Diversity of gastropods in Turnbull National Wildlife Refuge, WA and the regional spread of the invasive European ear snail (*Radix auricularia)*

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Abstract

Establishing base-line knowledge of native snails in an area and their relative abundance is important in understanding the role of snails in their environment and the possible use of snails as a bioindicator. This information can also be used to determine how an invasive species is affecting the native populations over time. Six genera of gastropods were found in the wetland ponds of Turnbull National Wildlife Refuge (TNWR) from April to June 2012, including the invasive European ear snail (*Radix auricularia*) which was found in four lakes it was not previously known to inhabit. A dominant snail genus was found for each lake, with Middle Pines Lake being dominated by *Radix auricularia*. Conductivity was the only abiotic factor studied that positively correlated with genera richness ($p = 0.035$).

Introduction

Freshwater mollusks (gastropods and bivalves) are a diverse group with over 7,000 species, more than 4,000 of which are gastropods (Lydeard *et al* 2004; Strong *et al* 2008). Freshwater gastropods are found on every continent except Antarctica and inhabit almost every type of freshwater source (Strong *et al* 2008). Freshwater mollusks often form narrow endemic ranges, including seasonal ponds and ground water sources (Strong *et al* 2008). Freshwater gastropods are so specific to their locations that even a single drainage or an isolated spring can be the sole habitat for some species (Brown *et al* 2008; Prezant and Chapman 2004).

Gastropods are also one of the most imperiled groups with over 700 species listed as threatened by the *IUCN Redlist of Threatened Species* (Lydeard *et al* 2004). Due to a lack of even base line data concerning gastropod distribution, abundance, and life history, this number may be a gross underestimate (Strong *et al* 2008). In North America, over 60% of freshwater snails are listed as imperiled or presumed extinct (Lysne *et al* 2008). The main factors for the drop off in snail biodiversity are habitat loss and degradation, water pollution, the introduction of fish and other invasive species (Lysne *et al* 2008; Strong *et al* 2008). Invasive snails compete with natives for habitat and sometimes food sources and may change the ecosystem functions or parasite populations in their new locations (Brown *et al* 2008). There are 37 nonnative gastropod species known in North America (Wesselingh *et al* 1999).

Most gastropods are important grazers in benthic freshwater ecosystems. Gastropods can reduce periphyton biomass by over 50% (Brown *et al* 2008) and change the community structure of algae through selective grazing (Gresens 1995, McCollum *et al* 1998, Kawata *et al* 2001, Liess and Kahlert 2007). Riley *et al* (2008) found that hydrobiidae snails reduced chlorophyll *a* by 66%, but had no significant reduction on the ash-free dry mass (AFDM) of periphyton indicating that these snails graze selectively on photosynthetic periphyton. Gastropod grazing creates

fragmented patches in algae that create dynamic habitats which influence periphyton diversity and growth (Kawata *et al* 2001). The high grazing rates of snails results in changes to the cycling of nutrients in freshwater ecosystems (Brown *et al* 2008) which can result in higher gross primary productive (Gresens 1995, Liess and Kahlert 2007).

Mollusks are often used as a bioindicator to help asses an index of ecological quality of aquatic ecosystems (Rosenberg and Resh 1993 cited by Prezant and Chapman 2004). A bioindicator is a species or group of species who are sensitive to changes in their habitat. While many sensitive species decline in the presence of only a small amount of a transient toxin, the change in the bioindicator population may indicate the toxins occurrence in the system. And some populations may actually increase in the presence of unfavorable conditions. *Physa,* for example, excel in septic conditions while Planorbids excel in low dissolved oxygen conditions (Goodnight 1973). These population shifts and declines can help indicate that something in the environment has changed and warrants further investigation. But in order to be used as a bioindicator, a base line study must be performed so that there is a relative amount of richness and abundance for future studies (Prezant and Chapman 2004). Our study of gastropods in Turnbull National Wildlife Refuge (TNWR) will be one of the few surveys conducted for Eastern Washington. This data is especially important due to the documented presence of the invasive snail, *Radix auricularia*, in TNWR (Kipp et al 2012).

Radix auricularia are native to Eastern Europe and prefer large lakes and rivers with hard water (Adam and Lewis 1992). They are a biennial iteroparous species that reproduces during the summer (Adam and Lewis 1992). *R. auricularia* reaches a maximum shell length of 40 mm. While *R. auricularia* prefer lentic environments, invasions do occurred in loctic environments; *R. auricularia* have established a population in the higher Columbia River (Sytsma *et al* 2004). *Radix auricularia* have been found in 22 states in America and 5 provinces in Canada (Brown *et*

73 *al* 2008). Adam and Lewis have shown that in permanent lentic environments, *R. auricularia* can
74 outcompete native Lymnaea species (1992). *Radix* have been established as a host species for a
75 variety of parasites that inhabit, humans, piscivorous birds, and livestock (Chai *et al* 2009;
76 Cipriani *et al* 2011, Yadav *et al* 2007).
77 The Turnbull National Wildlife Refuge (TNWR) was formed 15,000 years ago by Ice Age
78 floods which scoured out sloughs, seasonally filled potholes and wetlands (Turnbull National
79 Wildlife Refuge 2007). This diverse area provides exceptional habitat for wildlife and plant
80 diversity; including ducks, geese and other waterfowl for nesting and during migration (Turnbull
81 National Wildlife Refuge 2007). In the 1920s much of the wetlands were filled in and
82 interconnected by drainage ditches (Turnbull National Wildlife Refuge 2007). These man-made
83 waterways still interconnect some of the wetlands. TNWR owns 15,859 acres of land, 16% of
84 which are encompassed by wetlands, over 2,500 acres (Turnbull National Wildlife Refuge 2007).
85 Ponds and lakes range in size from 0.1 to >400 acres (Turnbull National Wildlife Refuge 2007).
86 The upper portions of three drainages are within the reserve's boundaries; Cow Creek, Hangman
87 Creek and Rock Creek (Turnbull National Wildlife Refuge 2007). This habitat is critical for many
88 animals especially waterfowl., Turnbull NWR has recently been shown to be an even more
89 productive areas for waterfowl than the Prairie Pothole region of North Dakota which is known
90 world-wide for waterfowl production (Turnbull National Wildlife Refuge 2007). Turnbull is one
91 of the last areas left for good breeding and resting areas for waterfowl in the area. For this reason
92 it is critical habitat that needs to be protected and productive (Davidson and Rule 2006).
93 In our current gastropod survey of ponds in TNWR we have several predictions. We
94 hypothesis that snail diversity will be higher in ponds with higher conductivity, pH, and dissolved
95 oxygen. We expect number of snail genera to range from two to four for each pond.
96 Additionally, ponds closer together will have more similar taxa of snails than those farther apart.

We expect to find snails from the Lymnaeidae, Physidae, and Planorbidae families. The invasive snail, *Radix auricularia*, will be found in ponds near Middle Pines Lake, but will be absent from other areas in the refuge. The null hypothesis is that no difference between snail diversity will exist between ponds.

Methods

Site Area – The following eight lakes were sampled on Turnbull National Wildlife Refuge: 30 Acre Lake, Blackhorse Lake, Crater Pond, Eagle Pond, Kepple Lake, Lower Finley Lake, Middle Pines Lake and Turnbull Lab Pond. Each lake had three sampling locations which were separated by at least 100 meters. GPS coordinates were taken at each site and recorded in decimal degrees. Samples were taken within the first meter of the shoreline.

Figure 1. Map of the study sites on Turnbull National Wildlife Refuge with dominant snail taxa represented by the outline color of each lake.

Diversity of gastropods- Snails were sampled using a D-frame dip net for a total of three minutes of sampling effort at each sample location for a total lake sampling effort of nine minutes. We used the shoreline sampling and preservation method outlined by Prezant and Chapman, although we used a stack of sieves from 500 micrometer to 2.8 mm instead of a single 5 mm sieve (2004). Specimens were preserved in 70% EtOH and stored at Eastern Washington University to be keyed to genus utilizing Thorp and Covich's Freshwater Invertebrate dichotomous key (2001). Snails measurements of total shell length (TSL) from apex to the

bottom of the aperture and aperture width (AW) were taken for at least 200 individuals for each genus in each sample.

Abiotic and Biotic Conditions - Temperature, pH, conductivity, turbidity, and dissolved oxygen were measured at each site using a YSI 6000 series environmental probe. Biotic factors including chlorophyll *a*, presence of fish, and presence of the invasive snail, *Radix auricularia* were also taken for each lake. Chlorophyll *a* was determined using a YSI 6000 series environmental probe. Presence of fish was determined by directly observing fish in a lake or incidental capture during snail sampling. The invasive snail was sampled as described in the previous section.

Statistical Analysis –The relative abundance for each genus of snails in each lake was determined by dividing the total number of snails of one genus by the total number of snails for that location (lake). Single linear regressions and general linear models were used to determine if any abiotic factors are correlated to snail richness. Regression data was transformed using natural log to meet normality. Mean total shell length for each pond was compared for each genus of snails using one-way ANOVA to determine if snails of the same genus have significant size differences between lakes.

Results

Six different genera from three families of snails were found in Turnbull National Wildlife Refuge. The mean number of snail genera/lake was 4.75 with a range of 3 to 6 genera. The three families of snails were Lymnaeidae, Physidae, and Planorbidae as hypothesized. Three genera of Lymnaeidae, one genus of Physidae, and two genera of Planorbidae were found at different relative abundance in the eight lakes surveyed (Table 1).

Relative Abundance						
Lake/Pond	Genera of Snails					
	Gyraulus	*Planorbella*	*Physa*	*Lymnaea*	*Fossaria*	*Radix*
Turnbull Lab	44.63%	0.91%	34.64%	17.10%	2.72%	0.00%
Eagle	16.66%	0.27%	73.01%	9.45%	0.14%	0.48%
Crater	91.79%	0.17%	7.86%	0.17%	0.00%	0.00%
Lower Finley	64.55%	0.02%	5.47%	15.32%	9.63%	2.63%
Middle Pines	0.00%	6.70%	7.24%	0.00%	2.41%	83.65%
30 Acres	10.14%	0.00%	21.96%	67.91%	0.00%	0.00%
Kepple	9.15%	2.44%	2.44%	75.00%	0.00%	10.98%
Blackhorse	14.17%	4.05%	3.64%	77.73%	0.00%	0.40%

Table 1. Relative abundance of snails surveyed from eight lakes on Turnbull National Wildlife Refuge.

Each lake had one predominant genus with greater than 40% of the relative abundance, however, the dominant genus varied between lakes (Figure 2). *Gyraulus* was the dominate genus in Turnbull Lab Pond, Crater Pond, and Lower Finley Lake while *Physa* was the most abundant snail in Eagle Pond. *Lymnaea* was the most abundant genus at 30 Acres Lake, Kepple Lake, and Blackhorse Lake. The invasive snail, *Radix auricularia*, was found in five lakes with relative abundance between 0.40% in Blackhorse Lake and 83.65% in Middle Pines Lake (Table 1). *Radix* was the predominant genus at Middle Pines Lake. Lakes in closer proximity had the same predominant genus (Figure 1).

Conductivity was the only abiotic factor that was significantly correlated ($p=0.035$) to snail genera richness (Figure 3). Conductivity predicted 54.9% ($R^2 = 0.549$) of the differences between snail genra richness for the eight lakes surveyed. Chlorophyll *a*, presence of fish, and presence of the invasive snail did not significantly impact snail richness.

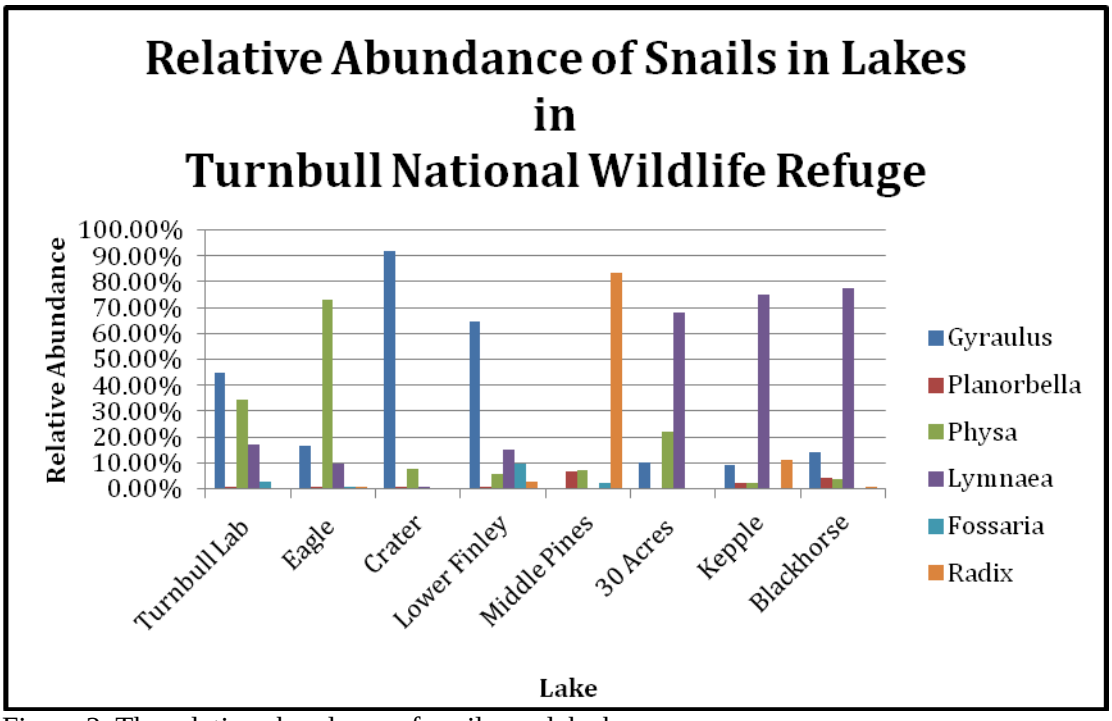

Figure 2. The relative abundance of snails per lake by genera.

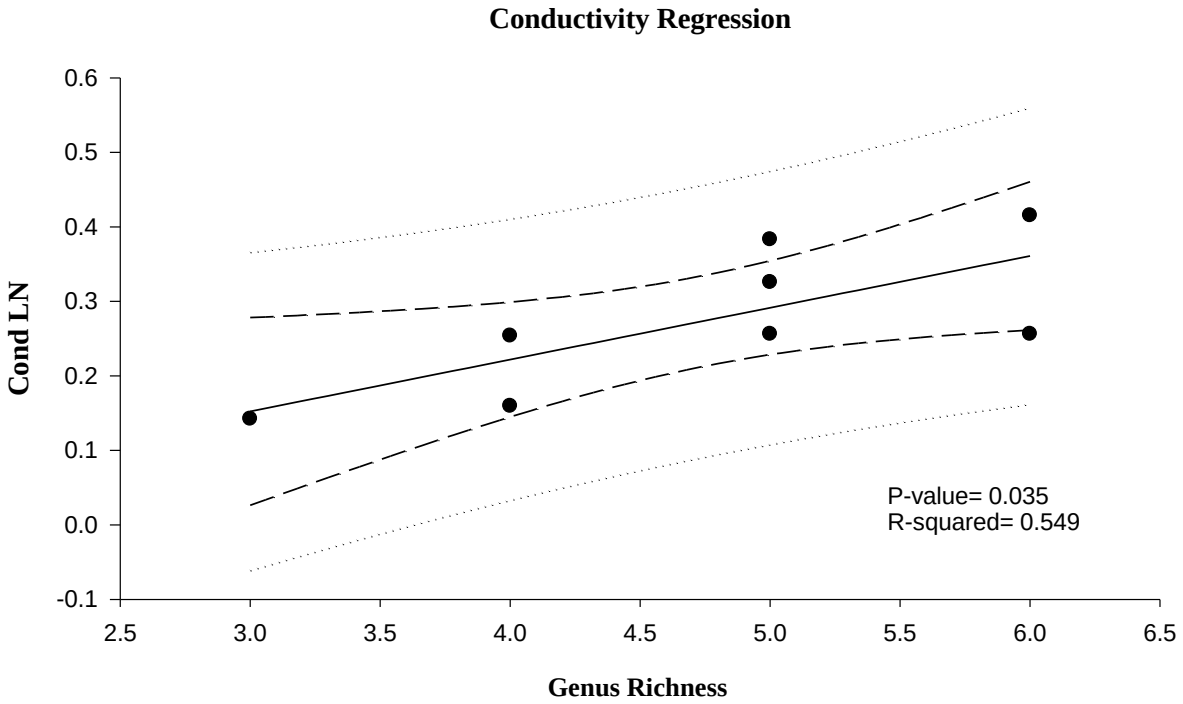

Figure 3. The relationship between genus richness and conductivity was shown to be significant ($p = 0.035$) and predicted 55% (R^2=0.549) of genus richness.

Two of the genra of snails showed a significant difference in size between lakes. *Physa* were significantly larger in total snail length ($p<0.05$) in Middle Pines Lake than in Turnbull Lab Pond, Eagle Pond, Crater Pond, and Lower Finley Lake (Figure 4). *Radix auricularia* were significantly larger in total snail length ($p<0.05$) in Middle Pines Lake than at any other location were these snails occurred (Figure 5). A significant difference in total snail length ($p<0.05$) for *Radix auricularia* was also seen between Lower Finley Lake and Blackhorse Lake with larger snails present in Blackhorse Lake (Figure 5).

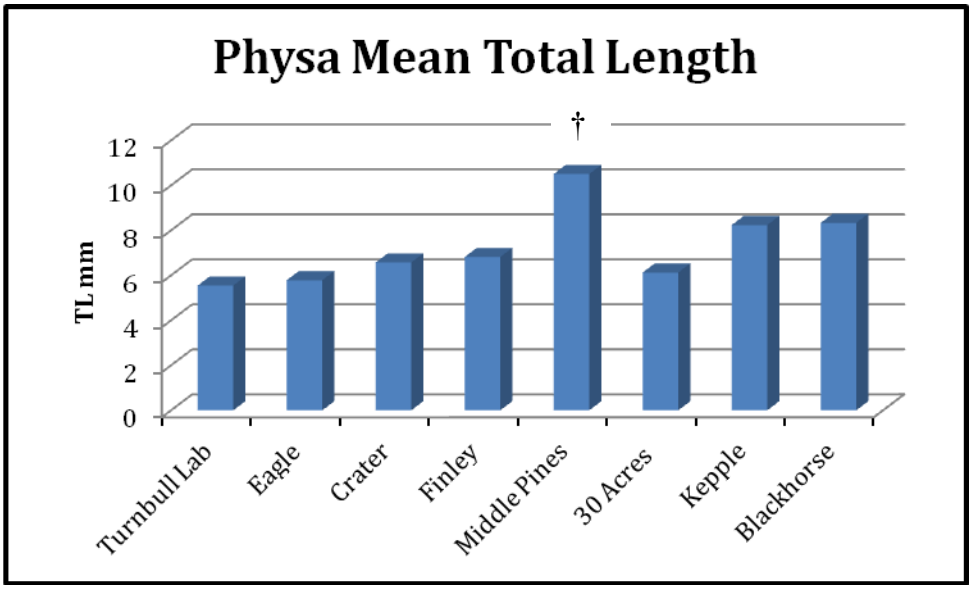

Figure 4. Graph illustrating the difference in *Physa* mean total length between ponds studied. The dagger represents the location of *Physa* with a significantly larger mean total length.

Discussion

We looked at the distribution of gastropods within TNWR in Eastern Washington. We found 3 – 6 genera of gastropods per lake with an average of 4.75 genera per lake. In a study in Wisconsin, Wojdak and Mittelback found an average of 2.25 species per lake (2005). Although we cannot make direct comparisons between these two studies, the Turnbull lakes may have higher species richness than what was seen in Wisconsin lakes.

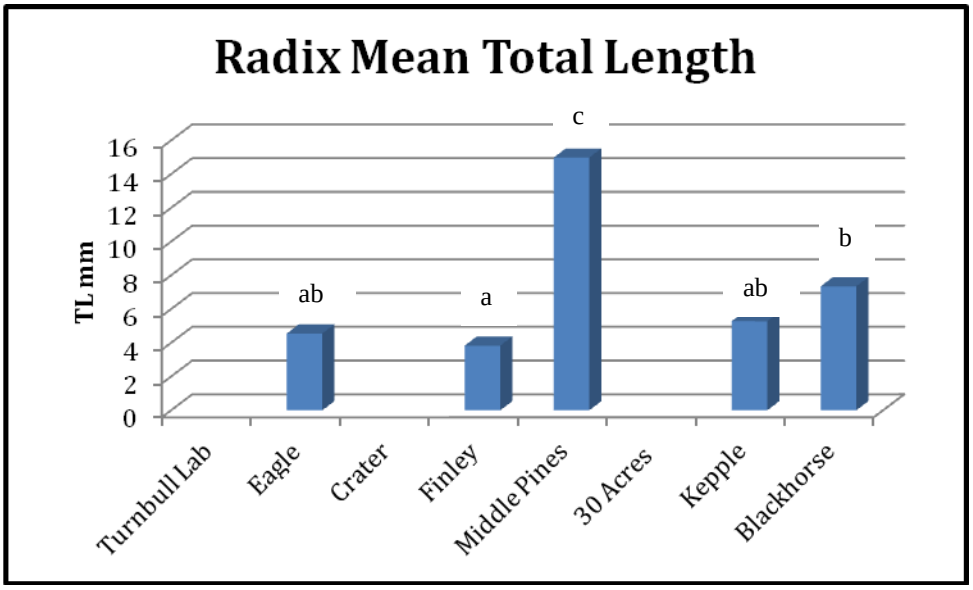

Figure 5. An average total length at each sample site of the genus *Radix*. The letters represent significant differences in mean total length of *Radix* between lakes.

The only significant abiotic factor in this study was conductivity, which was positively correlated with genera richness ($p = .035$, $R^2 = 0.549$). Conductivity is a measure of ions present in the water, including calcium ions. As calcium concentration is positively correlated with species richness of mollusks worldwide, if TWNR has high calcium ion concentrations, this may explain its relatively higher diversity compared to Wojdak and Mittelback's 2005 study (Dillon 2000).

A dominant snail genus was found for each lake. Eagle Pond was dominated by Physa while Turnbull Lab Pond, Crater pond and Lower Finley contained a majority of Gyraulus. 30 Acres Lake, Kepple Lake and Blackhorse contained Lymnaea and Middle Pines Lake was dominated by *Radix.* Possible explanations for the differences in dominant genera include the presence of flowing water in Middle Pines Lake, substrate variance between lakes, differences in dominant vegetation, lake surface area or the presence of another unknown organism. However these topics and variables were beyond the scope of our research. While the presence and absence

of fish was not found to be significant in our study, an exhaustive search for the presence of fish was not preformed. A more in-depth investigation as to the presence of fish in these lakes would need to be performed in order to confirm their influence is not a factor.

The invasive European ear snail was only known to inhabit Middles Pines Lake previously from a report in 1968 (Kipp *et al* 2012). From this survey we have determined that *Radix auricularia* is present in an additional four lakes; Eagle Pond, Kepple Lake, Lower Finley and Blackhorse Lake. It is now the dominant genus in Middle Pines Lake (83.65% relative abundance). Most freshwater pulmonate snails feed on periphyton and detritus and studies have shown that pulmonate snail survival rates decline during times of low food (Brown 1982; Osenburg 1989). If Radix and the native snails were competing for the same food source, but Radix has a better survival rate when food is scare, it could be outcompeting the native pulmonates.

The patchy distribution of some gastropods is attributed to the disbursal of these organisms via migratory birds (Boag 1986; Wesselingh *et al* 1999). Eggs and newly hatched juvenile gastropods can attach securely to duck's feet and feathers and be dispersed in this manner to nearby uncolonized lakes for up to 10 km (Boag 1986). As TNWR is a refuge for migrating waterfowl, this method of dispersal may account for *Radix's* dispersal to ponds not interconnected during high flow events. According to Hershler and colleagues, human activities can also transport gastropods effectively (2005). Middle Pines Lake, the first Lake reported to contain Radix is within the 2,200 acre public use area, which hosts approximate 30,000 visits per year (Turnbull National Wildlife Refuge 2007). This may account for how *Radix* initially made its way to Middle Pines Lake which is surrounded by a walking trail and is a popular bird watching site.

More research on the presence of *Radix* in Eastern Washington lakes would give a better understanding of the scope of this invasives' presence in these systems. The extent to which they compete with native snails could be determined through competition experiments, grazing trials, and stable isotope analysis. It would also be highly beneficial to determine if *Radix* established in Eastern Washington are harboring invasive trematode parasites that can affect waterfowl, livestock or humans to determine if there could be potential risks of transmission to these species.

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