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CONFIRMATION OF HYBRIDISATION BETWEEN RIVER LAMPREY *L. FLUVIATILIS* AND BROOK LAMPREY *L. PLANERI* FROM *IN SITU* EXPERIMENTS

Robertas Staponkus, Vytautas Kesminas

Nature Research Centre in Vilnius

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A b s t r a c t

Interspecific mating and taxonomic status of river lampreys *Lampetra fluviatilis* and brook lampreys *Lampetra planeri* has been repeatedly discussed in recent years. Molecular studies fail to deliver clear separation between two species and the number of reported communal spawning cases across the natural range is constantly increasing. Here, we present results of interspecific breeding experiment *in situ* involving both species of *Lampetra* genus in Lithuanian lowland rivers. For the experiment, we collected the spawning individuals in communal redds and manually striped them into modified salmonid incubation boxes to incubate lamprey eggs in the same redds under ambient conditions. We have received viable reciprocal crosses of ♂ *L. planeri* × ♀ *L. fluviatilis*, ♂*L. fluviatilis* × ♀ *L. planeri*, as well as viable ammocoetes in the control group of *L. fluviatilis* × *L. fluviatilis*. The experiment suggests that, in case of communal spawning, the production of viable hybrids is possible. The results as well supports hypothesis of two different ecotypes of a single *Lampetra* species.

HYBRYDYZACJA MINOGA RZECZNEGO *L. FLUVIATILIS* Z MINOGIEM STRUMIENIOWYM *L. PLANERI* POTWIERDZONA DOŚWIADCZENIEM *IN SITU*

Robertas Staponkus, Vytautas Kesminas
Centrum Badań Przyrodniczych w Wilnie

S ł o w a k l u c z o w e: *Petromyzontidae*, larwy minogów, krzyżowanie międzygatunkowe, specjacja.

Address: Robertas Staponkus, Nature Research Centre in Vilnius, Akademijos str. 2, LT-08412 Vilnius-21, Lithuania, phone: +370 5 272 92 92, e-mail: robertas.staponkus@gmail.com

Abstrakt

Status taksonomiczny i zjawisko hybrydyzacji międzygatunkowej minoga rzecznego *Lampetra fluviatilis* z minogiem strumieniowym *Lampetra planeri* były wielokrotnie dyskutowane w ostatnich latach. Badania molekularne nie dostarczyły wyraźnych dowodów na przynależność tych bezszczękowców do oddzielnych gatunków, co więcej rośnie liczba doniesień o wspólnie odbywanym tarle na terenie całego zasięgu naturalnego występowania tych ryb. W pracy prezentujemy wyniki eksperymentu krzyżowania międzygatunkowego *in situ* z udziałem obu gatunków z rodzaju *Lampetra* z litewskich rzek nizinnych. Do eksperymentu użyto tarlaków złowionych podczas tarła. Ikrę umieszczono w zmodyfikowanym aparacie wylęgarniczym, zapładniano i umieszczano w okolicy już zbudowanych gniazd. Uzyskano krzyżówki *L. planeri* × *L. fluviatilis* oraz L. *fluviatilis* × L. *planeri*. Wykazano, że w przypadku wspólnego tarła minogów rzecznych i strumieniowych mogą powstawać żywotne mieszance obu gatunków. Wyniki mogą też potwierdzać hipotezę o dwóch różnych ekotypach jednego gatunku w obrębie rodzaju *Lampetra*.

Introduction

According to HARDISTY and POTTER (1971), the lack of observation of successful interspecific mating of brook lampreys *Lampetra planeri* (Bloch, 1784) and river lampreys *Lampetra fluviatilis* L. in the field supports the theory of two distinct species. However, such communal spawning has been observed in a number of rivers across Europe (HUGGINS and THOMPSON 1970, LASNE 2010). Additionally, post-zygotic viability of *L. fluviatilis*, *L. fluviatilis* landlocked individuals and *L. planeri* has been shown via in *in vitro* experiment by HUME et al. (2013a). This phenomenon of communal spawning is also frequently observed in rivers of eastern and western Lithuania. According to LASNE (2010) interspecific communal spawning in the same redds is the first prerequisite for hybridisation to occur between *L. fluviatilis* and *L. planeri*. Therefore, we carried out an experimental *in situ* hybridisation between *L. fluviatilis* and *L. planeri* individuals collected in communal redds to test whether crossbreeding is possible under natural conditions.

Materials and Methods

The experiment was carried out in three rivers in the North West Lithuania: Šventoji, Blendžiava, Salantas. As *L. fluviatilis* and *L. planeri* were already described by ZHUKOV (1965) to occur sympatrically in the upper tributaries of the rivers Nemunas and Vistula, two study sites were selected in the Nemunas river basin. One site was situated in the Salantas River 1st order tributary and one in the Blendžiava River 2nd order tributary of the Minija River which is one of major tributaries of river Nemunas. The third site was selected in the Šventoji River which is a cross border river of Lithuanian and Latvia, a direct tributary of the Baltic Sea, sustaining abundant spawning populations of *L. fluviatilis*.

L. fluviatilis and *L. planeri* individuals were captured in three communal redds (one redd per study site) in early May 2012, during the peak of spawning period. Majority of lamprey spawners were individually collected by dip net as this was the least stressful method to avoid any undesirable effect on egg fertility. Lampreys that displayed avoidance reaction were trapped in fyke nets installed 1–1.2 m downstream of the redd tailspills. In order to eliminate any possibility of egg fertilization by undetected *L. planeri* males, we thoroughly checked substrate in the redds and redd tailspills. *L. planeri* individuals are known to be found burrowed in red substrate (personal observation).

All lampreys were collected while spawning in redds. Females and males of both species possessed distinctive sexual features typical for mature individuals: females – post-cloacal fin fold, males – genital papilla extending from the cloaca (RENAUD 2011). All crosses were performed between unique pairs of lampreys. As spent females were also present in redds, females were selected according to body fill and visible eggs through translucent skin near the cloaca. Males for crossbreeding were selected randomly. *L. planeri* and *L. fluviatilis* spawners were anaesthetised by 2-phenoxy ethanol 0.5 ml l^{-1}. Gametes of both sexes were hand stripped into modified salmonid incubation boxes (RUBIN 1995, NIKA 2011). Incubation boxes prior to realise of gametes were placed in 0.75 l beaker filled with stream water taken above redds. Hybridisation was tested among reciprocal crosses of *L. planeri* × L. fluviatilis and control group crosses of *L. fluviatilis* × *L. fluviatilis*. Each cross group was placed in individual incubation box. Incubation boxes were made of a PVC cylinder (diameter: 8 cm; height: 10 cm; volume: 500 cm^3). The box was sealed with double layer of mesh: outer flexible protective PVC mesh (mesh size 6 x 6 mm) and inner mesh (mesh size 200 μm) to secure egg compartments from fine sediment and to prevent hatched larvae from escaping (Figure 1a). PVC hoops were tightened with clamps on the top and bottom parts of the box to support its structure.

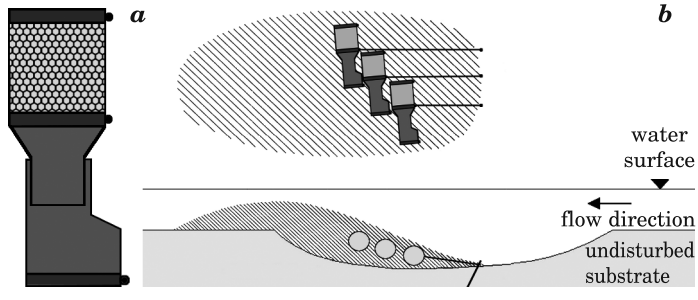

Fig. 1. Experimental egg incubation box (*a*) and scheme of incubation box placement in redd tailspills (*b*)

We applied minimal force to strip out only ovulated eggs into incubation boxes as ovulation in lampreys is known to start at the posterior end of the ovary (LARSEN 1970). Two thirds of an ovulated egg is covered with a thick layer of sticky jelly (LARSEN 1970); this enables instant fixation of eggs to the surface of the inner mesh leaving the animal pole exposed for spermatozoa to enter. The number of retrieved eggs varied considerably from a few dozen of *L. planeri* to several thousands of *L. fluviatilis*. According to KILLE (1960), lamprey spermatozoa can survive in fresh water for about 50 seconds. Considering this fact boxes were kept in the water reservoir and hand stirred for a minute.

The boxes with fertilized eggs were positioned at the front of the redd tailspill or crest and anchored to the bottom to minimize losses (Fig. 1b). Despite our preventive measures, all three incubation boxes were lost in the Salantas River and one incubation box in the Sventoji River, either due to the current or retrieved by local anglers. Thus, the results are presented of only two study sites – Šventoji and Blendžiava Rivers. The remaining boxes were checked every third day for hatched larvae.

Results and Discussion

In the three communal redds selected for this experiment we caught a total of 46 lampreys. Redds were dominated by river lamprey, the ratio of brook to river lampreys in redds varied between 0.35–0.45. Both male and females of *L. planeri* and *L. fluviatilis* were present in redds. The males to females ratio of both species in the study sites was close to equal, in *L. fluviatilis* it varied between 0.45–0.55 and in *L. planeri* between 0.25–0.71. Overall 188 larvae hatched during the study period of which 19 were hybrids either ♂ *L. planeri* × ♀ *L. fluviatilis* or ♂ *L. fluviatilis* × ♀ *L. planeri* and 169 *L. fluviatilis* larvae which were used as a control group (Table 1). The numbers of hatched larvae in each box ranged due to a different amount of eggs in every box. The experiment was designed to meet conditions closest to natural ones without the use of any egg de-adhesion compounds. Due to highly adhesive properties of eggs we stripped them directly into incubation boxes. Therefore, it was impossible to quantifying the number of eggs and later on estimate hybrid success between cross groups. The emergence of larvae in all incubation boxes was observed at the end of May after 21 days of incubation. We kept larvae in boxes for 41 day after hatching and during this time no mortality of hybrids were observed which indicates no increased mortality of hybrids in initial life stage.

Table 1

Number of lamprey larvae retrieved from incubation boxes

River	Hybridisation directions	Number of incubation boxes	Number of larvae
Salantas	♂ *L. planeri* × ♀ *L. fluviatilis**	1	–
	♂ *L. fluviatilis* × ♀ *L. planeri**	1	–
	♂ *L. fluviatilis* × ♀ *L. fluviatilis**	1	–
Blendžiava	♂ *L. planeri* × ♀ *L. fluviatilis*	1	18
	♂ *L. fluviatilis* × ♀ *L. fluviatilis*	1	21
Šventoji	♂ *L. planeri* × ♀ *L. fluviatilis**	1	–
	♂ *L. fluviatilis* × ♀ *L. planeri*	1	1
	♂ *L. fluviatilis* × ♀ *L. fluviatilis*	1	148

* lost incubation box

Our experiment shows that communal spawning of *L. planeri* and *L. fluviatilis* could produce interspecific hybrids, and they could stay alive for a prolonged period of time. These findings may also be applicable to possible hybridisation among other known paired species of lampreys such as *Ichthyomyzon unicuspis* and *I. fossor* (MORMAN 1979) or *Ichthyomyzon gagei* and *I. castaneus* (COCHRAN et al. 2008). During the collection of data, we observed *L. planeri* individuals burrowed in tailspills of redds what can be seen as one of strategies to fertilize eggs in a redd substrate and an argument in support of sneaky male hypothesis (HUME et al. 2013b). However, we observed both sexes of *L. planeri* in substrate of communal redds and consider this to be energy efficient behaviour as sexually mature adults also burrow in fine sand in pre-spawn period (RENAUD 2011). The ammocoetes require prolonged fluvial conditions for growth and later on access to large water bodies. Life history strategy makes it difficult to determine fertility of supposedly anadromous hybrids, thus, limiting a range of methods that can be applied to assess taxonomic status of the two *Lampetra* species.

Conclusions

We have confirmed that *L. fluviatilis* and *L.planeri* not only spawn in communal redds, but are also potentially capable of producing hybrids under natural condition. This is highly probable in case of simultaneous egg and milt release of the two species. The taxonomic status both species is intensively discussed across the Europe with little direct evidence to support or refuse that these are either one or two species. This can hardly be determined without hybridisation and gene flow studies between a number of sympatric popula-

tions. The Nemunas and Vistula River basins are one of the few regions where the ranges of both species overlap. This area provides a great opportunity to study these two species *in situ* and help determine they are truly separate species or not.

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