigas* and *Crassostrea sikamea* (Breese and Malouf, 1977). No Asian Oyster population have established on the west coast of the United States because the water temperature is too low (National Research Council, 2004).

Asian Oyster can survive in a temperature and salinity range of 14 - 32 °C and 7-30 ppt and settle and support larval growth at about 28°C and 20-30 ppt (Cai et al. 1992). The feeding rate is highest at 10 - 12°C and 15 - 30 ppt and is not affected by high levels of suspended materials (Zhang and Lou 1959). They reproduce from April to June in China and September to October in Pakistan (Cai et al. 1992). There is relatively little information about the ecology of the Asian Oyster in the native habitat. In China, they are found to build reefs and have larval settlement on the shady sides of hard surfaces (Cai et al., 1992). In Japan, they are only found on muddy surfaces (Amemiya, 1928; Hirase, 1930) and in Pakistan, they are found in both muddy and hard surfaces (Patel and Jetani, 1991; Ahmed et al., 1987).

The main food supply for the Asian Oyster includes phytoplankton and detritus. Asian Oyster will probably have the same predators as *Crassostrea virginica*, the Eastern Oyster, which includes sponges, annelids, gastropods and crabs (National Research Council, 2004). Several diseases have been documented to infect Asian Oyster. A Rickettsia-like organism might have caused an 80-90% mortality in the China population since 1992 (Wu and Pan 2000). Some Asian Oyster under quarantine in France was infected with *Bonamia* parasite (Cochennec et al., 1998). Asian Oyster can also be infected by *Perkinsus marinus* but there were no effects on growth and survival.

#### **3.2 Risk Assessment Methods**

The risk assessment was conducted using a conceptual model, developed by Colnar and Landis (in press), and designed to evaluate the regional risks of an invasive species. The model incorporated the Hierarchical Patch Dynamic models (Wu and David, 2002) and the Relative Risk Model (RRM) (Landis and Wieggers, 1997). The risk assessment included three parts: problem formulation, analysis and risk characterization.

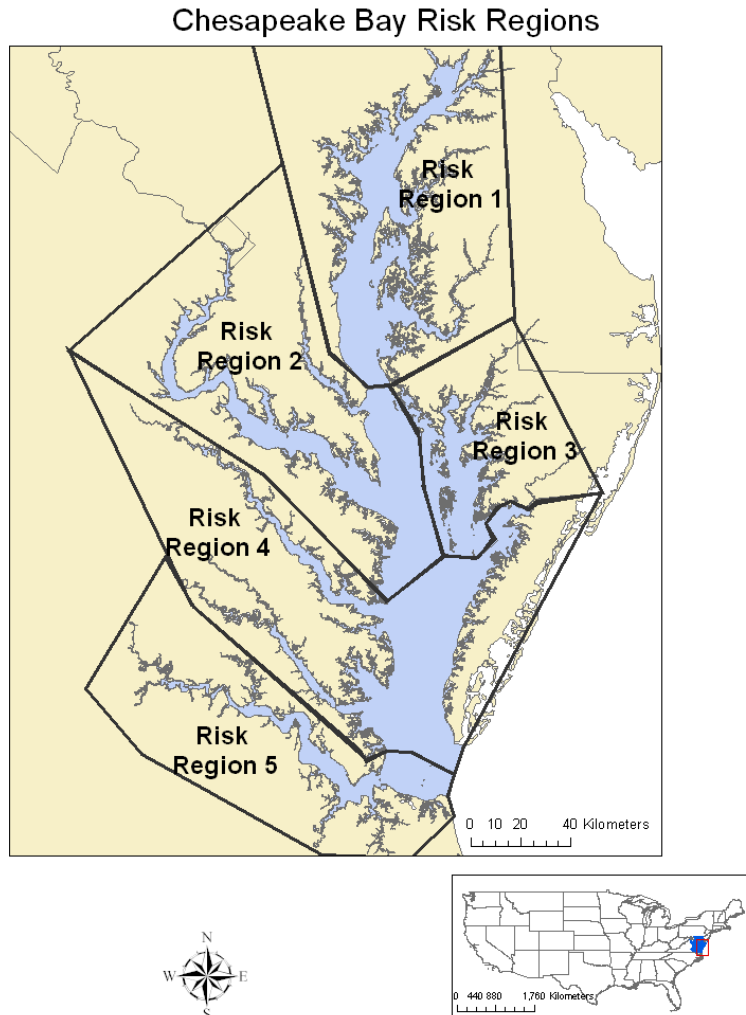
##### **3.2.1 Problem Formulation**

During the problem formulation, information about the study area and the components of sources, stressors, habitats and assessment endpoints was incorporated into a conceptual model. The conceptual model illustrates the potential exposure and effects pathways between all components.

#### **Description of Study Area – Chesapeake Bay**

The Chesapeake Bay watershed contains the Chesapeake Bay and is located in parts of New York, Pennsylvania, West Virginia, Delaware, Maryland and Virginia and the entire District of Columbia. The study area for this risk assessment only included the Chesapeake Bay (Figure 3.1). The bay is the largest estuary in the United and States, has 4,400 miles of shoreline and is important for fisheries, shipping, and industries and provides habitat for various organisms and recreations (EPA, 1996). Recently, oyster and blue crab populations have

declined dramatically because of over harvesting, diseases and degradation of habitat. The bay was once the dominant oyster source in United States but now it provides only 3% of the total supply. Between 1974-2000, Maryland and Virginia had a 65% drop in the number of processing plants, which affected oystermen, processors (National Research Council, 2004).



**Figure 3.1.** The location of the study area and the various risk regions.

The study area was divided into sub-regions mostly by the salinity of the bay and partly by the types of land-use. The salinity was considered because the bay has a large salinity gradient and salinity affects the survival and reproduction of oysters. The upper part of the bay has much lower salinity than the lower part. The land-use types were also considered when dividing the sub-regions because there is a difference between the eastern and western shore. The eastern shore has more urban development and the western shore has more agricultural land.

## Identification and Description of Potential Sources of Stressors

Two possible sources for the Asian oyster are aquaculture and larval current dispersal. There are efforts by groups such as the Virginia Seafood Council to start aquaculture in order to re-build the oyster industry in the Chesapeake Bay. As of July 2004, about 860,000 triploids have been tested. The triploid oyster is infertile while the diploid oyster is fertile. Asian Oyster can also be introduced through illegal introduction not in compliance with the International Council for the Exploration of the Sea. Accidents such as storms can destroy aquaculture bio-security measures and spread the triploids. The triploids might then be converted to the fertile form.

## Description of Habitats

The Chesapeake Bay consists of subtidal and intertidal habitats. Smith et al. (2001) identified six subtidal benthic habitats: 1) sand, 2) sand and shell, 3) mud, 4) mud and shell, 5) hard bottom and 6) oyster rock.

## Description of Assessment Endpoints

Assessment endpoints were chosen based on their importance economically, ecologically and culturally and suggestions from the project manager, Daniel Kluza. Five endpoints were chosen and they are the native eastern oyster (*Crassostrea virginica*), striped bass (*Morone saxatilis*), blue crab (*Callinectes sapidus*), piping plover (*Charadrius melodus*) and eelgrass (*Zostera marina* L.).

Native Eastern oyster (*Crassostrea virginica*). The Eastern Oyster is found on the Atlantic Coast from the Gulf of St. Lawrence to the Bay of Campeche in Mexico (Carriker and Gaffney 1996). The average life-span for the oyster is six to eight years but they have been found to live for as long as twenty-five years (National Research Council 2004). The presence of sperm causes the females to release eggs. A close proximity of oysters increases the chances of successful spawning and fertilization. The larvae must settle on clean and solid surfaces in order to grow. Some predators of the oyster include worms, crabs, oyster drills, starfish and finfish. Oyster reefs provide habitat for fish such as the striped bass and invertebrates such as shrimps and blue crabs (Coen and Luckenbach, 2000; Bahr and Lanier, 1981). The oyster population in the mid-Atlantic region has declined due to commercial harvesting, parasites and the increase in human disturbances.

Striped bass (*Morone saxatilis*). Striped bass are anadromous fish that spawn once a year. The inhabitant range of striped bass on the Atlantic coast extends from St. Lawrence River in Canada to the St. Johns River in Florida (Magnin and Beaulieu, 1967; McLane, 1955). Mid-Atlantic region is important to the striped bass because it provides spawning grounds and large amount of recreational fishery activities occur in the region (Fay et al. 1983). All striped bass are mature by age 6 but the years to maturity can vary between 2-3 for male and 4-5 for female (Pearson 1938; Bason 1971; Wilson et al. 1976). The larval stage generally remain near the areas spawned and is considered crucial for future population abundance (Bain, 1982; Setzler-Hamilton et al., 1981).

Blue crab (*Callinectes sapidus*). The Blue crab is found in the estuaries between Massachusetts Bay and the Eastern coast of South America (Piers 1923; Scatter-good 1960). It is important in the mid-Atlantic region because it is important in the structure and functions of estuarine communities and it also supports a commercial fishery. It preys on clams and oysters and is preyed by different estuarine and marine animals, including the striped bass (Newcombe, 1945; Manooch, 1973). Mating occurs in low-salinity waters and the females migrate to higher-salinity waters afterwards (Pyle and Cronin 1950; Churchill, 1919). Hatching

of the blue crab eggs only occur at salinities and temperature of 22-33 ppt and 19-29 °C (Sandoz and Rogers, 1944). Salinity levels are not critical for post larval crabs (Odum 1953; Costlow 1967). The optimal substrate habitat is soft detritus, mud or mud-shell for small crabs and hard bottom for large crabs.

Piping plover (*Charadrius melodus*). The piping plover, a small North American migratory shorebird, was listed on January 10, 1986 under the U.S. Endangered Species Act of 1973. There are three breeding piping plover populations and they are at the beaches of the Atlantic Coast, shorelines of the Great Lakes and along wetlands and rivers in the Northern Great Plains (Ferland and Haig 2002). Piping plovers prey on larvae and adult macro-invertebrates, mollusks, crustaceans as well as other small marine animals (Bent 1929; Shaffer and Laporte 1994; Cuthbert et al. 1999). Possible predators of the piping plover include various avian species as well as animals such as red fox and skunk (Germain and Struthers 1994). Piping plover in the Atlantic Coast mainly breeds on small sand dunes (U.S. Fish and Wildlife Service 1988).

Eelgrass (*Zostera marina L.*). Eelgrass is found on the eastern coast of North America from Nova Scotia to the Carolinas. It is the dominant species of submerged aquatic marine vegetation (SAV) in its range. Eelgrass can tolerate a wide range of environmental parameters. It is found in areas with soft mud to coarse sand substrates, salinity of 10‰ to 30‰ and temperature range of approximately 0 to 30°C (Ostenfeld 1908; den Hartog 1970). Eelgrass can reproduce both sexually and asexually. Both types of reproduction are largely affected by factors such as light, temperature and salinity (DeCock 1981; Phillips et al. 1983; Lamounette 1977). Some important factors of eelgrass as listed by Thayer et al. (1975) include: 1) support for epiphytic organisms, 2) leaves produce large amount of organic material that can decompose and be transported to other systems, 3) detritus supports local communities and 4) the shoots help stabilize sediments.

### **3.2.2 Conceptual Model**

We adopted the conceptual model developed by Colnar and Landis (in press) for the Green Crab into this study (Section 2, this document). The model addressed the different scales that can affect the succession of invasion. At the local scale, physical and biological parameters as well as disturbances or alterations can affect the local habitat suitability (Figure 3.2 and Figure 3.3). The physical parameters include local substrate, local depth, local salinity range and local temperature range. The biological parameters include local resource competition and local predation. Some disturbances that can occur include land use change and xenobiotics.

At the regional scale, there can be ocean regime and climate changes that alter the western Atlantic temperature and salinity range, Gulf Stream, Chesapeake Bay currents and estuarine-forced water flow which make up parts of the physical parameters that affect the habitat suitability at a regional scale.

Some possible undesirable effects of Asian oyster include resource competition, reproductive interference, disease transmission, physical habitat alteration and habitat destruction. Some potential desirable effects of Asian oyster include increased reef habitat and increased prey availability (Figure 3.3)



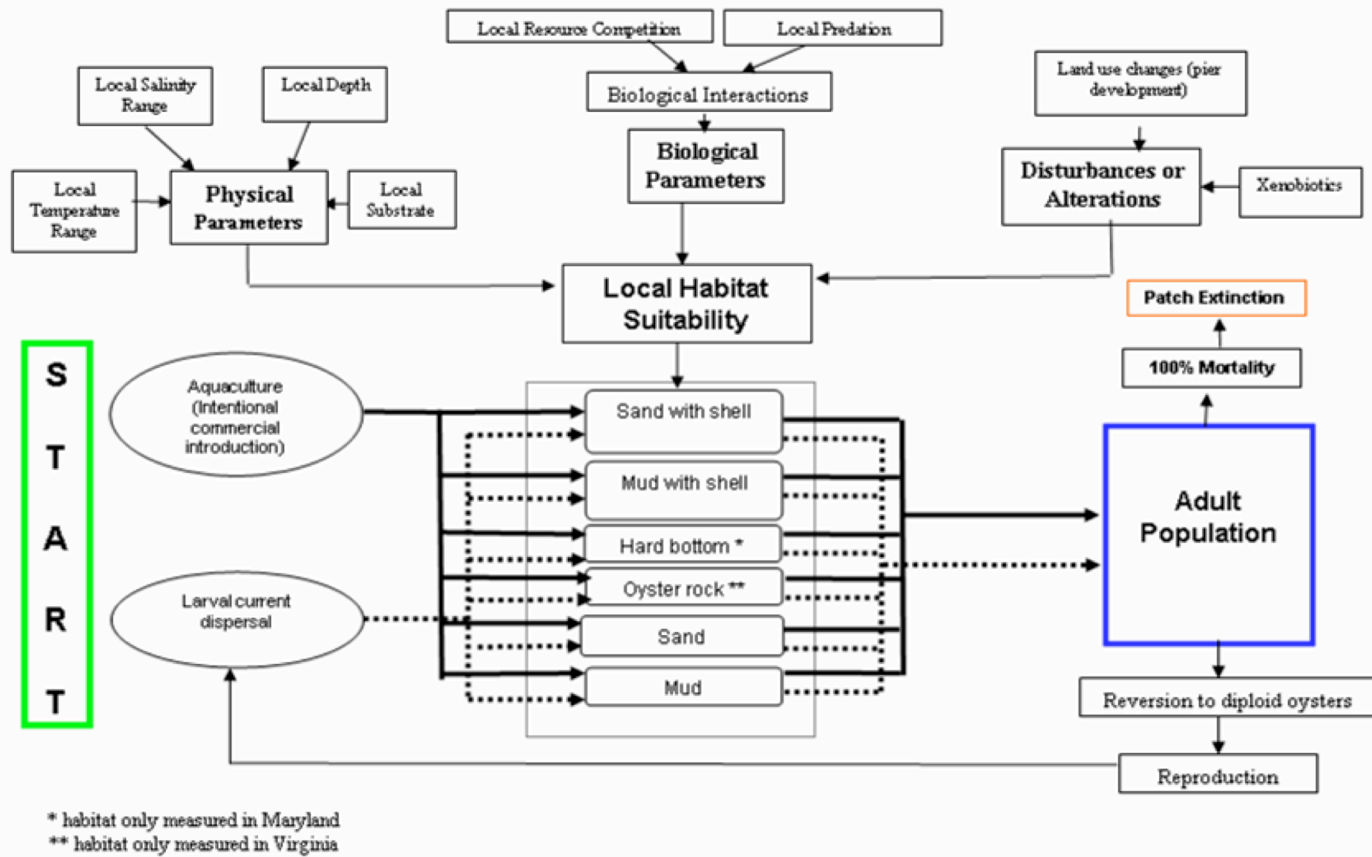
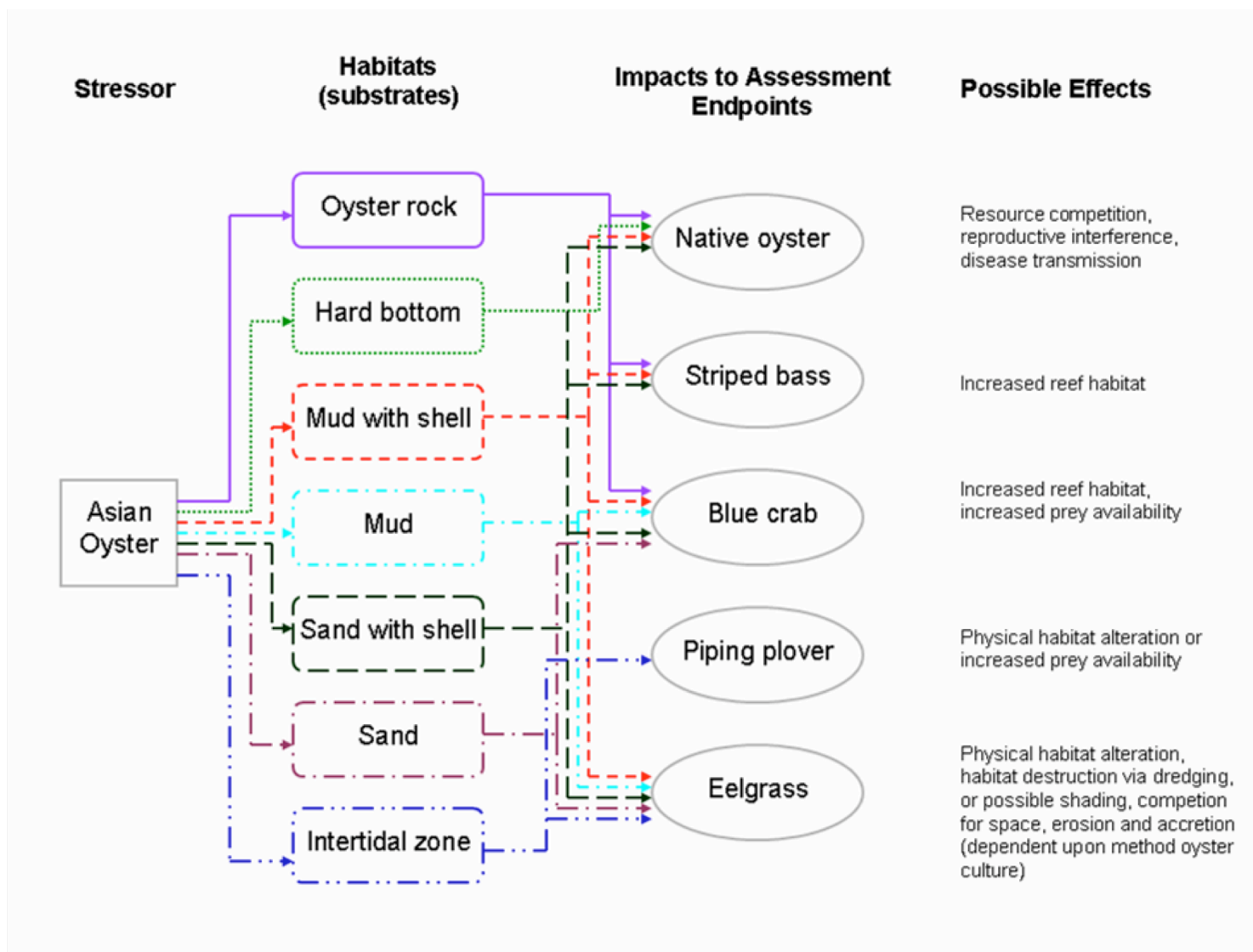


Figure 3.2. The local scale juvenile and adult life stages exposure portion of the hierarchical conceptual model.



**Figure 3.3.** The local scale effect of the Asian oyster to the various endpoints in the hierarchical conceptual model.

### 3.2.3. Analysis

The second phase in an ecological risk assessment, the analysis phase, involves relating exposure and effects to each other (U.S. EPA 1998) and investigating each route to the impact. To analyze the risk of exposure and effects, we used the Relative Risk Model developed by Landis and Wieggers (1997) and Wieggers et al. (1998). This methodology has been used numerous times to comparatively determine risk at a large scale (Hart Hayes and Landis 2004; Moraes et al. 2002; Obrey and Landis 2002; Wieggers et al. 1998). The RRM ranks risk components and filters for each possible pathway. The following assumptions were considered (Landis and Wieggers 1997; Wieggers et al. 1998):

1. The type and density of assessment endpoints is related to the available habitat;
2. The sensitivity of receptors to stressors varies between habitat; and
3. The severity of effects in sub-regions of the Chesapeake Bay region depends on relative exposures and the characteristics of the organisms present.

### Development of Habitat and Source Ranks

The subtidal habitats were ranked by the areas (Km<sup>2</sup>) in each sub-region and the ranks ranged from zero to six on a two-point scale. Intertidal habitat was ranked by whether or not the habitat is present and the ranks were either a zero or a six. The ranking categories were determined by using natural breaks in GIS datasets (Table 3.1). A rank of zero represents no habitat present and six represents the greatest relative amount of habitat present (Table 3.2).

The source ranks were based solely on presence/absence of each source with zero representing absence of source (low exposure potential) and six indicating presence of source (high exposure potential) (Table 3.3). To be consistent with the habitat-ranking scheme, zero and six were again used as the minimum and maximum ranks possible for the sources.

**Table 3.1.** Geographic information used in this risk assessment.

Name	Data Description	Data Source
Va176507	Predicted distributions of the piping plover	USGS GAP Analysis Program
Savdensities	Submerged aquatic vegetation (SAV) bed locations, classified into 4 density classes.	Virginia Institute of Marine Science (VIMS) (2003)
Cbseg2003	A segmentation scheme in which the Chesapeake Bay is divided into subunits based on similar criteria including salinity.	Chesapeake Bay Program
Countyp020	All U.S county boundaries	National Atlas of the U.S. (2004)
BBsurvey	Bottom (substrate) types for the MD portion of the Chesapeake Bay.	Maryland DNR (2003)
Bottom type (botall83)	Delineates the size and shape of productive and potentially productive bottoms for oyster reefs.	Comprehensive Coastal Inventory, VIMS (2001)
Statesp020	U.S. state boundaries	USGS (2002)

**Table 3.2.** Habitats ranking criteria.

Habitats (substrates)	Ranking Criteria	Range	Ranks
<b>Sand</b>	Area (km <sup>2</sup> )	0	0 (Zero)
		0.001 - 14.972	2 (Low)
		14.973 - 251.978	4 (Med)
		251.979 - 344.289	6 (High)
<b>Sand with shell</b>	Area (km <sup>2</sup> )	0	0 (Zero)
		18.825 - 26.316	2 (Low)
		26.317 - 47.001	4 (Med)
		47.002 - 112.340	6 (High)
<b>Mud</b>	Area (km <sup>2</sup> )	0	0 (Zero)
		0.001 - 10.711	2 (Low)
		10.712 - 141.391	4 (Med)
		141.392 - 425.437	6 (High)
<b>Mud with shell</b>	Area (km <sup>2</sup> )	0	0 (Zero)
		31.531 - 34.784	2 (Low)
		34.785 - 43.171	4 (Med)
		43.172 - 124.272	6 (High)
<b>Hard bottom</b>	Area (km <sup>2</sup> )	0	0 (Zero)
		0.001 - 20.099	2 (Low)
		20.100 - 73.015	4 (Med)
		73.016 - 75.336	6 (High)
<b>Oyster rock</b>	Area (km <sup>2</sup> )	0	0 (Zero)
		0.001 - 3.285	2 (Low)
		3.286 - 18.030	4 (Med)
		18.031 - 18.989	6 (High)
<b>Intertidal (shoreline) *</b>	Presence/Absence	Absent	0 (Zero)
		Present	6 (High)

**Table 3.3.** Sources ranking criteria

Sources	Ranking Criteria	Range	Ranks
<b>Aquaculture (intentional introduction)</b>	Presence/absence of aquaculture of Asian oyster	Absence	0
		-----	
		Presence	6
<b>Larval current dispersal</b>	Presence/absence of currents suitable for transport of Asian oyster larvae from areas with an existing population	Absent	0
		-----	
		Present	6

## Development of Exposure and Effects Filters

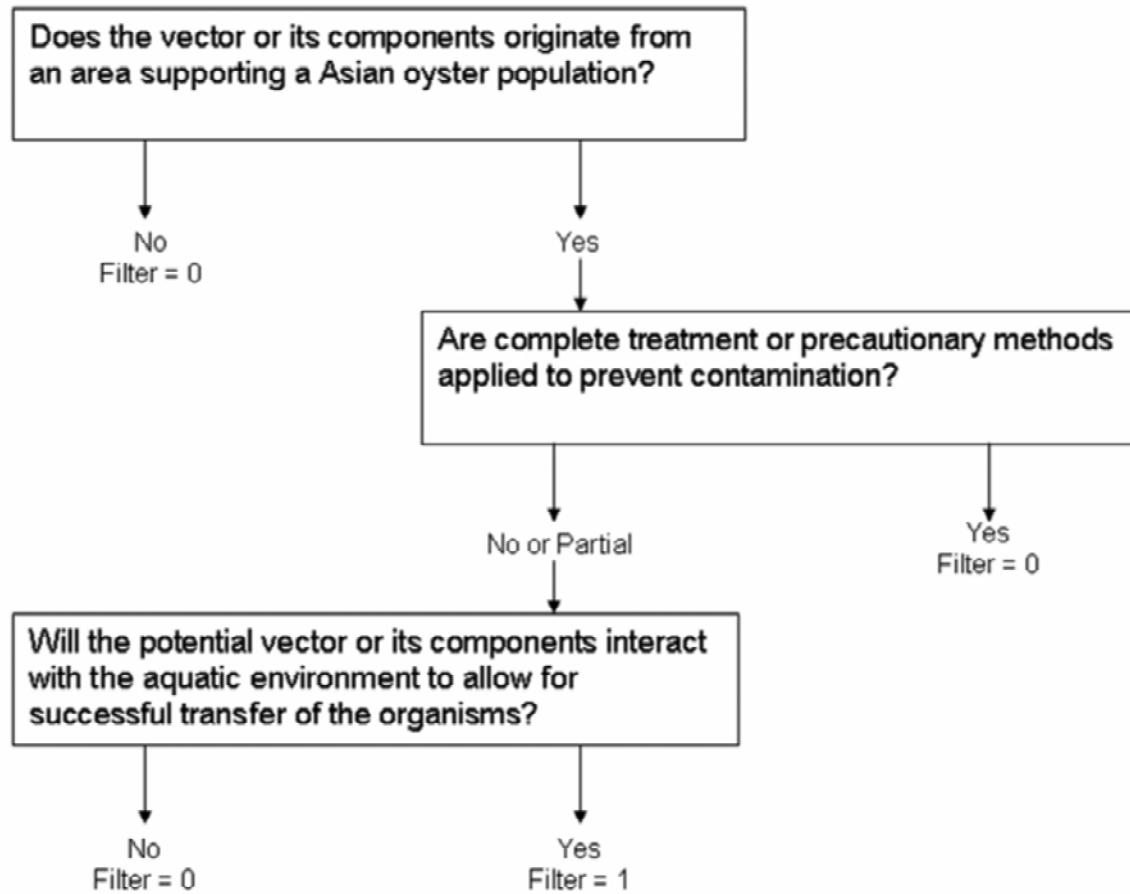
Filters are weighting factors used to determine the relationship between risk components: sources, habitats and impacts to assessment endpoints (Wieggers et al. 1998). The exposure and effects filters were determined with similar criteria as developed by Colnar and Landis (in press 2005). The filter values ranged between zero to one, with zero indicating an incomplete pathway and one indicating a complete pathway. We developed different decision trees to provide guidelines for filter assignments. When multiple components were present for the filter, all components were multiplied together to give individual filter values.

Two exposure filter components were used to indicate whether or not the source would release the stressor and whether the stressor could occur and persist in the habitat. Exposure filter component A considers whether or not the source will release the stressor and it is dependent upon the origin of the source, application of treatment or precautionary methods and the interaction of the source with the aquatic environment (Figure 3.4). Exposure filter component B considers the habitat suitability and is dependent upon temperature, salinity, substrate type and predation (Figure 3.5).

Three effects filter components were used to indicate whether or not the endpoint occurs in and utilize the habitat, if there is seasonal overlap in habitat usage between the stressor and the endpoint and are effects to the endpoint possible from interaction with the stressor (Table 3.4) Effects filter component A considered whether or not the endpoint occurs in and utilizes the habitat. For blue crab, striped bass and Eastern oyster, rank categories were determined using natural breaks on landing (pounds) GIS data (Table 3.1). There was not specific distribution data for eelgrass and piping plover, so we estimated whether or not the endpoint was present or absent (Table 3.4). Effects filter component B was determined by whether or not there was seasonal habitat overlap between the stressor and each endpoint. A value of zero indicates no overlap and a value of one indicates there is possible overlap. Effects filter component C was determined by whether or not there are possible effects to the endpoints by the stressor. A value of zero indicates no effects possible, a value of 1 indicates possible undesirable effects and a value of -1 indicates possible desirable effects.

## Exposure Filter Decision Tree

Criterion A:



**Figure 3.4.** Exposure filter criterion A decision tree.

Criterion B:

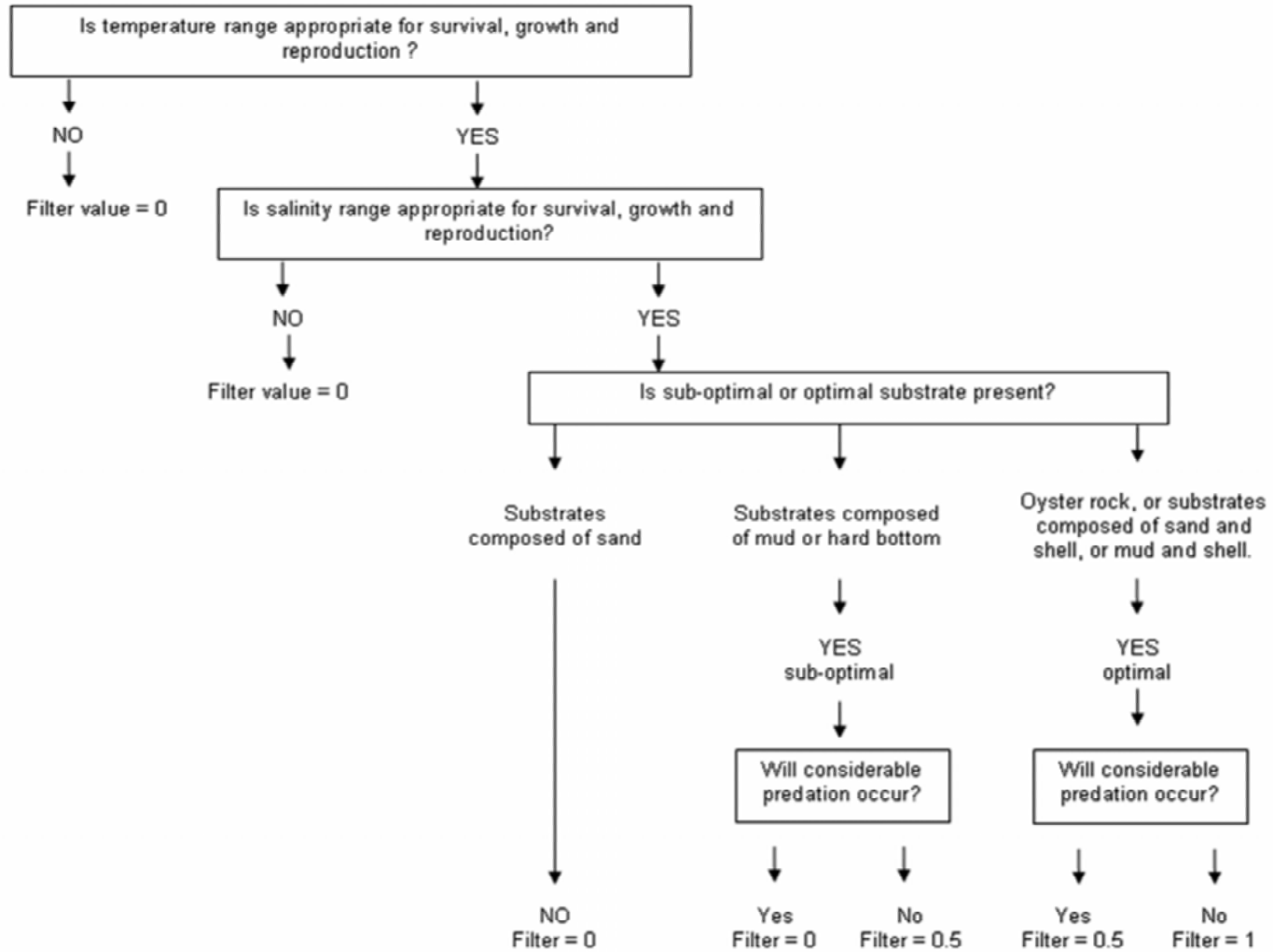


Figure 3.5. Exposure filter criterion B decision tree.

**Table 3.4.** Effects filter criteria A (a), B (b) and C(c).

(a)

Endpoint	Filter Criteria	Range	Filter Value
Blue Crab	Landings (pounds)	0	0
		3966290 - 7866447	0.5
		7866448 - 18684404	1
Striped Bass	Landings (pounds)	0	0
		124618 - 357783	0.5
		357784 - 1009787	1
Eastern Oyster	Landings (pounds)	0	0
		4456 - 33768	0.5
		33769 - 596699	1
Eelgrass *	Presence/absence of SAV in habitats	Absent	0
		Present	1
Piping Plover **	Presence/absence	Absent	0
		Present	1

(b)

Filter Values	Seasonal overlap in habitat usage with the Asian oyster and the endpoint?
0	No overlap
1	Overlap

(c)

Filter Values	effects (either undesirable or beneficial) to the assessment endpoints possible from interaction with the Asian oyster
0	No effects possible
1	Undesirable effects possible
-1	Beneficial effects possible

### 3.2.4. Risk Characterization

Risk characterization is the final phase of a risk assessment and is the process of integrating exposure and effects data to estimate the risk. All ranks were converted into a point system. To generate the exposure and effects filters for each pathway, the filter components of the exposure and effects filters were multiplied. Finally the source and habitats ranks were integrated with the exposure and effects filter products to generate the risk for each source-habitat-endpoint pathway. The risk scores were then summed to produce the following risk predictions: 1) risk in sub-regions, 2) risk in habitats, 3) risk to assessment endpoints and 4) risk from each source.



## Uncertainty Analysis

The amount of uncertainty for the ranks of components in the model was classified as low, medium or high based on the amount and the types of data available. The uncertainty was addressed using Monte Carlo analysis, a probabilistic approach. A discrete statistical distribution was assigned to components where the uncertainty was medium or high. The ranks or filters with low uncertainty retained their original values and were not assigned a distribution.

All source ranks were assigned low uncertainty because it was assumed that aquaculture would most likely occur in all risk regions. All habitat ranks were assigned with high uncertainty for different reasons. Sand, sand and shell, mud and shell, hard bottom and mud habitat for RR1, RR2 and RR3 were assigned high uncertainty because the data was based on 1975-1983 Maryland Department of Natural Resource survey to reassess oyster bottom and describe substrate (Table 3.1). The data may be outdated and not representative of the current habitat. Also, the habitats containing shell may be incorrect because in many areas the shell was actually covered by varying depths of sediment so it might not represent the habitat on the surface. Sand, sand and shell, mud, oyster rock and mud and shell habitats for RR4 and RR5 were assigned high uncertainty because the data was based on a survey of public oyster grounds that could be outdated and not representative of the total bay habitat. The intertidal habitat for all risk regions were assigned high uncertainty because it is known that inter-tidal areas are the primary habitat for Virginia's eastern shore but the exact location and areas were unknown. The hard bottom habitat for RR4 and RR5 were assigned high uncertainty because that specific habitat type was not measured but could be present.

All values for exposure filter component A for larval current dispersal had high uncertainty because the reversion of triploid state to diploid state is possible but only <1% of the Asian oyster would probably be able to produce normal gametes after being in the field for 3-4 years. All exposure filter component B values had low uncertainty for the aquaculture source and some had medium or high uncertainty for larval current dispersal source. The exposure filter component B values for mud and hard bottom in risk region 1 to 5 had medium uncertainty because we only know that Asian oyster can grow on mud and hard bottom substrates in Japan and Pakistan but there is not any information indicating whether or not they can grow on those types of substrates in the Chesapeake Bay. The exposure filter component B values for all habitat types but the sand and oyster rock in risk region 1 and the intertidal zone in risk region 2 to 5 had high uncertainty because the minimum salinity required for reproduction is approximately 15ppt while the salinity in risk region 1 is in the range of 10-15ppt and the substrate type within the intertidal zone is also unknown.

All exposure filter component A values had low uncertainty except for the Asian oyster in hard bottom habitat, the striped bass in sand and shell, mud and shell and oyster rock habitat and eelgrass in sand, sand and shell, mud, mud and shell and intertidal zone. The Asian oyster in hard bottom habitat had high uncertainty because it is unknown whether it is able to attach to hard bottom substrates. The small bass in sand and shell, mud and shell and oyster rock habitats had medium uncertainty because the striped bass are known to be present near oyster reefs and are assumed to utilize the habitats containing oyster shells. The eelgrass in sand, sand and shell, mud, mud and shell and intertidal zone had medium uncertainty because the dataset used to estimate the distribution of eelgrass contained all types of SAV not just eelgrass. All exposure filter component B values had low uncertainty based on the data available. The exposure filter component C values for blue crab, Eastern oyster and striped bass from both types of sources had medium uncertainty because the effects were probable

only. The exposure filter component C value for the effects of larval current dispersal source of the stressor on the piping plover has high uncertainty because the effects are only suspected.

### Sensitivity Analysis

We conducted a sensitivity analysis using the Crystal Ball®2000 software. The analysis examines the sources of uncertainty, influenced by either the model sensitivity or parameter uncertainty (Goulet 1995; Warren-Hicks and Moore 1988). Model sensitivity is the influence of a parameter within a model and parameter uncertainty is the influence of the range of possible parameter values. During sensitivity analysis, correlation coefficients are generated to rank each model parameter's contribution to predict uncertainty. A high rank correlation indicates that the uncertainty within the model parameter has great importance in influencing the uncertainty within the model.

### 3.3. Results

#### 3.3.1. Risk Characterization

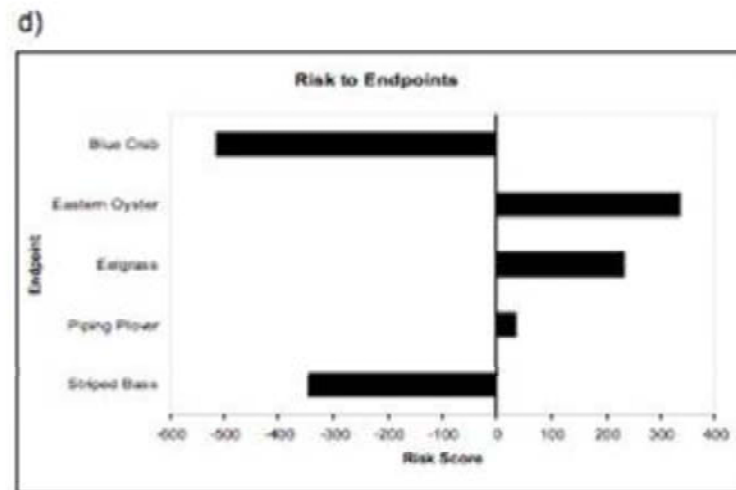
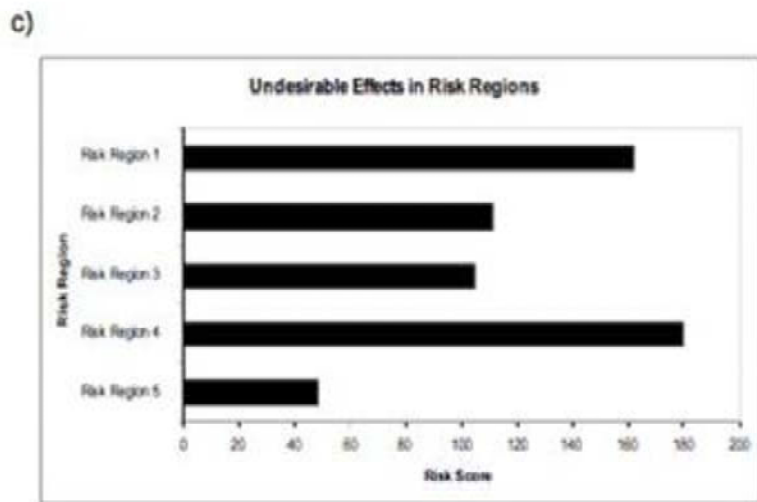
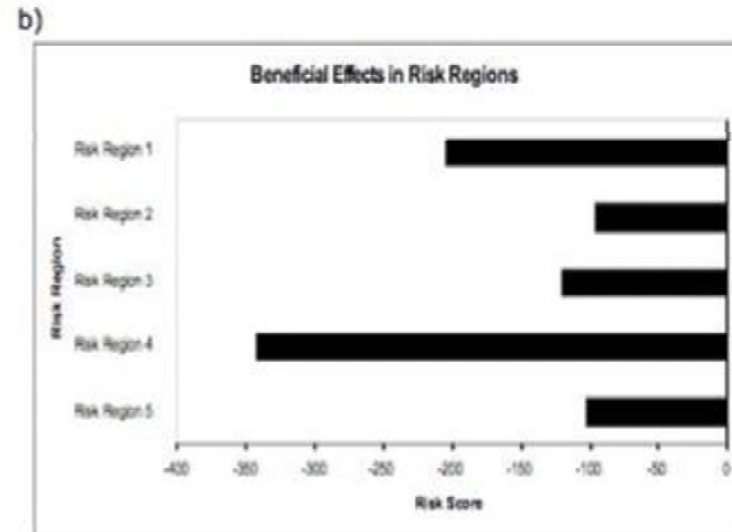
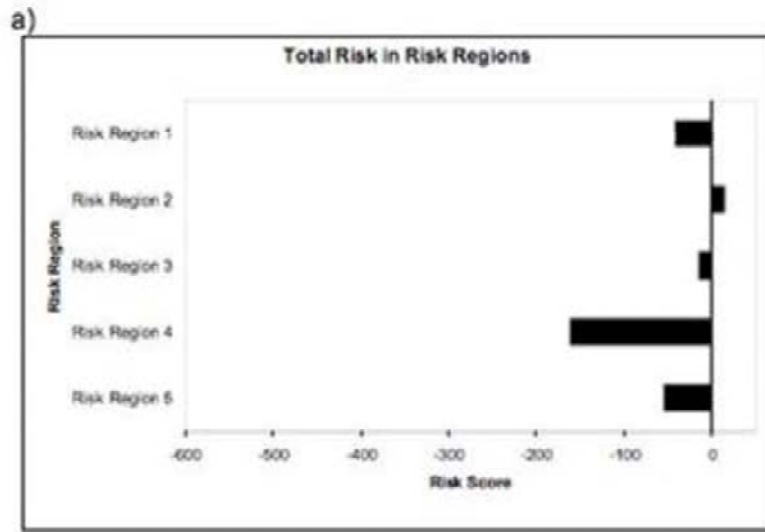
The total risks scores for scenario 1 and 2 were identical in risk region (RR) 1 and different in the other RR. RR2 had the highest overall risk in both scenarios. In scenario 1, RR1, RR4 and RR5 had beneficial effects and in scenario 2, RR3 also had beneficial effects (Table 3.5, Figure 3.6 and Figure 3.7). The overall risk is lower in scenario 2 when compared to scenario 1. The endpoints of blue crab and striped bass had negative risk scores in both scenarios, indicating the possibility of beneficial effects with Asian oyster introduction. The endpoints of Eastern oyster, eelgrass and piping plover had positive risk scores, indicating the possibility of undesirable effects caused by the introduction of the Asian oyster (Table 3.6). The risk scores of risk to endpoints in various habitats were different between the two scenarios in RR2, RR3, RR4 and RR5 (Table 3.7). The source of aquaculture contributed relatively more to the total risk than the source of larval current dispersal. The scores of risk in habitats are positive only in hard bottom and intertidal zone.

**Table 3.5.** Scenario 2 risk to each habitat type in various risk regions.

Risk Region		Risk Region 1	Risk Region 2	Risk Region 3	Risk Region 4	Risk Region 5	SUM
Habitat	Sand	-12	0	0	-18	-6	-36
	Sand and Shell	-18	0	0	-30	-12	-60
	Mud	-18	-6	-6	-18	0	-48
	Mud and Shell	-18	-6	-6	-60	-12	-102
	Hard Bottom	24	27	9	0	0	60
	Oyster Rock	0	0	-12	-108	-24	-144
	Intertidal zone ?	0	0	0	72	0	72
	<b>SUM</b>	-42	15	-15	-162	-54	-258

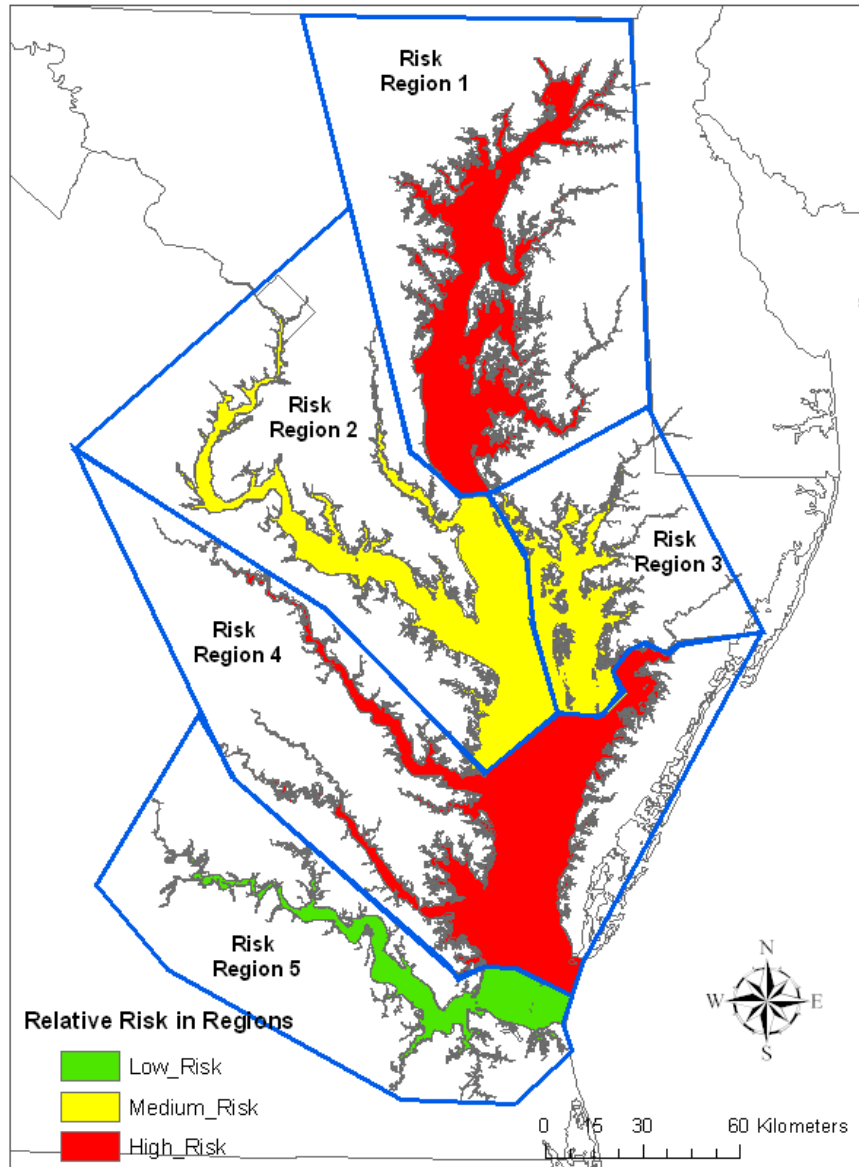
**Table 3.6.** Scenario 2 risks to each endpoint in various risk regions.

Endpoint		Blue Crab	Eastern Oyster	Striped Bass	Eelgrass	Piping Plover	SUM	SUM - only beneficial effects exhibited per region	SUM - only undesirable effects exhibited per region
<b>Risk Region</b>	Risk Region 1	-132	96	-72	66	0	-42	-204	162
	Risk Region 2	-60	63	-36	48	0	15	-96	111
	Risk Region 3	-72	57	-48	48	0	-15	-120	105
	Risk Region 4	-198	72	-144	72	36	-162	-342	180
	Risk Region 5	-54	48	-48	0	0	-54	-102	48
	<b>SUM</b>	-516	336	-348	234	36	-258		



**Figure 3.6.** (a) Total risks in various risk regions, (b) beneficial effects in various risk regions, (c) undesirable effects in various risk regions and (d) total risks to endpoints.

## Relative Risk in Regions



**Figure 3.7.** Total risk in each risk region for scenario 2.

**Table 3.7.** Scenario 2 risks to each endpoint in various habitats.

Endpoint		Blue Crab	Eastern Oyster	Striped Bass	Eelgrass	Piping Plover	SUM	SUM - only beneficial effects exhibited per habitat	SUM - only undesirable effects exhibited per habitat
Habitat	Sand	-102	0	0	66	0	-36	-102	66
	Sand and Shell	-96	108	-120	48	0	-60	-216	156
	Mud	-90	0	0	42	0	-48	-90	42
	Mud and Shell	-120	96	-120	42	0	-102	-240	138
	Hard Bottom	0	60	0	0	0	60	0	60
	Oyster Rock	-108	72	-108	0	0	-144	-216	72
	Intertidal zone ?	0	0	0	36	36	72	0	72
	<b>SUM</b>	-516	336	-348	234	36	-258		

### 3.3.2. Uncertainty Analysis

#### Scenario 1

**Sub-regions.** The risk scores for different sub-regions consisted of both negative and positive values. The uncertainty analysis indicated that for each risk score, there is a possibility that it is positive instead of negative and vice versa. RR1 had a risk score of -42, indicating that there would be possible beneficial effects but there is still a 30% probability that the risk score is actually a positive value. RR2 had a risk score of 30 but the distribution for the risk score indicated that it could be between -48 and 138. RR3 had a distribution with a range from -72 to 156, with a 50% probability that the risk score would be higher than the calculated risk score of 12. RR4 had a risk score of -84 and the range was between -234 and 126. RR5 had risk score distribution between -108 and 120. There is a 20% probability that the risk scores for RR4 and RR5 are actually positive.

**Sources.** The total risk scores in various sub-regions from aquaculture source were -114 and the range of the distribution was from -492 to 540. There is approximately a 40% probability that the actual risk score is positive.

**Habitats.** The total risk in sand habitat had a risk score of -36 and a distribution range from -78 to 120. The total risk in sand and shell habitat had a calculated risk score of -12 and there is a 50% probability that the actual risk score is higher than the calculated risk score. The risk in mud habitat had a range between -72 and 78 and there is a 20% probability that the actual risk score is positive. The total risk in mud and shell habitat was -48. The range is between -192 and 180 and the distribution is bi-modal. The risk in hard bottom habitat had a risk score of 48 and the risk in the intertidal zone is 36. There is a 0% probability that the actual risk score for hard bottom habitat and intertidal zone is negative. The risk to oyster rock habitat had a calculated risk score of -72 and there is a 10% probability that the actual risk score is positive.

**Endpoints.** The calculated risk to blue crab was -360 with a distribution range of -450 to 0 (Figure 3.8). The risk to Eastern oyster is 222 and there is a 0% probability that the risk would be a negative value. The risk score for striped bass was -210 with a range of -300 to 0. The calculated risk score for eelgrass was 234 and there is a 0% probability that the risk score is

negative. The calculated risk for piping plover is 0 and there is no uncertainty distribution for the piping plover.

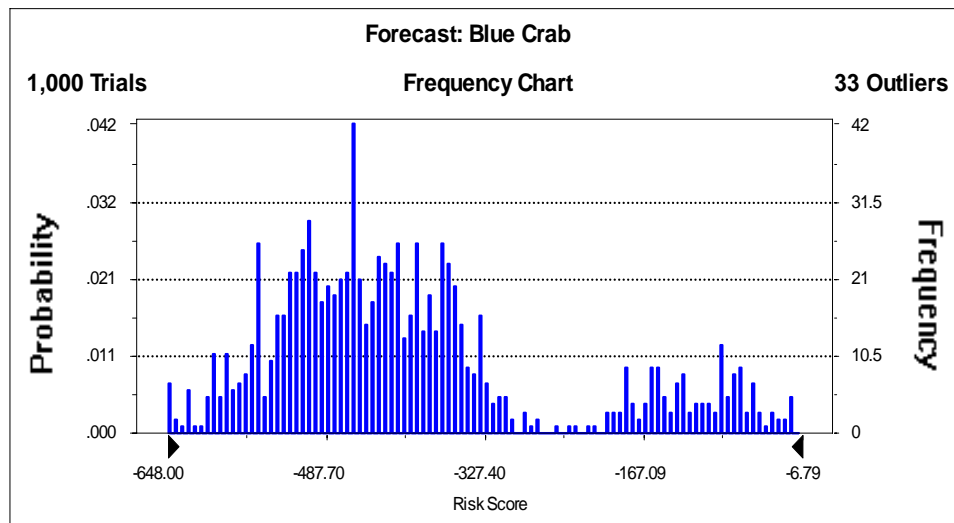
**Scenario 2**

Sub-regions. The ranges on the uncertainty distribution for all RR were wide and span across negative and positive risk values. In RR1, there is a 60% probability that the risk score will actually be higher than the predicted risk score of -42 from the RRM model. In RR2, there is about a 55% probability that the risk score will be higher and a 45% probability that the risk score will be lower than the predicted score of 15. In RR3, there is about a 70% probability that the risk score will be larger than the predicted score of -15 and about a 50% probability that the risk score will actually be positive. In RR4 and RR5, there is about a 15% probability that the risk score will actually be positive.

Sources. The aquaculture uncertainty distribution has multiple nodes and a wide range of 1,014. The predicted risk score was -114 and there is approximately a 65% probability that the risk score will be higher than the predicted score. The uncertainty distribution for larval current dispersal indicated that there is about a 15% probability that the risk score will be above 0.

Habitats. The uncertainty distributions for sand and mud were noticeably made up of multiple distributions. There is a 20% probability for sand and approximately a 15% probability for mud that the risk scores would be 0 or above. There is a 70% probability that the risk score for sand and shell habitat would be higher than the risk score of -60 as predicted in the RRM. There is a 0% probability that the risk score for hard bottom and the intertidal zone would be lower than 0.

Endpoints. The uncertainty distribution for blue crab indicated two slightly overlapping distributions. There is an 80% probability that the risk score would actually be higher than the risk score of -516 predicted from the RRM. The risk score, however, has a 0% probability of being above 0 (Figure 3.8). The uncertainty distribution for the Eastern Oyster and eelgrass indicated that there is a 0% probability that the actual risk scores would be below 0 whereas there is a 0% probability that the risk score for striped bass and piping plover would be above 0.



**Figure 3.8.** Uncertainty forecast for Blue Crab in Scenario 2. Risk Score = -516.

### 3.3.3. Sensitivity Analysis

#### Scenario 1

Sub-regions. The effects filter C that represents the effects from aquaculture source to blue crab (EffectsC.AQ.BC) is the model parameter that contributed the most to uncertainty in all RR. RR1 and RR4 were slightly more sensitive to EffectsC.AQ.BC relative to all other model parameters.

Sources. Aquaculture source was especially sensitive to EffectsC.AQ.BC, with a rank correlation of 0.45. It was also sensitive to the effects C filter for aquaculture to Eastern Oyster and to striped bass with rank correlations of 0.29 and 0.28.

Habitats. EffectsC.AQ.BC was the dominant parameter in sand and mud. There was not any parameter that contributed most to uncertainty in mud and shell, hard bottom and oyster rock. The effects filter from aquaculture to Eastern Oyster contributed the most to the hard bottom habitat.

Endpoints. There was a parameter that the endpoint of blue crab, Eastern Oyster and striped bass was especially sensitive to. For blue crab it was the effects filter C for aquaculture to blue crab, for Eastern Oyster it was the effects C filter for aquaculture to Eastern Oyster and for the striped bass it was the effects filter C for aquaculture to striped bass. For the endpoints of eelgrass and piping plover, there was not a parameter that the endpoints were especially sensitive to.

#### Scenario 2

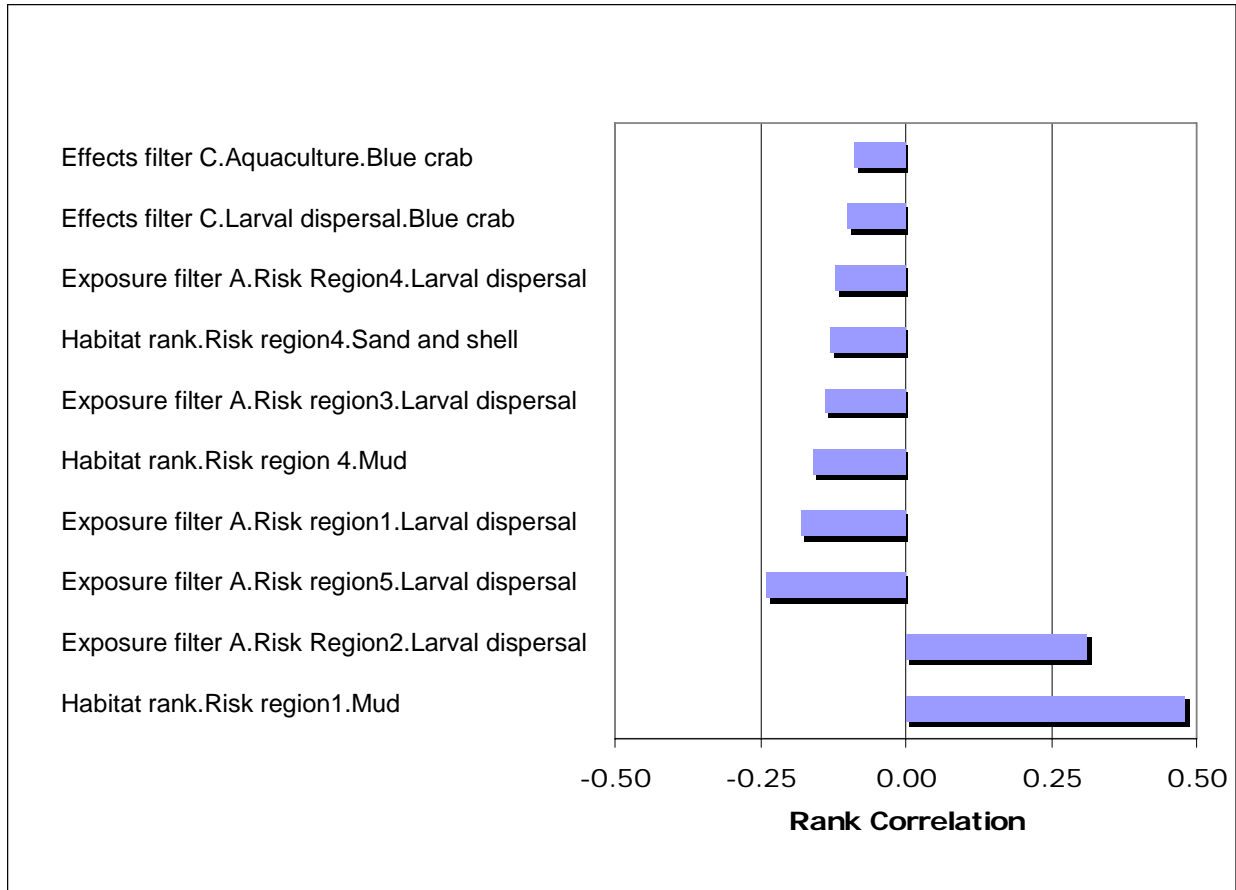
Sub-regions. The EffectsC.AQ.BC was the model parameter that contributed the most to uncertainty in all RR. RR1 and RR4 were more sensitive to EffectsC.AQ.BC than the other regions. RR1 and RR4 had EffectsC.AQ.BC rank correlation values of 0.40 and 0.43 while the other regions had a rank correlation value 0.29.

Sources. Aquaculture and larval current dispersal were most sensitive to the parameters of EffectsC.AQ.BC, effects filter C for aquaculture to Eastern Oyster and effects filter C for aquaculture to striped bass. Those parameters were especially dominant in aquaculture, where the other parameters all had rank correlation values lower than |0.09|.

Habitats. EffectsC.AQ.BC was the dominant parameter in sand and mud. There was not any parameter that contributed most to uncertainty in mud and shell, hard bottom and oyster rock. The habitat ranking for intertidal zone in RR4, with a rank correlation value of 0.53, was the parameter that contributed most to the uncertainty in intertidal zone risks.

Endpoints. Blue crab, Eastern oyster and striped bass had the effects C filter from aquaculture to endpoint as the parameter contributing most to uncertainty (Figure 3.9). Eelgrass was not particularly sensitive to any of the parameters. Piping plover was most sensitive to the effects C filter from larval current dispersal to piping plover.





**Figure 3.9.** Sensitivity analysis chart for blue crab in scenario 2.

### 3.4 Discussion

From the risk characterization, RR2 had the highest overall risk while RR1, RR4 and RR5 had beneficial effects in both scenarios. The uncertainty analysis must be considered along with the calculated risk scores. With the range of distributions for the various RR risk scores being very wide and with many of the distributions overlapping in large amounts, it would be inappropriate to conclude that any of the RR will especially have beneficial or undesirable effects. One way to reduce the uncertainty would be to gather more data regarding the effects of aquaculture to blue crab as indicated in the sensitivity analysis. That would reduce the uncertainty in all risk regions especially in RR1 and RR4 because those are the risk regions that are more sensitive to the effects filter from aquaculture to blue crab.

The risk from sources in scenario 2 indicated that both sources overall contributed beneficial effects. The uncertainty analysis indicated that both sources had high uncertainty and there is actually a high probability that the actual risk scores are much higher than the calculated risk scores. In aquaculture source, there is approximately a 30% probability that the actual risk score would be higher than 0, which would cause undesirable effects. The sensitivity analysis indicated that more data should be acquired for the effects of aquaculture to blue crab in order to reduce the uncertainty for the aquaculture source.

In both scenarios, the endpoints of blue crab and striped bass had negative risk scores, indicating possible beneficial effects. The uncertainty analysis indicated that the distributions

for the risk scores in both scenarios and endpoints had wide ranges. The distribution does not reach a positive risk score, however, indicating that the potential beneficial effects of Asian oyster might not be as large as predicted. There could be a possibility that there would be no effect from the Asian oyster to the blue crab or striped bass but there probably would not be a probability that there would be undesirable effects to blue crab or striped bass. The sensitivity analysis indicated focus should be placed on predicting the effects of Asian oyster to the endpoints in order to reduce uncertainty.

There were undesirable effects of Asian oyster to the Eastern oyster and eelgrass in both scenarios. The uncertainty analysis indicated a large range for the risk score distributions. There is a possibility that the actual risks to those endpoints are not as large as expected. However, there is no probability that there would be potential beneficial effects to those endpoints. The sensitivity analysis indicated that focus should be placed on determining the effects Asian oyster to Eastern oyster and eelgrass in order to reduce the uncertainty.

The exact risk scores for the different habitats between scenarios were not the same but still had similar qualities in the risk distributions. The risk scores were negative for all habitats except for hard bottom and intertidal zone. The risk in habitat was highest in hard bottom and intertidal zone in both scenarios. The negative risk scores for habitats indicate that there are potential beneficial effects to the endpoints in those habitats. However, the uncertainty analysis must also be considered. The ranges shown in the uncertainty analysis for all the negative risk scores habitats were wide, extending from negative to positive risk scores. There is a probability that there are actually undesirable effects to the endpoints in those habitats, which was consistent with the endpoints risk results. The hard bottom and intertidal zone had positive risk scores, indicating undesirable effects. The uncertainty distributions showed that there is a possibility that the risks to endpoints in those habitats might not be the exact calculated risk scores, but there is no probability that there would be beneficial effects. More information should be acquired for the effects from aquaculture to blue crab in order to reduce the uncertainty in sand and mud habitats. An overlay plot of the risks in habitats showed that the habitats of hard bottom and intertidal zones were noticeably separated from the other habitats. This is very important to consider because it shows how different components interact differently in various habitats and it is essential to consider the differences in habitats when conducting a region risk assessment.

The sensitivity analysis indicated that the effects filter component C was the most sensitive parameter in the model. The effects filter component C considers whether or not the Asian oyster have potential undesirable or beneficial effects to the endpoints. Focus should be placed on gathering more information about how the Asian oyster affects the various endpoints in order to reduce the uncertainty with various risk scores and to be able to indicate the differences between various risk distributions.

## **4. Ecological Risk Assessment for the Nun Moth in the Mid-Atlantic States**

### **4.1 Introduction to the Nun Moth (*Lymantria monacha*)**

The Nun moth, *Lymantria monacha*, is considered to be one of the most damaging pests in its native range, which extends from Portugal to Japan south of 60° latitude (Novak 1976, USDA 1991). In the southern part of its range, nun moth lives at higher elevations on conifers and broadleaf trees, while in the north it is found in lowlands, mainly on Norway spruce and Scotch pine (Novak 1976). During the largest outbreak in history, which occurred between 1978 and 1984, 3.7 million hectares of Scots pine and Norway spruce forests in Poland were defoliated (Glowacka 1998). The U.S. Department of Agriculture Forest Service (1991) predicts that introduction of nun moth would lead to high mortality in North American forests. Nun moth populations can grow on a wide range of host plants, and eggs are laid in several clusters that can be spread over a wide area (Keena 2003). A USDA Animal and Plant Inspection Service draft risk assessment (2000) predicts that the moth could spread up to 15 km / year in the worst case scenario.

Nun moth adults emerge and actively disperse between July and September, depending on weather conditions and elevation (Novak 1976 USDA 2000). Approximately 200 eggs are laid in clusters of 20-50 between or under the scales of bark (Novak 1976). Prolonged flight has been documented for adult female nun moth, permitting wide dispersal of egg clusters (USDA 1991). Larvae emerge the following year between April and May, and first and second instars can be transported several hundred meters by wind if adequate foliage is not available (USDA 1991 USDA 2000). Development through five to seven instars averages 63 days, with a minimum of 52 days in conditions with optimal food availability and quality (Novak 1976). Pupation begins in July; after emergence, adult females live approximately 10 days, while adult males live 20 days (USDA 2000). Adults do not feed (Keena 2003).

Flying nun moths are attracted to artificial lights, and have been found at ports in the Russian Far East (Wallner et al 1995, Keena 2003). Eggs normally laid in bark crevasses can also be laid in solid wood packing material (Keena 2003). Because eggs are laid in small clusters hidden in cracks and crevasses, and because the larvae do not emerge until up to nine months after laying, nun moth is highly suited to transport with logs, wood packing material, and with transport vessels. Diapausing egg masses can tolerate extreme variation in temperature and moisture that can occur during transport (USDA 1991). Larvae do not need to feed immediately after hatching, and they have the ability to wind disperse to find suitable habitat near ports in the United States (Keena 2003 USDA 2000).

Nun moth larvae feed preferentially on the young needles and male cones of conifers, but they are highly polyphagous and can also utilize the leaves of deciduous trees and shrubs (Keena 2003). In northern Europe, Norway spruce, Sitka spruce, Scots pine and lodgepole pine have been most seriously defoliated, although larvae have also been found on larch, white pine, silver fir, Douglas fir, beech, hornbeam, birch, Norway maple red oak, hazelnut, alder, and aspen (Jensen 1991, Novak 1976). Trees in the United States that could support an invading nun moth population include several species of spruce and oak, Scots pine with male cones, fir, and apple. Several other species found in North America, including western larch, were shown to provide forage for nun moth, but with high mortality and slow development in larvae, and lower fecundity rates in adults. These results are based upon laboratory findings where 34 North American tree species were tested individually for ability to support nun moth larvae. It is possible that a mixture of the 21 species that were determined to be moderately likely or unlikely to support a population would be more effective in combination (Keena 2003).

Phenological synchrony between nun moth larvae and host plants is important. First and second instars cannot feed on growth from previous years because the needles are too tough and contain secondary compounds that can be harmful (Keena 2003). If the larvae hatch before budburst, they may feed on male cones or deciduous foliage, or they may wind disperse (Keena, 2003 USDA 2000).

The preference for conifer trees makes nun moth a serious threat; conifers generate new growth slowly and they are more likely than hardwoods to die after defoliation (USDA 1991). Conifers are also more devastated than broadleaf trees because nun moth destroys more needles than it consumes. Keena (2003) observed poor survival in laboratory when grown on a limited supply of larch because the larvae begin feeding at the base of the needle so that most of it falls to the ground uneaten. In spruce, as little as 50% defoliation will cause mortality within a year (USDA 2000).

Outbreaks are centered in homogeneous stands of 40 to 60 year old Norway spruce or Scots pine, at approximately 500 meters above sea level or higher in warm years (Novak 1976). Outbreak centers often occur in stands with poor soils (Maksimov 1999, Jensen 1991). Maksimov (1999) found a correlation between the vertical gradients of nun moth survival and water deficiency in pine trees. He suggests that water stress caused by winter-spring droughts cause an imbalance between the size of the tree crown and the feeding roots that is the physiological basis for a focal state in host trees. When trees reach this state there is a sharp increase in nun moth survival leading to an outbreak which persists for approximately four years (Maksimov 1999). Although Maksimov suggests that water stress is a necessary condition for an outbreak, many studies have emphasized the importance of summers with above average temperatures the year of or the year preceding an outbreak (Jensen 1991, Klimetzek and Yue 1997, Liska and Strutka 1998).

In Europe, population levels are monitored with pheromone-baited sticky traps, although very intensive trapping is needed to detect near outbreak population levels (Jensen 1991). Alternative censusing methods include counting the number of female moths on several individual trees, and counting larval frass on sticky boards (Jensen 1991).

Outbreaks have been controlled using lindane, endosulphane, fenitrothion, and trichlorfon, although the use of these chemicals is declining due to negative environmental impacts (Jensen 1991). A comparison of treatment methods in Poland showed that mortality to beneficial or indifferent species was 10 times higher in stands treated with pyrethroids than those treated with stomach insecticides or left untreated (Glowacka 1998). More recently, populations have been controlled with diflubensuron, an insect growth regulator hormone analogue with high levels of mortality, although there is a time lag between application and the next molt (Jensen 1991). The nuclear polyhedrosis virus (NPV), naturally occurring in nun moth, has been applied for control purposes. The virus affected most moths as pupae, allowing the larvae to complete development and cause severe defoliation. Populations treated with NPV crash the following year due to the lack of emerging adults; however, NPV has been implicated in parallel population crashes in untreated areas as well (Jensen 1991). *Bacillus thuringiensis* has been applied to low density populations, although sometimes with limited or no success (Jensen 1991, Liska and Strutka 1998). The effectiveness of this method depends on the weather following application, and Jensen (1991) suggests that a cold spell resulted in 0% larval mortality after *B.t.* treatment in Denmark. Mating disruption has been used at an experimental scale. Tree trunks were sprayed with the sex pheromone disparlure; in treated areas, few or no males were captured in pheromone traps, and the number of egg masses per female were greatly reduced (Jensen 1991).

Integrated control of nun moth populations requires monitoring, and treatment according to the current population size and developmental stage (Jensen 1991). Treatment according to population size can be hazardous: between 1993 and 1994 the infested area in an outbreak in Poland increased sevenfold despite control measures that resulted in approximately 87% mortality. The following year, all areas with nun moth were treated and the population declined (Glowacka 1998).

## **4.2 Risk Assessment Methods**

### **4.2.1 Problem Formulation**

#### **Identification of Potential Sources**

Nun moth eggs can be laid in cracks and crevasse in wood packing material, logs, and transport vessels from Europe and Asia. In the current risk assessment, we considered three potential sources of nun moth larvae to the study area: international airports and maritime ports, and natural dispersal. The relative risk model calculates risk from all sources at once, so in order to include the risk due to spread of nun moth we need to either use two scenarios, or calculate the risk assuming that populations have become established. Solid wood packing materials could become sources for nun moth populations near airports and maritime ports (USDA 2000). Once populations became established, natural dispersal would become an additional source of nun moth larvae.

#### **Risk Regions**

The study area includes five states: Pennsylvania, Virginia, West Virginia, Maryland, and Delaware and encompasses several distinct landscape types. We defined seven risk regions using US EPA level III ecoregion data (Figure 4.1). Ecoregions have been defined for North America based upon geological, physiographical, vegetative, climactic, soil, land use, wildlife, and hydrological information. Level III ecoregions is the most detailed level available for the entire United States, including 84 different regions (USEPA 1997).

Risk Region 1 is a combination of the glaciated Erie Drift Plains, characterized by low rounded hills, moraines, kettles, and areas of wetlands, and the Western Allegheny Plateau, characterized by more rugged, forested hills. This region has mixed oak and mesophytic forests. In the northern portion of this region the weather is influenced by Lake Erie, which increases both the growing season and the winter snowfall.

Risk Region 2 consists of the North Central Appalachian ecoregion, with plateaus, high hills, and low mountains, with more forested areas than adjacent ecoregions. Land use in this region is primarily forestry and recreation.

Risk Region 3 is the southern section of the Northern Appalachian Plateau and Uplands ecoregion, a transitional area between the urban and agricultural lowlands to the north and west, and the more mountainous, forested regions to the south and east. Much of the land is farmed and in pasture, but large areas remain forested in oak and northern hardwoods.

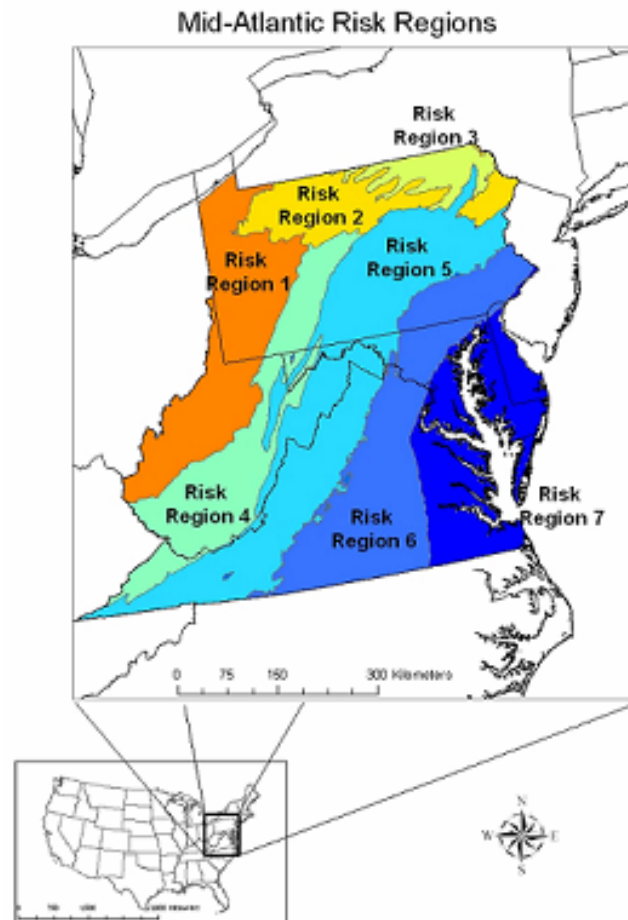
Risk Region 4, the Central Appalachian ecoregion, has rugged high hills and mountains. Appalachian oak and northern hardwood forests are the main land cover. This region has a cool climate and infertile soils. Streams have been polluted by coal mining.

Risk Region 5 consists of the Blue Ridge and the northern tip of the Ridge and Valley ecoregions. This region is the most diverse, with oak, northern hardwood, southeastern

spruce-fir, hemlock, and oak-pine forests. The terrain is mountainous and rugged, with many forested slopes.

Risk Region 6 is a combination of the Northern Piedmont and the northern third of the Piedmont ecoregions. This area has been largely cultivated, although in the south it has successional pine and hardwood forests.

Risk Region 7 is a combination of the northern sections of the mid-Atlantic and Southeastern coastal plain ecoregions. Land cover consists of cropland, pasture, woodland, and forest. In the east, native vegetation is longleaf pine, oak and hickory, and southern mixed forest, while the west has loblolly and shortleaf pine, oak, and gum forest.



**Figure 4.1.** Map of the study area, which includes Pennsylvania, Virginia, West Virginia, Maryland, and Delaware. The study area was divided by habitat type into seven risk regions using U.S. EPA level III ecoregions.

### Habitats

The diverse land cover found in the Mid-Atlantic States region was divided into five habitat types. These were conifer forest, mixed forest, deciduous forest, woody wetlands, and rivers and streams. Geographical information system data was used to define habitat boundaries

(USGS 2003 USEPA). In the conceptual model development, the impact on conifer, mixed, and deciduous forests are considered only as direct effects. Nun moth may have both direct, through removal of canopy cover and changes in litter composition, and indirect effects, though changes in water quality, on the woody wetlands. In the rivers and streams habitat, only indirect effects are considered.

### **Assessment Endpoints**

The assessment endpoints used in this study were threatened or endangered species selected by Daniel Kluza at USEPA. These endpoints were 1) Swamp pink (*Helonias bullata*), 2) Dwarf wedgemussel (*Alasmidonta heterodon*), 3) Southern water shrew (*Sorex palustris punctulatus*), 4) Shenandoah salamander (*Plethodon shenandoah*), 5) Cheat Mountain salamander (*Plethodon nettingi*), 6) Northern flying squirrel subspecies (*Glaucomys sabrinus coloratus* G. s. *fuscus*), 7) Red-cockaded woodpecker (*Picoides borealis*), and 8) Duskytail darter (*Etheostoma percnurum*).

The swamp pink is listed as a federally threatened perennial plant species. It is found along streams in Virginia, Maryland, and Delaware, in meadow, cedar swamp, and forested wetlands. There is a strong correlation between the presence of swamp pink and several conifer species. This plant has limited seed dispersal and viability, and spreads mainly through clonal rhizomal growth. These shade tolerant plants are inferior competitors in direct sunlight. The swamp pink requires a near constant water level, and the main threats to this plant include habitat loss due to wetland drainage and water quality degradation through sedimentation (US FWS 1991b).

The federally endangered dwarf wedgemussel live in areas of streams and rivers that have a muddy sand, sand, or sand and gravel substrate, low to moderate current and low turbidity. The historical range of the dwarf wedgemussel is Virginia, Maryland, and Pennsylvania; currently no populations exist in Pennsylvania. This mussel is sensitive to light penetration and dissolved oxygen. Threats to dwarf wedgemussel persistence include channelization, removal of shoreline vegetation, and polluted runoff from agriculture, industry, and homes (US FWS 1993b).

The southern water shrew is considered a vulnerable species in its range in Maryland, Pennsylvania, Virginia, and West Virginia. This shrew is found near streams in areas with low vegetation, rocks, and logs, which provide shelter, protection, and a high humidity microclimate. The Southern water shrew is threatened by warming and siltation of streams, habitat loss, and toxicity due to pesticide control of forest insect pests (NatureServe 2005).

The main threat to the federally endangered Shenandoah salamander is interspecific competition with the red-backed salamander. The Shenandoah salamander is found in only three metapopulations within the Shenandoah National Park in Virginia (Griffis and Jaeger 1998). More drought tolerant than other lungless salamanders, the Shenandoah Salamander inhabits dry, rocky talus slopes above 800 meters. Forest cover is required to maintain adequate moisture levels on the ground, and previous defoliation by the gypsy moth (*Lymantria dispar*) and the hemlock wooly adelgids (*Adelges tsugae*) have reduced the suitable habitat area. Defoliation may result in drying of the forest floor, as well as soil chemistry changes due to high composition of needles in the floor litter. Acid precipitation is an additional threat to the Shenandoah salamander. However, lowering soil pH, which may result as a combination of acid rain and defoliation, dramatically reduced red-backed salamander survival while effects on the Shenandoah are not known (US FWS 1993). If the Shenandoah is less sensitive, its competitive ability may be enhanced.

The Cheat Mountain salamander, endemic to West Virginia, is federally listed as threatened. These salamanders live in spruce and mixed forests above 2980 feet in cool, humid microclimates with moist soil and litter cover. Main food items include mites, springtails, beetles, flies, and ants. The home range of this salamander is 13 to 25 m<sup>2</sup>. In competition with other salamanders, the cheat mountain salamander will aggressively defend its territory, but it will usually not be successful. This salamander is probably limited to higher elevations due to a competitive disadvantage lower in its potential range. The main threats include habitat reduction through removal of forest canopy, fires, and alteration in the forests floor by road and trail development. Removal of canopy is the biggest factor affecting survival because increased sunlight alters the microhabitat. Roads and trails isolate diminishing populations (US FWS 1991a).

The northern flying squirrel subspecies, *G. s. coloratus* and *G. s. fuscus*, both federally endangered, are found in western Virginia and eastern West Virginia in boreal habitat, especially spruce-fir and northern hardwoods (US FWS 1990). Sites occupied by the squirrels have relatively more conifers, with little or no northern red oak. Understory components of forest habitat are not significant in determining habitat usage (Ford et al 2004). Diet consists of tree buds, lichens, epigeous and hypogeous fungi, and beechnuts; at times, the population may be entirely supported by fungi (US FWS 1990). Mycorrhizal fungi spore dispersal, facilitated by squirrels, may contribute to tree health in high altitude forests (Mitchell 2001). The individual home range requirement is 5-7 hectares, and nesting habitat appears to be a limiting factor, with nests often containing several adults. These squirrels exist in fragmented relic populations, and are at risk due to further habitat degradation that may result from insect pests. Risk also is possible due to the chemicals used to control insect pests, such as lindane which is used to control the balsam woody adelgid (*Adelges piceae*) (US FWS 1990).

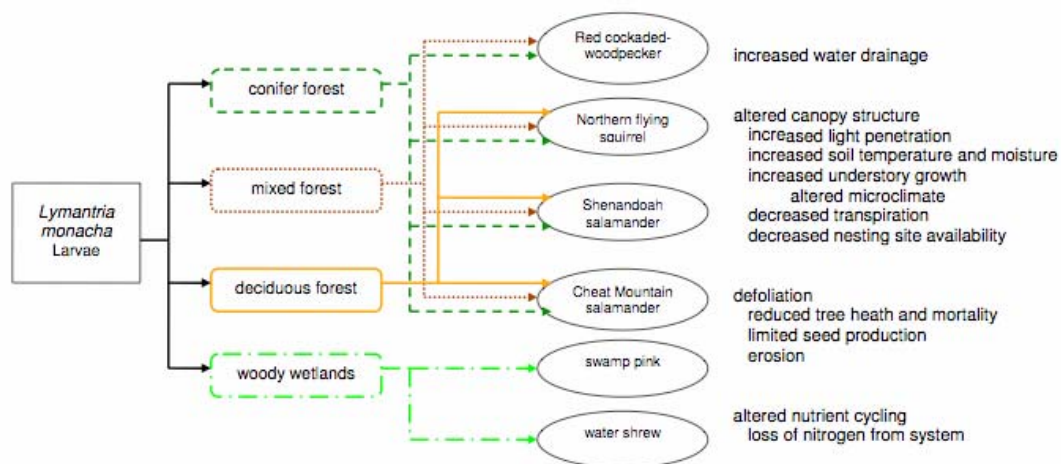
The range of the federally endangered red-cockaded woodpecker is determined by the distribution of southern pines, with current populations in the study area being fragmented and isolated. These birds breed in family units, called groups, consisting of a monogamous pair and the male offspring of the previous year. The required breeding clusters are open stands of pines or savannahs with large pines at least 80 years old, with little to no hardwood understory. Birds forage on insects, including ants, beetles, wood boring insects, and caterpillars, in pine or pine-hardwood forests at least 30 years old. Their diet also includes seasonal wild fruit. Home range requirements vary greatly, between 40 and 160 hectares per group. The main threat is due to loss of older pine forests, and growth of the hardwood midstory due to fire suppression (US FWS 2003).

The federally endangered duskytail darter is very selective in microhabitat choice, utilizing only pools with moderate to fast current where the substrate contains a mixture of pea gravel, cobble, and boulders (US FWS 1993). These fish are only found in large creeks and rivers in areas with little to no siltation (Powers and Mayden 2003). Because of specific habitat requirements, duskytails don't disperse, and existing populations are fragmented and isolated (US FWS 1993). Water quality impairment, including siltation, is the major cause of decline (Powers and Mayden 2003; US FWS 1993).

#### **4.2.2 Conceptual Model Development**

We built a conceptual model to illustrate the pathways from the stressor to the endpoints (Figure 4.2 a and 4.2b). In this model, nun moth can directly impact the woody wetland, conifer, deciduous, and mixed forest habitats, while it indirectly impacts the woody wetland and rivers and streams habitats.





**Figure 4.2.** Conceptual model for Nun Moth Risk Assessment

In the calculation of risk, the exposure assessment is incorporated in the connection between the stressor and the habitats: if there is a high probability of introduction and reproduction there will be a connection between the moth and the habitat. Each source is considered to have an equal chance of exposing any habitat within a particular risk region, so the source is not included in the model. In the case of the rivers and streams and woody wetland habitats, the stressors are indirect effects due to nun moth exposure in any other habitats. However, because the model only looks at one moment in time, potential for exposure to indirect effects is calculated at the same time as the potential for exposure to nun moth.

The effects assessment is diagrammed as the connection between the habitats and the endpoints, with possible effects listed after the endpoints. General effects following defoliation include tree mortality, increased light penetration, soil temperature and moisture, and water drainage, and decreased transpiration (Lovett et al 2002 Russell et al 2004). The gypsy moth, *Lymantria dispar*, has altered canopy structure, increased understory growth, and affected avian nest site availability (Crooks 2002). In individual trees, changes in nutrient allocation under

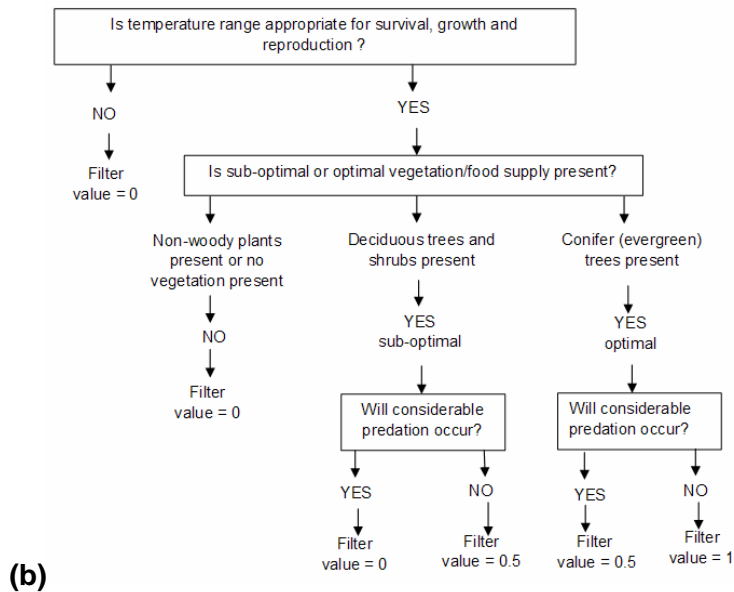
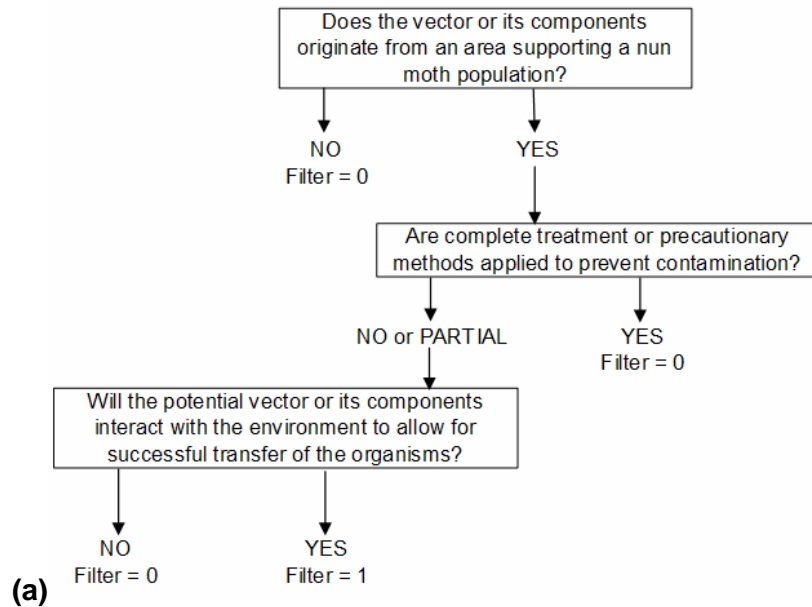
heavy defoliation may lead to limited mast production for many years in surviving trees (Lovett et al 2002). In addition, the addition of green leaf litter and moth frass to the forest floor can alter the nitrogen cycle, possibly leading to nitrogen loss from the system, and acidification or eutrophication in streams (Lovett et al 2002). In a study of a poplar plantation, Russel et al (2004) found that nitrogen redistributed by defoliation remained in the system, but noted that insect outbreaks in Appalachian Mountain forests were linked to elevated nutrient loads in rivers and nitrogen loss.

#### **4.2.3 Analysis**

A risk score combines the probability of establishment and effects to assessment endpoints with the consequences of establishment; in the relative risk model, the probabilities of establishment and effects are represented by the filters, while the extent of the stressor at establishment and the consequences of effects are represented by the rankings. Risk scores were calculated by following the flow through the conceptual model for each risk region.

For the exposure assessment, the potential sources of nun moth larvae were ranked independently within each region. Source ranks were based on presence – absence criteria (Table 4.1 a and b); while natural dispersal from established populations was considered a source in all of the risk regions. Each of the five habitats was ranked according to the percentage of that habitat type in the risk region out of the total amount of that habitat type in the study area (Table 4.2 a and b). The probability of habitat-exposure was calculated by multiplying two filters: one representing the likelihood of introduction from the source and the other representing the potential for survival in the habitat. The values for the exposure filters were assigned using decision trees (Figure 4.3 a and 4.3b). The exposure assessment results in a risk score for introduction of nun moth larvae to each habitat from each source, within each risk region.

Effects risk scores were assigned using the results of the exposure assessment, with the addition of three filters. Each non zero value for risk of exposure was multiplied by three exposure filters: one to indicate if the endpoint lives in and utilizes the habitat, one to indicate that there will be a temporal and spatial overlap between the endpoint and the stressor, and one to indicate whether it will be possible for the stressor to affect the endpoint. The first filter is based upon presence – absence of the endpoint within each habitat in each risk region (Table 4.4). The second filter was assigned a value of one for all endpoints because it was assumed that defoliation could either directly or indirectly alter any habitat, and that these effects would



**Figure 4.3.** (a) Decision tree for exposure filter A, representing the likelihood of introduction from an airport, maritime port, or natural dispersal. (b) Decision tree for exposure filter B, representing the potential for survival and reproduction. This decision tree assumes that if habitat is optimal, predators will not be able to control a nun moth population. In sub-optimal habitat, predation will slow the growth of the nun moth population. Rivers and streams are subject to indirect effects, which will vary according to the terrestrial habitat type that the stream flows through. Because the highest percentage of habitat cover is deciduous forest, the streams are expected to flow through this habitat most often, and all streams are given an exposure filter B value of 0.5. Optimal foraging habitats are the conifer habitat and the mixed forest habitats (Novak 1976). In this risk assessment, we assumed predation will be minimal during an outbreak because there was not specific data on potential predators.

**Table 4.1.** (a) Ranking criteria for the three potential sources of nun moth larvae is based on the presence / absence of the stressor in each region. (b) Results of source ranking for nun moth larvae. Data for the location of airports and maritime ports in the study area came from USGS (2001). All values have low uncertainty except the rank for maritime ports in risk region six: we were uncertain whether the ports at the top of region 7 could be direct sources of nun moth larvae to region 6.

(a)

Sources	Ranking Criteria	Range	Rank
<b>International Airports and Bases*</b>	Presence/absence of international airports and bases	Absent	0
		Present	6
<b>Maritime Ports</b>	Presence/absence of maritime ports	Absent	0
		Present	6
<b>Natural Dispersal</b>	Will the moth be able to disperse to the area through Either wind drift or adult migration?	No	0
		Yes	6

(b)

	RR1	RR2	RR3	RR4	RR5	RR6	RR7	RR6	RR7
airports (number)	6	0	0	0	6	6	6	6	6
maritime ports	6	0	0	0	0	6	6	6	6
Natural dispersal	6	6	6	6	6	6	6	6	6

be lasting. The third filter also received a value of one for each endpoint because all endpoints are threatened or endangered species, and further loss of habitat due to defoliation would likely effect population levels.

**Table 4.2.** (a) Ranking criteria for habitats. Ranks are based on percentage of total habitat type represented in each risk region. Rank values were assigned to percentages based upon natural breaks in the percent values, identified using GIS software. (b) Percentages and ranking results for each habitat type in each risk region. Habitat ranks are based on 10-year old GIS data, so we assumed medium uncertainty associated with those values.

(a)

Habitats	Ranking Criteria	Range	Ranks
<b>Conifer forest</b>	% of total conifer forest	0	0
		4.350 - 5.985%	2
		5.986 - 10.530%	4
		10.531 - 26.027%	6
<b>Mixed forest</b>	% of total mixed forest	0	0
		0.001 - 2.883%	2
		2.884 - 15.510%	4
		15.511 - 24.005%	6
<b>Deciduous forest</b>	% of total deciduous forest	0	0
		3.006 - 10.805%	2
		10.806 - 19.634%	4
		19.635 - 28.988%	6
<b>Woody wetlands</b>	% of total woody wetlands	0	0
		0.931 - 4.503%	2
		4.504 - 14.550%	4
		14.551 - 70.483%	6
<b>Rivers &amp; streams</b>	% of total rivers and streams	0	0
		3.33 - 9.97%	2
		9.98 - 19.22%	4
		19.23 - 29.68%	6

(b)

	RR1	RR2	RR3	RR4	RR5	RR6	RR7
Conifer Forest	5.99 (4)	9.13 (4)	4.35 (2)	10.53 (4)	22.1 (6)	26.03 (6)	21.87 (6)
Mixed Forest	11.79 (4)	10.12 (4)	2.88 (2)	13.02 (4)	24.01 (6)	22.68 (6)	15.51 (4)
Deciduous Forest	17.85 (4)	10.8 (2)	3.01 (2)	19.63 (4)	28.99 (6)	14.24 (4)	5.47 (2)
Woody Wetlands	4.01 (2)	4.5 (2)	0.93 (2)	1.78 (2)	3.74 (2)	14.55 (4)	70.48 (6)
Rivers & Streams	17.32 (4)	6.78 (2)	3.33 (2)	13.7 (4)	29.68 (6)	19.22 (4)	9.97 (2)

**Table 4.3.** (a) Exposure filter A values, representing the probability that the source (airport, maritime port, or natural dispersal) will be a transport vector for the stressor (nun moth larvae) in each region. These values were assigned using the decision tree in figure 4.3a. (b) Exposure filter B values, representing the probability of nun moth survival in each habitat within each risk region. Filter values were assigned using the decision tree in figure 3b.

(a)

	RR1	RR2	RR3	RR4	RR5	RR6	RR7
airports	1	0	0	0	1	1	1
maritime ports	1	0	0	0	1	1	1
Natural Dispersal	1	1	1	1	1	1	1

(b)

	RR1	RR2	RR3	RR4	RR5	RR6	RR7
Conifer Forest	1	1	1	1	1	1	1
Mixed Forest	1	1	1	1	1	1	1
Deciduous Forest	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Woody Wetlands	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Rivers & Streams	0.5	0.5	0.5	0.5	0.5	0.5	0.5

**Table 4.4** Exposure filter A values, based upon the presence – absence of the endpoint within the risk region (nature serve counties listings, GAP Analysis Project GIS dataset). In the relative risk model calculations, filter values are further divided into endpoint presence – absence in each habitat within each risk region. Endpoint usage of habitat is based on nature serve and USFS recover plan descriptions. Filter values are based on known or likely presence in state counties, and it is assumed that if a species is present in at least one county, then it could possibly be present throughout the risk region.

	RR1	RR2	RR3	RR4	RR5	RR6	RR7
Northern Flying Squirrel	0	0	0	1	1	0	0
Red-cockaded Woodpecker	0	0	0	1	0	1	1
Water shrew	1	0	0	1	1	0	0
Swamp pink	0	0	0	0	1	1	1
Dusky-tailed Darter	0	0	0	1	1	0	0
Dwarf Wedgemussel	0	1*	0	0	1*	1	1
Cheat Mountain Salamander	0	0	0	1	1	0	0
Shenandoah Salamander	0	0	0	0	1	1	0

\* Recently discovered in the upper Delaware River. Exact locations unknown.

### Uncertainty and sensitivity analysis

We used Monte Carlo analysis (Crystal Ball® 2000) to assess the variability in potential risk score values. Each rank and filter value was assigned an uncertainty level of high, medium, or low, and a distribution of possible values. For example, the conifer forest rank value in risk region one was 4, but it was assigned with medium uncertainty. In the Monte Carlo risk score calculation, 80 out of 100 times that rank will take a value of 4; for the other 20 iterations, this

rank will take a value of 2 for 10 iterations and a value of 6 for 10. The distributions for each input variable are given in the appendix A Part 3 for the Nun moth.

**Rank value uncertainty:** All source ranks have low uncertainty except the rank for maritime ports in region six, which was assigned medium uncertainty. We were uncertain whether the ports at the top of region seven could be a direct source of nun moth larvae to region six. Habitat rank values were based on 10-year old GIS data, so we assumed medium uncertainty associated with those values.

**Filter value uncertainty:** We did not use specific import data for each port, so all exposure filter A values for air and maritime ports have high uncertainty. The regions without a port source have a filter value of zero with low uncertainty. In addition, due to the large range of potential dispersal rates given by the USDA APHIS risk assessment (2001), we assigned natural dispersal exposure filter A values were assigned with high uncertainty in each region. All exposure filter B values were assigned with medium uncertainty. Effects filter A, indicating habitat usage by endpoints, was assigned a value of zero with low if the endpoint does not utilize the habitat. However, if the endpoint does utilize the habitat type, it is assigned a filter value of one or zero with medium uncertainty. If the species is present in at least one county in a risk region, then it could be present throughout the risk region, with medium uncertainty. If the endpoint is not found in any county in a risk region, then it will probably not be present in any habitat in that risk region and it is assigned a filter value of zero, with medium uncertainty. Effects filter B, representing that there will be temporal and spatial overlap between the stressor and endpoints, was assigned a value of one with low uncertainty for all endpoints. Effects filter C was assigned a value of one, with low uncertainty for all endpoints except the duskytail darter and the dwarf wedgemussel, which had medium uncertainty, indicating that all endpoints can potentially be impacted by defoliation.

In order to determine which input variables had the greatest impact on the risk score, we used a rank correlation sensitivity analysis (Crystal Ball® 2000).

## **4.3 RESULTS**

### **4.3.1 Risk Characterization**

The deterministic results of the risk calculation are shown in Table 4.5. The risk to each of the seven risk regions is shown in Figures 4.4 and 4.5a. Natural breaks in the risk score, found using GIS, were used to define low (0-54), medium (55-252) and high (252-744) risk. The low risk regions were one, two, and three, with risk scores of 54, 6, and 0. The regions with medium risk were four and seven, with risk scores of 198 and 252. The high risk regions were five and six with risk scores of 744 and 576.

**Table 4.5.** Deterministic risk scores for each risk region, habitat, endpoint, and source. Endpoint abbreviations: DTD – duskytail darter (*Etheostoma percnurum*) DW – dwarf wedgemussel (*Alasmidonta heterodon*) SP – swamp pink (*Helonias bullata*) WS – southern water shrew (*Sorex palustris punctulatus*) NFS – Northern flying squirrel subspecies (*Glaucomys sabrinus coloratus* G. s. *fuscus*) CMS – Cheat Mountain salamander (*Plethodon nettingi*) RCW – Red-cockaded woodpecker (*Picoides borealis*) SS – Shenandoah salamander (*Plethodon shenandoah*)

Risk Region		Endpoints	
Risk Region 7	252	SS	504
Risk Region 6	576	RCW	444
Risk Region 5	744	CMS	294
Risk Region 4	198	NFS	240
Risk Region 3	0	WS	120
Risk Region 2	6	SP	102
Risk Region 1	54	DW	90
		DTD	36
Habitats		Sources	
Woody Wetlands	138	Natural Dispersal	870
Rivers & Streams	336	Maritime Ports	294
Mixed Forest	576	Airports	666
Deciduous Forest	168		
Conifer Forest	612		

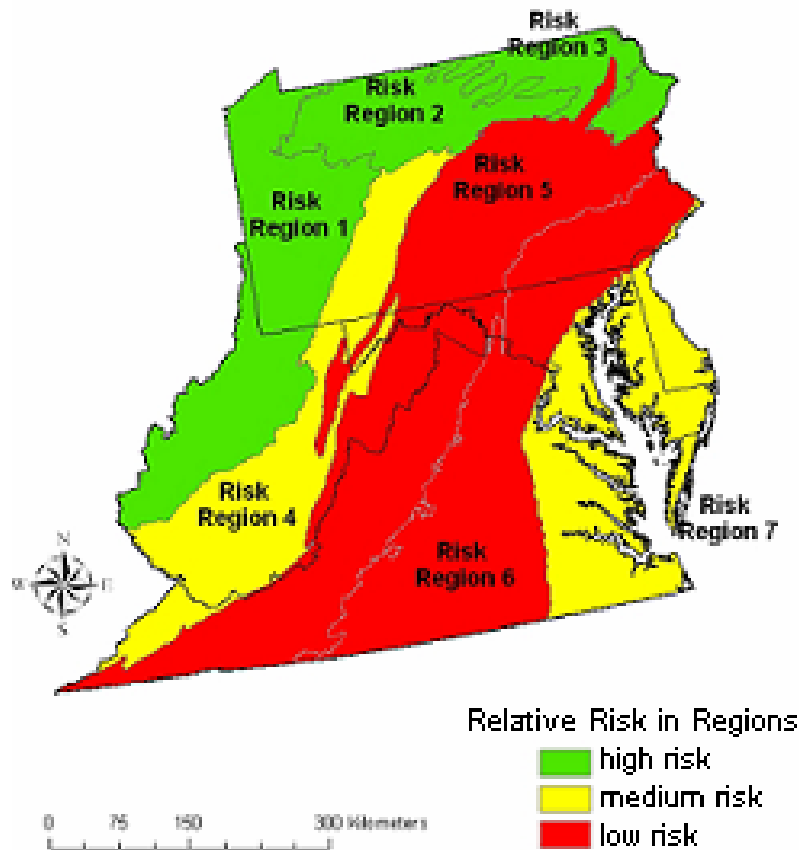
The breaks in the risk scores to endpoints were less clear (Figure 4.5b). The Shenandoah salamander and the red cockaded woodpecker had the highest risk scores; the cheat mountain salamander and northern flying squirrel had medium risk scores; and the water shrew, swamp pink, dwarf wedgemussel, and duskytail darter had low risk scores. Table 2 in Appendix A Part 3 shows the break down of risk to each endpoint in each habitat for the entire study area (a) and for risk region five (b).

The habitats most at risk were the conifer and mixed forest types (Figure 4.5c). The rivers and streams habitat had the next highest risk due to indirect effects, while the deciduous forest and the woody wetlands had the lowest risk scores.

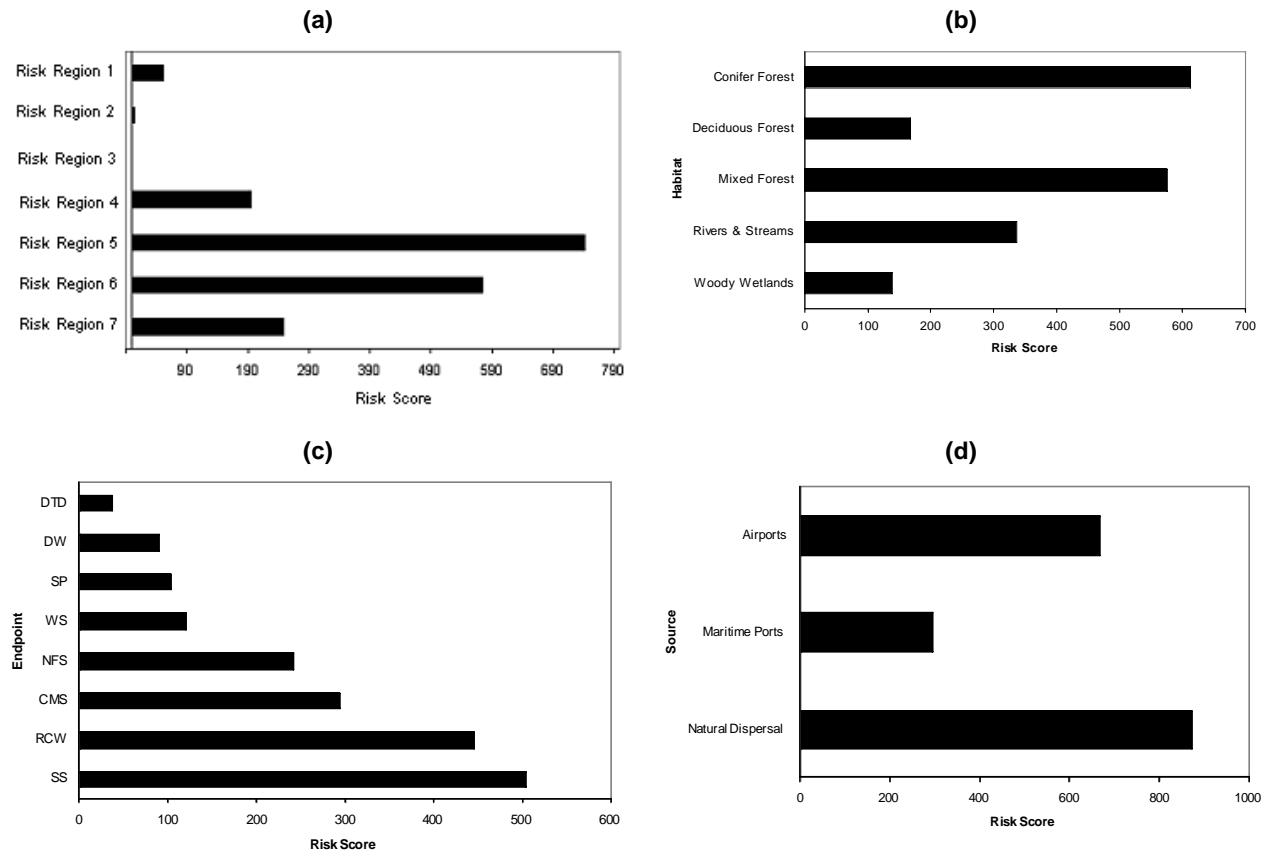
The highest risk of nun moth larvae introduction came from natural dispersal (Figure 4.5d). Airports and maritime ports came in second and third, respectively.



## Relative Risk in Regions



**Figure 4.4.** Map of the risk regions showing the location of the high, medium, and low risk regions. Natural breaks in the risk score, found using GIS, were used to define low (0-54), medium (55-252) and high (252-744) risk. The low risk regions were one, two, and three, with risk scores of 54, 6, and 0. The regions with medium risk were four and seven, with risk scores of 198 and 252. The high risk regions were five and six with risk scores of 744 and 576.

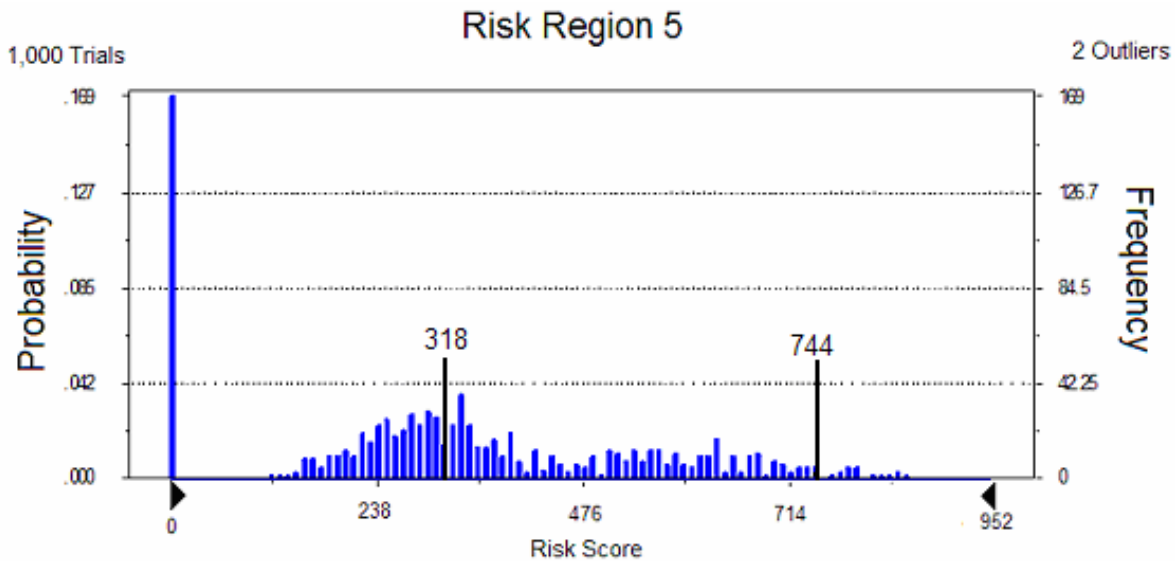


**Figure 4.5.** Deterministic risk scores for each of the seven risk regions (a), five habitats (b), eight endpoints (c), and three sources (d). Endpoint abbreviations: DTD – duskytail darter (*Etheostoma percnurum*) DW – dwarf wedgemussel (*Alasmidonta heterodon*) SP – swamp pink (*Helonias bullata*) WS – southern water shrew (*Sorex palustris punctulatus*) NFS – Northern flying squirrel subspecies (*Glaucomys sabrinus coloratus* G. s. *fuscus*) CMS – Cheat Mountain salamander (*Plethodon nettingi*) RCW – Red-cockaded woodpecker (*Picoides borealis*) SS – Shenandoah salamander (*Plethodon shenandoah*)

### 4.3.2 Uncertainty Analysis

Monte Carlo analysis produced probability distributions for each deterministic risk score calculated. Risk regions 1, 2 and 3 had deterministic risk scores that were less than or equal to the median risk score from their respective probability distributions. All other deterministic risk scores calculated were in the 90<sup>th</sup> percentile of the Monte Carlo-generated distribution, so there was at most a 10% chance for the risk score to occur. The probability distribution for risk to region 5 is shown in Figure 4.6 as an example.

All risk scores had a positive probability of being zero, and most had a positive probability of being close to zero, so distributions with high deterministic risk scores covered a very large range of values. The risk range of possible risk scores calculated for risk region 5, for example, was from a score of 0 to a score of 952.



**Figure 4.6.** Probability distribution for risk region 5 showing the range of possible risk scores, from 0 to 952. The median risk score, 318, and the deterministic risk score, 744, are shown on the graph.

### 4.3.3 Sensitivity Analysis

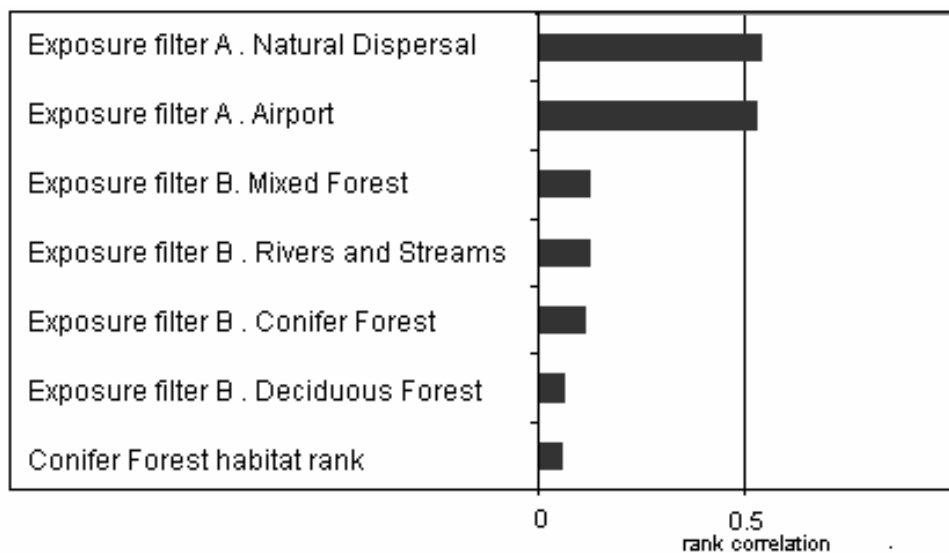
Most risk scores for region, source, habitat, and endpoint were sensitive to whether or not nun moth would be released.

The scores for risk regions were most sensitive to the first exposure filter, which indicates whether or not one or more of the sources would release the stressor within the region. The highest rank correlation values were for 0.74, 0.77, and 0.54 for natural dispersal as a source in risk regions 2, 4, and 5. Risk region scores were also sensitive to whether or not the endpoints exist in habitat in the risk region, and whether or not nun moth would survive in the region. The sensitivity chart for the risk score to risk region five is shown as an example in Figure 4.7.

Habitat risk scores were not sensitive to any specific input parameters, with no rank correlation value greater than 0.42. Habitat risk scores were most correlated with whether or not nun moth would be released by airports or natural dispersal, and whether or not nun moth would survive in the habitat.

Endpoint risk scores were also insensitive to input parameters, with the highest rank correlation being 0.47 for uncertainty associated with the potential for nun moth to affect the dwarf wedgemussel. The risk score for the swamp pink was most correlated with the likelihood that nun moth will survive in the woody wetlands of risk region 7. All the other endpoints were most sensitive to the likelihood that a source would release the stressor.

Source risk scores were sensitive to exposure filter A, which indicates the likelihood that the source will release the stressor. The risk score for airports was sensitive to whether or not airports would release nun moth in risk region 5, with a rank correlation value of 0.68. The score for maritime ports was sensitive to the uncertainty in whether or not ports in region 7 could be direct sources to region 6. The score for natural dispersal was sensitive to whether or not nun moth could disperse to region 5.



**Figure 4.7.** Sensitivity chart for risk region five, where sensitivity is determined by rank correlation. The risk score to risk region five is most sensitive to the probability that natural dispersal will be a source of nun moth larvae in this risk region.

#### 4.4 Discussion

The risk regions most at risk in this study were risk regions five and six. These regions, which contain the Blue Ridge, ridge and valley, and piedmont ecoregions (USEPA 1997), have the largest amount of high risk conifer and mixed forest habitat. More detail could be incorporated into the risk assessment to include the fact that risk region five contains a varied topography, which may affect nun moth rate of spread. Conifer and mixed forests were the habitats most at risk in these regions. The assessment endpoints most at risk were the Cheat Mountain and Shenandoah salamanders in region five and the Shenandoah salamander in risk region six; these endpoints utilize the largest number of habitats. Risk region three, which had the lowest risk of exposure, has a zero risk because none of the assessment endpoints are found there. Risk region one, which had the third highest risk of exposure, is also a low risk region because it has only a moderate amount of habitat and just one endpoint is present.

Risk scores to regions in this model are determined by the amount and type of habitat, and the number of endpoints present, so that highest risk region is the most-to-lose region.

Although the risk scores to regions follow the ranking patterns for habitats and endpoints and not the pattern of exposure risk, all risk regions are most sensitive to the likelihood that nun moth will be released by sources in that region, indicating that to narrow the range of possible risk scores, more information about potential sources is necessary. Jensen et al (1991) state that forest type and quality, as well as soil type could be used to estimate susceptibility to an outbreak; incorporating this level of detail into the present risk assessment would lower the uncertainty associated with the survival. More closely examining the materials coming through airports and maritime ports as well as wood treatment procedures in use in the study area would lower the uncertainty associated with source rankings and exposure filter A values. However, the exposure filter value for natural dispersal had the largest influence on most of the risk scores, so further study should attempt to more accurately portray the potential for nun moth dispersal in North American habitats.

The main outputs from the relative risk model are the patterns of risk scores and sensitivity, and the range of possible risk scores, as an indication of uncertainty. In the Mid-Atlantic States study the, all but one of the rankings remains the same whether the deterministic or median risk score is used. Even though there is high uncertainty in most of the risk scores calculated, the pattern of risk is not affected. In the present study, the pattern of risk followed the pattern of possible effects – the values of the habitat ranks and effects filter A. An invasive species risk assessment is different than a chemical risk assessment because the effects to endpoints are unknown and often indirect, and quantitatively portraying the risk due to invasive species is very difficult (USDA 1991). On the other hand, data about forest susceptibility and habitat suitability for many invading organisms are available (ie Keena 2003, Maksimov 1999). While describing the potential effects due to exposure to the stressor is one of the most important features of risk assessment, in an invasive species risk assessment more attention should be given to the predicting the extent of the exposure. Russell et al (2003) found that percent defoliation was not linearly related to nun moth density, emphasizing the importance of understanding exposure before attempting to predict effects. The flexible nature of the relative risk model will allow future assessors to implement a more exposure based risk assessment.

The present study is an ecological risk assessment: the endpoints are all organisms with little or no economic value. Given that monetary interests play a large roll in management decisions, it is perhaps a shortcoming of the study that economic endpoints were not considered. Our model also does not consider the efficacy of eradication programs, which might in themselves have negative ecological and economic impacts, with variable success rates.

Assessing the risks associated with Invasive species is an important task as global trade continues to increase. Decisions involving trade regulations are very time sensitive and as more global free trade markets develop these decisions also become more closely scrutinized. A standardized and efficient process for assessing the risk of invasive species is needed to aid decisions concerning importation of goods which may harbor potentially invasive species. The relative risk model has been applied to several invasive species on the east and west coasts. Variations include the Asian oyster, which might be intentionally introduced in the Chesapeake Bay, sargassum, which is already present at Cherry Point, Washington, and the green crab, which has been found along the Washington state coastline (Asian oyster in press Colnar and Landis in press). The study of the mid Atlantic states is a fourth demonstration that the relative risk model works for invasive species.

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## Appendix A Uncertainty Input Distributions Part 1. European Green Crab

### Uncertainty analysis input distributions for European green crab risk assessment

**Table 1.** Uncertainty analysis input distributions for the habitat ranks, originally designed by Hart Hayes and Landis (2004).

Assigned Value	Uncertainty	Probability (%) for Each Possible Value			
		0	2	4	6
0	Medium	80	10	10	0
	High	60	20	20	0
2	Medium	0	80	10	10
	High	0	60	20	20
4	Medium	0	10	80	10
	High	0	20	60	20
6	Medium	0	10	10	80
	High	0	20	20	60

**Table 2.** Uncertainty analysis input distributions for the exposure filter component B values (Is the habitat suitable enough to allow for survival and growth of the green crab?).

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		0	0.5	1
0	Medium	80	10	10
	High	60	20	20
0.5	Medium	10	80	10
	High	20	60	20
1	Medium	10	10	80
	High	20	20	60

**Table 3.** Uncertainty analysis input distributions for the effects filter component A values (Does the endpoint occur in and utilize the habitat?), originally designed by Hart Hayes and Landis (2004).

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		0	0.5	1
0	Medium	80	10	10
	High	60	20	20
0.5	Medium	10	80	10
	High	20	60	20
1	Medium	10	10	80
	High	20	20	60

**Table 4.** Uncertainty analysis input distributions for the effects filter component B values (Is there seasonal overlap in habitat usage between the green crab and the endpoint?).

Assigned Value	Uncertainty	Probability (%) for Each Possible Value	
		0	1
0	Medium	80	20
	High	60	40
1	Medium	20	80
	High	40	60

**Table 5.** Uncertainty analysis input distributions for the effects filter component C values (Are effects, either beneficial or undesirable, to the endpoint possible from interaction with the green crab?).

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		0	1	-1
0	Medium	80	20	0
	High	60	40	0
1	Medium	20	80	0
	High	40	60	0
-1	Medium	20	0	80
	High	40	0	60

## Appendix A Part 2. Asian Oyster

### Uncertainty analysis input distributions for Asian Oyster risk assessment

**Table 1.** Uncertainty distributions for habitat ranks

Assigned Value	Uncertainty	Probability (%) for Each Possible Value			
		0	2	4	6
0	Medium	80	10	10	0
	High	60	20	20	0
2	Medium	0	80	10	10
	High	0	60	20	20
4	Medium	0	10	80	10
	High	0	20	60	20
6	Medium	0	10	10	80
	High	0	20	20	60

**Table 2.** Uncertainty distributions for source ranks

Assigned Rank	Uncertainty	Probability (%) for Each Possible Rank	
		0	6
0	Medium	80	20
	High	60	40
6	Medium	20	80
	High	40	60

**Table 3.** Uncertainty distributions for exposure filter component A

Assigned Value	Uncertainty	Probability (%) for Each Possible Value	
		0	1
0	Medium	80	20
	High	60	40
1	Medium	20	80
	High	40	60

**Table 4.** Uncertainty distributions for exposure filter component B

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		0	0.5	1
0	Medium	80	10	10
	High	60	20	20
0.5	Medium	10	80	10
	High	20	60	20
1	Medium	10	10	80
	High	20	20	60



**Table 5.** Uncertainty distributions for effects filter component A

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		<b>0</b>	<b>0.5</b>	<b>1</b>
0	Medium	80	10	10
	High	60	20	20
0.5	Medium	10	80	10
	High	20	60	20
1	Medium	10	10	80
	High	20	20	60

**Table 6.** Uncertainty distributions for effects filter component B

Assigned Value	Uncertainty	Probability (%) for Each Possible Value	
		<b>0</b>	<b>1</b>
0	Medium	80	20
	High	60	40
1	Medium	20	80
	High	40	60

**Table 7.** Uncertainty distributions for effects filter component C

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		<b>0</b>	<b>1</b>	<b>-1</b>
0*	Medium	80	20	0
	High	60	40	0
0**	Medium	80	0	20
	High	60	0	40
1	Medium	20	80	0
	High	40	60	0
-1	Medium	20	0	80
	High	40	0	60

\*if undesirable effects are possible

\*\*if beneficial effects are possible

## Appendix A Part 3. Nun Moth

### Uncertainty analysis input distributions for nun moth risk assessment

**Table 1.** For habitat ranks

Assigned Value	Uncertainty	Probability (%) for Each Possible Value			
		0	2	4	6
0	Medium	80	10	10	0
	High	60	20	20	0
2	Medium	10	80	10	0
	High	20	60	20	0
4	Medium	0	10	80	10
	High	0	20	60	20
6	Medium	0	10	10	80
	High	0	20	20	60

**Table 2.** For Source ranks

Assigned Rank	Uncertainty	Probability (%) for Each Possible Rank	
		0	6
0	Medium	80	20
	High	60	40
6	Medium	20	80
	High	40	60

**Table 3.** For exposure filter component A

Assigned Value	Uncertainty	Probability (%) for Each Possible Value	
		0	1
0	Medium	80	20
	High	60	40
1	Medium	20	80
	High	40	60

**Table 4.** For exposure filter component B

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		0	0.5	1
0	Medium	80	10	10
	High	60	20	20
0.5	Medium	10	80	10
	High	20	60	20
1	Medium	10	10	80
	High	20	20	60

**Table 5.** For effects filter component A

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		<b>0</b>	<b>0.5</b>	<b>1</b>
0	Medium	80	10	10
	High	60	20	20
0.5	Medium	0	80	20
	High	0	60	40
1	Medium	0	20	80
	High	0	40	60

**Table 6.** For effects filter component B

Assigned Value	Uncertainty	Probability (%) for Each Possible Value	
		<b>0</b>	<b>1</b>
0	Medium	80	20
	High	60	40
1	Medium	20	80
	High	40	60

**Table 7.** For effects filter component C

Assigned Value	Uncertainty	Probability (%) for Each Possible Value		
		<b>0</b>	<b>1</b>	<b>-1</b>
0*	Medium	80	20	0
	High	60	40	0
0**	Medium	80	0	20
	High	60	0	40
1	Medium	20	80	0
	High	40	60	0
-1	Medium	20	0	80
	High	40	0	60

\*if undesirable effects are possible

\*\*if beneficial effects are possible

