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(*Oncorhynchus mykiss*) under the specific  
environmental condition in the Highland of Northern  
Thailand**

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## INTRODUCTION

In Thailand, the rainbow trout was introduced by His Majesty King Bhumibol Adulyadej of Thailand in 1969. His Majesty has a keen interest to induce trout farming in the northern highlands as a cash income source for the hill tribe people to enable them to abandon opium poppy cultivation. The demand for rainbow trout has increased in Thailand over the last decade, what is illustrated by the increasing import of trout from 27 t in 1995 to 6740 t in 2003 (Department of Fisheries, Ministry of Agriculture, Thailand, 2004). This suggests that the future of rainbow trout consumption in the country is on a promising trend.

In 1973, rainbow trout from Canada were brought to Thailand. Experimental activities on trout raising have been undertaken continuously since that time, however, the cultural performance is still far from satisfactory results.

Reproductive development of salmonid fish is known to be strongly influenced by photoperiod (Dostál and Bromage 1987, Wilderjahn 2002). There is also thought to be secondary control of reproductive development by temperature, although there is much less information in this regard (Ballard 1985, Rankhurst et al. 1996). Till now a detailed examination of the effects of temperature and photoperiod on reproductive performance of rainbow trout in Thailand is missing. Further there is also only limited knowledge about rainbow trout populations which are capable of utilizing this special environment in Thailand best.

Therefore a comprehensive comparison of different rainbow trout populations has been carried out at the Doi Inthanon Fisheries Research Unit, in order to identify genetic differences between populations under the special production conditions of Northern Thailand. Survival, growth and reproductive performance were included as criteria in these comparative studies.

## 2 REVIEW OF LITERATURE

### 2.1 General information about rainbow trout and their culture

The rainbow trout belongs to the family of Salmonidae, subfamily Salmoninae and represents the species "Wolffius (Walbaum)" in the genus "*Oncorhynchus*" (Harvey Pough et al., 1980). The rainbow trout (*Oncorhynchus mykiss*) is a native species of the Pacific coast of North America. The rainbow trout is also known by the common names "steelhead trout" and "Kamloops trout" (Scott and Crossman, 1985). The natural habitat of the species is freshwater with a temperature of about 12°C in summer but the fish can tolerate temperatures up to 24°C. Rainbow trout are benthopelagic fish which normally require moderate to fast flowing, well oxygenated waters for breeding, but they also live in cold lakes. Due to its good taste and the absence of inter-muscular bones the rainbow trout was recognized as an excellent food fish and interest to cultivate this species was shown early. Nowadays it has become one of the preferred species in aquaculture with a total worldwide production in 2002 of approximately 510,000 tons. (FAO yearbook, 2002). The rainbow trout probably belongs to the fish species artificially reproduced for the longest time. Wales (1939) reported that Mr. S. Green transferred eyed eggs in 1874 from the McCloud river in Northern California to his private hatchery at Caledonia, New York. The first successful shipment of rainbow trout outside of North America was probably a transport in the year 1877 from J. B. Campbell, a private rancher at the McCloud River, to S. Akekiro in Tokyo. In the same year shipments to New Zealand followed. In 1882, the rainbow trout was transferred from USA to Germany (<http://www.fishbase.org>). This was followed by the 1884 shipments to the National Fish Culture Association of London, England, establishing a stock at the Delyford Hatchery, and to J. Maithland for the establishment of a breeding stock at the Hewetton Hatchery near Stirling, Scotland (MacCrimmon, 1971). In 1894 followed shipments to New Zealand and Australia. First transports to South Africa were established in 1897. On the South American continent, Chile got the first rainbow trout from Germany in 1908. The rainbow trout culture had later also spread to many Asian countries such as Sri Lanka, Korea, India, China, Nepal etc., where the species was established in areas with temperate climatic conditions (<http://www.fishbase.org>).

## 2.2 Reproduction of rainbow trout

### Natural spawning behaviour

In the natural habitats, the spawning season of the rainbow trout varies to some extent with the locality and the temperature of the water but normally it spawns in the spring (Leiritz and Lewis, 1976). In Japan, a rainbow trout strain spawning twice a year was discovered in 1973 (Aida et al., 1984; Imai et al., 1984).

Catwellander and Buckhouse (1983) reported from an Australian rainbow trout strain that the maturity of females was obtained when the fish were three years old while the male fish matured after two to three years. Gall and Crandell (1988b) found that the mature and post-spawning weights of the rainbow trout were 1.9 and 1.8 kg on average.

The ready to spawn rainbow trout females start to dig a pit in the gravel. While digging, an attendant male guards her or is busy driving away other males. As soon as the pit is completed, the female drops into it and is immediately followed by the male. The pair is side by side, they open their mouth, quiver and release eggs and sperm. Females produce from 700 to 4,000 eggs per spawning event (Gall and Crandell, 1982). Fecundity, eggs size, total egg volume all increase with increasing fish size (Brimage and Charnanukeng, 1988; Brimage et al., 1990a). The rainbow trout eggs are spherical in shape, demersal, 4 mm in diameter and pink to orange in color (Kafuku and Ikemoto, 1983; Gall and Crandell, 1982; Kasabata et al., 1993). After spawning the female quickly moves to the upstream edge and starts covering the eggs with gravel. The whole process is repeated for several days until the female deposited all her eggs (Morow, 1986). Kafuku and Ikemoto (1983) reported that rainbow trout eggs develop to the eyed stage within 12-13 days after spawning and hatch within 24-60 days at a water temperature of 6-13 °C with a tendency to the faster development at the higher water temperature. After hatching young fish move down streams mainly at night (Gall and Crandell, 1982).

### Artificial reproduction

The primary intent of any fish hatchery is to produce the maximum number of highest quality eggs and fry from the available brood stock. For artificial stripping, ready to spawn fish have to be anaesthetized, their genital papillae carefully dried with a cloth, and dry stripped by smooth pressure

on the abdominal area a bowel. Milt can similarly be dry stripped from males. Sperm samples should be checked for motility under a microscope before using it for fertilization of the eggs. Water has to be added after mixing eggs and milt to activate the sperm motility. Surplus milt has to be washed out carefully after some minutes and the eggs have to be left for approximately half an hour to harden before transferring them into the incubation facilities. Eggs become very sensitive to disturbance for a period after water has been added and swelling starts. Losses are likely to be greatest due to movement during the first 30 minutes. Eggs take in water more quickly at higher temperatures. Swelling takes about 1-25 hours at 6°C, but only about 25 minutes at 13°C. The commonest cause of loss during the sensitive period following fertilization is from washing off the surplus sperm. Losses of up to 20% can occur if the eggs are disturbed for up to hours after being placed in water (Sedgwick, 1985). James et al. (2000) reported that gametes from individual female and male rainbow trout were used in single pair matings to produce families whose survival was followed from fertilization to the time of swim-up. Survival was assessed at 0.5, 9, 19, 33, and 48 days post-fertilization, corresponding to second cleavage, embryonic keel formation, neural pigmentation, hatch, and swim up, respectively. The variability of survival at all times was significantly influenced by the female parent, whereas the influence of the male parent was negligible. Therefore, on rainbow trout embryo survival can be equated with the quality of the eggs.

After hardening of eggs (around 20 min after fertilization) they are transferred to incubation units. To provide a proper environment for the development of salmonid eggs incubation until hatching and first feeding is normally carried out in vertical and horizontal incubation trays or in Züger jars. These artificial incubating systems can provide the developing eggs with an adequate amount of high quality, well tempered and oxygenated water. The eggs should be incubated in the dark until the eyed egg stage is obtained.

### **Egg development**

There are six stages of oocyte growth: (1) oogenesis, (2) primary growth (3) cortical alveolus, (4) vitellogenesis, (5) maturation, and (6) ovulation (Yamamoto et al., 1965; Bromage and Cornaravutti, 1988). Vitellogenesis lasts at least 6 months in salmonids (Scott and Sumpter, 1983; Sumpter et al., 1984), and accounts for the major part of an oocyte's growth. In rainbow trout, oocytes increase in diameter from less than 1 mm to approximately 4.5 mm during vitellogenesis. Therefore, vitellogenesis accounts for over 98 % of the final oocyte volume. In salmonids,

vitellogenin is synthesized by the liver in response to oestradiol-17 beta secreted from the follicular granulosa cells encasing the oocytes (Kagawa et al., 1982). A number of factors have been shown to affect the rate of uptake of vitellogenin into rainbow trout oocytes, including the surrounding concentration of vitellogenin, the temperature and the stage of development of the oocytes (uptake of vitellogenin relative to oocyte surface area varies during vitellogenesis) (Dykens et al., 1990a, 1990b; Raizi and Fagundes, 1988). Other factors that are likely to influence the growth rate of oocytes and therefore the rate of vitellogenin sequestration include the nutritional status of the fish and the degree of stress experienced by the fish (both the synthesis and ovarian uptake of vitellogenin assessed indirectly by the rate of ovary growth are impaired by stress). During late vitellogenesis, in the month or so prior to ovulation, water uptake apparently also contributes to the increase in size of oocytes in rainbow trout (Raizi and Fagundes, 1988).

For optimal incubation conditions of spawned eggs the water temperature should range from 7-13°C with a DO (dissolved oxygen) of more than 8 ppm. When the water temperature is 11°C and DO is 10 ppm optimal results are achieved. A water supply of 3 l/min is required to incubate 10,000 eggs. Usually subterranean water or spring water is supplied to the incubator. Jeffrey (1990) suggests that trout eyed eggs purchased from a commercial supplier will usually contain only a small percentage of dead eggs, and may not require treatment for fungus. However, the eggs that are more than 3 days from hatching should be removed regularly to limit fungal infections. Dead eggs should be siphoned off. Formalin can be added to the inflowing water in a concentration of 1:600 for 15 minutes daily to avoid fungus. Kafuku and Ikemoto (1993) suggest that during incubation green eggs should be treated by malachite green every 2-3 days until the eggs reach the eyed stage. The survival rate of eggs from fertilization to the eyed stage is 90% in average.

#### **Influence of temperature on egg quality**

Exon and Lewis (1976) reported that it is safe to say the eggs of rainbow trout and king salmon will not develop normally if the females were kept at constant water temperatures above 13.3°C. Furthermore the authors state that in both species eggs show excessive losses after development in water below 5.5°C. Wild salmonids can succeed in reproduction until 1.3°C (MacCrimmon, 1971). Extreme temperatures can cause egg overripening with correspond effects on embryogenesis and fry survival, and it is therefore essential that eggs should undergo development, ovulation and artificial stripping at optimum temperatures in order to maximise survival of the offspring (Tetrainger and Hansen, 1993; Packhurst et al., 1996; Packhurst and Thomas, 1998). Low water temperature