

Regional ecological risk assessment for the introduction of *Gambusia affinis* (western mosquitofish) into Montana watersheds

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Abstract Qualitative risk assessment methodologies were used to assess the risk of establishment and consequent impacts on native minnows and species of concern (SOC) associated with the intentional or unintentional introduction of the mosquito biological control agent, *Gambusia affinis*, to various Montana watersheds. *Gambusia affinis* introductions for mosquito control have been made throughout the world; some introductions have resulted in deleterious effects on native species. We used average January water temperatures, the presence of dams, and the presence of native minnows and SOC to define endpoints for our assessment. Our results suggest that a section of the Missouri River running between Wolf Creek and Landusky had the highest overall risk score, which corresponds to the highest likelihood of establishment and effects on native minnows and SOC. We also demonstrate how rivers with the highest temperatures are not necessarily at the highest risk of having deleterious effects on minnows and SOC; conversely, these rivers were found to be at highest risk of a population of *G. affinis* establishing.

Keywords *Aedes* · Biological control · *Culex* · Geographical Information Systems (GIS) ·

Introduced fishes · Invasive species · Non-indigenous species · Qualitative risk assessment · Relative risk assessment · Risk analysis

Abbreviations

EA	Environmental assessment
<i>G. affinis</i>	<i>Gambusia affinis</i>
GIS	Geographical Information Systems
MFWP	Montana Department of Fish, Wildlife, and Parks
NRC	National Research Council
ORS	Overall risk score
SOC	Species of concern
USGS	United States Geological Survey
USOSTP	United States Office of Science Technology and Policy

Introduction

Increasingly stringent standards to ensure the safety of pest management practices, along with the current popularity of management directives favoring the inclusion of integrated pest management, drive the ongoing need for risk assessments of biological control agents and invasive species (USOSTP 1999; Andersen et al. 2004a, b; Peterson and Sing 2007). Although it is well known that accidental introductions of non-native species can result in serious environmental problems, the potential also exists for

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deleterious ecological consequences associated with the intentional release of biological control agents (Louda et al. 2003). Therefore, there is a distinct need to assess the risks that arise when biological control agents are released.

The mosquito biological control agent, *Gambusia affinis*, also known as the western mosquitofish, has been introduced throughout the world for mosquito control (Rees 1934; Krumholz 1944; Brown and Fox 1966; Crivelli and Boy 1987; Haynes 1993). *Gambusia affinis* has often been released and re-distributed indiscriminately, causing negative impacts on native fish species (Schoenherr 1981; Meffe 1984; Bence 1988; Courtenay Jr. and Meffe 1989; Komak and Crossland 2000; Ayala et al. 2007).

In Montana, *G. affinis* are currently stocked in ponds by the Montana Department of Fish, Wildlife, and Parks (MFWP) through a program maintained by the Montana Department of Public Health and Human Services. The program targets the control of mosquito larvae, because of recent concerns about West Nile virus and the nuisance mosquitoes cause. Currently, *G. affinis* can be released anywhere in the state of Montana, however they can only be stocked by a mosquito control district or other authority, they must be released in an isolated pond or pool, and permits are required for every release. The MFWP Environmental Assessment (EA) for *G. affinis* states that, if released, the fish would have a minor impact on terrestrial and aquatic life and it would not over winter in Montana (MFWP 2004). However, this EA does not cite any data supporting the findings of a minor impact.

To address the lack of evidence supporting the MFWP's findings on the relative safety of releasing *G. affinis* in Montana watersheds, we performed a risk assessment using a qualitative risk ranking system. Qualitative risk assessments are often used when quantitative risk assessment methodologies may not be practical, generally due to data inadequacies. Many qualitative risk assessments utilize a risk ranking system, which assigns values to data that corresponds and signifies an increase in risk as the value becomes larger. The approach we took was similar to that of Landis and Wieggers (1997), Andersen et al. (2004c), and Colnar and Landis (2007). We made changes in the structure and ranking protocols of these studies to address

fundamental mathematical problems arising from the use of zero in qualitative risk assessments (Cox Jr. et al. 2005). Therefore, the objectives of our study were to use qualitative risk assessment methodologies to determine the risk of establishment and over wintering for *G. affinis* in rivers and creeks in Montana, and to assess the consequences to species of concern (SOC) and native minnows if *G. affinis* were to become established.

Materials and methods

Problem formulation

Ecological risk assessment can be described in quantitative terms as a function of effect and exposure (NRC 1983). The assessment flows in a logical, stepwise fashion, proceeding in four well-defined phases. First, the organism of interest or ecosystem stressor, *G. affinis*, was identified through a stressor description and an effects assessment of known or potential effects. Second, assessment endpoints, the SOC and native minnows currently occurring in the rivers, river sections, or creeks of interest and that might be affected by the ecological stressor were identified. Third, we performed an exposure assessment by identifying characteristics that potentially open habitats to invasion and establishment by *G. affinis*. Fourth, we combined the outcomes of the second and third steps to generate a risk characterization of *G. affinis* establishment and effects to SOC and minnows.

Stressor description

The native range of *G. affinis* is from the Gulf Coast of northeastern Mexico, through Texas, and Louisiana including the Mississippi River and its tributaries (Krumholz 1944). The northern edge of its native range extends into the southern parts of Illinois and Indiana (Krumholz 1944). *Gambusia affinis* is considered to be an invasive species outside of its native range (Courtenay Jr. and Meffe 1989). It has been documented to have self-sustaining populations in Utah, Nebraska, northern Indiana, northern Illinois, Michigan, and Montana (Rees 1934; Krumholz 1944; Brown and Fox 1966; Haynes 1993).

A live-bearing fish, *G. affinis* can produce multiple broods in a year due to its short gestation period ranging from 1–3 weeks, and reproduction occurring every 3–4 weeks (Turner 1937; Krumholz 1948). Females have 5–205 fry per brood, and those offspring become reproductively mature at about 4 weeks of age (Krumholz 1948; Hughes 1985). Average reproduction over an 8–10 week period results in 2–3 broods, while a single female can give birth to 4–5 broods during a 14–15 week period (Krumholz 1948). *Gambusia affinis* reproduce by internal fertilization and possess the ability to store sperm through the breeding season and winter, allowing dispersal to new locations and reproduction without males (Robbins et al. 1987; Haynes 1993).

Along with the ability to store sperm, *G. affinis* travels great distances; it has shown a greater dispersal capacity than non-invasive related species, with 76% of the dispersing *G. affinis* being females (Robbins et al. 1987; Rehage and Sih 2004). *Gambusia affinis* in the Platte River, Nebraska demonstrated an average downstream dispersal rate of approximately 17–18 km per year. Their annual minimum upstream dispersal rate, in the Republican River, Nebraska from October to July was 9.5 km (Lynch 1988).

Gambusia affinis has demonstrated the ability to seek out optimal microsites, and using a range of strategies adapt according to the demands of local environmental conditions to successfully colonize a variety of marginal habitats. In Utah, *G. affinis* survive in pools varying in temperature from 23–28°C which are fed by a warm-water spring (Rees 1934). Individuals in Nebraska collected in March when water temperatures were 6–7°C with populations surviving the winter (Haynes 1993). *Gambusia affinis* in Montana survive the winters in a warm spring-fed pond with a low water temperature of 22°C in January (Brown and Fox 1966). Currently, the population in Boulder Hot Springs is the only population in Montana, and is where the MFWP obtains the fish for release.

Gambusia affinis can grow to a maximum length of 49 mm in 36–75 days depending on environmental conditions, and can tolerate salinities of up to 50‰ sea water (Krumholz 1948; Al-Daham and Bhatti 1977). Cold-tolerant *G. affinis* from Utah can survive temperatures above 0.5°C when acclimated to 5–10°C water, and *G. affinis* from Indian Hot Springs, Arizona

have a lower lethal limit of 2.7°C when acclimated to 5–10°C (Otto 1973; Al-Daham and Bhatti 1977; Lynch 1988; Meffe and Snelson Jr. 1989).

Effects assessment

Effects, as defined in our assessment, occur when *G. affinis* is able to survive the winter and establish self-sustaining populations in a Montana river or creek which can potentially cause deleterious effects. Negative impacts can include out-competing native fish for food, causing stress or death through aggressive behavior, direct consumption of SOC or minnows, decreasing the growth of native fish, consuming native fauna that will result in decreases in populations or elimination from that habitat, and out-competing native fish for hiding places.

Gambusia affinis is an omnivore, with the largest portion of its diet consisting of aquatic and terrestrial invertebrates. *Gambusia affinis* have significantly lowered the densities of invertebrate prey after introduction into mesocosms (Walton and Mulla 1991). Omnivorous invasive species such as *G. affinis*, have been shown to have higher feeding rates than non-invasive species in the same genus (Rehage et al. 2005). They also feed on larvae, juveniles, or small adults of other fish species (Courtenay Jr. and Meffe 1989; Ayala et al. 2007). Mosquitofish were shown to be a significant predator of all developmental stages of two frog species *Limnodynastes ornatus* and *Bufo marinus* and a newt species *Taricha torosa* (Gamradt and Kats 1996; Komak and Crossland 2000). *Gambusia affinis* is extremely aggressive, with their attacks on other fish causing stress or even physical damage (Lloyd et al. 1986). This species is also known to become cannibalistic at higher densities (Krumholz 1948; Dionne 1985; Crivelli and Boy 1987; Garcia-Berthou 1999). *Culex tarsalis* one of the main vector of West Nile virus, developed faster in the presence of *G. affinis*, due to lowered abundance of invertebrate competitors (Blaustein and Karban 1990). Decline in water quality, measured in decreased water clarity and dissolved inorganic phosphorus, and increased water temperature, pH, and oxygen have been correlated with *G. affinis* introductions (Hurlbert et al. 1972; Hurlbert and Mulla 1981).

Some *G. affinis* introductions are thought to have resulted in population reductions or extirpation of native invertebrates, fish, and frogs (Hurlbert and Mulla 1981; Miura et al. 1984; Courtenay Jr. and Meffe 1989). In the case of the endangered Gila topminnow (*Poeciliopsis occidentalis*), *G. affinis* predation has caused a major decline in populations (Meffe 1985). In some cases, native fish species are as, or more, efficacious than *G. affinis* at controlling mosquitoes (Nelson and Keenan 1992; Offill and Walton 1999; Childs 2006; Billman et al. 2007).

In addition to deleterious effects, it is possible for *G. affinis* to have beneficial ecological effects after introduction. *Gambusia affinis* can serve as prey for largemouth bass (*Micropterus salmoides*), little blue herons (*Egretta caerulea*), and green-backed herons (*Rutorides striatus*) (Swingle 1949; Niethammer and Kaiser 1983).

Another beneficial effect of *G. affinis* is its ability to suppress mosquito populations by consumption of larvae depending on what type of environment they are released. *Gambusia affinis* continue to be introduced because they consume 42–167% of their body weight per day (Chipp and Wahl 2004). *Gambusia affinis* have been shown to be effective in the control of *Culex* and *Aedes* larvae in abandoned swimming pools (Duryea et al. 1996). In California rice fields, *G. affinis* were found to significantly reduce *C. tarsalis* larvae (Hoy and Reed 1970; Hoy et al. 1971; Hoy and Reed 1971; Hoy et al. 1972; Miura et al. 1984; Kramer et al. 1988). However, in southern California, *G. affinis* stocked in duck club ponds did not significantly control *C. tarsalis* (Walton et al. 1991).

In a mesocosm study, *G. affinis* did not significantly reduce the larval abundance of mosquitoes until 3 weeks after their introduction (Walton and Mulla 1991). Bence (1988) found that although *G. affinis* stocked in rice paddies reduced *C. tarsalis* larvae, they also reduced the abundance of other invertebrates and invertebrate predators.

Assessment endpoints

To stakeholders in a given area, assessment endpoints represent ecological, economic, or social values. We chose fish that are SOC in the state of Montana and

native minnows as our assessment endpoints. We chose these species because their maintenance serves the ecological, economic, and social values of diverse stakeholders. Table 1 lists all species assessed, and is limited to those SOC occurring in at least one river or creek used in this assessment, that could potentially interact with *G. affinis*. For example, our rationale for choosing Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) was that both the young and adults could potentially compete with *G. affinis* for invertebrate prey. Pallid sturgeon (*Scaphirhynchus albus*) were not included in the study because, unlike *G. affinis*, these are large fish that live in large rivers and have a benthic lifestyle.

Exposure assessment

We used ArcView[®] 9.2 (ArcGIS[™] 9.2; ESRI[®], Redlands, CA) to analyze data and display rivers, river sections, and creeks that are potentially at risk of establishment from *G. affinis*. We obtained GIS data from the MFWP (MFWP 2006) and the University of Montana's SpatialSci (SpatialSci 2007). We used average river temperature data for 42 rivers or river sections and creeks for the month of January in Montana from the United States Geological Survey National Water Information System (USGS 2007) (Table 2).

The Gallatin River provides an example of what we defined as a river section. The Gallatin River has two different temperature points, one near Gallatin Gateway and the other near Logan. The river above Gallatin Gateway and the section below Gallatin Gateway until Logan had a January average temperature of 1.1°C. The section of the Gallatin River below Logan had an average January temperature of 0.2°C until it reached the Missouri River.

The temperature rating was scored based on the known range of river temperatures. We distinguished between *G. affinis* that are cold tolerant (survive $\geq 0.5^\circ\text{C}$) and hot springs adapted *G. affinis* (survive $\geq 2.7^\circ\text{C}$) (Otto 1973). Cold tolerant *G. affinis* can also survive where hot springs adapted fish can persist. If average monthly January river temperatures were $<0.5^\circ\text{C}$, the river, river section, or creek received a temperature score of 1 (minimal risk), if the temperature was $\geq 0.5^\circ\text{C}$ and $\leq 2.6^\circ\text{C}$ the river, river section, or creek received a temperature score

Table 1 List of species of concern (SOC) or native minnows (M) for the state of Montana used in the assessment

Common name	Scientific name	SOC or M
Arctic Grayling	<i>Thymallus arcticus montanus</i>	SOC
Blue Sucker	<i>Cycleptus elongatus</i>	SOC
Bull Trout	<i>Salvelinus confluentus</i>	SOC
Northern Redbelly × Finescale Dace (hybrid)	<i>Phoxinus eos</i> × <i>phoxinus neogaeus</i>	SOC
Pearl Dace	<i>Margariscus margarita</i>	SOC
Sauger	<i>Stizostedion canadense</i>	SOC
Shortnose Gar	<i>Lepisosteus platostomus</i>	SOC
Sicklefin Chub	<i>Macrohybopsis meeki</i>	SOC
Sturgeon Chub	<i>Macrohybopsis gelida</i>	SOC
Westslope Cutthroat Trout	<i>Oncorhynchus clarki lewisi</i>	SOC
Yellowstone Cutthroat Trout	<i>Oncorhynchus clarki bouvieri</i>	SOC
Creek Chub	<i>Semotilus atromaculatus</i>	M
Northern Redbelly Dace	<i>Phoxinus eos</i>	M
Flathead Chub	<i>Platygobio gracilis</i>	M
Lake Chub	<i>Couesius plumbeus</i>	M
Peamouth	<i>Mylocheilus caurinus</i>	M
Emerald Shiner	<i>Notropis atherinoides</i>	M
Sand Shiner	<i>Notropis stramineus</i>	M
Brassy Minnow	<i>Hybognathus hankinsoni</i>	M
Plains Minnow	<i>Hybognathus placitus</i>	M
Fathead Minnow	<i>Pimephales promelas</i>	M
Longnose Dace	<i>Rhinichthys cataractae</i>	M
Redside Shiner	<i>Richardsonius balteatus</i>	M
Northern Pike Minnow	<i>Ptychocheilus oregonensis</i>	M

of 2 (medium risk), and if temperatures were $>2.7^{\circ}\text{C}$ the river, river section, or creek received a temperature score of 3 (high risk).

Altered flow in river systems is thought to increase the likelihood of an invasion of freshwater fish, including *G. affinis* (Baltz and Moyle 1993; Moyle and Light 1996; Gido and Brown 1999; Marchetti and Moyle 2001; Marchetti et al. 2004a). If a dam was present, the section of river or creek below that dam was given a dam score of 2; if there was no dam present, the river, river section, or creek received a dam score of 1.

Exposure of a SOC or minnow occurs when they are in the presence of *G. affinis* and could compete directly or indirectly with or be consumed by *G. affinis*. If a SOC was present in the river, river section, or creek we gave it a SOC score of 2, if they were not present we gave it a score of 1. We used the same scoring system for minnows to assign a minnow

score. Species distributions were obtained from MFWP GIS data (MFWP 2006).

Risk characterization

We summed the temperature score and dam score for each river, river section, or creek to generate an exposure score. We summed the SOC score and minnow score in a river, river section, or creek generating a species score. The exposure score was multiplied by the species score to generate an overall risk score (ORS) for that river, river section, or creek. The minimum possible ORS was 48 while the maximum possible score was 240. We used ArcView[®] 9.2 to spatially display ORS with respect to sites in Montana. For organization of ORS into visual categories, we used natural breaks (Jenks) to partition the data into four risk classifications.

Table 2 Montana rivers, river sections, or creeks, location where average January temperatures were taken, average January temperatures (°C), number of years temperature data was taken (Years), temperature score, dam score, species of concern (SOC) score, minnow score, and overall risk score (ORS) used in the assessment

Site name	Location	January average temperature	Years	Temperature score	Dam score	SOC score	Minnow score	ORS
Dearborn River	Craig, MT	0.2	10	1	1	12	14	52
Little Blackfoot River	Garrison, MT	0.1	4	1	1	13	13	52
Rock Creek	Red Lodge, MT	0.2	1	1	1	12	14	52
Shields River	Livingston, MT	0.2	4	1	1	12	14	52
Big Hole River	Melrose, MT	0	8	1	1	12	15	54
East Gallatin River	Bozeman, MT	0.3	2	1	1	13	14	54
Gallatin River	Logan, MT	0.2	1	1	1	13	14	54
Jefferson River	Three Forks, MT	0.1	3	1	1	11	16	54
Jefferson River	Twin Bridges, MT	0.3	2	1	1	11	16	54
North Fork Flathead River	Columbia Falls, MT	0.2	4	1	1	14	13	54
Smith River	Fort Logan, MT	0	10	1	1	11	16	54
Blackfoot River	Bonner, MT	0.3	6	1	1	13	16	58
Blackfoot River	Helmville, MT	0.4	2	1	1	13	16	58
Judith River	Winifred, MT	0.1	2	1	1	12	17	58
Teton River	Loma, MT	0	5	1	1	13	21	68
Little Prickly Pear Creek	Wolf Creek, MT	1.3	2	2	1	11	13	72
Missouri River	Toston, MT	0.3	26	1	1	16	21	74
Boulder River	Big Timber, MT	0.8	1	2	1	12	14	78
Gallatin River	Gallatin Gateway, MT	1.1	3	2	1	13	14	81
Nevada Creek	Helmville, MT	0	4	1	2	12	15	81
Sun River	Vaughn, MT	0.1	7	1	2	11	16	81
Warm Springs Creek	Warm Springs, MT	0.8	6	2	1	14	13	81
Rock Creek	Clinton, MT	0.7	7	2	1	13	16	87
Bitterroot River	Darby, MT	0.7	3	2	1	13	17	90
Bitterroot River	Missoula, MT	1.8	4	2	1	13	17	90
Clark Fork	Galen, MT	1	12	2	1	13	17	90
Clark Fork	Deer Lodge, MT	0.5	3	2	1	13	17	90
Swan River	Bigfork, , MT	1.2	4	2	1	13	17	90
Gardiner River	Mammoth, YNP ^a	6.9	1	3	1	12	13	100
Beaverhead River	Twin Bridges, MT	0.7	4	2	2	12	14	104
Yellowstone River	Corwin Springs, MT	1.7	1	2	1	13	22	105
Yellowstone River	Livingston, MT	0.9	4	2	1	13	22	105
Madison River	McAllister, MT	1.2	21	2	2	13	14	108
Madison River	Cameron, MT	1.6	7	2	2	13	14	108
Musselshell River	Harlowton, MT	0	1	1	2	14	22	108
North Fork Blackfoot River	Ovando, MT	4.1	1	3	1	14	13	108

Table 2 continued

Site name	Location	January average temperature	Years	Temperature score	Dam score	SOC score	Minnow score	ORS
Missouri River	Wolf Point, MT	0.1	4	1	2	16	21	111
Missouri River	Landusky, MT	0	1	1	2	16	21	111
South Fork Flathead River	Columbia Falls, MT	3.9	7	3	1	14	14	112
Flathead River	Perma, MT	2	1	2	2	13	17	120
Flathead River	Columbia Falls, MT	2.4	21	2	2	13	17	120
Missouri River	Wolf Creek, MT	1.3	3	2	2	16	21	148

^a Yellowstone National Park

Results

We found that 21 of 42 rivers, rivers sections, or creeks are at risk of establishment by *G. affinis* based on January minimum average water temperature. There were no rivers, river sections, or creeks that had the minimum or maximum ORS (Table 2). We determined that the Missouri River section near Wolf Creek to Landusky was at highest risk for *G. affinis* establishment and impacts on SOC and minnows of all locations assessed, with an ORS of 148 and receive the classification representing the highest ORS (121–148; Table 2, Fig. 1). The Dearborn River near Craig, Rock Creek near Red Lodge, Little Blackfoot River near Garrison, and the Shields River near Livingston all had the lowest ORS of 52 (Table 2). The South Fork of Flathead River near Columbia Falls, North Fork Blackfoot River near Ovando, and the Gardiner River near Mammoth in Yellowstone National Park had the highest temperatures but had an ORS of 112, 108, and 100, respectively (Table 2). Figure 1 displays locations of rivers with respect to large cities in Montana. In some cases, lower risk rivers feed into higher risk rivers like the Missouri River section near Wolf Creek, which could also increase the risk of establishment and effects, because of the potential for *G. affinis* movement downstream into the Missouri, or upstream out of the Missouri after an introduction (Fig. 1).

Uncertainty analysis

There are several areas of uncertainty related to water temperature. *Gambusia affinis* may not be able to

survive at the point of introduction, but a kilometer up or down stream might provide suitable habitat for these fish to survive the winter. Temperature data are not available for some major rivers in Montana, such as the Milk, Kootenai, Big Horn, Tongue, and Powder. There are also a large number of thermal springs in the state, which could provide suitable habitat for establishment. During low temperatures, *G. affinis* have been documented to burrow in the silt on the bottom of ponds, which could protect them from cold water temperatures (Lloyd 1984).

No data currently exist explicitly reporting the effects of *G. affinis* on SOC and minnows used in our assessment. There also are no data on the mosquito control efficacy of *G. affinis* in Montana, or if this species is more efficacious than native species present in Montana. The majority of studies on efficacy have occurred in California rice fields, which are not adequately representative of habitats for introduction in Montana.

Discussion

We have identified locations in Montana where *G. affinis* can potentially establish a population and cause negative effects on SOC and minnows. Higher scores should represent areas that are at higher risk of establishment and impacts on SOC and minnows. For example, the Dearborn River near Craig has a temperature of 0.2°C, no dam, 1 SOC, and 1 minnow species and received an ORS of 52 (Table 2). The Missouri River near Wolf Creek has an average January temperature of 1.3°C which would be suitable for cold-tolerant *G. affinis*, a dam, 5 SOC,

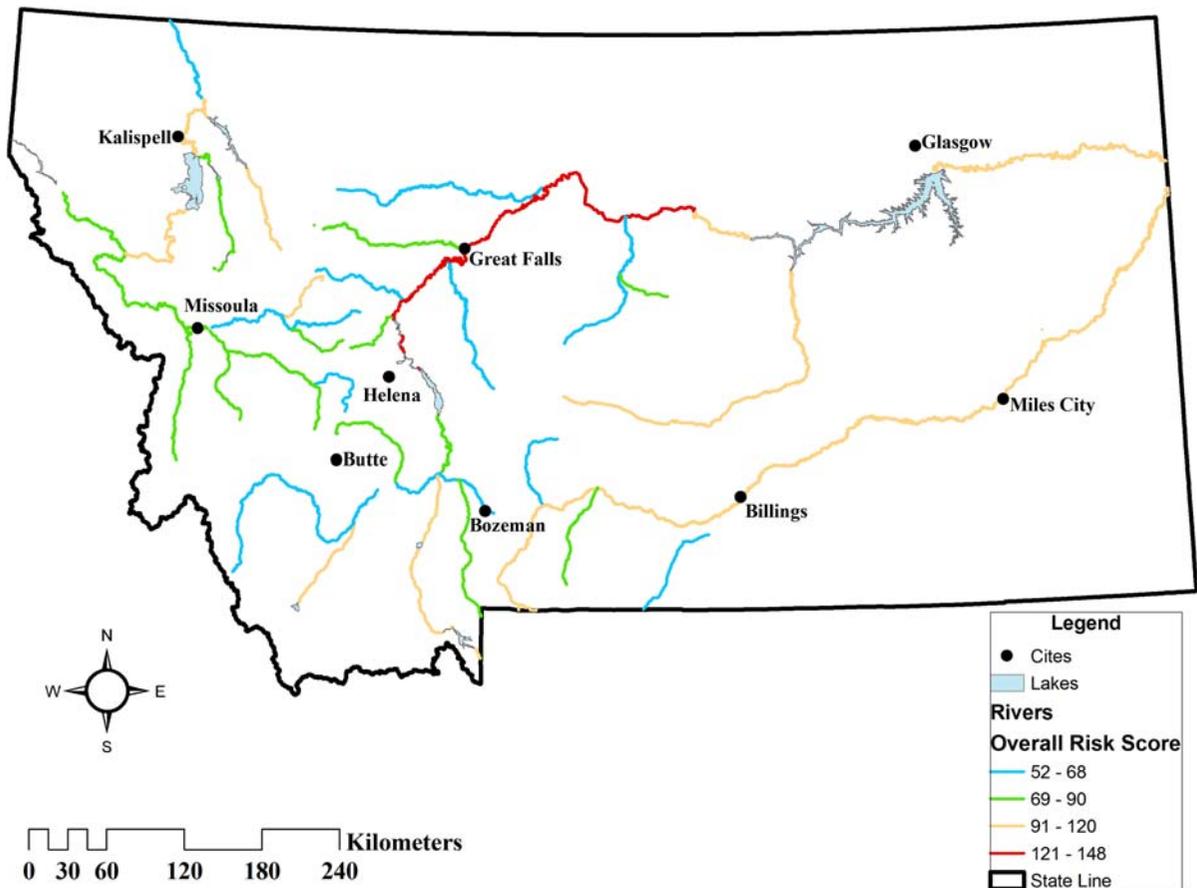


Fig. 1 Spatial display of overall risk scores (ORS) for each river assessed with respect to large cities in Montana

and 8 minnow species and received an ORS of 148, the highest ORS of any river, river section, or creek assessed (Table 2). We also determined through this analysis that the rivers with the highest temperature scores are not necessarily the locations with the highest risk of deleterious effects on SOC and minnows; however, these rivers are at highest risk of establishing a population of *G. affinis*.

Qualitative risk ranking models have shown versatility for the assessment of chemicals and invasive species and have been a valuable tool in ecological risk assessment (Landis and Wiegiers 1997; Wiegiers et al. 1998; Andersen et al. 2004c; Colnar and Landis 2007). One technique associated with qualitative risk ranking approaches is to assign the value of zero to specific factors. However, Cox Jr. et al. (2005) demonstrated fundamental mathematical problems with using the value of zero in qualitative risk assessments.

To illustrate a limitation of using zero, Little Prickly Pear Creek near Wolf Creek has no SOC or minnows, so if it were to receive a value of zero for the species score; it would have received an ORS of zero. However, the habitat of Little Prickly Pear Creek is at risk of establishment from cold-tolerant *G. affinis*, so an ORS of zero is not appropriate. There is little risk to SOC and minnows used in our assessment, but establishment of an invasive species is still considered a negative impact on that river, given the movement potential of these fish.

Uncertainty in the risk assessment for *G. affinis* could be reduced considerably by generating data on water temperature variability within a river, river section, or creek. If the daily water temperature does fall below the lower lethal limit of *G. affinis*, this could result in elimination of the population in that area. However, environmental variability could lead to microhabitats that support these fish during the

winter, allowing these fish to survive much like they do in Nebraska. Determination of the effects *G. affinis* have on SOC and minnows in this assessment would also reduce uncertainty. Data on the efficacy of *G. affinis* in Montana needs to be determined with respect to mosquito species as well, to judge if these fish are worth the risk of introduction.

Although quantitative risk assessments are almost always preferable to qualitative assessments (Cox Jr. et al. 2005), they are not always possible. In risk assessments of biological invasions, there may be insufficient quantitative data to estimate the probability of establishment (Drake et al. 2006; Drake and Lodge 2006). Consequently, qualitative risk assessments fill this void and can be quickly adaptable as new information becomes available. Qualitative risk models can incorporate inputs from experts as well as from the public, which can strengthen public trust in the decision-making process (Gentile and Harwell 2001). Further, these risk assessments can be used to guide future research (Landis and Wiegiers 1997).

Gambusia affinis has been documented to be invasive in other locations. Because of the high intrinsic rate of population increase of *G. affinis*, wide physiological tolerance, favorability by humans, large native range, and previous invasion history, we believe *G. affinis* may pose an unacceptable risk to species of economic, ecological, and social importance in certain locations if it were to become established in Montana rivers and creeks (Moyle and Light 1996; Goodwin et al. 1999; Kolar and Lodge 2001, 2002; Marchetti et al. 2004b, c).

There is a considerable need for the discipline of biological control to incorporate more rigorous methods to evaluate risks of deleterious effects on the environment. We have demonstrated here how the risk assessment paradigm can be used for biological control agents, much like it has been for invasive species (Colnar and Landis 2007). Steps should be taken by managers and public health officials to address the uncertainties in our assessment with respect to efficacy against mosquito species (specifically *C. tarsalis*) and possible deleterious effects on species of concern as well as native minnows. We believe that the qualitative risk model we used for *G. affinis* is a straight forward approach for stakeholders and managers to evaluate the risks and benefits posed by the release of biological control agents into new environments.

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