Artificial propagation of the indigenous Tor species, empurau (*T. tambroides*) and semah (*T. douronensis*), Sarawak, East Malaysia

Sena S. De Silva[1], Brett Ingram[2], Stephen Sungan[3], David Tinggi[3], Geoff Gooley[2], Sih Yang Sim[4,1]

1. School of Ecology & Environment, Deakin University, Warrnambool, Victoria, Australia 3280; 2. Department of Primary Industries, Snobs Creek, Private Bag 20, Alexandra, Victoria, Australia 3714; 3. Indigenous Fisheries Research & Production Centre, Inland Fisheries Division, Department of Agriculture, Tarat, 94700 Serian, Sarawak, East Malaysia; 4. Network of Aquaculture Centres Asia-Pacific, PO Box 10400, Kasetsart Post Office, Bangkok 10903, Thailand.

There is an increasing realisation, a need and a trend to explore possibilities of culturing indigenous species. This becomes a reality only if species with a culture potential can be artificially propagated. This article summarises the success story of artificial propagation of two very important mahseer species, of high value and with high culture potential, carried out in Sarawak, East Malaysia. Furthermore, this is a good example of an international collaboration of research & development, in which NACA also played a role.

Introduction

Tor species, commonly known as mahseers, are widely distributed in Asia, in the Himalayan and South-east Asian regions, in the trans-Himalayan region (of Pakistan, Nepal, India and Myanmar) and in South-east Asia (including Thailand, Lao PDR, Cambodia, Vietnam, southern China, Peninsular Malaysia, Borneo, Sumatra and Java). The habitats of *Tor* species range from mountainous streams and rivers to fast flowing rivers in the plains, often preferring clear, swift-flowing waters with stony, pebbly or rocky bottoms (Shreshtha 1997). Currently about 16 species of *Tor* have been described, but the taxonomy of some remains uncertain and controversial. Some of the *Tor* species are considered to be excellent sport fish and Ogale (2002) described *T. khudree*, "as a sport fish, that provides unparalleled recreation to anglers from all over the world, better than salmon". Mahseers are often mooted as potential candidates for aquaculture, especially in the wake of the recent push toward the culture of indigenous species; mahseers are highly prized in most of Asia, and are sought after as food fish.

One possible factor hindering the culture of *Tor* species is the dependence of wild caught mature fish for artificial propagation, which lays a further stress on ever dwindling natural populations due to habitat degradation arising from forestry and associated developments. Breeding techniques are being developed for several *Tor* species in a number of countries. Most hatchery production of *Tor* juveniles,

94 hours post fertilisation, 1 post-hatch (total length = 9.82 mm).

5 days post-hatch (total length = 12.24 mm).

Pond-reared empurau at 60 weeks old (total length=250 mm, weight = 180 g).

Above & below: Empurau brood fish can be up to 20 kg.

Stripping and dry fertilizing.

including *T. khudree*, *T. mussullah*, *T. putitora* and *T. tor* for stocking programs, are derived from hand-stripping mature spawners caught from reservoirs, lakes and rivers during spawning seasons with or without use of hypophysation (Kulkarni 1971; Tripathi 1977; Sehgal 1999; Ogale 2002). More recently, research with these species has focused on inducing spawning in captive, pond-held broodstock. Joshi et al. (2002) and Gurung et al. (2002), successfully hand-stripped domesticated *T. putitora* broodfish without use of hormones while Nandeesha et al. (1993) induced spawning in pond-reared *T. khudree*. Joshi et al. (2002) also reported natural spawning in a pond-held T putitora.

Empurau, *T. tambroides* also known as kelah or belian, and semah, *T. douronensis* are indigenous to Sarawak, East Malaysia, and are the most valued freshwater fish species of the State. These two species are distributed throughout southeast Asia from Indonesia to southern China (Kottelat et al. 1993; Zhou and Cui 1996; Roberts 1999). Empurau and semah have significant cultural and economic importance in Sarawak, being highly prized and valued by indigenous communities. However, both species are now threatened in the wild due to environmental degradation and over-fishing. Consequently the Government of Sarawak embarked on a research and development program on the artificial propagation of the two species, when it established a special facility in this regard, the Indigenous Fisheries Research & Production Centre (IFRPC), in Tarat, Serian. Many attempts were made to artificially propagate these two species at this facility, using pond-reared broodstock, but with little success. The pond reared broodstock was originally caught from the wild as fingerlings and some of the broodstock were over eight years old, and weighed more than 20 kg.

In the above context the Division of Inland Fisheries, Department of Agriculture, Sarawak, in conjunction with the School of Ecology & Environment, Deakin University, Victoria, Australia, commenced a R & D project on the artificial propagation / captive breeding of these two species, under the leadership of Professor Sena S. De Silva. The project commenced in July 2001 and this paper entails the approach undertaken and the successes of the project.

Empurau and semah were caught from both the Limbang (4°4.6'N; 115°17.7'E to 3°52.6'N; 115°21.7' E) and Adang (4°4.54'N; 115°18.92' E to 4°6.27' N; 115°21.5' E) rivers, and transferred to the IFRPC. The bulk of the fish were collected over the period 1990 to 1993 and ranging in weight from about 200 g to 2.5 kg. These stocks, considered to be the broodstock were grown and maintained in either concrete or plastic-lined ponds (0.014-0.145 ha, 1.6-1.86 m deep), or circular tanks (1.9-3.0 m diameter, 0.9-1.1 m deep) with a

Table 1: Dimensions of semah and empurau broodfish, eggs and larvae

Parameter	Semah		Empurau	
	Mean	Range	Mean	Range
Mature females (induced to ovulate)				
Total length (mm)	519	440-635	678	610-749
Fork Length (mm)	456	385-565	575	500-660
Weight (g)	1499	600-3,300	3817	2,500-4,900
Mature males (running ripe)				
Total length (mm)	402	265-520	599	545-660
Fork Length (mm)	345	210-457	515	465-580
Weight (g)	851	300-2,300	2414	850-3,900
Eggs				
Diameter of stripped eggs (mm)	2.48	2.00-2.90	2.69	2.20-3.08
Weight of stripped eggs (mg)	11.1	6.8-16.1	13.3	9.5-18.8
Diameter of water-hardened eggs (mm)	2.92	2.52-3.28	3.21	2.88-4.44
Larvae				
Total length (mm)	8.79	7.29-9.38	9.08	7.40-10.21
Notochord length (mm)	8.78	8.54-9.07	9.37	8.96-9.69
Yolk -sac length (mm)	5.06	4.56-5.33	5.79	5.42-6.15
Yolk-sac breath	2.90	2.46-3.23	2.95	2.40-3.23
Yolk-sac depth	1.76	1.38-2.03	1.92	1.67-2.19
Yolk-sac depth	0.84	0.75-0.90	1.09	0.94-1.25

constant flow water and supplementary aeration. Ponds and tanks were stocked with fish of both sexes. Stocking densities ranged from 0.25 to 0.5 fish m^{-2}, depending on size and condition of the facilities.

The approach

The first step under the collaborative project was to get an insight into all forms of information that were available with the IFRPC. As always, there was a large amount of data that had been collected over the years, most from landings. The archival data was sieved through and analysed, particularly in relation to gonadal maturity. The gonadal maturation data, and estimations of gonadosomatic index (GSI), indicated, as expected of most riverine species, that the main spawning season of the two species was related to the rainy season, perhaps with two peaks in the year. The data also enabled a deduction of the mean maturation size of the two species, and it was also evident that mature, running ripe males were present in the riverine populations to some extent almost throughout the year. Egg diameter distributions of preserved ovaries indicated that semah and empurau, in all probability, are serial spawners capable of spawning several times in a season, provided that key environmental stimuli (i.e. monsoonal rains, etc.) are present. However, it is uncertain which is/are the real cues, except to suggest that rains/flow rate in the river had a major influence.

Previous artificial induction attempts using hypophysation techniques, which are routinely effective for most cultured finfish species, have not been successful. The primary reason for this appears to be that the ova did not mature beyond stage IV and hence the ineffectiveness of hypophysing. It was considered that the inability to reach maturation could be related to nutritional factors and so the diets on which the potential broodstock were maintained were analysed. This revealed that the fish were maintained on sub-optimal diets and that none of the diets given to the broodstock had sufficient amounts of highly unsaturated fatty acids, in particular, eicosapentaenoic acid (EPA: 20:5n-3), docosahexaenoic acid (DHA: 22:6n-3) and arachidonic acid (AA: 20:4n-6). Consequently, from January 2002, the fish were placed on a diet formulated for Murray cod, *Maccullochella peelii peelii* from Australia (De Silva et al. 2004), which was thought to fulfil the nutrient requirements of the two *Tor* species, of which little is known, and to have had a fatty acid profile suitable for maturation of most fish species. The broodstock condition improved and in April 2002 a few individuals when cannulated were found to have ova in Stage IV and V maturity stages.

The next step was to determine which was the most effective hormone to be used. In this regard different dosages of the hormones, and different methods of introduction were assessed through a series of controlled trials on both empurau and semah. In the trials mature females (induced to spawn) from 600 g and from 2,500 g and running ripe males, from 300 g and 850 g for semah and empurau respectively were used. All broodfish were individually pit-tagged (passive implant transponder), so as to enable reliable and accurate inventory control.

The various hormone treatments and dosages used were: Carp Pituitary Gland (CPG) (4 mg/kg followed by 8 mg/kg 17-27 hours later) human chorionic gonadotropin (HCG) (Pregnyl , Organon Laboratories Ltd) (300 iu/kg); and Ovaprim (Syndel Laboratories Ltd) (0.5 ml/kg) and Ovaplant (Syndel Laboratories Ltd) (75 and 150 mg pellets, 21-71 mg/kg). Combinations of hormones were administered in separate intra-musculature injections below the second dorsal fin. Males were given a single injection of either HCG (250 iu/kg) or Ovaprim (0.2-0.25 ml/kg), or not injected. After injection, females and males were placed together in 2000 litre

Above & left: Make-shift hatchery jars used in the trials

covered concrete tanks that were supplied with a constant flow of water and aerated. In all instances broodfish were handled with anaesthetic (MS 222; 1: 10,000) and in saline baths for prophylactic purposes, to reduce stress and reduce risk of infection.

The latent period was defined as the time between injection and stripping of gametes. To determine if ovulation had occurred, female semah and empurau were anaesthetised and pressure was applied to the abdomen to determine whether eggs could be stripped from the fish. These examinations commenced as early as 17.75 hours, though the bulk were 22-28 hours after the first injection, while latest examination occurred as late as 67 hours. During this period fish were examined up to four times.

Spawning induction

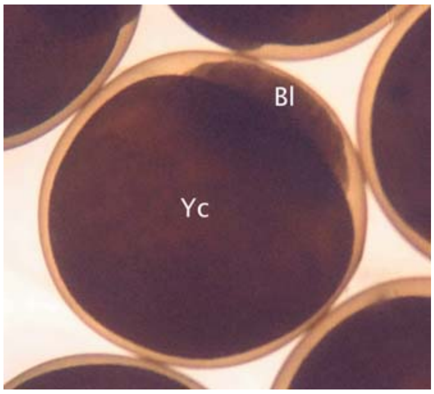
A total of 66 female empurau and 139 female semah were treated with hormones. Both pond-held and tank-held empurau and semah were induced to ovulate. Ovaprim was the most successful treatment for inducing ovulation in both species. Of the 130 females that were injected with Ovaprim, 55% of empurau and 24% of semah ovulated, while 42% and 10% of empurau and semah that were injected with Ovaprim, respectively, produced eggs that hatched (Fig. 1). In contrast, eleven females were injected with CPG, but one semah only ovulated, and ten females were injected with HCG but none ovulated. Of the 54 females that were implanted with Ovaplant, three semah ovulated and were stripped, but no eggs hatched.

Ovaplant pre-treatment

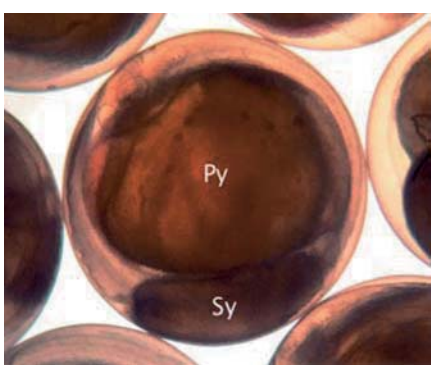
Thirty-six female empurau and 98 female semah were pre-treated with Ovaplant, 14-50 days (mean 31 days) prior to commencing spawning trials. Use of Ovaplant greatly improved the success rate of spawning induction using Ovaprim (Fig. 1). A total of 60% of female empurau received both Ovaplant and Ovaprim treatments ovulated, and 50% of injected fish produced eggs that hatched. In contrast, 38% of female empurau that were treated with Ovaprim only ovulated, while 13% of injected fish produced eggs that hatched. This trend was less clear for semah, 24% of fish ovulated for each treatment, while 9% and 12% produced eggs that hatched.

Ovaplant dose rates ranged from 21 to 71 mg/kg, though fish that received dose rates of 31-60 mg/kg (empurau) and 28-68 mg/kg (semah) ovulated following Ovaprim injection. A higher percentage (31%) of semah that received a high dose of Ovaplant (>45 mg/kg) ovulated following Ovaprim injection, but for empurau there was no clear relationship between dose rate of Ovaplant and spawning induction following Ovaprim injection.

Empurau that were implanted with Ovaplant 25-50 days were more likely to be induced to ovulate following Ovaprim injection (67-69% of fish), and produce eggs that hatched (56%-67% of fish) than did fish implanted 14-24 days prior to Ovaprim injection (ovulation = 38% of fish, produce eggs that hatch = 25% of fish) (Fig. 2). Although 40% of semah that were implanted with Ovaplant 14-24 days prior to Ovaprim injection ovulated following Ovaprim injection, no eggs hatched. A lower proportion of semah that were implanted with Ovaplant 25-50 days were induced to ovulate following Ovaprim injection (20-22% of



3.7 hours post fertilisation (3.13 mm diameter). Bl= Blastodisc; Yc = Yolk cell.



72 hours post fertilisation (3.29 mm diameter). Py = Primary yolk sac; Sy = Secondary yolk sac.

fish), but some fish produced eggs that hatched (9-15% of fish) (Fig. 2).

Eggs were hand-stripped from fish 23-53 hours post-injection (at 26-30 C), but hatch rates were greatest in eggs stripped 23-26 hours post-injection. The number of eggs stripped ranged from 30 to 2,150/kg (mean 875 eggs/kg) and 45-4,460 eggs/kg (mean 960 eggs/kg) for empurau and semah, respectively.

The number of eggs that could be stripped from the one female following initial stripping declined significantly with time. The number of eggs in subsequent strippings was <1% to 56% (mean 24%) of initial stripping.

Eggs and embryonic development

Embryonic development of other *Tor* species have been described previously (Kulkarni 1971; Desai 1972; Kulkarni 1980). Stripped eggs, prior to fertilisation were firm, and pale yellow to dark golden orange in colour. Semah eggs were "pale" in colour whereas "pale", "medium" and "dark" coloured eggs were stripped from empurau. There was no obvious relationship between broodstock diet and egg colour in the present study. Further, there was no apparent relationship between hatch rate of empurau eggs and egg colour.

Semah eggs were slightly smaller than empurau eggs. Prior to fertilisation eggs were 2.0-2.90 mm (mean 2.48 mm) and 2.20-3.08 mm (mean 2.69 mm) for semah and empurau, respectively. Within 15 minutes of fertilisation, eggs swelled in diameter by up to 20%. After this, egg diameter did not change over the remaining incubation period. Water-hardened eggs were spherical, demersal, non-sticky and translucent. The surface of the eggs was crenulated. Development rates were similar for semah and empurau.

Eggs were incubated in tilted plastic jars with flow-through water directed to the bottom of the jar to ensure continuous rolling motion of the eggs; thereby avoiding fungal infection. Hatching occurred 69-90 hours post-fertilisation. At hatch larvae were 7.3-10.2 mm TL and commenced feeding 4-5 days post-hatch. Juveniles were reared in static greenwater ponds. For fish up to 18 weeks of age, SGR's were 1.5-3.4%/day and for older fish (18-52 weeks of age) were 0.1-1.2 %/day (Fig. 3). At 60 weeks of age, empurau reared in one pond (AP11) were 100-270 g in weight (mean 179 g) (Fig 3). These results represent the first successful captive spawning and rearing of both species.

Conclusions

Historical date (from Sungai Adang Station, Sarawak), anecdotal reports and results from the current study support the view that both empurau and semah are asynchronous or intermittent spawners capable of spawning at several times per year, providing that key environmental stimuli are present. In the present study, one female semah was induced to ovulate four months after being stripped. Studies of pond-reared fish have shown that *T. putitora* can breed most months of the year (Gurung et al. 2002), but the frequency at which single females could spawn in a single year is unknown. Subhan and Hafeez (1998) suggested *T. putitora* undertook at least two spawning episodes each season. Shreshtha (1986) found that *T. putitora* ovaries possessed three size classes of eggs, the proportions of which varied throughout the year. Asynchronous spawners possess both primary oocytes and heterogenous populations of vitellogenic oocytes that may undergo final oocyte maturation on several occasions during the annual spawning season (Tyler and Sumpter 1996). Results from the present study support the view that mahseer are asynchronous spawners and that single females could spawn at least twice in a season.

Further research is required to improve and refine spawning and rearing techniques and associated facilities to mass-produce juveniles for stock enhancement purposes. In the present study inducement of spawning in semah was considerably more difficult than for empurau, and the quantity of deformed larvae (>14%) in each spawn was more than desirable. In particular research should focus on broodstock nutrition and refinement of hormone treatments. Joshi et al. (2002) fed an artificial diet to domesticated *T. putitora* that contained 35% protein at 2%/day, and subsequently reported natural spawning in a pond-held fish. It is likely that the ability to induce spawning in captive, domesticated stocks of empurau and semah will improve over time as the nutritional requirements of broodstock are met and spawning and associated husbandry techniques are improved. Also, there is a need to determine optimal rearing protocols for juveniles and yearlings. While the present study showed that empurau and semah can be grown in earthen ponds, there is a need to evaluate stocking densities, dietary requirements and feeding strategies to enhance growth and survival. Only after these aspects have been determined should commercialisation of the culture of these two species be promoted.

The captive breeding study is supplemented with a study on the genetics of the broodstock, using molecular genetic techniques, together with the genetic diversity of other *Tor* species. It is believed that this study will enable to provide useful insights into management practices for sustaining the genetic diversity of broodstock, ensure that genetic diversity of wild stocks are not overly influenced by stock enhancement using captive bred young for future conservation purposes, and that it will also shed light on the taxonomy and phylogeny of *Tor* species. In this regard this will be the first time in the region when the aquaculture development of an indigenous species has taken in to consideration genetic aspects, and therefore biodiversity issues, from inception.

This article is based on extracts from a manuscript submitted to the journal Aquaculture Research by these authors.

References

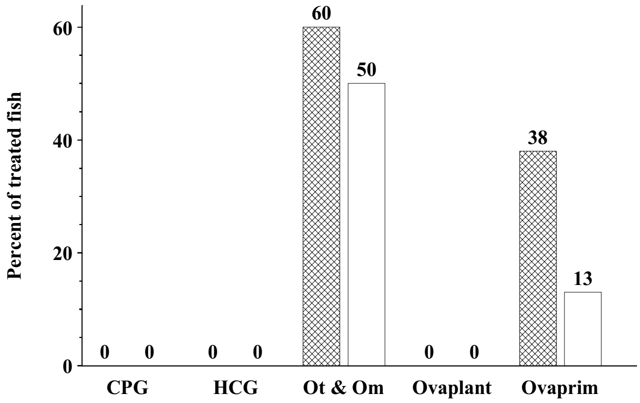
De Silva, S.S., Gunasekera, R.M., and Ingram, B.A. (2004). Comparisons of the performance of intensively farmed Murray cod in response to newly formulated diets versus commercial diets, and fillet chemical composition of farmed and wild fish. Aquaculture Research 35: 1039-1052.

Desai, V.R. (1972). Notes on the early larval stages of *Tor putitora* (Ham.). Journal of Zoological Society of India 24: 47-51.

Gurung, T.B., Rai, A.K., Joshi, P.L., Nepal, A., Baidya, A., Bista, J., and Basnet, S.R. (2002). Breeding of pond reared golden mahseer (*Tor putitora*) in Pokhara, Nepal. FAO Fisheries Technical Paper 431: 147-159.

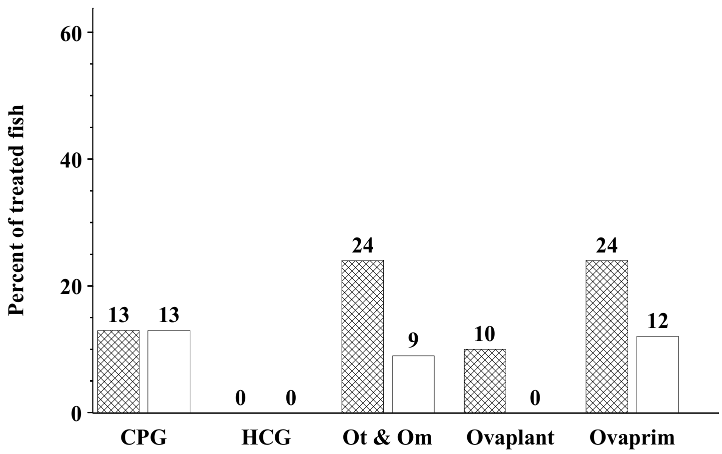
Joshi, P.L., Gurung, T.B., Basnyat, S.R., and Nepal, A.P. (2002). Domestication of wild golden mahseer (*Tor putitora*) and hatchery operation. FAO Fisheries Technical Paper 431: 173-178.

Figure 1a:



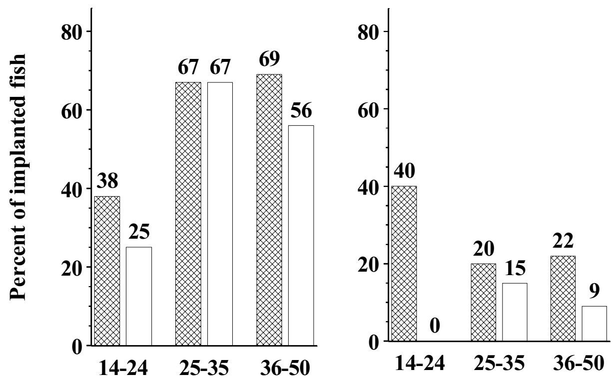
Effects of different hormone treatments on induction of spawning (shaded) and hatching success (clear). (a) Empurau. (b) Semah. (Ot & Om = Pre-treatment with Ovaplant followed by spawning induction with Ovaprim).

Figure 1b:



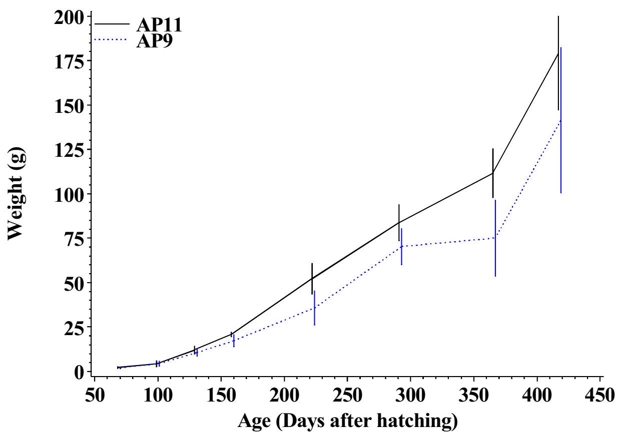
Percent of fish induced to spawn (shaded) and percent of fish induced to spawn and successfully hatch (clear) for fish that received Ovaplant at different time (14-24, 25-35 & 36-50 days) prior to induction of spawning with Ovaprim. (a) Empurau (b) semah

Figure 2:



Growth (mean weight SE) of empurau in two ponds, AP9-with bamboo poles and AP11 without bamboo poles

Figure 3:



Growth (mean weight SE) of empurau in two ponds, AP9-with bamboo poles and AP11 without bamboo poles

Kottelat, M., Whitten, A.J., Kartikasari, S.N., and Wirjoatmodjo, S. (1993). Freshwater Fishes of Western Indonesia and Sulawesi. Periplus Editions, Hong Kong. 221 pp.

Kulkarni, C.V. (1971). Spawning habits, eggs and early development of Deccan Mahseer, *Tor khudree* (Sykes). Journal of the Bombay Natural History Society 67: 510-521.

Kulkarni, C.V. (1980). Eggs and early development of *Tor tor* Mahseer. Journal of the Bombay Natural History Society 77: 70-75.

Nandeesha, M.C., Bhadraswamy, G., Patil, J.G., Varghese, T.J., Sarma, K., and Keshavanath, P. (1993). Preliminary results on induced spawning of pond-raised mahseer, *Tor khudree*. Journal of Aquaculture in the Tropics 8: 55-60.

Ogale, S.N. (2002). Mahseer breeding and conservation and possibilities of commercial culture. The Indian experience. FAO Fisheries Technical Paper 431: 193-212.

Roberts, T.Y. (1999). Fishes of the cyprinid genus *Tor* in the Nam Theun watershed (Mekong Basin) of Laos, with description of a new species. The Raffles Bulletin of Zoology 47: 225-236.

Sehgal, K.L. (1999). Coldwater fish and fisheries of the Indian Himalayas: Culture. FAO Fisheries Technical Paper 385.

Shreshtha, T.K. (1986). Spawning ecology and behavior of the mahseer *Tor putitora* (Hamilton) in the Himalayan waters of Nepal. In: J.L. Maclean, L.B. Dizon, and L.V. Hosillos (Eds), The First Asian Fisheries Forum. Proceedings of the First Asian Fisheries Forum, Manila, Philippines, 26 31 May 1986. Pp 689-692.

Shreshtha, T.K. (1997). The Mahseer in the Rivers of Nepal Disrupted by Dams and Ranching Strategies. Mrs. Bimala Shrestha, Kathmandu, Nepal. 259 pp.

Shrestha, T.K. (1994). Migration and spawning of golden mahseer in Himalayan waters of Nepal. Journal of Freshwater Biology 6: 71-77.

Subhan, F., and Hafeez, M.A. (1998). Gonadal maturation and breeding biology of the Putitora mahseer, *Tor putitora* Hamilton (Teleostei) from Rawal Lake, Islamabad. Pakistan Journal of Zoology 30: 341-353.

Tripathi, Y.R. (1977). Artificial breeding of *Tor putitora* (Ham.). Journal of the Inland Fisheries Society, India 9: 161.

Tyler, C.R., and Sumpter, J.P. (1996). Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6: 287-318.

Zhou, W., and Cui, G.-H. (1996). A review of *Tor* species from the Lancangjiang River (Upper Mekong River), China (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters 7: 131-142.