Do disinfectants against chytrid fungus affect amphibians?

Great barred frog (*Mixophyes fasciolatus*) with severe Chytrid infection — note lethargic attitude and sloughing skin. Photo: L. Berger

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Introduction

Climatic changes and diseases are both great threats to amphibians and it is not clear whether they act together or separate (Lips et al. 2008). Particularly the epidemic fungus *Batrachochytriumdendrobatidis* (Order Chytridiales, Longcore et al., 1999) causing the disease chytridiomycosis is a growing danger to amphibians worldwide and effective methods to prevent further spreading into pathogen-free populations are necessary and recommended.

The origin of *B. dendrobatidis* is considered to be Africa, where the disease is common in *Xenopus* frogs without causing mass mortality or declines(Weldon et al. 2004; Lips et al. 2008). The first evidenceis from 1938 and before 1961 no detection of the pathogen outside Africa was made(Weldon et al. 2004). The global trade of these frogs for medical and research purposes leads to a dispersal of the pathogenall over the world. Additionally, the American bullfrog (*Ranacatesbeiana*) acts as a vector and the earliest evidence for the occurrence of *B. dendrobatidis*in *R. catesbeiana* is from South Carolina in 1978(Weldon et al. 2004). This species as well has been traded worldwide, this time as food item.

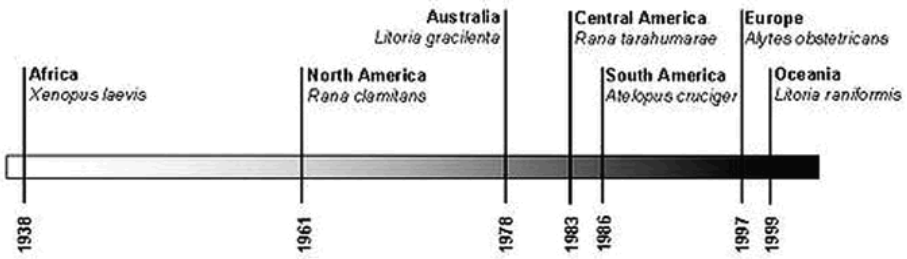

Figure 1: First evidence of *B. dendrobatidis* in different countries (Weldon et al. 2004)

The disease can spread wave like and the detection of declines and extinctions of local populations is often delayed by years, which makes it difficult to detect the initial introduction of the pathogen (Lips et al. 2008). The speed of expansion at local natural dispersion is low (>25 km/year) and high at continental human caused dispersal (>282 km/year) (Lips et al. 2008). Little is known whether the pathogen is present in nondeclining populations and therefore the recent distribution area is underestimated

(Lips et al. 2008). This underestimation of the infected area makes it crucial to take action against further spreading of the disease.

It has already been found in North, Central and South America, Australia, New Zealand and Europe causing sometimes sudden mass mortalities in some species or even extinctions (Bosch, Martinez 2006). The current distribution of *B. dendrobatidis*in Europe as well is not exactly known but at least three species are directly endangered through the disease due to die-offs or declines(Garner et al. 2005). Evidence for the occurrence in Europe is known from Spain, Portugal, Italy, Switzerland and Great Britain where the chytrid fungus has been found in 20 of 28 amphibian species examined since 1998(Garner et al. 2005).

Massive die-offs of midwife toads (*Alytesobstetricans*), common toads (*Bufobufo*) and salamanders (*Salamandrasalamandra*) have been reported from Spain (Bosch et al. 2001; Bosch, Martinez 2006). The infection prevalence in Spain and in Switzerland is exceptionally high but in the latter no die-offs have been reported until today and all infected animals were adults in good breeding condition(Garner et al. 2005).

The complex disease dynamics involves multiple factors between the host, the pathogen and the environment and factors like humidity and temperature affect both amphibians and chytridiomycosis (Lips et al. 2008). Certain factors like cool temperatures and moisture promote the survival and spread and thus the epidemic could not spread autonomously through lowland areas (Lips et al. 2008). Habitat destruction mainly takes place in lower elevations and many species find refuges in montane regions. Chytridiomycosis poses new threats because it favours lower temperatures and affects particularly these undestroyed habitats (Bosch, Martinez 2006). Therefore human dispersal of this pathogen is the main reason for further spreading and effective measures have to be applied to prevent further damage. Conservation actions increasing connectivity and thus the genetic exchange may improve the resistance against this infection (Bosch, Martinez 2006).

The chytrid fungus infects only the keratinized tissue of amphibians like the skin of adults and the mouthparts of tadpoles, humans are not threatened. The two life stages

of the fungus, intracellular sporangium and free-swimming zoospores, enables it to survive in water bodies without hosts. How the disease kills amphibians is currently not known. It is possible that the fungus either releases a toxin or it changes the skin of the host resulting in disordered functions concerning respiration or water uptake. Infected amphibians can effectively be treated with fungicide but that is not applicable to wild populations (ZSL 2008).

Useable and effective procedures have to be provided to all who work regularly with amphibians in the field like researchers and other amphibian enthusiasts to prevent the introduction or transmission of the pathogen into uninfected sites. Each individual water body has to be considered as a separate site and sites where the chytrid fungus is not known to be present should be sampled first, followed by sampling of infected areas(NSW National Parks and Wildlife Service 2001). Footwear, all the equipment and eventually even car tires must be well cleaned and disinfected after and between each visit of every site. Disinfection solutions must be effective against bacteria and both the vegetative and spore stages of fungi. Any release of the disinfecting solution into the environment should be avoided because common used disinfectants pose a potential threat due to their toxic properties. Similar field studies, which tested possible impacts of insecticides on amphibians showed both direct and indirect effects, for example changes in the food resources due to reduced zooplankton and increased algal growth (Boone et al. 2004).

Particularly for scientific research where multiple sites are sampled it is important to disinfect the equipment to prevent further spreading of the pathogen. As disinfectants are toxic and some people fear that the toxic effects of the disinfectants could be worse than chytridiomycosisitself, it is important to find out if conventional disinfectants against the chytrid fungus affect the larvae of amphibians negatively.

We tested if common multi-purpose disinfectant agents against the chytrid fungus affect the larvae of two species of amphibians negatively.

Methods and Materials

Study species and disinfectants

For the experiment we used larvae from the common toad (*Bufo bufo*, Linnaeus 1758) and the grass frog (*Rana temporaria,* Linnaeus 1758) because of their broad distribution and availability.

Common multi-purpose disinfectant agents against the chytrid fungus are bleach (Javelle water) and Virkon S® (Antec International - A DuPont Company, Sudbury GB). Both are effective virucidal, bactericidal and fungicidal products. Javelle water is an aqueous solution of potassium or sodium hypochlorite, used as a disinfectant and bleaching agent. We used a standard dilution of 2.5%. Virkon S® is an oxygen based disinfectant, active at low temperatures and very hard water and is considered biosafe because it does not display effects of acute toxicity by exposure to skin or by ingestion and is readily biodegradable within 7 days. We used a standard dilution of 10 grams Virkon S® per litre water, which is used for instrument disinfection.

Mesocosm set-up

Tadpoles were held in plastic tubs (0.28 m^2, 80 L) located outdoors near Strickhof at the University of Zurich. These tubs were filled with city tap water and enriched with 40 g of dry leaves and 2 g of Chinchilla food. In addition, 1 litre of phytoplankton rich water and two aliquots of zooplankton from nearby ponds were added. In each tub one pond snail *Lymnaea* sp. was placed. As a result, a small self-sustaining pond ecosystem was mimicked. This also provides near natural conditions for the testing of the disinfectants.

After a few days tadpoles were added. They were taken from various ponds located on the Zürichberg and randomly distributed in the tubs. From each species ten individuals were added in each tub. The *B. bufo* tadpoles had a mean mass of 17,6 mg and the *R. temporaria* tadpoles of 20,2 mg.

Treatments

Overall, there were six different treatments: two different disinfectants and one control, each applied at a high and a low dose. These six treatment combinations were replicated five times. For the treatment combinations we used following abbreviations: Ch=Control in a high dose; Cl= Control in a low dose; Jh= Javelle in a high dose; Jl= Javelle in a low dose; Vh= Virkon S in a high dose; Vl= Virkon S in a low dose.

In order to simulate the amount of disinfectant that could enter a pond on the field through disinfected materials, we immersed a gumboot in water and then measured the volume of dripping water. This was replicated three times and with boots of different sizes. All the repetitions gave about 0.4 dl per boot pair as a result. Since on the field the material is dried before the next usage, this measured amount presumably too high and represents a worst-case dose. Therefore this amount was defined as the amount in the treatment with high dose and a tenth of it with low dose (i.e., 0.04 dl). Consequently, the results from our tests were 0.4 dl and 0.04 dl. The respective amounts of disinfectants were always filled up with tap water to 1 dl, so that all tubs received the same amount of liquids. In the tubs of the controls 1 dl of tap water was added. Like this the effect of the addition of a liquid is controlled for.

The treatments were repeated each week over a period of three weeks. The first treatment was applied one day after having added the tadpoles.

Collecting data

The tadpoles were controlled once a day whereas the visible ones were counted and the activity of each was recorded. Species were counted separately. The activity of tadpoles was divided into “swimming”, “feeding” and “resting”.

The mass of each species was determined at the beginning and the end of the experiment. Therefore, at the beginning of the experiment 40 individuals where weighed at once in order to obtain a better value, since they showed a very small mass. This procedure was repeated three times for each. For the data at the end of the experiment,

all surviving tadpoles of one species in one tub were weighed together and an averaged value was calculated.

However, for statistical analysis only final mass were used because the tadpoles had very similar mass at the beginning of the experiment. Like this a comparison between the tadpoles reared under different conditions were compared and not growth itself.

Additionally, the zooplankton and phytoplankton were measured in order to examine whether the disinfectants also affect other aspects of the mesocosm. For the zooplankton, one litre of water was taken from the centre of each tub and sifted with a plankton filter. The zooplankton, kept in a solution of 94% ethanol and some sugar, was counted under a binocular. Here daphnids, ostracods and copepods were counted separately.

For the analysis of the phytoplankton the same litre was taken and filtered through a paper filter with a vacuum pump. The filters were previously dried for 17 hours at 60ºC and weighed before filtration. They were dried and massed again after the filtering in order to obtain the biomass of present phytoplankton.

Statistical analysis

We analysed several traits that describe the response of tadpoles to experimental treatments: survival, mass and behaviour (swimming, resting, feeding).

The values for visible tadpoles were transformed into a new proportional value by diving them by the expected surviving tadpoles of that day. The expected number of surviving tadpoles for each day was calculated with a linear regression through the starting number of tadpoles (in our experiment always ten) and the surviving number of tadpoles at the end of the experiment. This was made for each tub separately. The activity values were divided by the visible tadpoles in order to obtain a proportion.

For statistical analysis the program R was used. The significance was tested with Anova except for the data for surviving. Here an analysis of deviance was applied, because these data are binomial. We defined a P-value lower than 0.05 as significant.

In order to test whether the density influences the mass of the tadpoles, we included the number of surviving tadpoles in the Anova as a covariate. This was done with the surviving tadpoles of the same species as well as with the total number of surviving tadpoles in one tub, i.e. of both species.

For the analyses of activities and weight the treatment Jh was excluded since there were no surviving tadpoles. For analyzing the survival and the zooplankton, the respective values were zero and included into the analyses.

Additionally, Excel was used to make the graphs concerning the behaviour.

After the experiments the tadpoles were released back in the ponds where they were caught.

Results

Survival of tadpoles of *R. temporaria* and *B. bufo*

The survival rate of *R. temporaria* tadpoles as well as *B. bufo* tadpoles was high in the control tubs and the tubs treated with Vh and Vl. The tadpoles of the tubs treated with Jl instead showed broad distribution of survivors varying from two up to ten survivors (Figure 2, Figure 3).

All of the tadpoles in tubs exposed to Jh died within a day after the first application of the disinfectant.

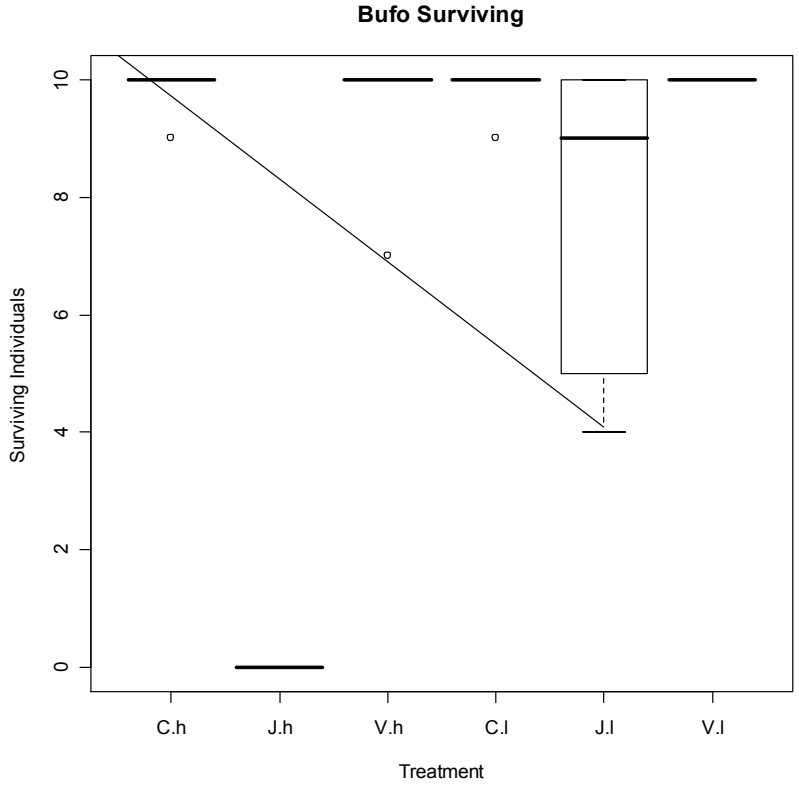

Figure 2: Boxplot of surviving individuals of *B. bufo* tadpoles with respect to the six different treatments.

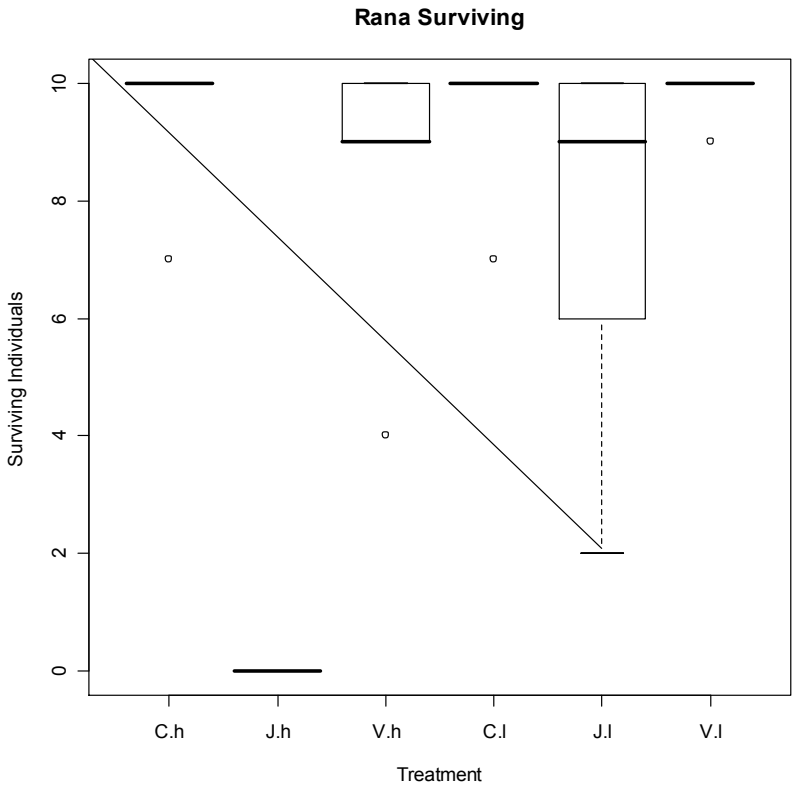

Figure 3: Boxplot of surviving individuals of *R. temporaria* tadpoles with respect to the six different treatments.

Testing the survival by using Anova revealed that the factors treatment and dose had a highly significant effect on the survival of *R. temporaria* and *B. bufo* tadpoles respectively (for P values see Table 1).

Furthermore, the interaction of treatment and dose displayed a significant effect on survival of *R. temporaria* tadpoles ($P<0.001$) whereas the interaction showed only a trend on the survival of *B. bufo* tadpoles ($P<0.1$).

Table 1: Summary of analysis of deviance for survival for tadpoles of *R. temporaria* and *B. bufo*.

Factor	Df	Deviance	F	P
R. temporaria				
Treatment	2	106.142	17.4729	**<0.0001**
Dose	1	60.507	19.9210	**0.0002**
Treatment x dose	2	20.715	3.4102	**0.0497**
B. bufo				
Treatment	2	139.288	42.7143	**<0.0001**
Dose	1	72.695	44.5854	**<0.0001**
Treatment x dose	2	9.262	2.8403	0.0781

Mass of *B. bufo* tadpoles

Analysis of the boxplot of the individual mass of *B. bufo* tadpoles (Figure 4) showed a similar distribution of mass in all the different treatments, excluding Jh. A slight increase of mass of tadpoles that were exposed to Jl could be found but the interaction treatment-by-dose was not significant. This trend is also visible in the Anova (Table 2) in which the influence of the treatment Jl on the individual mass was nearly significant ($P<0.1$).

Table 2: Summary of Anova for the individual mass [in g] for *B. bufo*.

Factor	Df	MS	F	P
Treatment	2	0.0028	2.8270	0.0829
Dose	1	<0.0001	0.0622	0.8055
Treatment x dose	2	0.0007	0.7633	0.3927
Residuals	20	0.0010		

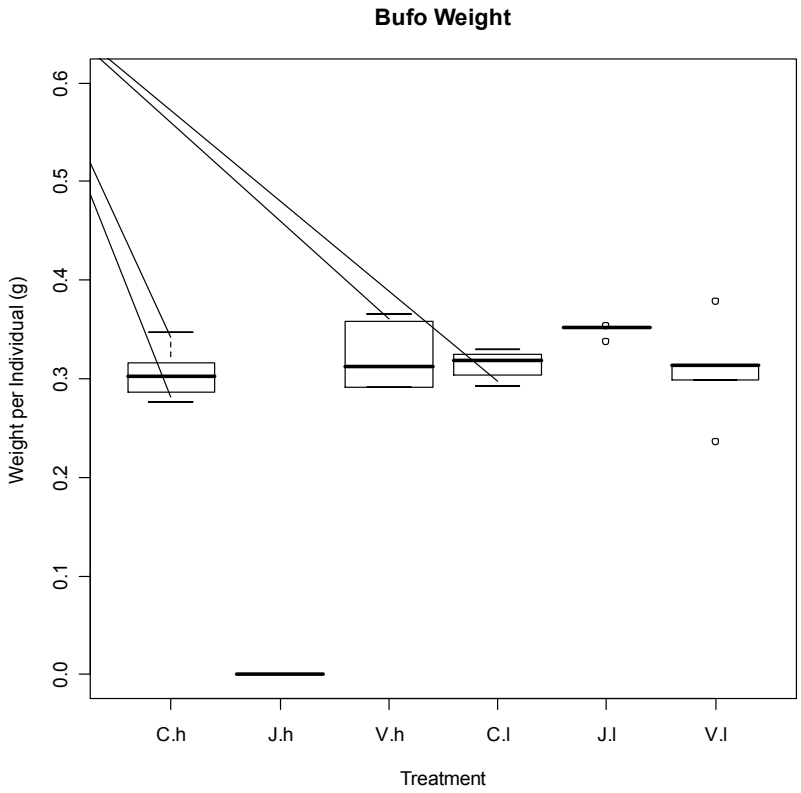

Figure 4: Boxplot of the mass distribution [in g] of B. bufo tadpoles with respect to the six different treatments

The Anova (Table 3) including the number of surviving tadpoles revealed that the body mass of a tadpole is significantly influenced by the number of surviving tadpoles of the same species ($P<0.05$). The analysis was conducted once again, but that time including the total number of surviving tadpoles (i.e. both species added up). This second analysis assumes that the total number of survivors determines the response to density and not simply the number of co-specifics. The results obtained (table is not listed) were significant as well but showed an almost equal P-value ($P<0.05$) compared to the above mentioned P-value of the number of surviving tadpoles.

Table 3: Summary of Anova for the individual mass [in g] for *B. bufo* considering density effects.

Factor	Df	MS	F	P
Number surviving	1	0.0047	4.8290	**0.0406**
Treatment	2	0.001	0.9895	0.3901
Dose	1	<0.0001	0.0194	0.8907
Treatment x dose	2	0.0006	0.5891	0.4522
Residuals	19	0.0010		

Mass of *R. temporaria* tadpoles

The boxplot of the individual mass of *R. temporaria* tadpoles (Figure 5) displayed a clearly higher mass per individual in tadpoles exposed to JI compared to all the other treatments. The results of Anova (Table 4) showed that the treatments differ significantly among each other and influence the individual mass of a *R. temporaria* tadpole ($P<0.01$).

Table 4: Summary of Anova for the individual mass [in g] for *R. temporaria*.

Factor	Df	MS	F	P
Treatment	2	0.0295	7.9925	**0.0028**
Dose	1	<0.0001	0.0001	0.9916
Treatment x dose	2	0.0011	0.2914	0.5953
Residuals	20	0.0037		

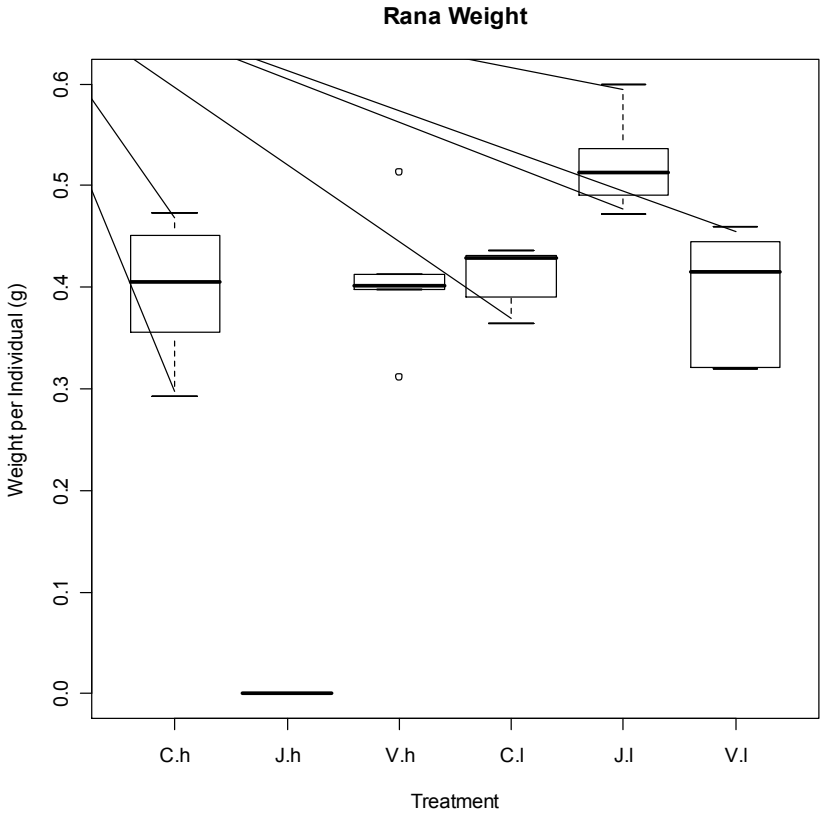

Figure 5: Boxplot of the mass distribution [in g] of *R. temporaria* tadpoles with respect to the six different treatments.

The results of Anova (Table 5) revealed that the mass of a tadpole is significantly affected by the number of surviving tadpoles of the same species (P<0.01). As for *B. bufo* a second analysis was conducted including the total number of surviving tadpoles of both species. The analysis (table is not listed) showed significant results and the P-value (P<0.001) was very similar compared to the P-value for the number of surviving tadpoles. Additionally, the analysis showed that the kind of treatment had a significant effect on the individual mass of a *R. temporaria* tadpole.

Table 5: Summary of Anova for the individual mass [in g] for *R. temporaria* considering density effects.

Factor	Df	MS	F	P
Number surviving	1	0.0418	13.6458	**0.0015**
Treatment	2	0.0166	5.4139	**0.0138**
Dose	1	0.0004	0.1338	0.7186
Treatment x dose	2	0.0001	0.0453	0.8337
Residuals	19	0.0031		

Activity

After we had analysed the exact data of weight and survival we wanted to examine the following behavioural features: visible, resting, feeding and swimming animals.

Regarding Figure 6 the fraction of visible tadpoles is clearly different between the two species, except for the treatment with Javelle water at a low dose. There are generally much more *B. bufo* visible than *R. temporaria*. The only significant result of Anova was a different visibility in relation to the different treatments for *R. temporaria* ($P<0.05$, Table 6). This species did not react significantly to the difference in dose and to the interaction of treatment and dose. *B. bufo* did not react to any of these factors (Table 6).

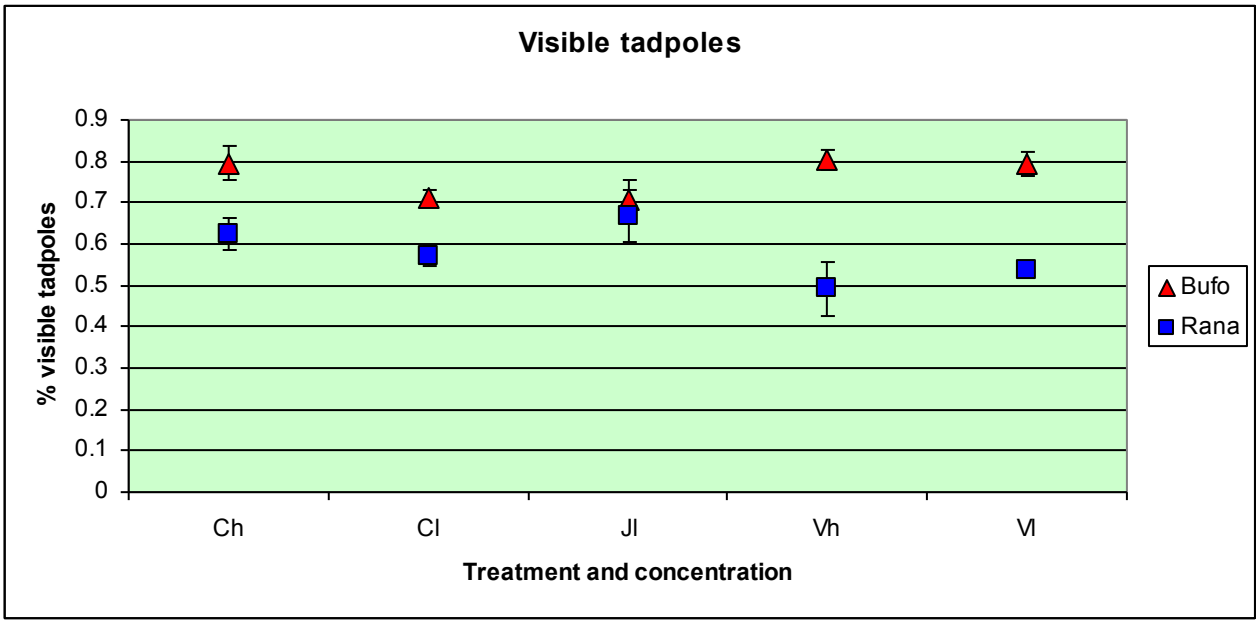

Figure 6: Fraction of visible tadpoles with standard errors.

Table 6: Summary of Anova of percent of visible tadpoles of R. temporaria and B. bufo

Factor	Df	MS	F	P
R. temporaria				
Treatment	2	0.0417	3.8617	**0.0381**
Dose	1	<0.0001	0.0034	0.9954
Treatment x dose	1	0.0127	1.1831	0.2896
Residuals	20	0.0108		
B. bufo				
Treatment	2	0.0143	2.4002	0.1116
Dose	1	0.0109	1.8294	0.1913
Treatment x dose	1	0.0066	1.1136	0.3039
Residuals	20	0.0059		

In Figure 7 and 8 the red arrows show the days when we added the different treatments. For *B. bufo* there was no visible trend to observe, except a decrease on day 19 and 20. For *R. temporaria* there is a decrease in visibility on the days 9 and 16,

which are the days after treatment. In addition a net decrease is to observe again in the last days, especially day 19 and 20 which are the days after the treatments.

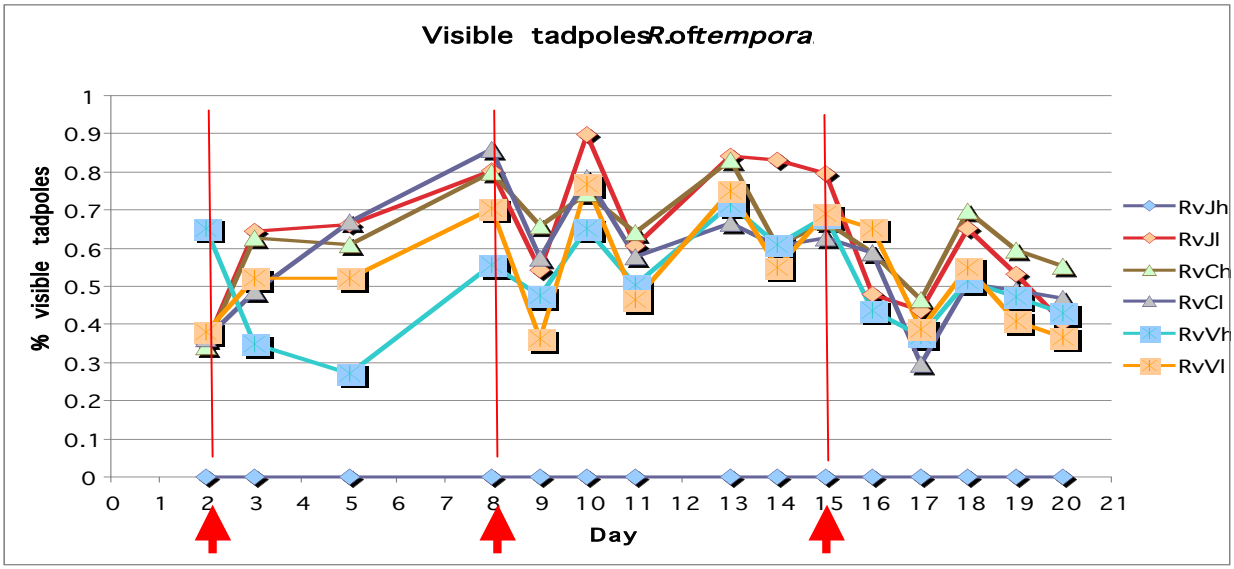

Figure 7: Percentage of visible tadpoles of *R. temporaria* in the course of days. The red arrows are the days of treatment (Rv: percent of visible *R. temporaria*).

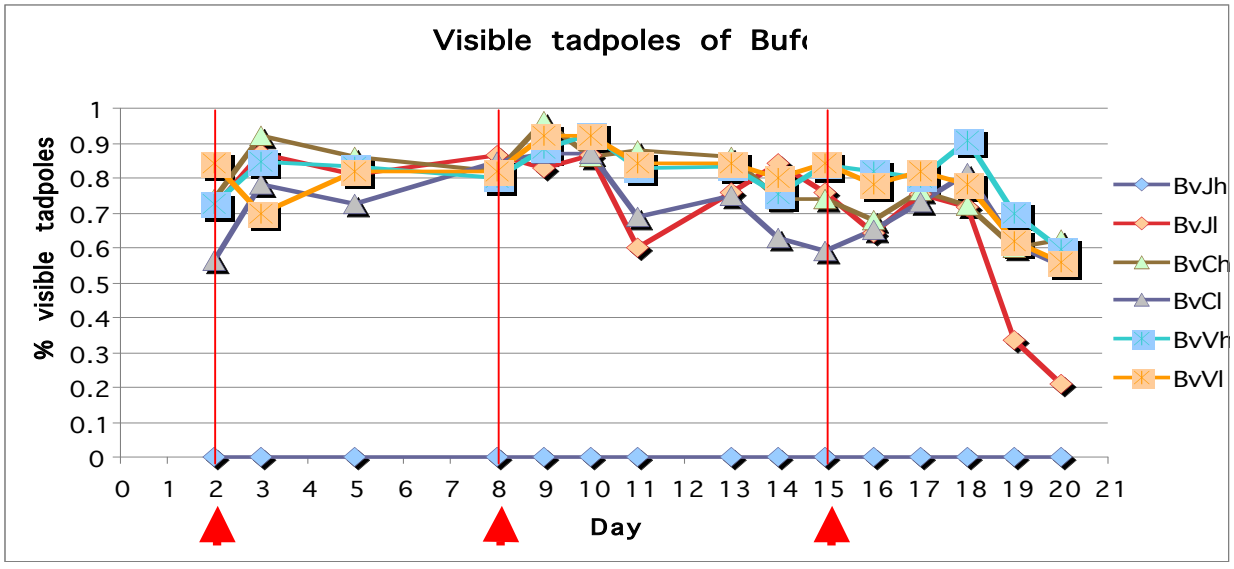

Figure 8: Percentage of visible fraction of tadpoles of *B. bufo* in the course of days. The red arrows are the days of treatment. (Bv: percent of visible *B. bufo*)

In Figure 9 we could see again a net difference between both species. Less *B. bufo* tadpoles were resting. Here again, after analysing the data with Anova, we found only a significant difference comparing the fraction of resting *R. temporaria* in the different

treatments (P<0.05, Table 7). For the other factors there was no significance. For *B. bufo* again there were no significant differences.

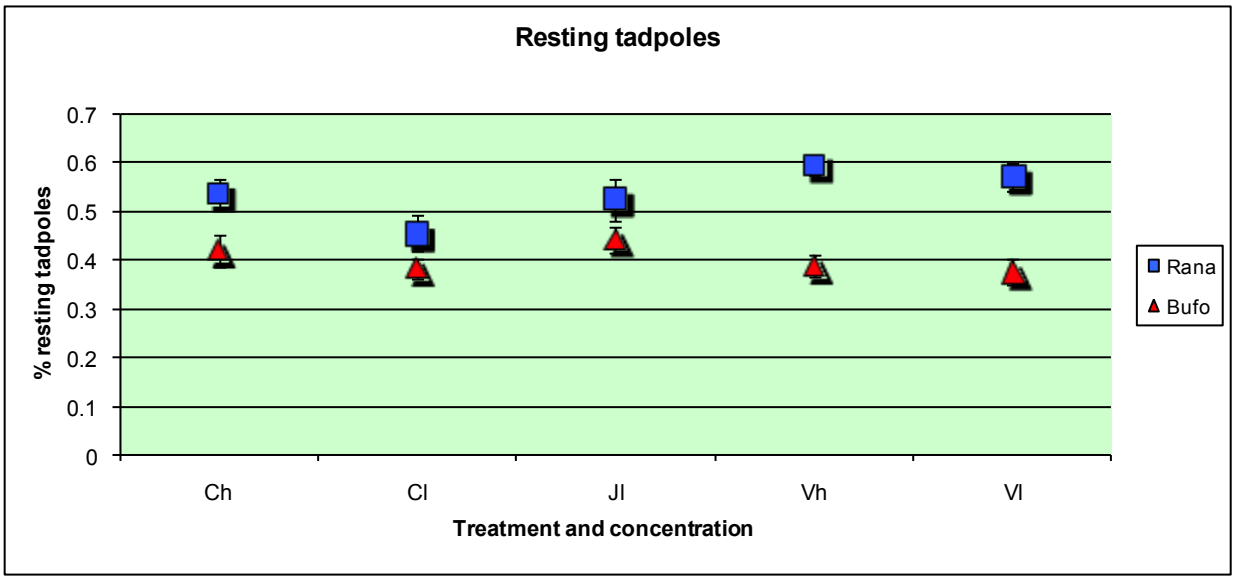

Figure 9: Fraction of the resting tadpoles with standard errors.

Table 7: Summary of Anova of percent of resting tadpoles of *R. temporaria* and *B. bufo*

Factor	Df	MS	F	P
R. temporaria				
Treatment	2	0.0193	3.5489	**0.0479**
Dose	1	0.0138	2.5483	0.1261
Treatment x dose	1	0.0041	0.7594	0.3393
Residuals	20	0.0054		
B. bufo				
Treatment	2	0.0059	1.4167	0.2658
Dose	1	0.0029	0.7111	0.4091
Treatment x dose	1	0.0008	0.191	0.6668
Residuals	20	0.0041		

In Figure 10 we could again clearly recognize a difference in percentage of feeding tadpoles between both species. The fraction of feeding *B. bufo* is higher than the one of *R. temporaria*. After analysing the data with Anova (Table 8) we found neither significance nor a trend.

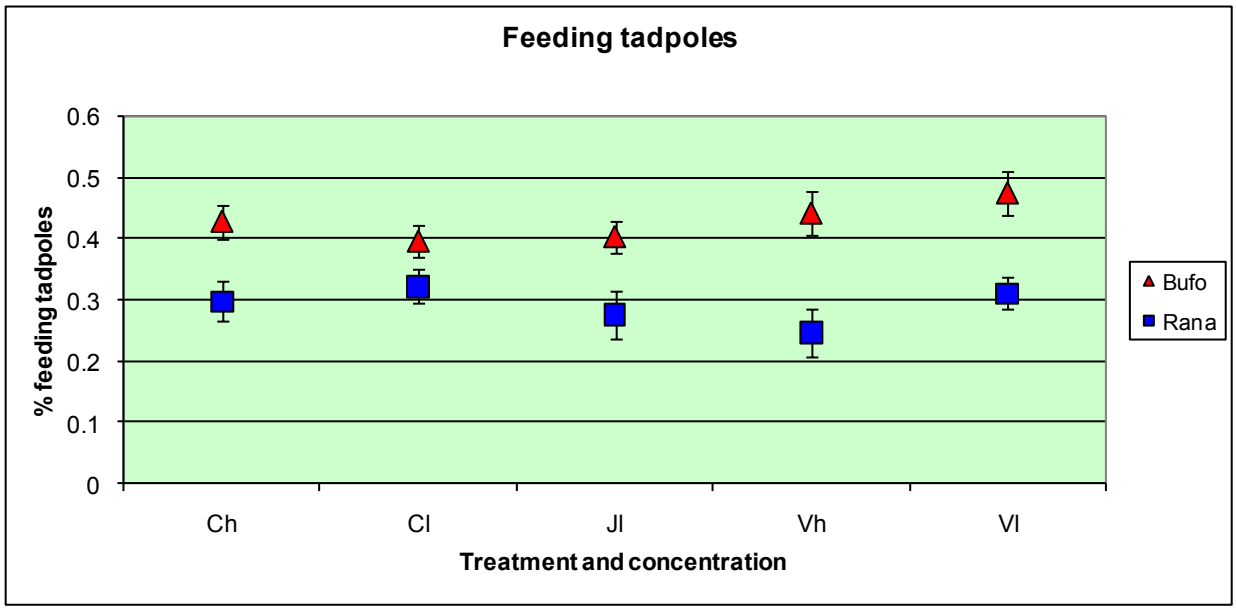

Figure 10: Fraction of feeding tadpoles with standard errors.

Table 8: Summary of the Anova of percent of feeding tadpoles of *R. temporaria and B. bufo*

Factor	Df	MS	F	P
R. temporaria				
Treatment	2	0.0026	0.4903	0.6204
Dose	1	0.0100	1.8422	0.1915
Treatment x dose	1	0.0018	0.3471	0.5631
Residuals	18	0.0054		
B. bufo				
Treatment	2	0.0071	1.5396	0.2388
Dose	1	<0.0001	0.0001	0.9943
Treatment x dose	1	0.0051	1.1194	0.3027
Residuals	20	0.0046		

Concerning the fraction of swimming tadpoles we did not really see differences between the species, except maybe in the treatment with Virkon at a high dose where there were relatively more *B. bufo* than *R. Temporaria* (Figure11). After the Anova analyses of both species separately (Table 9) we found no significant differences for the factor in none of the species. Indeed, we found a trend for both species ($P<0.1$) regarding the differences between the different treatments.

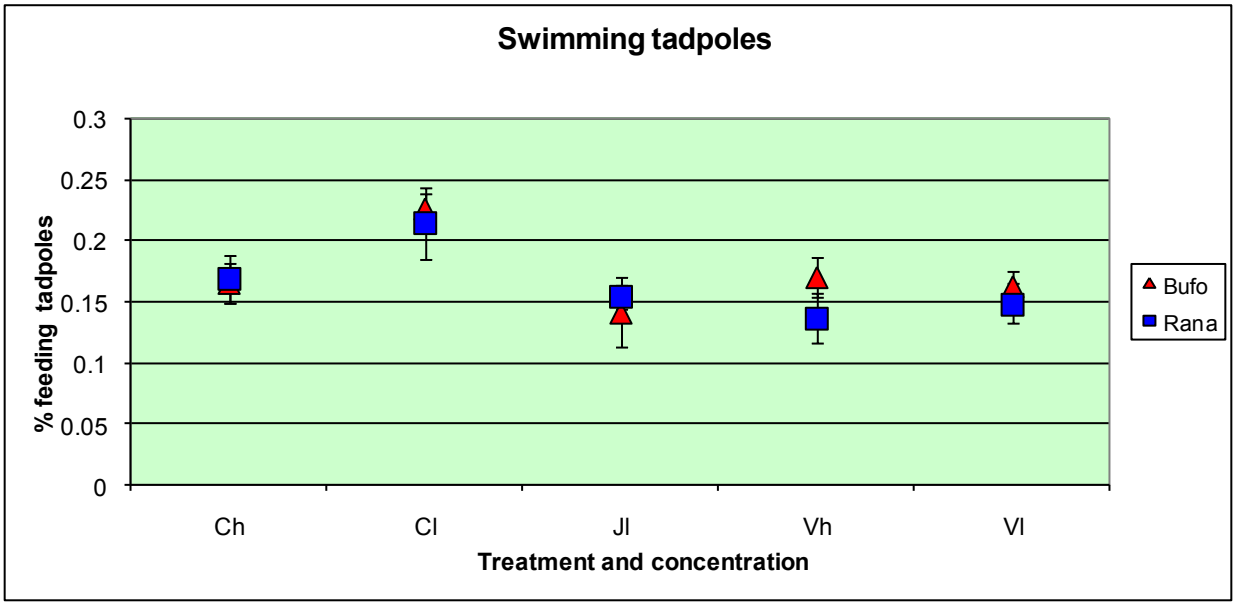

Figure 11: Fraction of swimming tadpoles with standard errors.

Table 9: Summary of Anova of percent of swimming tadpoles of *R. temporaria* and *B. bufo*

Factor	Df	MS	F	P
R. temporaria				
Treatment	2	0.0064	3.2521	0.0598
Dose	1	0.0041	2.0754	0.1651
Treatment x dose	1	0.0013	0.6934	0.4148
Residuals	20	0.0019		
B. bufo				
Treatment	2	0.0053	3.3738	0.0546
Dose	1	0.0035	2.2448	0.1496
Treatment x dose	1	0.0058	3.6662	0.0699
Residuals	20	0.0015		

Zooplankton

The abundance of zooplankton presented in the boxplot (Figure 12) was determined mainly by Copepoda as it was the most abundant and varying species. In the boxplot we can observe far less zooplankton in tubs exposed to Jl compared to both controls and Vl. Tubs treated with Vh seemed to have a slightly reduced number of zooplankton as well.

Analysis of variance (Table 10) revealed that the treatments differ significantly among each other ($P<0.001$). Hence the disinfectant Jl had an effect on the total number of zooplankton. The dose instead only showed a slight tendency ($P<0.1$) to influence the total number of zooplankton. The interaction of the treatment and dose ($P<0.05$) was significant.

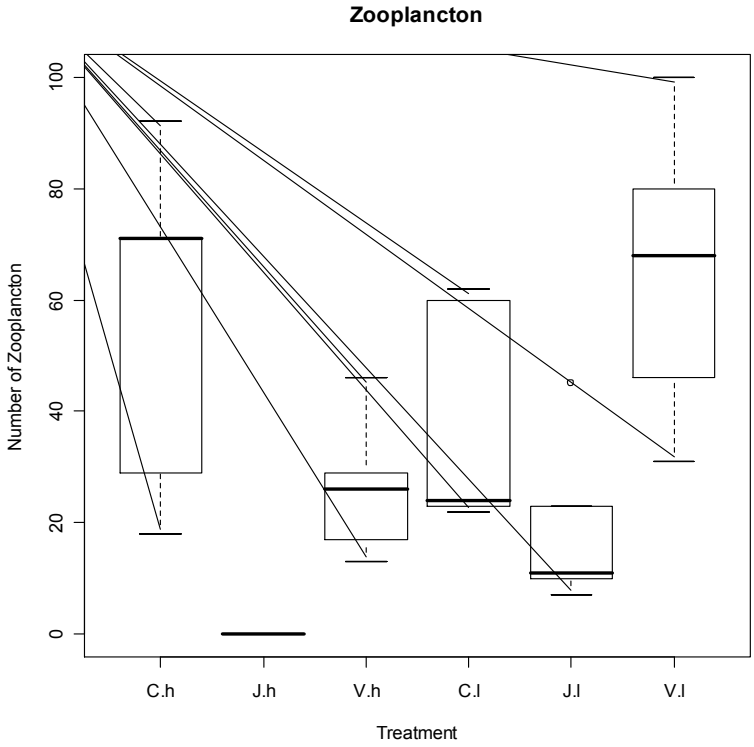

Figure 12: Boxplot of zooplankton abundance (Copepoda and *Daphnia*) with respect to the six different treatments.

Table 10: Summary of Anova for zooplankton.

Factor	Df	MS	F	P
Treatment	2	4520.5	10.5628	**0.0005**
Dose	1	1333.3	3.1155	0.0903
Treatment x dose	2	2080.9	4.8624	**0.0169**
Residuals	24	428.0		

Discussion

To put the whole in a nutshell there were differences between the treatments Javelle water and Virkon, the latter showing similar results as the controls. Javelle water affected mainly the survival. Especially in a high dose since there were no surviving tadpoles after the first treatment. Furthermore, Javelle water in a low dose slightly reduced the survival rate of tadpoles but positively influenced indirectly the mass of the remaining tadpoles. Both disinfectants (J and V) had no influences on the activities of a tadpole neither in high nor in low doses.

Survival of tadpoles of *R. temporaria* and *B. bufo*

The survival rate of tadpoles in all the treatments (except Jh) was very high compared to a normal survival rate of about 5% that can be observed in natural ponds (Alford and Wilbur 1985). The increased survival rate is due to the fact that in the artificial mesocosm were no predators present and the algae provided ensured enough food.

The fact that all tadpoles exposed to the disinfectant Javelle water in high dose died shortly after being exposed to the disinfectant shows that it was too large dosed in relation to the 80 litres tubes. One explanation could be that in our pre-experiment the measured volume of water dripping of the gumboots was unrealistic calculated because the testing tubs just contain a volume of 80 litres compared to natural ponds that are much bigger. Therefore adding Javelle water in high dose was not a representative simulation of a natural contamination because under natural conditions it will rarely happen that such a high dose can enter a natural pond. In natural ponds the disinfectant can distribute over a larger scale and hence the disinfectant will be diluted which results in a weakened effect on aquatic organisms like tadpoles. Nevertheless, the 100% mortality of tadpoles exposed to Jh demonstrates its dangerous and even lethal effect on tadpoles. Besides, the treatment Jl showed negative effects on survival of tadpoles as well. Furthermore, in two of five Jh treated tubs the *Lymneae* sp. died and in all Jh treated tubs no zooplankton could be found which represents further evidence for the

deleterious effect of Javelle water and implies that its use to disinfect field equipment should be avoided.

The Anova of the survival of both *R. temporaria* and *B. bufo* tadpoles (Table 1) showed that the factors treatment and dose had a highly significant influence on tadpole survival. It can therefore be concluded that tadpoles show a reaction to the different treatments, especially to Jh. The level of dose is an important factor for survival as well. The high dose of Javelle water killed all the tadpoles whereas the low dose revealed only a decreased survival rate compared to the other treatments. Tadpoles exposed to Virkon, high and low dose, did not show a significant increase in mortality so that it can be concluded that Virkon is less dangerous to tadpoles than Javelle water when used in the recommended dose. Therefore our experiment recommends using Virkon as disinfectant against the chytrid fungus.

Mass of tadpoles of *R. temporaria* and *B. bufo*

Our results obtained for the individual mass (Figure 3, Figure 4) indicate that *R. temporaria* tadpoles in Jl treated tubs were significantly heavier than in the other treatments. Combined with the result that Jl treated tadpoles showed higher a mortality rate compared to the other treatments one explanation could be that because of the reduced density the surviving tadpoles had more food and could therefore grow faster. It can be assumed that Jl causes stress in tadpoles but the surviving tadpoles can compensate this stress factor when more food is available so that they can gain in mass and in size. As more tadpoles die the density and hence the competition on food resources within and between the species is reduced. This results in more food available per individual. Additionally, in Jl treated tubs the number of zooplankton per litre was lower compared to the other treatments. This fact is consistent with our observations: exposed to Jl the zooplankton declines which can result in an increased algal growth. We could observe an increased growth of algae in three of five tubs exposed to Jl as they were much greener than the other tubs.

Our mass data including density effects suggests that density plays an important role in how heavy a single tadpole can get. Increasing density means increasing competition on resources but our results do not show that the mass of one species is affected by the presence of the other species. 0It seems that the mass and growth of an individual tadpole of *R. temporaria* or *B. bufo* is mostly controlled by the extent of density.

For *B. bufo* tadpoles JI has only an indirect influence on the mass by affecting the density.

For *R. temporaria* tadpoles we found that the disinfectant Javelle water in low dose has a direct effect on the mass of a tadpole as well as an indirect effect by affecting the density (survival of the tadpoles) and hence the growth and mass.

These findings imply that *R. temporaria* tadpoles react more sensitive to the disinfectant Javelle water than *B. bufo* tadpoles as the disinfectant has a direct influence on the tadpole itself. In order to find out more about these internal, physiological damages a disinfectant can cause further experiments are necessary. Thus it would be possible to get not only externally visible signs as an increased mortality rate or a reduced mass compared to healthy, untreated tadpoles but it would also detect how a disinfectant acts in the body of a tadpole.

Activity

The fact that the *B. bufo* tadpoles are far more visible than these from *R. temporaria* can probably be explained by the different behaviours of the two species. As a matter of fact, the tadpoles of *R. temporaria* tend to hide for protection. On the contrary, because of their toxicity the *B. bufo* tadpoles do not behave the same way. That is probably why they are more visible while being counted. However, *R. temporaria* is less visible firstly because it hides, secondly because they have a similar colour as the leaves. The fact that we found a significant difference in visibility from *R. temporaria* in the different treatments probably shows a sensibility of this species to Javelle water and Virkon. Apparently there is no such a sensibility in *B. bufo*.

Concerning the course of days we did not carry out a statistical test. We found no real trend in relation with treatments for *B. Bufo* species. The decrease of visible tadpoles on day 19 and 20 is correlated with the bad weather conditions on these days for both species. The decrease of visibility of *R. temporaria* after the second and third time we applied the treatments to the tubs seems to match quite good with the observation mentioned before that *R. temporaria* seems to be more sensitive to Javelle water and Virkon.

The fact that there are more *R. temporaria* than *B. bufo* which seem to be resting can be again explained by their protection behaviour, they try not to move so that predators do not detect them. We found that the *R. temporaria* reacted, concerning resting, differently to the different treatments, but not to the dose. *B. bufo* did not show a similar reaction. This strengthens the hypothesis that *R. temporaria*, but not *B. bufo*, showed a reaction in behaviour depending on the treatments.

The two species seemed to show no significantly different feeding reactions regarding the different treatments or doses.

Interestingly, for swimming behaviour, the two species reacted quite similarly. That is probably due to the fact that only very few swimming tadpoles were counted which would mean that this data can not be analysed for its siginificance.

Zooplankton

The fact that in Jl treated tubs less zooplankton is present than in the other treatments suggests that zooplankton is affected negatively by the disinfectant. Together with the observation that Jl tubs contain heavier tadpoles, especially of *R. temporaria*, it can be concluded that the reduction of the zooplankton resulted both in more algal mass which provided more food for the remaining tadpoles and in a reduced competition between zooplankton and tadpoles on algal food. Furthermore, the slightly reduced number of zooplankton in Vh treated tubs indicates that the zooplankton reacts probably more sensitive to the disinfectants than the tadpoles.

Phytoplankton

By measuring the amount of phytoplankton we used filters with too large pores so that probably most of it went through. Therefore the obtained data was useless and we exluded it from further analyses.

Outlook

Our experiment can be seen as a pilot study to test possible negative effects of the two most common used disinfectants against the chytridiomycosis disease. Therefore, it is certainly necessary to repeat the experiment in order to confirm the existing evidence that Jh negatively affects tadpoles whereas Virkon has no effects. This is important because our finding that Javelle water is very dangerous for tadpoles and other aquatic organisms could act as a guide for people using disinfectants in the field. Additionally, repeating the experiment over a longer time span could reveal if the disinfectants also influence amphibians negatively in later life stages.

We could show that the disinfectant Javelle water in high dose does affect amphibian tadpoles negatively. Our findings strongly indicate that it is worth reconsidering the nationally and internationally recommended use of Javelle water as a disinfectant against the fungus *B. dendrobatidis.*

Therefore we suggest to use Virkon as a disinfectant against the chytridiomycosis disease and to avoid using Javelle water at all.

Literature

ALFORD, R. A.; WILBUR, H. M. (1985): Priority effects in experimental pond communities: competition between Bufo and Rana. Ecology 66:1097–1105

BOONE, M. D.; SEMLITSCH, R. D.; FAIRCHILD, J: F.; ROTHERMEL, B. B. (2004): Effects of an insecticide an amphibians in large-scale experimental ponds. Ecological Applications 14: 685–691

BOSCH, J.; MARTINEZ, I.; GARCIA-PARIS, M. (2001): Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytesobstetricans*) in protected areas of central Spain. Biological Conservation 97: 331-337

BOSCH, J.; MARTINEZ, I. (2006): Chytrid fungus infection related to unusual mortalities of *Salamandrasalamandra* and *Bufobufo* in the Peñalara Natural Park, Spain. Oryx Vol. 40 No 1: 84-89

GARNER, T.: WALKER, S.; BOSCH, J.; HYATT, A.; CUNNINGHAM, A.; FISHER, M. (2005): Chytrid fungus in EuropeEmerging Infectious. Diseases 11:1639–1641

LIPS, KR.; DIFFENDORFER, J.; MENDELSON, JR. III; SEARS, MW. (2008): Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. PLoS Biology 6: 441-454.

NSW National Parks and Wildlife Service (2001): Hygiene protocol for the control of disease in frogs. NSW NPWS, Hurstville, New South Wales, Australia, 16 p.

The Zoological Society of London. UK Chytridiomycosis survey (A ZSL/NE partnership). Available from https://www.zsl.org/field-conservation/uk-native-species/ukchytridiomycosis,842,AR.html

SCHMIDT, B (2007): Die Chytridiomykose: Eine neue gefährliche Pilzerkrankung der Amphibien. Available fromhttp://www.karch.ch/karch/d/ath/chytri/media/Chytridiomykose_PDF_De.pdf

WELDON, C.; du PREEZ, L. H.;HYATT, A. D.; MUELLER, R.; SPEARE, R. (2004): Origin of the Amphibian Chytrid Fungus. Emerging Infectious Diseases. Vol. 10, No. 12: 2100–2105