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Application of QBRAT for a Risk Assessment of the Invasive Tunicate *Didemnum* sp. in British Columbia

Application de l'OQRB à l'évaluation du risque de l'espèce de tunicier envahissante *Didemnum* en Colombie-Britannique

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ABSTRACT

The invasive tunicate *Didemnum* sp. has been reported in Canadian waters and has the potential to negatively impact native flora and fauna. In British Columbia, limited surveys have found *Didemnum* sp. in the Strait of Georgia and at sites along the west coast of Vancouver Island. This tunicate possesses several traits that likely enhance its invasion success including its ability to grow and sexually reproduce quickly, smother competing or co-occurring organisms, and its tolerance of a wide variety of environmental conditions. *Didemnum* sp. has the potential to negatively impact water quality, macrophytes, invertebrates, fishes, and aquaculture facilities. This report summarizes available information on *Didemnum* sp. including a taxonomic description, biological characteristics, its distribution (native and non-native ranges), and potential impacts of its invasion.

We also present a biological synopsis and a risk assessment for the British Columbia coastline. The risk assessment is based on estimates of propagule pressure, which combine the spatial distribution of aquaculture and boating facilities. Additionally, it incorporates measures of environmental suitability from a environmental niche model. Impact is measured based on the sensitive habitat and shellfish aquaculture facilities. Overall, the Strait of Georgia (the current location of most *Didemnum* sp sightings) has the highest total biological risk as calculated by QBRAT. The next highest risks are predicted for Johnstone Straight and three different areas along the west coast of Vancouver Island.

A sensitivity analysis of different methods of determining impact, discovered a very strong effect on the risk ranking for the different study areas. This highlights the great importance of developing clearer guidelines on quantifying impact for comparable results.

RÉSUMÉ

L’espèce de tunicier envahissante *Didemnum* a été signalée dans les eaux canadiennes; elle aurait le potentiel de nuire à la faune et à la flore indigènes. En Colombie-Britannique, des relevés limités ont permis de déceler la présence de l’espèce *Didemnum* dans le détroit de Georgia et à certains endroits le long de la côte ouest de l’île de Vancouver. Cette espèce de tunicier possède plusieurs caractéristiques qui augmentent ses chances de se propager, notamment de croître et de se reproduire rapidement, étouffant les organismes concurrents ou cooccurents, et une tolérance à un large éventail de conditions du milieu. L’espèce *Didemnum* peut avoir des effets néfastes sur la qualité de l’eau, les macrophytes, les invertébrés, les poissons et les installations aquacoles. Le présent rapport résume l’information disponible sur l’espèce *Didemnum*, donnant sa description taxonomique, ses caractéristiques biologiques, sa répartition (aires indigènes et non indigènes) et les effets possibles de sa présence envahissante.

Les auteurs présentent aussi un résumé biologique et une évaluation des risque pour la zone littorale de la Colombie-Britannique. L’évaluation des risques est basée sur une estimation de la pression propagulaire, combinant la répartition spatiale de l’aquaculture et des installations de plaisance. De plus, elle inclut des résultats sur la mesure dans laquelle l’environnement est approprié, obtenus à l’aide d’un modèle de la niche environnementale. Les effets sont mesurés en fonction de la sensibilité de l’habitat et des installations de conchyliculture. Dans l’ensemble, le détroit de Georgia (emplacement où la plupart des membres de l’espèce *Didemnum* ont été signalés) comporte le plus haut risque biologique total, calculé à l’aide de l’OQRB. Au deuxième rang viennent les risques prévus pour le détroit de Johnstone et trois zones différentes le long de la côte ouest de l’île de Vancouver.

Une analyse de sensibilité de différentes méthodes de calcul des effets a permis de définir un effet très marqué sur le classement des différentes zones d’étude en fonction du risque. Ce résultat fait ressortir la grande importance d’établir des lignes directrices plus claires pour la quantification des effets afin d’obtenir des résultats comparables.

INTRODUCTION

Aquatic invasive species (AIS) pose an enormous risk to native biodiversity and can compromise ecosystem function (e.g., Sala *et al.* 2000). For marine ecosystems invasive tunicate species are a global concern and Canadian waters are no exception. Some invasive tunicates are solitary (e.g., *Styela clava*, *Molgula manhattensis*, *Ciona intestinalis*) while others are colonial (e.g., *Botryllus schlosseri*, *Bottryloides violaceus*, *Didemnum* sp.). Most of these invasive tunicate species have been identified in both Atlantic and Pacific Canadian waters but with different distributions and impacts. *Didemnum* sp. has been identified as a species of concern in the United States, New Zealand and Canada but appears to have been present in British Columbia, Canada for a number of years. The smothering capabilities of *Didemnum* sp. and other invasive tunicate species have potential negative impacts on both native species and human-mediated activities (e.g., commercial shipping and aquaculture) (Lambert and Lambert 1998, Dunstan and Johnson 2004, Carman 2005). Once AIS become established there are often significant economic impacts, as is the case for biofouling organisms like tunicates that are responsible for fouling aquaculture gear and product (Carver et al 2003).

In order to characterize the potential risk posed by a new invader to Canadian waters or the spread of an existing invader to other locations, a formal risk assessment is conducted. This risk assessment is adapted from the process outlined in the Canadian National Code on Introductions and Transfers of Aquatic Organisms and contains two-parts. Part I evaluates the probability of establishment and consequence of establishment of an aquatic organism while Part II evaluates the probability of establishment and consequence of establishment of a pathogen, parasite or fellow traveler of the aquatic organism. Since one of the goals of this risk assessment is to evaluate a new semi-quantitative tool for conducting risk assessments, we focus only on Part I here but the tool would be equally applicable to Part II. Within each Part of the national framework two component ratings are determined (the probability and consequences of establishment) and each are assigned ratings of high (risk is likely, or very likely, to occur), medium (there is probability of negative impact), or low (risk is considered to be insignificant). In addition, a level of certainty is also assigned as a gradient from very certain (scientific basis), reasonably certain, reasonably uncertain, to very uncertain ("best guess"). This differs from the tool being evaluated which allows the investigator to assign an estimated probability value (0 to 1) and an impact score (0 to X) rather than simply the risk score. As with the traditional framework, an overall risk potential or score is determined (separately for Parts I and II).

Here, we present the results of the risk assessment for the spread of the colonial tunicate Didemnum sp across the British Columbia coastline. The risk assessment is based on predictions of environmental suitability, obtained from environmental niche modelling, and estimates of human transport vectors associated with recreational boating and aquaculture.

BIOLOGICAL SYNOPSIS

Background

A biological synopsis for *Didemnum* sp. recently was completed by Daniel and Therriault (2006) so the synopsis presented here should be considered a summary. It is important to note that the Family Didemnidae remains taxonomically complex and controversial and no general consensus exists on what species of *Didemnum* is invading coastal waters worldwide (e.g., Valentine 2003, Cohen 2005, Daniel and Therriault 2006). For this report we adopt the view that *Didemnum* sp. is native to Japan, with one known type-location at Ise Bay, near Nagoya (Gretchen Lambert, pers. comm.).

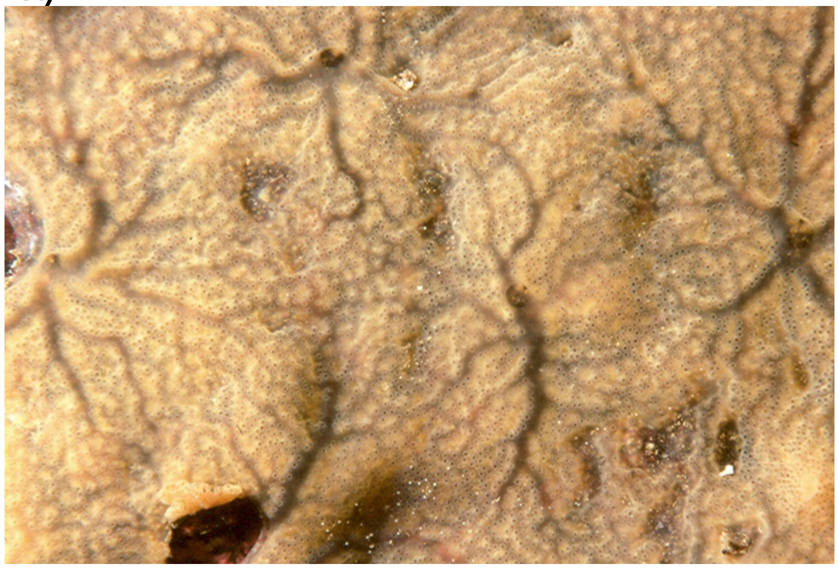
Didemnum sp. (Figure 1) is characterized by many small zooids (1-2 mm in length) embedded in a sheet-like, gelatinous matrix called a tunic (Kott 1989, Monniot *et al.* 1991, Kott 2001, Tyree 2001, Pederson 2004, Cohen 2005, Lambert 2005a, Lambert and Lambert 2005). Embedded within the tunic are white, calcareous stellate spicules that give the colony a white dot appearance (Kott 2004, Cohen 2005, Lambert and Lambert 2005, Geerlofs and Gordon 2005a). These colonies form thin encrusting sheets or irregularly lobed encrusting mounds depending on colony location and age (Valentine 2003, Cohen 2005, Geerlofs and Gordon 2005a).

Biology

Colonial ascidians typically live from 1 to 3 years (Berrill 1950, O'Clair and O'Clair 1998) but age determination is difficult due to the periodic regeneration and reduction colonies undergo (Millar 1971, Tyree 2001). Colonial tunicates can grow and spread both by sexual reproduction via tadpole larvae and asexual reproduction via propagative budding, making them good invaders. Also, unlike other ascidians, didemnids can bud while the gonads are maturing (Monniot *et al.* 1991) and can undergo precocious budding where blastozooids are produced in the larvae within the tunic (Kott 2001) further enhancing their invasion potential. Factors such as season, temperature, and habitat type each affect the extent of *Didemnum* sp. colony growth. *Didemnum* sp. has been reported to grow rapidly during summer months (Valentine *et al*. 2005a) but slowly during winter months as the colony often reduces to a dormant bud (Millar 1971, Nakauchi and Kawamura 1990, Monniot *et al.* 1991). Habitat type also affects *Didemnum* sp. colony growth with faster growth reported from open coastal habitats due to an apparent competitive advantage over other co-occurring species that can become overgrown by the colony (Osman and Whitlatch 2005).

Didemnum sp. is hermaphroditic and ovoviviparous producing tadpole larvae via sexual reproduction (Van Name 1945, Monniot *et al.* 1991, Tyree 2001). Ascidian gonad development and spawning are controlled by several factors with light and temperature, which change seasonally, apparently most important (Millar 1971, Berrill 1975, Svane and Young 1989, Forward *et al.* 2000, Bates 2005, Lambert 2005a). For ascidians in temperate waters spawning generally occurs during summer months (Cohen 2005, Lambert 2005b).

A)



B)

Figure 1 *Didemnum* sp. from Agamemnom Channel, British Columbia. A) Closeup of *Didemnum* sp. colony surface, and B) *Didemnum* sp. colony. Both photos by Bernard Hanby. (http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/htm/brit4a.htm)

Released larvae only swim in the water column for minutes to hours before settling (Monniot *et al.* 1991, Cohen 2005, Lambert and Lambert 2005, Valentine *et al.* 2005b) thereby lowering their ability to spread quickly naturally. Larval dispersal away from the parental colony is affected by currents, wave action, wind exposure, angle of the sun, and water temperature (Hurlbut 1992, Stoner 1992, Forward *et al.* 2000) but the short time ascidian larvae spend in the water column may limit the impact such factors exert on larval dispersal thereby limiting spread.

As a sessile marine invertebrate, movement of the adult colony of *Didemnum* sp. is limited, although the colony can expand through larval dispersal, fragmentation or moving along with its habitat (e.g., rafting). Modes of transport, and possible introduction, include hull fouling of ships and boats, fouling of fishing gear, fishing trawls, dredges, colony fragments in ballast water, movement of oyster and other shellfish stock or gear, and natural process like currents (Millar 1971, Monniot *et al.* 1991, Lambert 2002, Cohen 2005, Lambert 2005b, Valentine *et al.* 2005b).

Didemnum sp. tolerates a wide range of environmental conditions including temperature, salinity, and water quality (Millar 1971, Lambert and Lambert 1998, Lambert 2002, Lambert 2005b). *Didemnum* sp. colonies are found in water temperatures ranging from -2^{o}C to 24^{o}C with 4^{o}C possibly representing a critical temperature where cooler temperatures limit spread or growth (Cohen 2005, Lambert 2005b, Valentine *et al*. 2005a). Ascidians tend to hibernate, die off, or go dormant when temperatures are not favourable and resume growth and reproduction when favourable conditions return (Millar 1971, Nakauchi and Kawamura 1990, Monniot *et al.* 1991). Colonies on Georges Bank are found in 4^{o}C to 15^{o}C water and apparently do not die off during the winter months, possibly because temperatures don't become cold enough to initiate the dormancy period (Valentine *et al.* 2005a).

Didemnum sp., like most ascidians, is rarely found in salinities less than 25‰ but it can tolerate wide fluctuations in salinity (Millar 1971, Vázquez and Young 2000, Lambert 2005b). However, at salinities lower than 20‰ ascidians tend to close their siphons which can lead to zooid death (Stoner 1992, Tyree 2001). Ascidian larvae also are affected by salinity as larvae remain below a halocline (Vázquez and Young 1996). In lower salinity waters, colonial tunicate larvae do not metamorphose or metamorphose slowly, resulting in reduced survival (Vázquez and Young 2000).

Generally, ascidians can not withstand extended periods of air exposure (desiccation) but can tolerate a wide range of water quality. For example, when exposed to air for more than 3 hours per day for 28 consecutive days *Didemnum* sp. died (Valentine *et al.* 2005a). Many ascidians are tolerant of high water pollution, high particulate and dissolved organic matter, and may even consume organic pollutants like heavy metals and hydrocarbons (Monniot *et al.* 1991, Tyree 2001, Lambert 2005b).

Didemnum sp. has the ability to colonize a variety of natural and artificial hard structures but not muddy or sandy substrates from the intertidal zone to about 65m as on Georges Bank (Coutts 2002, Valentine 2003, Cohen 2005, Valentine *et al.* 2005b). *Didemnum* sp. grow on natural substrates such as rock outcrops, gravel seabeds, pebbles, cobble, boulders, tunicates, sponges, macroalgae, hydroids, anemones, bryozoans, polychaetes, scallops, mussels, oysters, limpets, barnacles, other ascidians, shell, and hard clay with stones (Berrill 1950 and 1955, Monniot *et al.* 1991, Lambert

2002, Valentine 2003, Cohen 2005, Gittenberger 2005, Valentine *et al.* 2005a and 2005b). *Didemnum* sp. can colonize artificial structures including docks, floats, wood and metal pilings, moorings, rope, steel chain, automobile tires, plastic, ship hulls, buoys, jetties, concrete, iron, and wood (Millar 1971, Monniot *et al.* 1991, Tyree 2001, Lambert 2002, Valentine 2003, Cohen 2005, Geerlofs and Gordon 2005a and 2005b).

Prior to settlement, the tadpole larvae do not actively feed but following settlement they become sessile marine filter feeders (Berrill 1955, Jeffery 1997, Monniot *et al.* 1991, Tyree 2001, Lambert and Lambert 2005a). Ascidian diets consist primarily of phytoplankton, suspended particulate matter, diatoms, invertebrate larvae, and suspended bacteria (Millar 1971, Monniot *et al.* 1991, Bak *et al.* 1998, O'Clair and O'Clair 1998, Tyree 2001). *Didemnum* sp. has few known predators possibly due to the low nutritive value of the tunic (Tarjuelo *et al.* 2002, Lambert 2005b). The common periwinkle, *Littorina littorea*, preyed upon a weakened *Didemnum* sp. colony (Valentine 2003, Carman 2005, Valentine *et al.* 2005a) and chitons, sea urchins and sea stars are reported predators of *D. vexillum* (Valentine 2003). Also, little is known about the diseases and parasites specific to *Didemnum* sp. In general, ascidians have communal, parasitic and symbiotic organisms living in their tunics, branchial chambers, gut tracts, or atrial chambers (Millar 1971, Monniot 1990, Monniot *et al.* 1991, O'Clair and O'Clair 1998, Kott 2001, Tyree 2001). Carman (2005) found 18 species of benthic foraminifera in *Didemnum* sp. samples but all were dead. Copepods, amphipods, shrimps, polychaetes, molluscs, decapods, hydroids, algae, nematodes, ciliates, protozoans (gregarines), suctorian ciliates, and pea crab have been found living on or in ascidians (Millar 1971, Monniot 1990, Monniot *et al.* 1991, O'Clair and O'Clair 1998, Kott 2001, Tyree 2001, Coutts 2002). These organisms are thought to do relatively little harm to the organism but some parasitic copepods, nemertean worms and decapod crabs could cause minor damage to the tunicate (Monniot 1990, Monniot *et al.* 1991, O'Clair and O'Clair 1998).

Distribution

Given the ongoing taxonomic debate concerning species identity, we have assumed that *Didemnum* sp. is native to Japan (Cohen 2005, Daniel and Therriault 2006). Further, we consider the type location for *Didemnum* sp*.* to be Ise Bay, near Nagoya (Gretchen Lambert, pers. comm.), but it is probable this species is more widely distributed. (Figure 2). In Ise Bay the temperature varies greatly over the year while salinity remains relatively constant. The mean annual water temperature is 19.9^{o}C at 0 m, with a range between 6.0^{o}C and 31.1^{o}C (Japan Oceanographic Data Center 2006). The mean annual salinity is 33.41‰ at 0 m with a range between 29.28‰ and 34.99‰ (Japan Oceanographic Data Center 2006). Moreover, at the mouth of Ise Bay a thermohaline front forms during the winter (Yanagi *et al.* 1997). For example, during February 1995 the temperature ranged from 14.0^{o}C at the mouth to approximately 8.5^{o}C at the head of the bay (Yanagi *et al.* 1997). Salinity ranged between 34.6‰ at the mouth to 32.0‰ at the head during the same period (Yanagi *et al.* 1997).

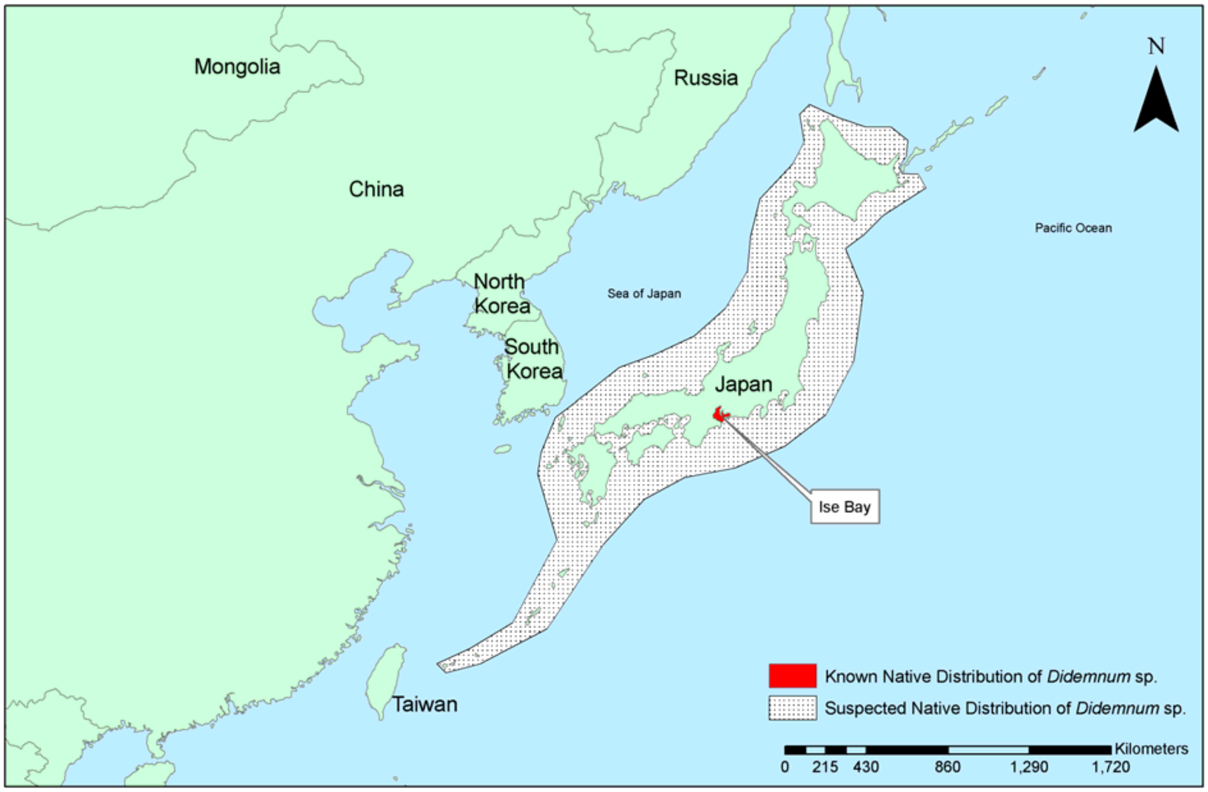


Figure 2. Known native distribution in Ise Bay, Japan and suspected native distribution of *Didemnum* sp. (Gretchen Lambert, pers. comm.)

Didemnum sp. has been introduced to four countries, excluding Canada, worldwide: France, the Netherlands, New Zealand, and the United States (Coutts 2002, Valentine 2003, Cohen 2005, Gittenberger 2005, Coutts and Taylor 2005). In the United States, *Didemnum* sp. has been introduced on both coasts (Valentine 2003, Carlton 2004, Auker 2005, Carman 2005, Cohen 2005, Pederson *et al*. 2005, Valentine *et al*. 2005a, deRivera *et al.* 2005) with offshore colonies identified on Georges Bank (Valentine *et al*. 2005b).

In Canada, *Didemnum* sp. has been found at several locations in British Columbia (Figure 3). The first documented occurrence of the tunicate was Okeover Inlet on mussel cages in 2003 (Valentine 2003, Cohen 2005). *Didemnum* sp. has since been reported from Agamemnon Channel, Pendrell Sound, Jedediah Island, Trevenen Bay, Jervis Inlet, Deep Bay, Lemmens Inlet, and Lions Rock (Valentine 2003, Cohen 2005). Six additional locations were identified in 2005 including Tyee Cove, False Bay, Gorge Harbour; Village Bay, Teakerne Arm, Deep Bay and Nanoose Bay (Debbie Palzat, pers. comm.). Nevertheless, we have to point out that the current distribution data is not based on large scale surveys, and might therefore introduce some bias into the analysis.

Potential Impacts

Didemnum sp. has many potential impacts on aquatic macrophytes, water quality, plankton, invertebrates, and fishes but most of these potential impacts are speculation as documented impacts of invasive species in general, and ascidians in particular, is minimal. Ascidians generally possess traits that allow them to be successful biofouling organisms including the ability to efficiently filter-feed, grow rapidly, reproduce rapidly at a young age, readily colonize both artificial and natural substrates, and tolerate a wide range of environmental conditions (Berrill 1950, Berrill 1955, Millar 1971, Berrill 1975, Monniot *et al.* 1991, Lambert and Lambert 1998, Tyree 2001, Coutts 2002, Lambert 2002, Valentine 2003, Dunstan and Johnson 2004, Carman 2005, Cohen 2005, Geerlofs and Gordon 2005b, Gittenberger 2005, Lambert 2005b, Osman and Whitlatch 2005, Pederson *et al.* 2005, Valentine *et al.* 2005a and 2005b).

In general most impacts on macrophytes and invertebrates is due to *Didemnum* sp.'s ability to overgrow and smother aquatic vegetation, sponges, hydroids, anemones, limpets, oysters, mussels, scallops, barnacles, bryozoans, corals, coelentrates, other ascidians, and other fouling community species (Birkeland *et al.* 1981, Monniot et al. 1991, Tyree 2001, Coutts 2002, Kott 2002, Cohen 2005, Lambert 2005b, Pederson *et al.* 2005, Valentine *et al.* 2005a). Additional impacts may include the ability to out compete native species for food (Lambert and Lambert 1998, Pederson *et al.* 2005), space (Osman and Whitlatch 1995b, Oren and Benayahu 1998, Pederson *et al.* 2005), or settlement substrates (Osman and Whitlatch 1995a, Cohen 2005, Pederson *et al.* 2005), effectively altering species composition within native biofouling communities (Valentine *et al.* 2005b) that could affect fish forage or rearing habitats (Pederson *et al*. 2005).

Didemnum vexillum has been found on salmon cages in East Bay, New Zealand, but no negative impacts were noted at the time (Sinner and Coutts 2003, Coutts and Sinner 2004). The ability to smother other invertebrates is the greatest potential impact of *Didemnum* sp., especially as it pertains to shellfish aquaculture. In the Netherlands there are reports of Didemnum sp. to impact mussel and oyster farms (Gittenberg 2005, in Bailey and Cameron 2005).

POTENTIAL DISTRIBUTION

Background

A successful invasive species is dependent on two main factors for its success, an existing dispersal vector and suitable habitat in the introduced region (herborg et al 2006), this study provided estimates for both factors. For the purpose of the risk assessment we divided British Columbia's coastline into eight separate areas of coastline: Strait of Georgia, Southern Vancouver Island, Central Vancouver Island, Northern Vancouver Island, Johnstone Strait, Queen Charlotte Sound, Hecart Strait, and Queen Charlotte Islands. Currently *Didemnum* sp are reported in 15 locations within the Strait of Georgia, and one location at Southern Vancouver Island (Figure 3).

Potential distribution of *Didemnum* sp will depend on the spatial distribution of dispersal vectors supplying a particular area with propagules. The two vectors believed to be most likely to transport *Didemnum* sp in BC coastal waters are small vessel transport and the movement of aquaculture product and equipment. The overall vector input into a particular area was estimated by creating a standardized density layer for each of the following potential vectors: marinas, small craft harbours, anchorages, aquaculture processor sites, shellfish, and finfish aquaculture sites. The first three vectors were used to provide estimates for the propagule pressure of small crafts, while the next three vectors capture the introduction pressure by aquaculture facilities. The layer density was calculated for a 2km cell size and a 20km search radius, and the output was standardized to a scale from 0-1 for each separate vector. The overall risk was calculated by combining the density value for all six layers for each raster cell and then dividing the result by the number of layers.

We measured the propagule pressure into our study areas by extracting the mean and standard deviation of vector density at 50 random locations within 2km from the shoreline (Figure 4 & 5). This limitation to 2km offshore was based on the maximum distance offshore current *Didemnum* sp reports are found within BC. For comparison the same values were extracted for the *Didemnum* sp reports within the Strait of Georgia.

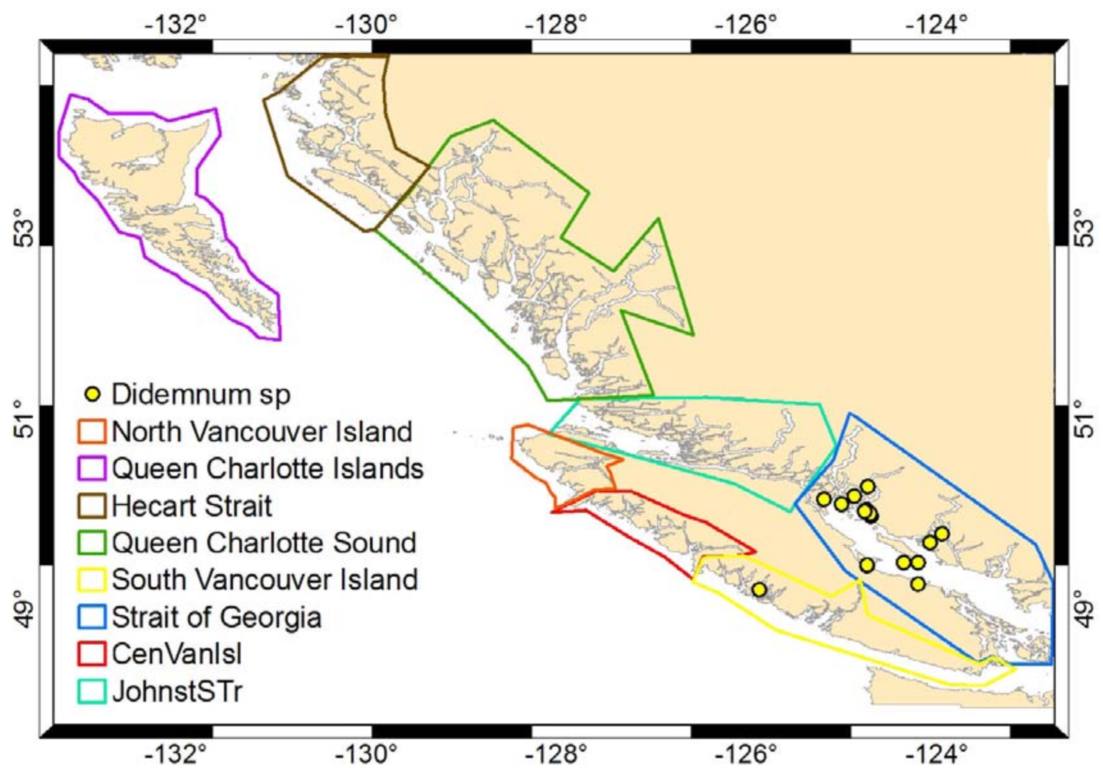


Figure 3: Outline of the study areas used in the risk assessment

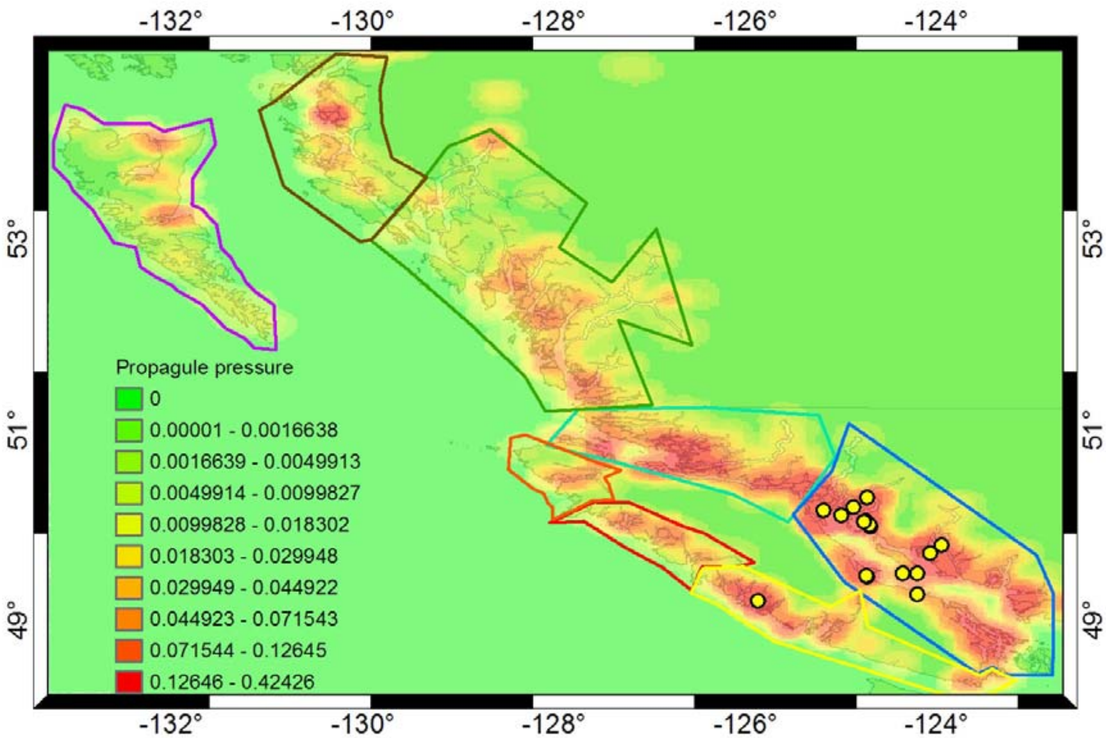


Figure 4: Spatial distribution of propagule pressure

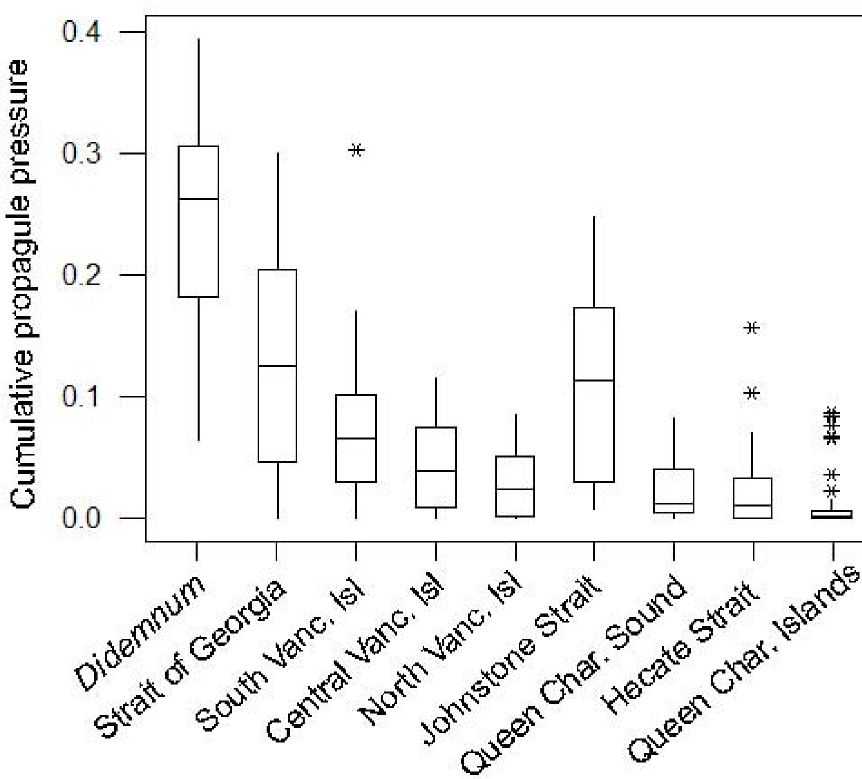


Figure 5: The cumulative propagule pressure for eight areas along the BC coastline (based on n=50 sub-sample) and reported Didemnum infestations (n=15).

Once a potential invader is released by a transport vector into a particular area, its invasive success is dependant on its ability to survive the local environmental conditions. In order to predict the likelihood of survival, we developed an environmental niche model using the Genetic Algorithm for Rule-set prediction (GARP) (Peterson and Cohoon 1999; Peterson and Vieglais 2001). This approach uses species' presence data and coverages of environmental data to develop a algorithm predicting areas with suitable environmental conditions for establishment. The environmental layers initially tested for their contribution were the seasonal salinity, temperature, and oxygen content up to the 200m depth contour along the US and Canadian coastline. These layers were generated by extrapolation of point measurements extracted from the NOAA world ocean database. After testing each individual layer for its contribution to the prediction accuracy (for details see Drake & Bossenbroek, 2004, Herborg et al, 2006). The final prediction included: temperature layers for April-June, July-September, and October-December; oxygen concentration for July-September; and salinity for January-March. Environmental suitability was based on the results of 100 individual predictions, selected out of 3000 prediction using the best-subset method (Anderson et al 2003).

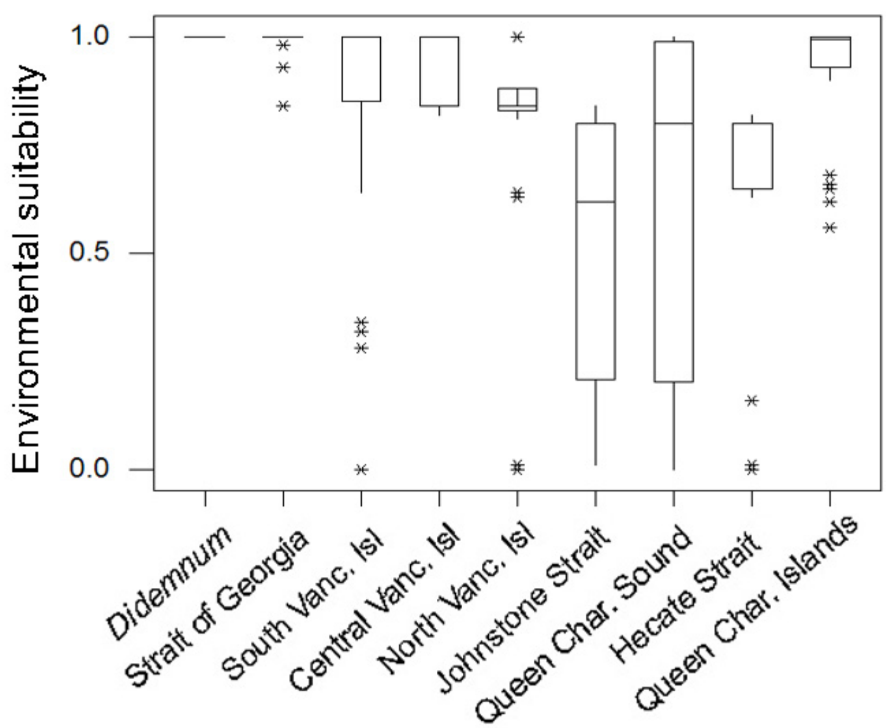


Figure 6: The environmental suitability for eight areas along the BC coastline (based on n=50 sub-sample) and reported Didemnum infestations (n=15).

The overall habitat match into a particular area was estimated by sub-sampling the level of environmental match for the same 50 random locations as in the previous paragraph. The mean and standard deviation was used as an estimate of the probability and standard deviation of survival. While most areas in this , study were predicted a high environmental match (>0.80) Johnstone Strait (0.48), Queen Charlotte Sound (0.63), Hecart Strait (0.68), and North Vancouver Island (0.75) had lower predicted environmental suitabilities (Figure 6).

Biological Risk Assessment (probabilities and uncertainties)

1. Arrival
Didemnum sp. has been reported from several locations around the Strait of Georgia in southern BC waters. This tunicate species likely has been present for some time owing to past practices related to Pacific oyster culture practices half a century ago (ref). Given no known uses for this species intentional introductions are unlikely. However, as with other biofouling organisms the risk of unintentional introductions is very high. It is probable that *Didemnum* sp. was introduced to British Columbia as a hitchhiker with Pacific oysters brought from Japan sometime prior to the 1960s. This practice is no longer allowed but *Didemnum* sp. could be spread via fouling on shellfish aquaculture gear either directly to a new location (e.g., growers with multiple leases) or indirectly via processing plants or harbours. Once established in a harbour or marina there is a high risk of further spread due to recreational boating activities, especially larger pleasure craft that remain in the water and can travel considerable distances. Therefore the risk of arrival was based on an estimate of propagule pressure into the areas of interest in this study, combining spatial data on aquaculture and boating fascilities (see previous section for more detail).
The actual probability of arrival for a particular area was calculated by dividing the mean propagule pressure for one particular study area by the mean propagule pressure value of reported Didemnum sp sites in order to obtain a value between zero and one. The standard deviation used in QBRAT, is the standard deviation of the propagule pressure for the 50 sample locations within an area (Table X).

2. Impact (I1 – does not arrive)
The impact of Didemnum not arriving in an area is considered as zero in this case study, in the absence of any mitigation efforts.

3. Survival
We defined the probability of *Didemnum* sp. to survive at one of these new locations by the mean environmental suitability for the particular area and the associated standard deviation (Figure 6, Table 1).

4. Impact (I2 – arrives but does not survive)
The impact of the species arriving and not surviving was considered as zero.

5. Establishment
Although the exact requirements for sexual reproduction are unknown, this colonial tunicate can grow (and spread) via asexual reproduction (budding). Thus, if this species is able to survive at one of these new locations then establishment is almost assured. Hence, the risk of establishment was calculated exactly the same as the risk of survival (Table 1).

6. Impact (13 – arrives, survives, but does not establish)
The impact of viable Didemnum sp arriving, surviving but not establishing was considered zero.

7. Spread
The ability of an invasive species to spread within a particular area is based on the wide availability of suitable habitat and transport vectors to disperse it. Since the natural ability of Didemnum sp for natural long distance dispersal seems to be very limited, human dispersal vectors are the driving factors. We based our estimate of spread for each area on the mean value of environmental suitability and presence of human dispersal vectors associated with aquaculture and small boat traffic (for both see ‘potential distribution’ section). The standard deviation is based on the sum of the individual standard deviations for each mean (Table 1).

8. Impact (14 – arrives, survives, establishes in a localized area)
We based the impact of the establishment of *Didemnum* sp in a particular area on several estimators of vulnerability. We used area of sea grass and kelp beds as indicators of the distribution of benthic communities at risk from overgrowth by the tunicate. Another estimator was the area of marine parks in the study area, as these areas are more likely to contain diverse biota this invasive tunicate would be particular deleterious there. The final measure is the number of shellfish farms in the area, representing the impact *Didemnum* sp. would have on shellfish farmers. In order to quantify the impact, the area covered of the number of locations in each study area was converted into a fraction of the total are or number of locations along the whole B.C. coastline. Clearly, these estimates of impact are not ideal, but they are the best available information available to our study. The individual impact measures for each of these four coverages were then added together and divided by four to obtain a value between zero and one (Table 1). The probability of the impact at the establishment stage was selected as very uncertain, due to the limited knowledge of *Didemnum* biology and its interaction with native species it cannot be discounted that it might carry pathogens could spread to native species. Additionally, depending on the location of establishment, it could have a serious impact on a species with a limited distribution.

9. Impact (15 – arrives, survives, establishes and spreads)
The impact of spread was assumed to be identical to the impact of establishment, only the certainty of the impact occurring was very certain, based on the effects invasive tunicates have on native species (Table 1).

Table1: Estimates of probabilities and impacts as used in QBRAT.

	P1	P1 StDev	P2	P2 StDev	P3	P3 StDev	P4	P4 StDev	I4/I5
Strait of Georgia	0.51	0.0869	0.99	0.0035	0.99	0.0035	0.75	0.0904	0.385
South Vancouver Island	0.28	0.0580	0.84	0.0406	0.84	0.0406	0.56	0.0986	0.097
Central Vancouver Island	0.18	0.0363	0.95	0.0106	0.95	0.0106	0.57	0.0469	0.090
North Vancouver Island	0.12	0.0299	0.75	0.0446	0.75	0.0446	0.44	0.0745	0.038
Johnstone Strait	0.45	0.0768	0.48	0.0459	0.48	0.0459	0.47	0.1227	0.043
Queen Charlotte Sound	0.08	0.0208	0.63	0.0546	0.63	0.0546	0.36	0.0754	0.054
Hecate Strait	0.08	0.0287	0.68	0.0362	0.68	0.0362	0.38	0.0649	0.065
Queen Chalotte Islands	0.04	0.0230	0.93	0.0175	0.93	0.0175	0.49	0.0405	0.228

RESULTS

Key for this risk assessment exercise is a realistic prediction of propagule pressure. In this study the cumulative pressure for *Didemnum* sp locations is significant higher than all areas except Johnstone Strait and Strait of Georgia (Table 2). The fact that currently *Didemnum* sp is found in areas which have a higher predicted propagule pressure than most other areas in the region indicate, within the limitations of the sampling effort for this invasive, that our predictors of propagule pressure are capturing spatial distribution of human vector transport.

Table 2: A comparison of estimated propagule pressure for eight areas along the BC coastline and current locations of Didemnum sp infestation within one area (Strait of Georgia). See figure 3 for more detail on the areas used here. (* is significant; NS is non-significant).

	Queen Chalotte Islands	Hecate Strait	Queen Charlotte Sound	John-stone Strait	North Vancouver Island	Central Vancouver Island	South Vancouver Island	Strait of Georgia
Didemnum locations	*	*	*	NS	*	*	*	NS
Strait of Georgia	*	*	*	NS	*	*	NS	
South Vancouver Island	*	*	*	NS	*	NS		
Central Vancouver Island	*	*	NS	*	NS			
North Vancouver Island	*	NS	NS	*				
Johnstone Strait	*	*	*					
Queen Charlotte Sound	NS	NS						
Hecate Strait	NS							

A comparison of the total biological risk (TBR) for each area and the individual risk and impact parameters reveal several interesting results. Strait of Georgia has the highest total biological risk (0.1933), the highest propagule pressure, and the highest environmental suitability of all areas, which supports the overall predictions, as 15 out of 16 reports of *Didemnum* sp are from this area (Table 3). The next highest TBR is predicted for Johnstone Strait (0.0443), where similarly high propagule pressure but a much lower habitat match is predicted. The most likely reason for this much lower TBR value is the lower predicted environmental suitability found here in comparison to the Strait of Gerogia. The three areas along the west coast of Vancouver Island (north, central, and south) have the next highest biological risks. The Queen Charlotte Islands were predicted as being the most severely impacted by a *Didemnum* sp invasion (Table 1) but still was predicted to have only the third lowest TBR. A further assessment of impact prediction is described at the beginning of the next section.

Table 3: Total biological risk for eight areas along the BC coastline based on the QBRAT output.

	Total biological risk	StDev
Strait of Georgia	0.1933	20.470
South Vancouver Island	0.0192	2.001
Central Vancouver Island	0.0146	1.511
North Vancouver Island	0.0259	2.745
Johnstone Strait	0.0443	4.646
Queen Charlotte Sound	0.0017	0.184
Hecate Strait	0.0024	0.258
Queen Charlotte Islands	0.0079	0.926

RECOMMENDATIONS FOR QBRAT

Impact sensitivity analysis

Due to the high degree of subjectivity involved in estimating and calculating the impact we decided to test the sensitivity of QBRAT to different methods. Using the same original data, we used three ways to calculate the relative impact:

1) Normalised impact = $(I_{Kelp}+I_{Seagrass}+I_{MPA}+I_{Shellfish})/N_{Impacts}$
2) Cumulative impact = $I_{Kelp}+I_{Seagrass}+I_{MPA}+I_{Shellfish}$
3) I_{max} normalised impact = $(I_{Kelp}+I_{Segrass}+I_{MPA}+I_{Shellfish})/I_{MAX}$

The impact value on each of the coverages (kelp beds, sea grass beds, marine protected areas, and shellfish aquaculture) for each particular area was calculated as described in point eight above.

The first method provides a value between 0-1 for each impact and a total combined risk for all each areas of one (as used in the risk assessment detailed above). The second formula results in impact values between 0.171-1.54, and a total risk of 4.00. The final method was normalized using the highest risk value (1.54), resulting in values between 0-1 and a total combined risk for all eight regions of 2.60 (see table 4).

Table 4: QBRAT input from three different approaches to impact quantification

	Normal. impact	Cumul. impact	I_{max} normal. impact
Strait of Georgia	0.385	1.540	1.000
South Vancouver Island	0.097	0.389	0.253
Central Vancouver Island	0.090	0.361	0.234
North Vancouver Island	0.038	0.153	0.099
Johnstone Strait	0.043	0.171	0.111
Queen Charlotte Sound	0.054	0.214	0.139
Hecate Strait	0.065	0.260	0.169
Queen Charlotte Islands	0.228	0.910	0.591

The comparison of the total biological risk for each area based on the three different measures of impact revealed some striking differences in the results (Figure 7, Table 5). While the Strait of Georgia has the highest risk in all cases, the order of the risk rank for all other locations varies considerable. The normalised impact based risk values identify Johnstone Strait, North, South, and Central Vancouver Island as having the highest risk. The cumulative and the I_{MAX} normalised impact measures both provide identical rankings, with South, and Central Vancouver Island, Queen Charlotte Islands, and Johnstone Straits as the highest risks of invasion.

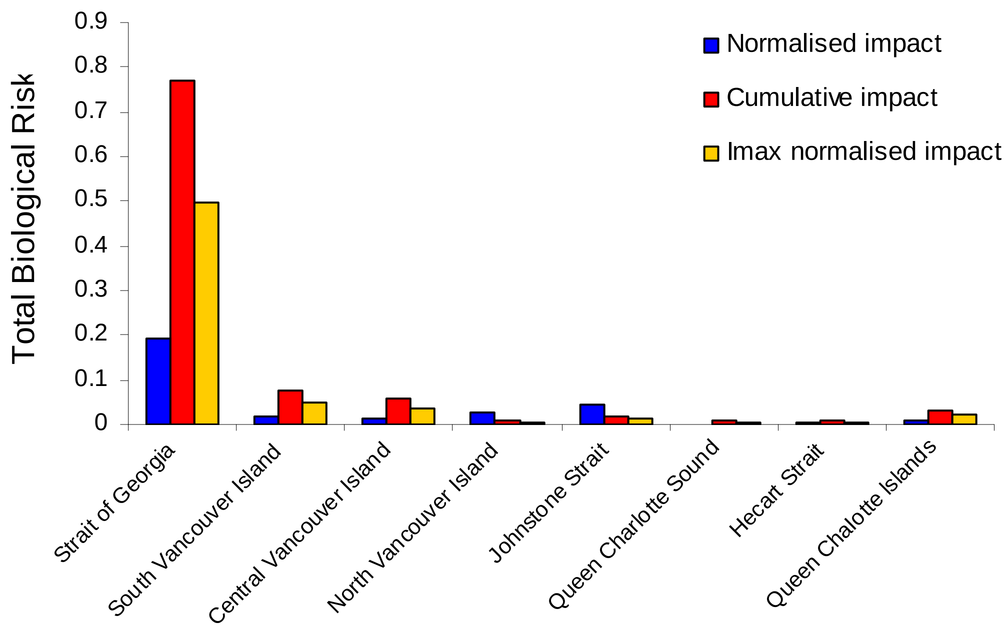


Figure 7: Total biological risk based on three different methods of estimating the impact.

Table 5: Ranking of eight areas along the BC coastline by their total biological risk obtained from QBRAT. Please see the text for the three different approaches in quantifying impact.

	Ranked total biological risk by impact assessment calculation		
	Norm. impact	Cumulative impact	I_{max} norm. impact
Strait of Georgia	1	1	1
South Vancouver Island	4	2	2
Central Vancouver Island	5	3	3
North Vancouver Island	3	6	6
Johnstone Strait	2	5	5
Queen Charlotte Sound	8	8	8
Hecate Strait	7	7	7
Queen Charlotte Islands	6	4	4

The marked difference in the QBRAT results based on the slight variations raise several important points. First of all it would be desirable to be able to run the tool without any impact, as it is possible to exclude the economical impact. This would allow a researcher reduce the margin of error in a case like the one presented here, where the risk can be estimated to some degree, but the impacts are very difficult to quantify. Secondly, if the impact is included some clearer guidelines need to be developed with regards to how to weigh different parts of the ecosystem or what range to the input variables have to cover. Based on this small sensitivity analysis it became very clear that it would be impossible to compare QBRAT results based on different approaches to impact quantification.

General recommendations

One potential limitation is that users become over-confident in the output of this tool, especially if the tool is provided with a detailed manual (see below). For example, QBRAT will provide an output of biological risk based user inputs regardless of the accuracy in these values. We believe that for cases where inputs are equivalent to guesses the quantitative nature of the tool would be misleading. However, in the few cases where more realistic input values can be obtained this tool is an improvement over traditional tools. Having the ability to transition from a qualitative framework to a semi-quantitative framework is advancement worth pursuing.

Improvement must be made to the accompanying manual. Although the tool provides information on the basic mechanics of the tool there is little information for potential users as to what various p-values or I-values should be. Also some further information how the actual risk is calculated, or at least how the different input variables are used or weighted in the model would be valuable.

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