

ACTION PLAN FOR THE FIRE-BELLIED TOAD *BOMBINA BOMBINA* IN LATVIA: ASSESSMENT OF THE IMPLEMENTATION FOR TEN YEARS, RELEASING FROM AQUACULTURE AND RESTORATION OF HABITATS IN 2006-2016

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Fire-bellied toad *Bombina bombina* (Linnaeus, 1761) inhabits only the Southern part of Latvia and is very rare and preserved species. *B. bombina* Action Plan in Latvia (BAP in the next text) was developed in 2006 and approved in 2007. The BAP described threats for *B. bombina* in Latvia (invasive fish *Perccottus glenii*, native fishes, degradation of biotopes etc.) and suggested measures for conservation (protection of territories, optimization of habitats, releasing in wild, education etc.). In the study implementation of BAP Sub-actions was estimated in points. The implementation of most BAP Sub-actions is notable increased after BAP.

Key words: *Bombina bombina*, conservation, Action plan, Latvia.

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INTRODUCTION

Fire-bellied toad *Bombina bombina* (Linnaeus, 1761) inhabits only the Southern part of Latvia (Siliņš, Lamsters 1934, Kuzmin et al. 2008). Populations of *B. bombina* are not big in number and are located in the distance of not more than 20 km from the Southern border of Latvia (Pupina & Pupins 2008). The rarity and paucity of *B. bombina* served as a reason for including the species in the list of Annexes to the Cabinet Regulations No.396 (Ministru kabinets... 2000) and in the lists of species for which it is allowed to create micro-reserves. Action plans for preserved species, which are called in Latvia “Plan of Conservation of Species” (“Sugas aizsardzibas

plans” in Latvian), are created for conservation of such species in Latvia according to the Latvian legislation (Saeima 2000). Such Plans reflect the state policies regarding the conservation of rare species, but are not provided directly with the state financing; but on their basis, during the execution of different projects, the plans for management of concrete populations are developed and realised (Pupina, Pupins 2012). Also, any actions related to the protected species in Latvia, should to be taken in accordance with the Action Plan for this species (Saeima 2000).

Bombina bombina Action Plan in Latvia (BAP in the next text) was developed in 2006 and approved in 2007 (Pupina & Pupina 2006).

In accordance with the Latvian legislation (Saeima 2000), BAP included the assessment of risks for the species and its habitats. In the BAP the negative factors were specified and ranked based on the risks for *B.bombina* population: 1) cold climate; 2) cold snowless winters; 3) cold short spring; 4) dry hot summer; 5) dilution of the population; 6) local species of fish; 7) uncontrolled introduction and relocation of fish in fishing farms; 8) introduction of new predatory species of fish (Chinese sleeper *Perccottus glenii*); 9) illegal introduction of new predatory species (*Trachemys scripta* and other alien freshwater turtles); 10) illegal introduction of other species of *Bombina* genus; 12) disturbance caused by human; 13) illegal capturing; 14) burning of dry grass in spring and fires.

As threats for *B.bombina* habitats in Latvia, the following negative factors were specified and ranked based on risk degree: 1) irrigation works; 2) extermination of beavers and their dams; 3) deforestation; 4) pollution of the environment; 5) construction of roads, buildings, settlements; 6) impact of roads; 7) clearing and modernization of ponds; 8) overgrowing of biotopes; 9) draining of ponds.

In the BAP in accordance with the Latvian legislation (Saeima 2000) these conservation actions were proposed: 1) Species Conservation; 2) Habitats Conservation; 3) Research and Monitoring; 4) Information and Education (Pupiņš, Pupina 2006).

This BAP should have been revised in 2011, but in accordance with the regulation in Latvia, provisions of the Plan remain in force until its revision. For ten years from 2006 to 2016 many of these recommended sub-actions were implemented, which led to changes in the condition of *B.bombina* population and its habitats in Latvia.

The assessment of the implementation of recommended activities in the BAP until 2016 can give important information for the *B.bombina* distribution, ecology and genetic researches, as well for practical protection of *B.bombina* in

Latvia, and for the development of a revised BAP, which serve as a basis for the following researches.

MATERIAL AND METHODS

The majority of the activities suggested in the BAP have a qualitative, not quantitative nature. It complicates a quantitative assessment regarding the implementation of BAP.

In this study, we provided the assessment only for those BAP sub-actions, for which it was possible to determine the exact quantitative results: the amount of dug out ponds, the area of the cut off bushes, the number of juvenile *B.bombina* which have been grown-up in the aquaculture and released into the nature, the amount of scientific publications etc. With the view of comparison of the implementation of different sub-actions for each implemented unit (one dug out pond, one cut off ha of bushes, one grown-up individual, one published publication, etc.), one point was granted (Table 1).

For the study, all the informational, educational and people targeted sub-actions from different actions were estimated as a part of "Information and Education" Action; all monitoring and research of the sub-actions – as a part of "Research and Monitoring" Action. Some similar Sub-actions were combined (described in the Results section). The difference between Sub-actions' implementation before and after BAP was calculated.

For the assessment of degree of the BAP implementation, we studied the *B.bombina* oriented projects' (5 projects) documentation, scientific articles and scientific-technical publications (47 issues), popular publications (3 issues), and conference materials (24 issues), analysed the three web-sites, related to the implementation of the BAP (the studied issues aren't in the references list, if not referenced). The final years of the Projects were considered to be the years of the implementation of any actions regarding the Project.

Table 1. System of the quantitative estimation of implementation of the BAP Actions and Sub-actions in real units and in points

Estimated Action and Sub-action	Sub-actions' index numbers in BAP, what were estimated	Real unit	Points for one unit
Species Conservation	1.		
Strengthening of weak populations	1.3	individual	1
Introduction of new populations	1.4	individual	1
Habitats Conservation	2.		
Establishing of the protected territories	2.1	protected territory	1
Land purchase	2.3	territory of subpopulation	1
Pond digging	2.4.1+2.7	pond	1
Bush cutting	2.4.2+2.7	ha	1
Research and Monitoring	3.		
Research in distribution, number and ecology <i>in-situ</i> and <i>ex-situ</i>	3.1+3.2+3.3+3.4+1.1	publication	1
Research in genetics	3.5	publication	1
Information and Education	4.		
Seminars	4.1	event	1
Brochure	4.2	piece	1
Web-site for landowners	4.3	IP address	1
Conferences	4.7+3.6	presentation	1

We conducted 6 interviews with project executors, researchers and workers of Zoos, 8 field expeditions to the *B.bombina* improved habitats, visited LIFE-HerpetoLatvia Breeding Centre, Daugavpils University, Rigas Zoo, Latgales Zoo and 6 sites of *B.bombina* releasing in wild.

RESULTS

As a result of the study, the estimation on the implementation of the “Species Conservation”, “Habitats Conservation”, “Research and Monitoring”, and “Information and Education” BAP Actions was obtained. It was discovered that, during the periods before and after BAP, the Actions and Sub-actions recommended in the BAP were implemented to different extent:

Action 1: Species Conservation

Sub-action 1.2: Creation of *B.bombina* population in aquaculture as a genetic reserve. Before and after BAP implemented by keeping and regular breeding of a group of *B.bombina* in Rigas Zoo (Helmanis 1990). After BAP small group of *B.bombina* kept in Latgales Zoo too. Due to the expansion of the known number of *B.bombina* populations in Latvia (Kuzmin et al. 2008; Pupiņa 2011), since 2011 the sub-action was recognized by the coordinators of the BAP as inexpedient for most of the populations and was not estimated in the study.

Sub-action 1.3: Strengthening of weak populations by individuals from aquaculture. The number of released individuals was estimated in the study. We didn't find any info about the Sub-action's implementation before BAP. After BAP 7 129

bred juveniles of *B.bombina* were released in the populations Ilgas, Ainavas, Bauska, Demene (LIFE-Bombina 2004, LIFE-Razna 2005, LIFE-HerpetoLatvia 2015, A.Pupina, pers.comm.) (Fig.1). The strengthening was successful, in all sites of releasing the stable reproducing of *B.bombina* was noted in the following years, tadpoles and juveniles were found by us in 2016.

Sub-action 1.4: Introduction and re-introduction of populations with individuals from aquaculture.

The number of released individuals was estimated in the study. Before BAP Rigas Zoo introduced in 1988 174 and in 1989 577 bred juveniles

B.bombina in Tervete Nature Park (Helmanis 1990), out-side of species natural area, also in Gobijas (G.Jansone, pers.comm. with no precise data), overwintered males were observed in Tervete Nature Park in 1989-1990, but no *B.bombina* were observed here in the next years (J.Zvirgzds, pers.comm.). After BAP introduction of 1 010 *B.bombina* juveniles to Nature Park Razna (LIFE-Razna 2005) was executed outside of *B.bombina* natural area, where overwintered adult *B.bombina* was registered in 2010 (Fig.2), the present situation needs for additional field research.

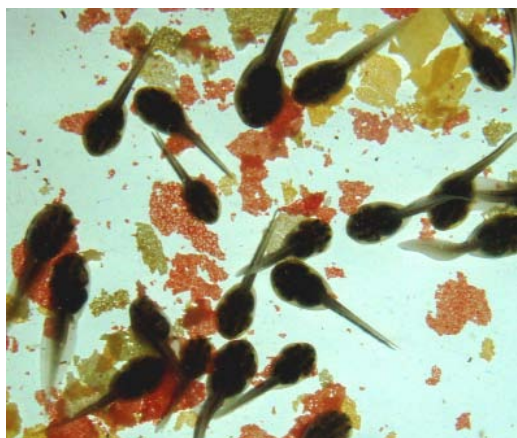


Fig. 1. *B.bombina* tadpoles in aquaculture in the Latgales Zoo.



Fig. 2. Overwintered adult of *B.bombina* in Nature Park Razna, 2010.06.11.

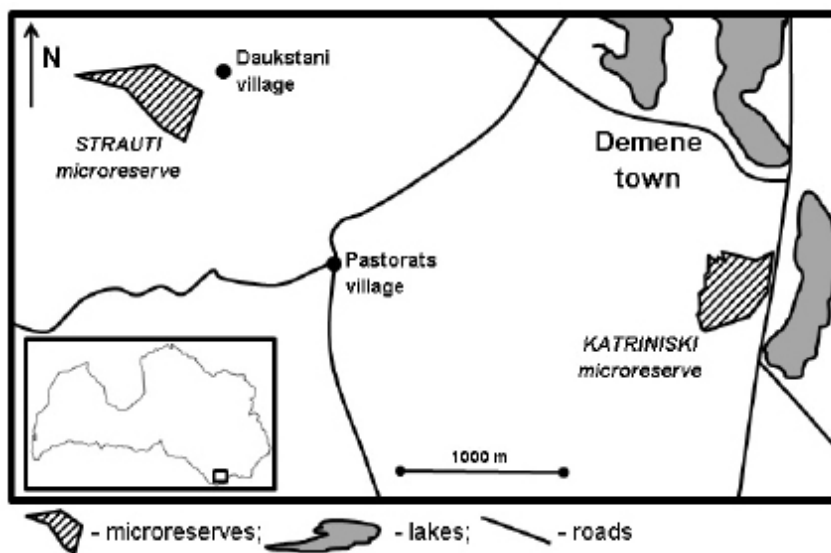


Fig.3. Two new micro-reserves Strauti and Katriniski, established for *B.bombina* after BAP.

Sub-action 1.5: Control of other species of *Bombina* genus importation in Latvia. The Sub-action was not estimated in the study, because there is no need for any additional control: the Sub-action is implemented within the framework of the current legislation, customs and veterinary control.

Action 2: Habitats Conservation

Sub-action 2.1: Establishing of the protected territories. The number of newly created protected territories was estimated in the study. Before BAP two Natura 2000 protected territories for *B. orientalis* were created in Bauska (Nature park Tervete) and in Silene Nature Park (micro-reserve Ilgas). After BAP two new Natura 2000 territories were created for *B. orientalis*: micro-reserves Strauti and Katriniski (both in Demene) (LIFE-HerpetoLatvia 2015) (Fig.3).

Sub-action 2.2: Regulation of human activities in the territories bordering with protected territories. The Sub-action was not estimated in the study, because it is implemented in accordance with the law regulations for these protected territories and doesn't need any additional measures.

Sub-action 2.3: Purchase of *B. orientalis* territories for state ownership. The number of purchased territories was estimated in the study. The Sub-action was not implemented before BAP, after BAP partly implemented by purchase in private ownership for conservation goals. Due to the lack of the state financing, the coordinator of the BAP purchased the land Strauti (Demene) for establishing of the micro-reserve Strauti, in which the land is the central part. Another BAP coordinator owns a plot of land in Daugavpils district, inhabited by the *B. orientalis* population Ainavas, which guarantees safety for these two populations.

Sub-action 2.4: Optimization of habitats. The Sub-action was divided into two parts: 2.4.1 – Digging of ponds and 2.4.2 – Cutting of bushes. The Sub-action 2.7 Creation of new biotopes was divided into 2.7.1 and 2.7.2 consequently and added to these Sub-actions.

Sub-action 2.4.1+2.7.1. Digging of ponds. Number of optimized or newly created ponds was estimated in the study. Before BAP there were created four new ponds in Nature Park Tervete (Helmanis 1990). After BAP 48 ponds were dug out or optimized in the areas of populations Ilgas, Ainavas, Bauska, Demene (LIFE-*Bombina* 2004; LIFE-Razna 2005; Project LVAFA 2006; LIFE-HerpetoLatvia 2015) (Fig.4).

Sub-action 2.4.2+2.7.2. Cutting of bushes. The area of bush cutting (in ha) was estimated in the study. We didn't find any data about the Sub-action implementation before BAP. After BAP 67.3 ha of bushes were cut in the areas of populations Ilgas, Ainavas, Bauska, Demene, and in Nature Park Razna (LIFE-Razna 2005; Project LVAFA 2006; LIFE-HerpetoLatvia 2015; authors' data). Sub-action 2.6: Arranging the places for overwintering. The Sub-action's implementation was not estimated in the study, because of the lack of information.

Sub-action 2.8: Protection of beavers *Castor fiber* in the areas of *B. orientalis* populations. Beaver protection is a difficult action, because it contradicts with pragmatic aims of land and forest owners. The Sub-action's implementation was not estimated in the study, because the activities regulated by law only.

Sub-action 2.9: Control of fish in the areas of *B. orientalis* populations. The sub-action is very important, especially for alien invasive



Fig. 4. Optimized pond for *B. orientalis* population in Katriniski.

Percottus glenii, which is found in *B.bombina* ponds (Pupiņš, Pupina 2012) (Fig.5), but it is practically implemented as a part of the Sub-action 2.4.1+2.7.1. Digging of ponds – as a creation of ponds, isolated from other water bodies. Therefore the Sub-action’s implementation wasn’t estimated separately in the study.

Sub-action 2.10: Control of potential alien predators. The Sub-action was not implemented purposefully before BAP. Creation and support of the shelter for *Trachemys scripta* and other alien freshwater turtles found in Latvia (Pupins 2007; Pupina, Pupins 2016) was implemented after BAP partially, because creation of special shelter for such turtles did not go through the projects’ competition. Therefore, facilities of Rigas Zoo

and Latgales Zoo, rather limited, were used for the keeping of these turtles, in Latgales Zoo kept 37 *Trachemys scripta* and other alien turtles in 2016. The Sub-action’s implementation wasn’t estimated in the study, because the number of the kept turtles is constantly changing.

Action 3. Research and Monitoring

Sub-action 3.1. Research of populations’ number and structure. In the study, all the estimated Research and Monitoring Sub-actions, excluding Genetic Research, were combined with Sub-actions 3.1: 3.2, 3.3, 3.4, 2.5, and 1.1. Number of scientific and technical publications was estimated in the study. The number was increased more for seven times after BAP.



Fig. 5. Invasive fish *Percottus glenii* from pond of *B.bombina* (Ainavas).

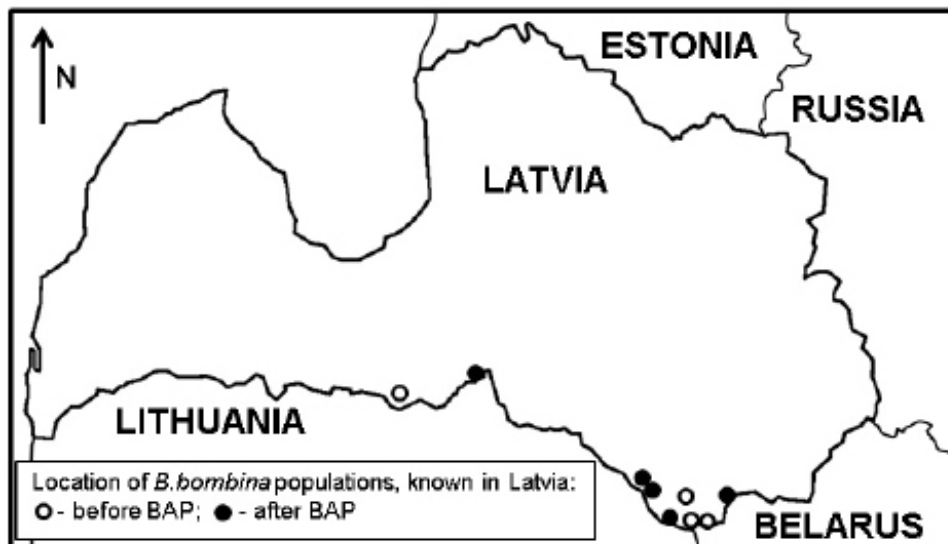


Fig. 6. *B.bombina* populations found in Latvia before and after BAP.

Before BAP four populations of *B. orientalis* (Bauska, Ilgas (Lipsbergs, Kasparsons 1977; Zirnīs 1980), Ainavas, Demene) were known in Latvia. After BAP till 2016, there were found six new populations of *B. orientalis* (Medumi, Kurmene, Sedere, Kaplava, Eglaine) (Pupiņa 2011; A.Pupina, pers.comm.), which all can be parts of one meta-population of Southern Latvia (Fig.6).

Sub-action 3.5. Genetic Research. The number of the investigated populations was estimated in the study. We have no information about implementation of the Sub-action before BAP. After BAP were investigated 3 populations: Bauska (Islice), Ilgas, and artificial population of Rigas Zoo (created mostly from Ilgas individuals caught by M.Pupins, M.Pupins, pers.comm.) (Fog 2007).

Action 4. Information and Education

Sub-action 4.1: Informative seminars. Number of seminars was estimated in the study. There were no seminars for land-owners before BAP. After BAP five seminars were held within implementation of the LIFE projects (LIFE-Bombina 2004; LIFE-Razna 2005; LIFE-HerpetoLatvia 2015; authors' data).

Sub-action 4.2. Informative brochure. Number of brochures or booklets was estimated in the study. The sub-action was not implemented before BAP.

Three issues were published after BAP in paper or PDF format in Latvia (LIFE-Razna 2005; Project LVAFa 2006; LIFE-HerpetoLatvia 2015).

Sub-action 4.3: Information in the Internet for landowners on arranging ponds for *B. orientalis*. Number of IP-addresses was estimated in the study. The Sub-action was not implemented before BAP. After BAP the information is published on Latgales Zoo website.

Sub-action 4.4: Scientific information on the Internet. The Sub-action wasn't estimated in the study, because full articles and books are free to access on websites of Latgales Zoo and Project LIFE-HerpetoLatvia, in the ResearchGate www.researchgate.net and Academia www.academia.edu, and don't need for additional implementation.

Sub-action 4.5. Spring informative campaign. The Sub-action wasn't estimated in the study, because it was impossible correctly to count all the mass-media publications about *B. orientalis* in Latvia before and after BAP.

Sub-action 4.6: Support of the local residents' activity. Acquired with Sub-action 1.6. The Sub-action wasn't estimated in the study, because we found not any statistics about number of consultations for residents about new *B. orientalis* findings in Latvia, suitable ponds for them, danger of fish releasing in these ponds etc.

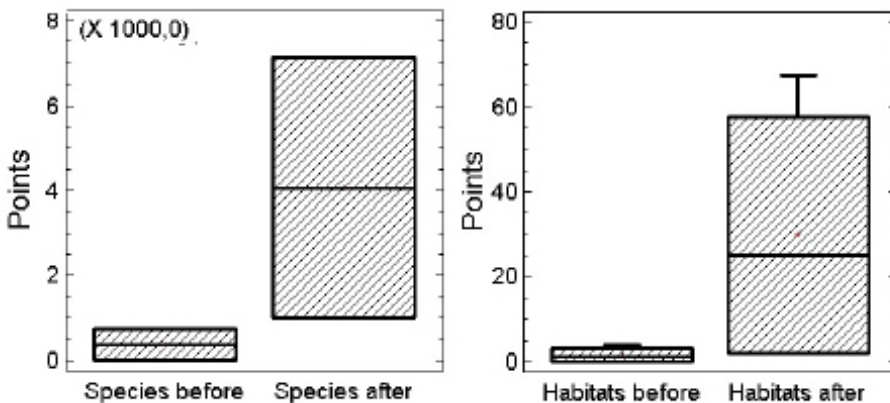


Fig. 7. Box-and-Whisker plots for Species Conservation and Habitats Conservation Sub-actions' implementation in points before and after BAP.

Table 2. Implementation of the BAP suggested actions and sub-actions in real units before and after BAP from 2006 and before and including 2016

Action and sub-action	Number in BAP	Real unit	Value before BAP	Value after BAP
Species Conservation	1.			
Strengthening of weak populations	1.3	individual	0	7129
Introduction of new populations	1.4	individual	751	1010
Habitats Conservation	2.			
Determining of the protected territories	2.1	protected territory	2	2
Land purchase	2.3	subpopulation	0	2
Pond digging	2.4.1+2.7	pond	4	48
Bush cutting	2.4.2+2.7	ha	0	67,3
Research and Monitoring	3.			
Research in distribution, number and ecology <i>in-situ</i> and <i>ex-situ</i>	3.1+3.2+3.3+3.4+1.1	publication	5	38
Research in genetics	3.5	publication	0	4
Information and Education	4.			
Seminars	4.1	event	0	5
Brochure	4.2	piece	0	3
Web-site for landowners	4.3	IP address	0	1
Conferences	4.7+3.6	presentation	5	76

Table 3. Summary statistics for the implementation of all 12 Sub-actions before and after BAP

	before BAP	after BAP
Count	12	12
Average	63.9167	698.775
Standard deviation	216.385	2044.89
Coeff. of variation	338.542%	292.64%
Minimum	0.0	1.,0
Maximum	751.0	7129.0
Range	751.0	7128.0

Table 4. Analysis of variance for the Sub-actions' implementation before and after BAP

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	1084.19	1	1084.19	0.02	0.8874
Residual	513963.	10	51396.3		
Total (Corr.)	515047.	11			

Sub-action 4.7: Dissemination of researches results. Acquired with Sub-action 3.6. Number of presentations in scientific conferences (or analogues) was estimated in the study. The results probably were presented five times before BAP. Sub-action's implementation is more for 15 times increased after BAP.

As a result of the study we obtained the numeral data on implementation of the "Species Conservation", "Habitats Conservation", "Research and Monitoring", and "Information and Education" BAP suggested Actions and its Sub-actions before and after BAP (Table 2).

The difference between the main part of the Sub-actions Species Conservation and Habitats Conservation estimated in points before and after BAP is notable (Fig.7).

The summary statistics for all four Actions' 12 Sub-actions before and after BAP is shown in the table (Table 3).

Kolmogorov-Smirnov test was used to compare the distributions of the Sub-actions' samples before and after BAP. There is a statistically significant difference between before and after BAP samples' distributions at the 95.0% confidence level ($P < 0.05$).

The relationship between Sub-actions' implementation before and after BAP was estimated by using the correlation coefficient and ANOVA (Table 4). The equation of the fitted linear model described the relationship between Actions' Sub-actions implementation before BAP and after BAP is: Sub-actions before = $60.5241 + 0.00485496 * \text{Sub-actions after}$ (Fig.8).

The correlation coefficient equals to 0.0458806, indicating a relatively weak relationship between the variables before and after BAP. Since the P-value in the ANOVA table is greater than 0.05, there is not a statistically significant relationship between Sub-actions' implementation before and after BAP at the 95.0% or higher confidence level.

DISCUSSION

The main goal of the following study was the assessment of the degree of implementation of BAP activities, by using the quantitative results, in order to make corrections for the development of a new revised Plan.

The results showed rather high degree of implementation of the Sub-actions, recommended in the BAP. In spite of this, it was discovered that the majority of the Sub-actions have to be continued. Due to the discoveries of new populations and localities of *B.bombina* after BAP, many sub-actions for conservation of *B.bombina* must be implemented permanently (informing the local population, control of fish, monitoring of populations and habitats, habitats improving etc.).

At the same time, one of the vital activities of the new BAP must be research and management of new invasive parasites and fish in habitats of *B.bombina*, especially of invasive *Batrachochytrium* sp., Ranaviruses, and Chinese sleeper *Perccottus glenii*, which is widely distributed in territories of *B.bombina* (Pupina et al. 2015; Pupiņš, Pupiņa 2012)). Such control shall be gained by creation of ponds, not related to other water bodies, also by informing the local population about prohibition to access the water plants, fish and amphibians releasing in ponds inhabited by *B.bombina*.

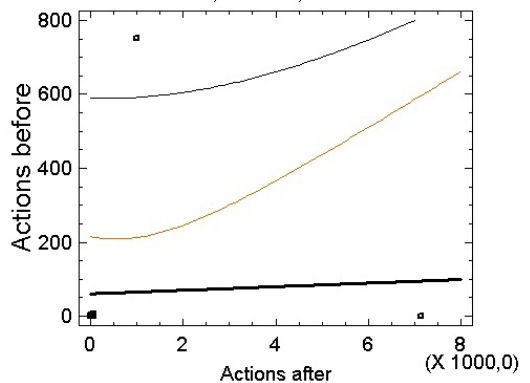


Fig. 8. Fitted linear model of the relationship between Actions' implementation before and after BAP.

CONCLUSIONS

Implementation of most Actions is notable increased after BAP and has statistically significant difference in comparison with before BAP implementation. There is not a statistically significant relationship between Sub-actions' implementation before and after BAP at the 95.0% or higher confidence level.

The new BAP has to suggest the most important sub-actions and actions for conservation of *B. bombina* in Latvia, directed towards the conservation of populations and their habitats: optimization of ponds, creation of genetic bridges for contacts between populations, management of invasive species, the impact of the climate change on the population and habitats of *B. bombina* in Latvia etc. The revision of the Action Plan for *B. bombina* (BAP) must take into consideration the results of implementation of the existed Plan.

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