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RECIPROCAL PREDATION BETWEEN PRESERVED AND INVASIVE SPECIES: ADULT *BOMBINA BOMBINA* PREDATE YOUNG WHITEBAITS OF ALIEN FISH *PERCCOTTUS GLENII*

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The invasive Amur sleeper *Perccottus glenii* is evolutionarily new predator and food competitor for rare and preserved *Bombina bombina* in Europe. We conducted this experiment for better understanding of interactions between these species. We placed 148 predator-naive young whitebaits of *P. glenii* and 4,4 fishing-naive adult *B. bombina* in four experimental boxes. Within 6 days, we counted the number of whitebaits (live, died/killed, and predated) in each box. On the fourth day the amount of *P. glenii* in each box was again increased to 20, modelling immigration from other populations. In a result of the experiment, we identified reciprocal predation of *B. bombina* in relation to the invasive fish *P. glenii*: adult *B. bombina* predated *P. glenii* whitebaits. In total, *B. bombina* in the experiment eliminated 97,97% of experimental population of *P. glenii*. Number of predated whitebaits correlated with the density of their population.

Key words: reciprocal predation, *Bombina bombina*, invasive fish, *Perccottus glenii*, predator-naive, fishing-naive, nature conservation, Latvia.

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INTRODUCTION

Bombina bombina (Linnaeus 1761) is a rare and protected amphibian species in European Union (Bern Convention 1979) and in Latvia (Ministru... 2000), living here on the extremely northern border of its European range (Kuzmin et al. 2008, Pupina & Pupins 2007, 2008, Drobenkov et al. 2005).

Main threats for *B. bombina* in Latvia are urbanization, overgrowing of ponds, amelioration, parasites, and native and invasive predators (Pupins & Pupina 2006, 2011, Pupina & Pupins 2016). One of a new species of fish predator in Europe and Latvia, which is dangerous for larvae and adults of many amphibians, including the *B. bombina*, is the invasive Amur sleeper *Perccottus glenii* (Dybowski 1877) (Reshetnikov 2005, Fog et al. 2011, Pupina & Pupins 2016b).

In present time *P. glenii* is occupied more than half of the territory of Latvia (Pupins & Pupina 2012, Pupina & Pupins 2012, Pupina et al. 2015), including territories inhabited by *B. bombina* (Fig. 1.) (Pupina et al. In press).

P. glenii is evolutionarily new predator and food competitor for *B. bombina* in Europe and it is officially considered to be an important threat for *B. bombina* in Latvia (Pupins & Pupina 2006).

P. glenii also is a host and can be new vector of expansion of parasites in Latvia (Kvach et al. 2013, Kirjušina et al. 2014). Global climate change can be trigger for changes in distribution of both species and for more closed interactions between them (Tytar & Nekrasova 2016, Tytar et al. 2018, Pupina et al. In press).

An additional question is efficiency of Bombina-related conservation projects and *B. bombina* releasing in wild (Pupina & Pupins 2016b). Do we need to train adult *B. bombina*, bred in captivity and fed only by insects, in hunting for other food objects before releasing in natural conditions (Sih et al. 2010)?

All these factors make the research on interactions between both species actual.

MATERIALS AND METHODS

The study was conducted in September 2015. Adult *B. bombina* (4 males and 4 females) and 148 young whitebaits *P. glenii* (~L = 10.7 ± 1.34 mm) were used in the experiment. Both species were born and cultivated in zooculture and used in the study in accordance with Latvian legislation. Before the experiment, fishing-naive *B. bombina* received only insects *Blatta lateralis* as a food and had no experience in hunting in water or for vertebrates, predator-naive young whitebait *P. glenii* did not have any experience in contact with predators.

We placed 20 young whitebaits of *P. glenii* in each of 4 experimental boxes with water (V=1l), air compressor, and green plastic fence - island. T° of water = ~23°-25°C. Thus, 4 initial model populations of *P. glenii* (*Pg-1*, *Pg-2*, *Pg-3*, *Pg-4*) were created, where in a first day of experiment were placed 4 model populations of *B. bombina* accordingly, each consisting of 2 (1,1) adult individuals (*Bb-1*, *Bb-2*, *Bb-3*, *Bb-4*). In 4 control aquariums were only *P. glenii* (20 in each). During the experiment *B. bombina* did not receive any additional food. *P. glenii* received "*Vipan*" aquarium fish food in the usual dosage. Every day we replaced the water in the boxes (Fig. 2).

Within next 6 days, we counted the number of whitebaits (live, died/killed, and predated) in each box. On the fourth day, after the counting, the amount of *P. glenii* in each experimental box was again increased to 20, modelling immigration from species other populations. The obtained results were processed using standard statistics methods and *Excel* for *Windows* and *StatGrafics* software.

RESULTS

In a result of the experiment, we discovered that *B. bombina* in all its model populations (*Bb-1*,

Fig. 1. Overlapping areas of *B. bombina* and *P. glenii* distribution in Latvia (Pupina et al. In press).

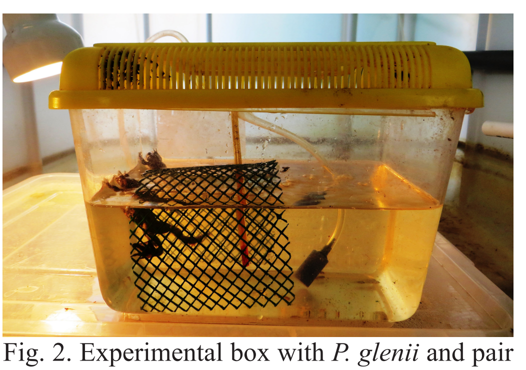

Fig. 2. Experimental box with *P. glenii* and pair of *B. bombina*.

Bb-2, *Bb-3*, and *Bb-4*) from the first day of the introduction into model populations of *P. glenii* began to hunt and eat *P. glenii*, also we found small number of dead/killed whitebaits (Fig. 3).

The density of the experimental model populations of *P. glenii* significantly decreased, while the number of control model populations of *P. glenii* in boxes without *B. bombina* remained unchanged (Fig. 4).

The main amount of *P. glenii* eliminated was predated, a small amount was died/killed, but was not eaten (Table 1).

Table 1. Number of *P. glenii*: live, predated, died/killed, and eliminated in total during the experiment

Parameter of the experimental group	**Number (individuals)**	**Comparison with the initial number (%)**
P. glenii used in the experiment in total	148	100
B. bombina predated *P. glenii* in total (*Npred*)	133	89,86
1 B. bombina predated P. glenii per day	2,77	1,87
B. bombina killed *P. glenii* in total (*Nkill*)	12	8,11
1 B. bombina killed P. glenii per day	0,25	0,17
B. bombina eliminated *P. glenii* in total (*Npred+Nkill*)	145	97,97
1 B. bombina eliminated P. glenii per day	3,02	2,04
P. glenii left alive after the experiment in total	3	2,03

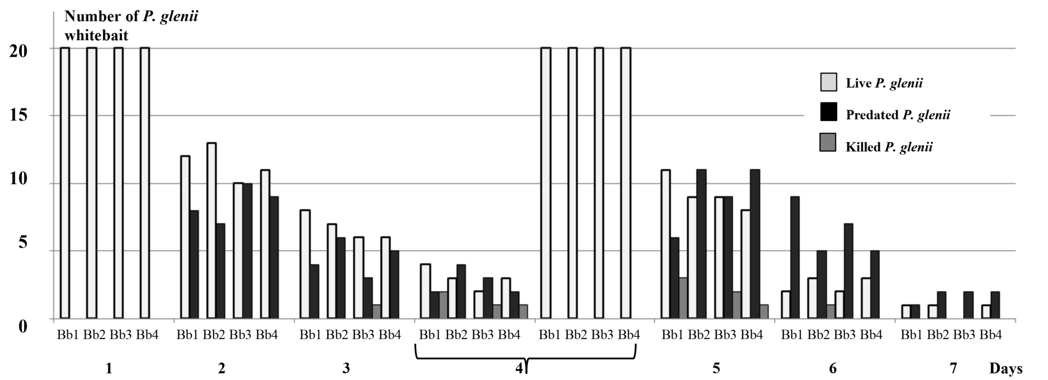

Fig. 3. Dynamics of number of live, predated, and died/killed *P. glenii* in different *B. bombina* model populations (*Bb-1*, *Bb-2*, *Bb-3*, and *Bb-4*).

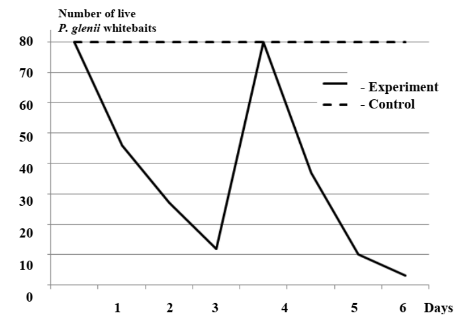

Fig. 4. Dynamics of quantity of live *P. glenii* in experiment and control groups in total.

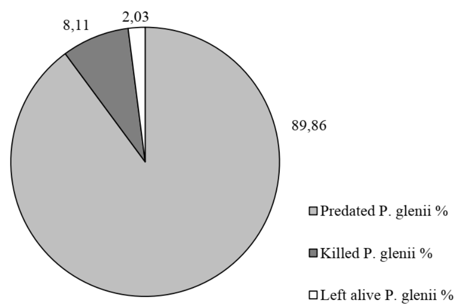

Fig. 5. Number of left live, predated, and died/killed *P. glenii* in all experimental groups in total after the experiment.

In total, *B. bombina* in the experiment eliminated almost all *P. glenii*, only 2.03% remained of their total number in the model population (Fig. 5).

There was a statistically significant relationship between the variables "*Number of live P. glenii whitebaits*" before predating and "*Number of predated P. glenii whitebaits*" at the 99% confidence level (p=0,0057<0.01). The correlation coefficient equals 0,9377 indicating a relatively strong relationship between the variables (Fig. 6).

Dynamics of relative predation (% from existed number of live whitebaits) for all model populations of *B. bombina* is shown in Fig. 7, and predating by individual populations *Bb-1*, *Bb-2*, *Bb-3*, and *Bb-4* – in Fig. 8.

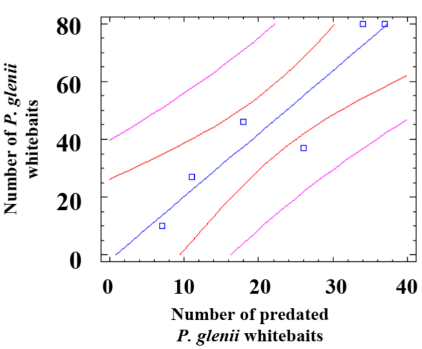

Fig. 6. Plot for fitted model of initial number of live and predated *P. glenii*.

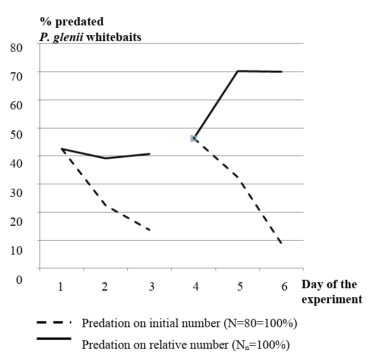

Fig. 7. Dynamics of relative predation (% from existed number of live whitebaits) for all model populations of *B. bombina*.

DISCUSSION

This study showed the existing of reciprocal predation of the rare and protected in Europe species *Bombina bombina* in relation to an important its threat in Latvia, the invasive fish *Perccottus glenii*.

In the experiment adult *B. bombina*, without any experience in fishing, successfully predate predator-naive young whitebaits of its evolutionarily new predator and food competitor alien fish *P. glenii*.

Therefore in Bombina-related conservation projects it is not necessary to train *B. bombina*, bred in captivity and fed only by insects, in hunting for whitebaits before releasing in wild.

Undoubtedly, the conditions of this experiment are very different from the natural conditions in which these two species meet each other in Latvia and in Europe. For example, only fishing-naive *B. bombina* and predator-naive *P. glenii* used in the study. The small size of the boxes and a small number of shelters did not allow *P. glenii* whitebaits to avoid predators or escape, or hide from them, the lack of alternative types of food stimulated *B. bombina*'s hunting for whitebaits, and the effects of other factors cannot be ruled out.

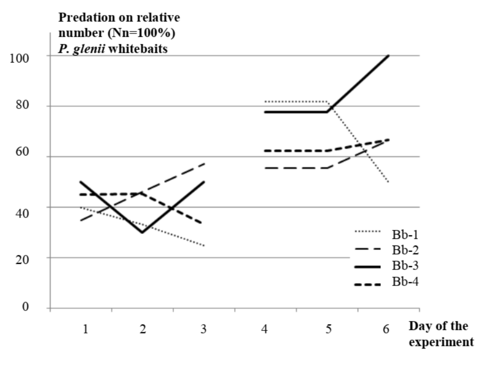

Fig. 8. Dynamics of relative predation (% from existing number of live whitebaits) in individual populations of *B. bombina*.

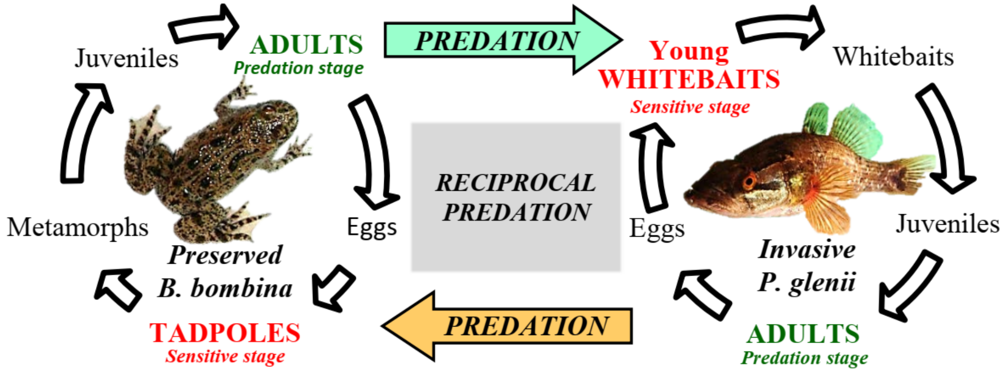

Fig. 9. Scheme of the reciprocal predation between *B. bombina* and its invasive threat *P. glenii* registered in the study.

Therefore, the purpose of this preliminary study was only to register the possibility of a reciprocal predation between these two species, but not a deep description of patterns of this predation. This reciprocal predation in natural conditions allows *B. bombina* to use *P. glenii* as a new type of food, which may be relevant in food competition with *P. glenii* for feeding aquatic invertebrates, as well as *B. bombina* trying to regulate the number of dangerous for themselves predators *P. glenii* at the preys sensitive developmental stage (Fig. 9).

As field studies have shown earlier (Fog et al. 2011, Pupina & Pupins 2016b), in nature *P. glenii* displaces *B. bombina* during invasion into its water bodies, therefore, the detected reciprocal predation possible does not have a significant deterrent effect when *P. glenii* invades into water bodies inhabited by *B. bombina* and therefore, the reciprocal predation does not reduce the importance of *B. bombina* protection from *P. glenii* in Europe.

At the same time, such reciprocal predation may form a new vector of distribution of new or native parasites from *P. glenii* to *B. bombina*, which is a prerequisite for further research in this area.

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