

Survival of Rainbow Trout *Oncorhynchus mykiss* Walbaum, 1792 (Salmoniformes - Salmonidae) Eggs in an Altitude Stream in Southern Brazil.

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ABSTRACT: Survival of Rainbow Trout *Oncorhynchus mykiss* Walbaum, 1792 (Salmoniformes - Salmonidae) Eggs in an Altitude Stream in Southern Brazil. The introduction of rainbow trout (*Oncorhynchus mykiss*) in a high altitude stream in Southern Brazil occurs every year in winter to support a put and take fishery of local economical importance. The effects of this introduction on the native fauna have not been investigated. To address the question whether rainbow trout might build up a self-sustaining population, we measured the survival of eggs in modified Whitlock-Vibert-Boxes. These artificial redds, filled with natural substrate and rainbow trout eggs, were incubated in the bottom substrate of the Silveira stream. After three weeks exposure mean survival was 10.7% (1.7 standard error). In the fourth week hatched larvae were found. The number of surviving larvae decreased in the fifth week, indicating high larval mortality in the post-hatching phase. The overall survival rate at this time was 3.5 % (1.1 s.e.). Considering the low survival of eggs and the high water temperatures during summer which increase the mortality of adults, the build-up of a self-sustaining population is not probable.

Key-words: *Oncorhynchus mykiss*, reproduction, exotic species.

RESUMO: Sobrevivência de ovos da truta arco-íris *Oncorhynchus mykiss* Walbaum, 1792 (Salmoniformes - Salmonidae) em um rio de altitude do Brasil. A introdução da truta arco-íris (*Oncorhynchus mykiss*) ocorre todos os anos durante o inverno num rio de alta altitude na serra Gaúcha, para viabilizar um "pesque-e-solte" de importância econômica local. Os efeitos desta introdução sobre a fauna nativa não são conhecidos. Para avaliar a possibilidade da formação de uma população auto-sustentável nós investigamos a sobrevivência de ovos em "Whitlock-Vibert-Boxes" modificados. Estes ninhos artificiais foram preenchidos de saibro natural e ovos fertilizados. Posteriormente, foram incubados no substrato do fundo do rio Silveira. Depois de três semanas de exposição, a sobrevivência média foi de 10,7% (1,7 erro padrão). Na quarta semana foram encontradas larvas eclodidas. O número de larvas diminuiu na quinta semana, indicando alta mortalidade na fase de pós-eclosão. A sobrevivência total neste período foi de 3,5 % (1,1 e.p.). Considerando a baixa sobrevivência e as altas temperaturas durante o verão, que aumentam a mortalidade dos indivíduos adultos, a formação de uma população auto-sustentável não parece provável.

Palavras-chave: *Oncorhynchus mykiss*, reprodução, espécie exótica.

Introduction

Every winter rainbow trout (*Oncorhynchus mykiss*) are introduced in the Silveira stream, a high altitude water course in southern Brazil to support a local put and take fishery without previous knowledge on its ecological consequences. Rainbow trout were introduced in all continents and today is one of the most widely distributed species in the world (Hershberger, 1992). In tropical countries, like Brazil, the most

limiting factor for dispersal is temperature, constraining introductions to mountainous areas higher than 1200 m. Thus, constant reintroductions on an annual basis may favor the permanent establishment of the species (Proença et al., 2001).

Headwaters usually show lower species richness than in lowland river sections and a higher degree of endemism (Lowe-McConnell, 1999). Due to the small number of studies, the fish community of the low-order streams in Brazil is poorly known. Bastos (2002), studying the headwater fish fauna in the Brazilian southernmost state of Rio Grande do Sul, found an assemblage of six species, of which three had not been described scientifically. Assemblages of low species richness, high degree of endemism and lacking a top predator, are especially vulnerable to invasion (Townsend, 1996).

For the introduced species, the invasion success is related to growth rate, age and size at sexual maturity, dispersal rate and fecundity (Laurenson et al., 1989). One of the key factors is the reproduction of the introduced species in the new environment. The spawning of rainbow trout in southern Brazilian streams has not yet been investigated, although the knowledge about natural reproduction and survival rates of eggs and larvae are crucial for management decisions.

The survival of salmonid eggs in streams has been estimated in several ways. A summary of artificial incubation methods used in rivers can be found in MacCrimmon et al. (1989). The Whitlock-Vibert-Box is most widely used to investigate survival of salmonid eggs by incubation in natural environment (Whitlock, 1978; Harshbarger & Porter, 1982; MacKenzie & Moring, 1988). Modifications were proposed by MacCrimmon et al. (1989) and Rubin (1995) to follow egg survival until the post-emergent larvae stage. Other methods used to evaluate the reproduction of salmonids in natural environments consist in excavating redds (Soulsby et al., 2001), placement of nylon mesh traps over nests (Phillips & Koski, 1969), and placing eggs in nests excavated by man in the river bed to count the emerging fry (Harshbarger & Porter, 1982).

In a natural environment, rainbow trout females excavate one or more redds at sites with gravel size of 2 to 3 cm, mean water depths of 20-30 cm, and water velocities of 25-77 cm/s (Stefferd, 1993; Muhlfeld, 2002). After spawning the female covers the eggs with gravel. Oxygenation of the interstitial water is a principal factor for egg survival. Larvae hatch after approximately 300 day degrees which means within 30 days at a water temperature of 10°C (Billard, 1992). After hatching, they still remain in the interstitial flow until the yolk sac is absorbed. After another 240 day degrees larvae are transformed in fry and start feeding (Tabata, 2004).

The objective of this study was to measure the survival of artificially incubated rainbow trout eggs in a high altitude stream in southern Brazil. The study is part of a larger project focusing the impacts of trout introduction on local fish fauna.

Material and methods

The study was conducted in the fifth order Silveira stream near in the city of São José dos Ausentes, northeastern region of Rio Grande do Sul State (Fig. 1). The altitude is higher than 1200 m. The annual mean air temperature is 14°C, but maximum temperatures in summer may come close to 35°C, causing water temperatures of up to 28°C. During winter air temperatures may fall to -8°C, with frequent frost formation and occasional snowfall (Nimer, 1989). The yearly rainfall varies from 1500 to 2000 mm, with well-distributed rainfalls during the year (Boldrini & Eggers, 1997).

During the experiment, 2400 eggs were incubated. They were obtained on July 31, 2003, at the "Casa de Pedra" Trout Hatchery in Paniel, Santa Catarina State, at about 140 km distance from the study area.

Half of the eggs was fertilized at the hatchery. The other half was transported unfertilized and mixed with trout sperm at the incubation site. During the transport of about four hours at 10°C air temperature, eggs and sperm were placed separately in plastic recipients in a styrofoam box with ice, and wrapped in glass fiber to avoid

major mechanical impacts. The total time span the eggs spent off the water was about six hours.



Figure 1: Location of trout introduction in Rio Grande do Sul.

The eggs were distributed in 12 hatching and 12 emergence boxes, forming batches of 100 eggs each. Six hatching boxes and six emergence boxes were filled with hatchery fertilized eggs. On site fertilized eggs were distributed in the same manner. The boxes were buried in a supposed trout spawning area in order to expose the eggs to similar conditions to those naturally spawned in the Silveira stream. The boxes were constructed according to the proposal of Rubin (1995). Hatching boxes consisted of a tube formed net cage 10 cm long and 7 cm in diameter, with 84% of the lateral surface covered by 1 mm mesh size PVC plastic screen. The emergence boxes consisted of two compartments, the incubation unit with the same dimensions of the hatching box, and an emergence unit on top, being 15 cm long and 11 cm in diameter (Fig. 2). All redds were filled with 100 eggs and natural gravel of 2 to 3 cm in diameter. They were embedded in the stream substrate at a site which corresponded to the physical structure of a natural salmonid reproduction site, with 20 cm depth at 14 cm/s flow velocity (MacCrimmon et al., 1989).

The incubation started on 31st of July 2003. During the next month, four redds per week were removed to count the surviving eggs. In the third week two redds were lost due to a spate. In the fifth week all remaining six redds were removed.

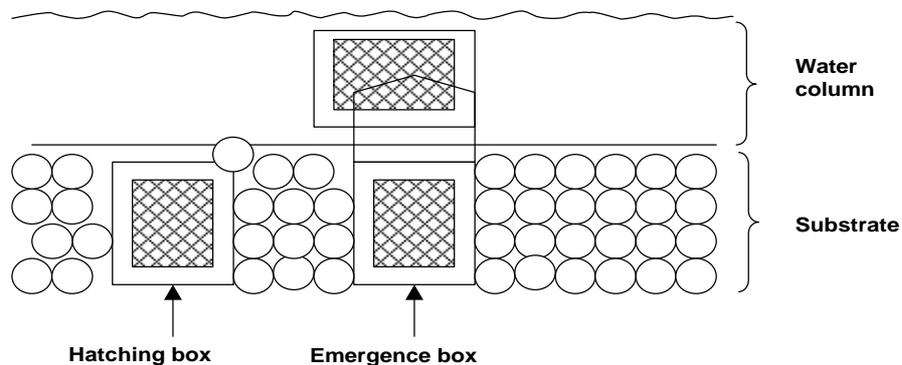


Figure 2: Positioning of hatching and emergence boxes in the substrate at the experimental site.

Each removal consisted of two box types (hatching and emergence) with on site fertilized and two box type of hatchery fertilized eggs.

The removed eggs were bleached in a solution of acetic acid (10%). White eggs without embryonic development were accounted as dead.

Differences in mean egg survival in onsite and hatchery fertilized batches were tested with Student-T-test. Since Levene's test showed the variances in weekly data sets were not homogenous, differences in weekly survival rates were tested pairwise by non-parametric Mann-Whitney-U-test. Data were processed and statistically tested with SPSS 10 (SPSS, 1999).

Results

No differences were detected comparing the overall survival rate of onsite (21.5%, standard error s. e. = 6.9) and hatchery (23.8%, s.e. = 8.8) fertilized eggs. For further data evaluation, both treatments were joined in one group.

After one week the mean survival rate of all four removed redds was 56.1% (s.e.=8.3; Fig. 3). At this time, the first embryos with pigmented eyes were found in the samples. During the following weeks mean survival decreased most drastically between the second and third week from 43% to 10.7%, which was the only significant decrease between consecutive weeks (Mann-Whitney-U=1.0, p=0.043). Although not measured quantitatively, the amount of fine sediments increased in the samples and on the plastic screens of the redds, showing a tendency of clogging them. After one week, the first eggs covered with fungus were detected. The decaying material made it difficult to count the eggs. In the third week sample, two hatched larvae were counted (out of 400 eggs). The following week 25 larvae were found, indicating that the major part of hatching process started after three weeks. Due to the weekly removal of the redds, it was not possible to calculate the exact amount of day degrees between fertilization and hatching. Thus, considering mean water temperatures of 11°C, 13°C, 10°C and 10°C during four consecutive weeks, the accumulated number of day degrees comes up to 308. During the fifth week, the number of larvae was lower (n=18 in 6 redds) indicating a possible problem of larval survival in the artificial redds.

The flow velocity and depth at the deployment site of the redds varied during the experiment. The flow during the first week (11 cm/s) was higher than during the rest of the experimental period (5 cm/s). The same occurred with depth, which decreased during the study from 13 to 8 cm.

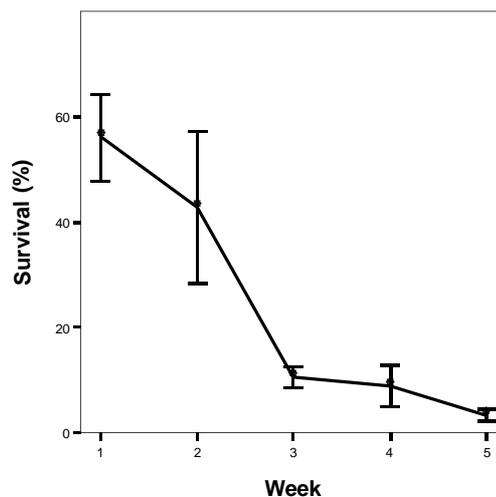


Figure 3: Mean survival of incubated rainbow trout eggs (vertical bars= standard error).

Discussion

The period in which the greatest accumulated mortalities occurred was the interval between fertilization and the end of the first week, which summed up to a mean survival rate of 55.6%. Since fertilization rate was not determined in the field, this value includes as well eggs which were not fertilized. The low survival indicates a possible effect of mechanical impacts caused by the egg transport to the experiment site. Jensen & Collins (2003) found that steelhead (*O. mykiss*) and pink salmon (*Oncorhynchus gorbuscha*) eggs exposed to mechanical shocks died by yolk coagulation, which may involve as much as 50% of the total yolk material. Losses begin one hour after fertilization, and four hours after fertilization this sensibility is doubled (Jensen & Alderdice, 1983). Since the eggs were handled until six hours after fertilization, the initial survival may have been compromised.

Goetz & Coffman (2000) obtained survival rates ranging from 55% to 66.7% until the beginning of the eyed stage in laboratory experiments with rainbow trout eggs fertilized soon after collecting. In Goetz and Coffman's study as in ours the eggs were obtained from commercial hatcheries. In Brazil, where rainbow trout is an exotic species and the import of new broodstock fish is restricted, consanguinity may have contributed to low survival. The reproductive capability, including fertility, egg size and hatching success is frequently affected by consanguinity (Kincaid, 1983).

During the experiment the redds accumulated fine sediment. Screens were partially clogged, hampering water flow into the redds. Several authors (Phillips & Koski, 1969; Harshbarger & Porter, 1979 and 1982; Rubin, 1995; Rubin & Glimsäter, 1996; Soulsby et al., 2001) considered the accumulation of fine sediment as a principal cause for salmonid egg mortality. Additionally, water flow outside the redds was reduced during the experimental period due to very low rainfall. Both factors, sediment accumulation inside the redds and reduced flow outside the redds probably reduced the oxygen supply of the incubated eggs, contributing to high mortality rates (Rubin, 1995; Rubin & Glimsäter, 1996).

Another key factor for salmonid survival is water temperature. The highest mean temperature during the experimental period was 13°C. Incubation experiments with *O. mykiss* eggs in Chile showed that the highest survival rates were obtained at 8°C or lower (Estay & Díaz, 1994). Although thermal limits may vary for salmonid populations in different ecoregions (Huff et al., 2005), the upper limit for egg development is between 14°C and 16°C in experiments with brown trout, *Salmo trutta*, a salmonid species considered to have slightly lower thermal limits than rainbow trout (Ojanguren & Brana, 1993; Molony 2001). Natural reproduction success of introduced rainbow trout under the thermal conditions in the Silveira stream must be very low for several reasons. During summer, water temperatures reaches 28°C. Sauter et al. (2001) reviewed publications about salmonid behavior in relation to temperature. They list acute preference temperatures (influenced by acclimatization temperatures prior to the experiment) and final preference temperature for several salmonid species. Maximum final preference temperature measured under laboratory conditions with well fed subyearlings of resident rainbow trout was 22°C. Trout under deprived feeding conditions or starving fish choose lower temperatures between 11.3°C and 19°C. Trout captured during electrofishing in the Silveira stream were in poor condition, not indicating favorable feeding conditions. This means that trout in this stream should prefer the lower temperature range. Thus, when temperature conditions exceeded the optimum and approach the thermal limits, appropriate available habitat area decreases and warm water adapted species may outcompete salmonids.

In the Silveira stream, where rainbow trout is introduced and all native species are warm water adapted, *O. mykiss* is in competitive disadvantage. When water temperatures increase in summer, cold-water refugia are of prime importance for rainbow trout survival. Sauter et al. (2001) emphasize the importance of cold-water

refugia for salmonids that a) "reside at the southern end of the range" (which corresponds to the northern end on the southern hemisphere) and b) inhabit marginally suitable habitat. Ebersole et al. (2001) showed that 10 to 40% of the rainbow trout populations in 12 Oregon streams used cold-water refugia of 1–10 m², where temperatures were 2–3°C lower than ambient temperatures. Since these areas or thermal refugia are scarce they may be the most limiting factor for salmonid populations outside their optimum thermal distribution range. In the Silveira stream, a limited number of thermal refugia may exist in some deep pools of 2 to 3 m depth. However, the small number of refugia can only support a few individuals surviving the summer. These fish exposed to high temperatures will probably produce offspring with higher mortality rates. Pankhurst et al. (1996) reported that females submitted to higher temperatures in summer and autumn would generate eggs with lower survival. Egg viability in the post-ovulatory period in the female abdominal cavity is reduced by 10 days, if they are kept at 10°C, and by 70 to 110 hours when kept at 20°C.

Due to geological characteristics and soil use in the surroundings of the hydrographic basins of the studied region, the possible spawning sites are very restricted. These are generally shallow places used for cattle passage, what could reduce much the success of natural reproduction due to mechanical disturbances.

Under the current conditions it seems reasonable to assume that natural reproduction of introduced rainbow trout may occur, but survival rates of eggs and all other stages of the life cycle are very low. The fact that no juvenile was ever captured during 20 electric fishing surveys supports this view. Considering the economical importance of the local put and take fishery, small scale trout stocking, accompanied by a management plan and regular monitoring of the stocks may be considered. It has to be emphasized that these results are only valid for the conditions encountered in the Silveira stream. In other water courses with lower temperatures during summer, natural reproduction with high survival rates may occur and interfere severely with the native fauna.

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