Guidelines for broodstock management, propagation and culture

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1 Introduction

Tor tambroides and *T. douronensis*, locally referred to as *semah* and *empurau*, respectively, are high valued mahseer species, indigenous to Sarawak, East Malaysia, with an aquaculture potential and of conservational value. These two indigenous species live in headwaters of most major river systems of this state. These two species also occur in Peninsular Malaysia and are distributed throughout south-east Asia from Indonesia to southern China (Kottelat et al. 1993, Roberts 1999, Zhou and Cui 1996). Semah is the State Fish of Sarawak, and both species currently fetch a very high market price are of high cultural value. Juveniles of these two species are also increasingly sought after by the aquarium industry (Ng 2004).

The Government of Sarawak, recognising the importance of these two species, made an attempt to evaluate their aquaculture potential, including captive breeding using long-term pond-reared broodstock, commencing in the 1990s. However, limited success was achieved until the period 2002-2004 when, in an international collaboration, researchers from Australia and Sarawak were able to captive breed both species using hormone induction techniques on long-term, pond-reared broodstock.

The Project "*Artificial propagation of empurau, Tor tambroides and semah, Tor douronensis, two species of commercial and conservation value to Sarawak, Malaysia*", commenced in April 2001, and was successfully carried out over a four- year period. The Project accomplished the primary objectives, and the most outstanding achievements were the success in the captive breeding of the *semah* and *empurau*, and the training of the Malaysian officers in adopting the techniques, as a routine, and the successes in the larval to fingerling rearing of the two species escaped/ released on the genetic diversity of the wild counterparts. As such, a genetic management plan is needed to warrant long-term maintenance of genetic diversity of cultured stocks, as well as to minimise potential adverse effects on the genetic integrity of the wild populations.

The recent successful hatchery production of *semah* and *empurau* brought to the forefront problematic questions regarding sustainability of the aquaculture development and stock enhancement program of these two species. From an environmental point of view, aquaculture and stock enhancement could be counter-productive if not properly managed, especially with regard to the potential negative impacts of hatchery-produced seed if

This document presents:

Better management practices for captive breeding and culture of *semah* and *empurau* which encompass broodstock managenment, artificial propagation and related husbandry activities.

In the above context an enhancement strategy for the long-term management of *semah* and *empurau* broodstock, and conservation of wild stocks, based on genetic findings will be discussed in this guidelines. In addition, the guidelines also incorporate best husbandry management practices to ensure a supply of physically and genetically healthy quality seed.

2 *Semah* and *empurau*

2.1 *Nomenclature* and *taxonomy*

Class	Actinopterygii (Ray-finned fishes)
Order	Cypriniformes
Family	Cyprinidae
Genus	*Tor* Gray, 1834
Species	*T. douronensis* (Valenciennes, 1842) (*semah*)
	T. tambroides (Bleeker, 1854) (*empurau*)

Taxonomy of the genus *Tor* is generally remains controversial and no accepted consensus is available at present.

As for the two species under consideration, they are morphologically similar and as such rather difficult to distinguish by their external appearance. Commonly, the two species could be distinguished by a few diagnostic characters, such as scale size and colour, the length of the median lobe and the thickness of the lip as shown in Table 1.

Taxonomy of semah and *empurau:*

Taxonomic status of *semah* and *empurau* is complicated. The two species show similar morphological characters and sometimes it is difficult to distinguish. However, molecular genetic tools can be used to identify the two species very efficiently.

Table 1. Characters used for identifying *empurau*, *T. tambroides* and *semah*, *T. douronensis*

Characters	***Empurau***	***Semah***
Scale size	Larger	Smaller
Scale colour	reddish, white	Yellowish, gold, silvery
Length of median lobe	short, not extending to a line connecting inner corners of mouth	long, extending to a line connecting inner corners of mouth
Thickness of lip	thicker and more fleshy	thinner and less fleshy

2.2 Distribution

Semah and *empurau* live in headwaters of most major river systems of Sarawak. These two species also occur in Peninsular Malaysia and are distributed throughout southeast Asia from Indonesia to southern China (Kottelat et al. 1993, Roberts 1999, Zhou and Cui 1996). *Semah* is the State Fish of Sarawak, and both species currently fetch a very high market and are of high cultural value. Juveniles of these two species are also increasingly sought after by the aquarium industry (Ng 2004).

Semah and *empurau* may be a useful indicator of environmental health (The Kuala Lumpur Declaration, 2006). In Sarawak, the two species is distributed in most river systems and often found in upper reaches of rivers and streams with fast, clear flowing waters of high oxygen content and stony or rocky substrates. Their natural food is mainly plant matter such as leaves, flowers and fruits (Ng, 2004). Sometimes they feed opportunistically on aquatic animals and fallen terrestrial insects (Sungan, 2006). The two species sometimes co-occur in the same river systems but not always.

As with many other *Tor* species, wild populations of *semah* and *empurau* are increasing exploited due to their high value and attraction as good sport fish. Pressures from fishing and modification of habitats in the watersheds have led to significant decline of the wild populations. The spawning habitat in clear, calm and slow moving upstream waters with pebble or sandy bottom, are much disturbed by logging-related activities (Sungan, 2006).

Distribution of semah and empurau in Sarawak:

1. *Semah* are found in almost all river systems in Sarawak while *empurau* has more limited distribution. The two species are found co-occurred in some rivers such as the Limbang and the Rajang rivers, but only *semah* is found in some rivers such as the Layar.
2. Wild populations of *semah* and *empurau* have declined as a result of anthropogenic activities.

3 Guidelines for broodstock management

The goal of the broodstock management strategy for *semah* and *empurau* is to assure the sustainable supply of genetically and physically good quality seed for aquaculture as well as for replenishing the depleted wild stocks in Sarawak.

3.1 Procurement of new broodstock

3.1.1 Number of broodstock

Number of broodstock required for beeding purposes will depend on the aims of the breeding programs. For details on determining suitable numbers of broodstock for each breeding plan please refer to pages 10-13 of the "Genetic management guidelines". It is important to use large number of broodstock, with male : female ratio of 1:1 in order to maintain high effective population size, hence maintaining high levels of genetic diversity.

3.1.2 Capture

Broodstock should be collected from the catchment or river system that is intended to be stocked to ensure the genetic structure of the population within the system is maintained and that restocking activities has minimal impact on that structure.

Capture and transport and handling techniques should minimise physical damage to captured fish.

3.1.3 Quarantine

An important source of pathogens in aquaculture facilities is new fish stock. To ensure that diseases are not introduced to aquaculture facilities, all new stock, regardless of the source, must be quarantined. All aquaculture facilities, regardless of size, must have a quarantine facility that is physically isolated from the other facilities (separate room or building, or separate location on the farm). Effluent water discharged from the quarantine facility may carry pathogens and so must not be allowed to mix with the water entering other culture systems within the facility. Equipment used in the quarantine facility must not be used in other areas of the aquaculture facility. Minimise traffic through the quarantine facility and disinfectant footbaths should also be used at entries/exists. New stock should be quarantined for at least 2 weeks before introduction to other facilities on the farm. During that time, fish should be checked regularly for signs of disease, and should be given prophylactic treatments, such as salt (10 g per liter for up to one hour) and formalin baths (50-100 ppm for up to one hour). New stock should appear healthy and ideally be inspected by a qualified fish health specialist before leaving the quarantine facility.

3.1.4 Microchipping

In order to effectively manage breeding programs, either for stocking or aquaculture, all broodstock must be individually identifiable. This can be achieved by implanting each fish with a microchip, a passive integrated transponder (PIT) that emits a unique alpha-numeric code that is detected by an electronic scanner. Microchips are implanted with a hypodermic syringe. Instructions for implanting fish are provided by the equipment supplier. However, locations for implanting the microchips within broodstock include, intramuscular sites and intraperitoneally. This should be done as soon as the new fish arrive on the farm.

Procurement of new broodstock:

1. Please refer to the "Genetic management guidelines" for details on number of new broodstock that should be procured for breeding programs, as well as appropriate locations to collect new broodstock.
2. Broodstock capture, handling and transport should be undertaken with care in order to minimize potential physical damage to the fish.
3. All newly procured fish should undergo a quarantine period of at least 2 weeks.
4. All newly procured fish should be tagged for identification and monitoring purposes.

3.2 Maintenance

3.2.1 Pond and tanks maintenance/ management

At the IFRPC, broodstock have been maintained in circular HDPE or fiberglass tanks (1.9-3.0 m dia, 0.9-1.1 m deep), square concrete tanks (13-14 m^2, 0.6-0.8 m deep), plastic-lined earthen ponds (0.014-0.145 ha^{-1}, 1.6-1.86 m deep) and mesh cages (4 m^2, 1-1.5 m deep) suspended in ponds. Tanks and ponds are provided with a constant flow water and supplementary aeration. Ponds and tanks are stocked with fish of both sexes.

Stocking fish in tanks and ponds at high densities can expose stock to constant, elevated concentrations of ammonia and/or nitrite, and low concentrations of DO, which can suppress growth. Optimal stocking densities for *semah* and *empurau* broodstock have not been determined. However, stocking densities used for broodstock at the IFRPC have ranged from 0.25-0.5 fish/m^2 (0.5-1.25 kg/m^2) in ponds and up to 10 kg/m^3 in tanks.

3.2.2 Sedation and anaesthesia

Semah and *empurau* are sedated or anaesthetised for a range of purposes, including measurement, tagging, spawning and transfer between holding facilities. It is important to handle fish as gently as possible to preserve the surface mucous coating and to prevent any physical damage. Handling of fish is always stressful, and may lead to lowered immune function and subsequent increased risk of infectious disease. Thus, for certain procedures, especially with larger fish, sedation is required to minimise this stress.

Common drugs used to sedate and anaesthetize fish include, Aqui-S, MS222 and benzocaine. These drugs are administered through the water and are taken up across the gills. Dose rates for these drugs depend on the species, size of fish water temperature and level of sedation required. Use of these drugs should follow the instructions provided by the supplier. Exposure time should be minimised where possible to avoid death from over exposure.

When using anesthetics, it is important that the water is well aerated or oxygenated to maintain dissolved oxygen concentrations. Fish condition should be monitored closely while under anaesthesia, for example, opercula movement should be regular, flaring of opercula may indicate over exposure and immediate steps should be taken to recover the animal. Following anaesthesia, fish should be placed in clean, well aerated water and monitored until they recover. Initial recovery should be quick, i.e. a few seconds to a few minutes. Full recovery may take several minutes or longer depending on species and drugs used. In some cases, “swimming” of fish to increase ventilation of the gills may facilitate recovery from deep anaesthesia.

3.2.3 Water quality

Water is a vital component of aquaculture operations as it is the medium in which the fish live and grow. Both the quantity and quality of water used in aquaculture are critical to the well-being of fish under culture. A regular and abundant water supply is essential for any aquaculture operation. Ideally the water supply should be regular and reliable, free of pathogens, and free of pollutants (organic, industrial and urban) and consequently, regardless of the water source, some form of pre-treatment will be required before it is used in the culture system. Untreated surface waters pose the greatest risk of pathogen contamination, especially if fish are present in the water supply. Treatment options may include combinations of:

- Mechanical screening (sand filters, carbon filters, screen filters) to remove particulate matter.
- Chemical treatment (chlorination followed by dechlorination and/or aging) to eliminate pathogens.
- Ozonisation to eliminate pathogens
- UV sterilisation to eliminate pathogens.
- Elimination of fish from the water source (where possible).

Many water quality parameters, if they are altered beyond acceptable limits for the fish being cultured, can influence fish health both directly and indirectly. Each species has a preferred range of water quality parameters and outside of this range will suffer stress, which may lead to reduced growth, disease and even death. There is a high degree of inter-relationship between water quality parameters, which means that a variation in one water quality parameter can influence the toxicity of others.

Fish can be affected by water-borne contaminants from the external environment as well as those arising from their own activities. Potential external sources of water contaminants include pollution from agriculture, sewage or industrial sources as well as natural variations in water quality caused by geology, soils and the climate. Internally, water quality may be influenced by farm management, fish husbandry (including stocking density and feeding) as well as excreted wastes of the fish. Culture of fish at high densities requires close monitoring of water quality, especially DO, pH, TAN (and unionised ammonia) and nitrite to ensure that critical levels are not reached or exceeded. The physical action of the fish in stirring up sediments can also have an impact on some parameters. To minimise the risk of water quality-related fish disease the potential hazards of any water supply should always be well understood. Farm management and fish husbandry should also reflect risks associated with the water supply.

Water quality requirements vary between species, but there is no information on the tolerance levels of *semah* and *empurau*. In the absence of specific water quality requirements for these species, historical data recorded in ponds and tanks used to rear *semah* and *empurau* may be used as a guide to acceptable water quality for aquaculture as well as published water quality guidelines for the production of other aquatic organisms (Table 2).

3.2.4 Nutritional requirement and feeding

Nutritional requirements of fish, especially for protein, amino acids, lipids, minerals and vitamins, varies from one species to another, and for some species the nutritional requirements will also vary with age (De Silva and Anderson 1995). There is a wide variation in the types and quality of diets used to feed farmed fish and broodfish nutrition and feeding can greatly affect gamete quality and seedstock production. Nutritional deficiencies or imbalances can reduce growth rates and even cause disease and death in farmed fish.

An improvement in broodstock nutrition and feeding has been shown to greatly improve not only egg and sperm quality but also seed production. Izquierdo *et al.* 2001). Gonadal development and fecundity in broodstock are affected by certain essential dietary nutrients, especially in continuous spawners with short vitellogenic periods such as *semah* and *empurau*. Therefore it is important to feed the broodstock of these species a high quality diet that meets their nutritional needs.

Table 2. Recommended acceptable water quality ranges for *semah* and *empurau* aquaculture

Parameter	**Suitable concentrations for continuous exposure**	**Recorded from *empurau* & *semah* aquaculture facilities[2]**	
	(Various sources[1])	**Range**	**Mean**
Water temperature (°C)	5-30	22.5-35.1	26.9
Dissolved oxygen (mg/L)	>5	2.4-15	6.2
pH	6.5-9	5.6-10.9	8.3
Total ammonia (TAN) (mg/L)	<3.0	0.02-2.1	0.23
Unionised ammonia (mg/L)	<0.02	0.0002-0.471	0.057
Total alkalinity (mg/L)	20-400	8-72	35
Carbon dioxide (mg/L)	<15	No data	
Chlorine (mg/L)	<0.03	No data	
Conductivity (us/cm)	<10,000	0.004-2.58	0.1
Total hardness (mg/L)	50-400	No data	
Hydrogen sulphide (mg/L)	<0.002	No data	
Nitrate (mg/L)	<100	No data	
Nitrite (mg/L)	<0.1	0.001-0.067	0.0063
Total Phosphorus (mg/L)	No data	No data	
Orthophosphate (mg/L)	No data	0-0.55	0.13
Turbidity (NTU or FAU)	<40	0-522	109
Metals Cadmium (mg/L)	<0.003	No data	
Calcium (mg/L)	10-160	No data	
Copper (mg/L)	<0.006	No data	
Iron (mg/L)	<0.5	No data	
Lead (mg/L)	<0.03	No data	
Manganese (mg/L)	<0.01	No data	
Mercury (mg/L)	<0.002	No data	
Zinc (mg/L)	<0.03	No data	

1. ANZECC 2000, Shepherd and Bromage 1988, Piper *et al.* 1998, Boyd 1990, Billard 1995.
2. Summary of data collected between 2003 and 2006 from ponds and tanks at the IFRPC Tarat, supplemented with data from ponds at Layar and Lundu.

Mahseer broodstock have been fed diets containing 30-40% crude protein (Gurung *et al.* 2002; Ingram *et al.* 2007a), or formulated diets containing rice bran, oil cake, fish meal, wheat flour, ragi flour, rice flour, horse gram and vitamin and mineral mix (Basavaraja *et al.* 2006). Keshavanath *et al.* (2006) found that *Tor khudree* feeding broodstock with a diet containing 24.5% crude protein resulted in higher ovulation response, shorter time for ovulation and higher fertilization rate of eggs, compared to 31.5% crude protein diet.

Unfortunately there are no commercially available artificial diets specifically formulated for *semah* and *empurau* species. Instead, artificial feeds formulated for other species, such as seabass and tilapia, are often used to feed mahseer in captivity. In previous research on *semah* and *empurau*, broodfish were fed commercially available fish diets (40-45% crude protein, 5-10% fat). However, these diets may not be ideal for feeding *semah* and *empurau* broodfish as their nutritional composition (protein, amino acids, lipids, etc) may not be appropriate for development of

reproductive gametes. Supplementing the diet of *semah* and *empurau* with fruits, nuts, seedlings and vegetables is thought to provide a more nutritionally varied diet that may be more conducive to gonadal development, maturation and spawning. In the wild, *semah* and *empurau* are known to feed on fruits and seeds that fall into the water (Tan 1980, Sungan 1994), whereas in captivity fish will feed on a range of food items, though acceptance of diets is variable (Table 3). For example, banana and kelampu, have a low acceptance whilst kelampai, carrot and rambutan have a medium acceptance, and papaya and an artificial seabass diet a high acceptance. Nutritional value of these diets is also variable.

Preliminary broodfish feeding experiments suggest that diet affects reproduction in captive *semah* and *empurau* (Ingram *et al*. 2007). After feeding broodfish on either seabass diet only or seabass diet supplemented with fruits and vegetables for four months, there were no significant differences in the condition of fish (estimated as weight in g ÷ length in cm^3 x 1000), proportion of fish that can be induced to spawn, and stripped fecundity between treatments for either species. However, the hatch rate in *empurau* fed sea bass diet only (79% hatch rate) was significantly greater than fish fed seabass diet supplemented with fruits and vegetables (34% hatch rate).

Table 3. Acceptance rating of various diets fed to *semah* and *empurau* broodfish (1 = poor acceptance to 5 =- high acceptance) (Nr = not recorded)

Diet	**Acceptance**
Mung beans	1
Banana (*Musa* spp.)	1
Kelampu (*Sandoricum koetjape*)	1
Kelampai (*Pimelodendron* sp.)	2-3
Tapioca	2-3
Carrot (*Duacus carota*)	3
Rambutans (*Nephelium lappaceum*)	3
Ara	3
Live shrimp	4
Papaya (*Carica papaya*)	4
Rubber seeds (*Hevea brasiliensis*)	Nr
Rapi (*Elateriospermum tapos*)	Nr
Soaked soy beans	4
Peanuts	4-5
Sea bass pellets (40-45% crude protein, 5-10% fat)	5

Since feed rates have not been determined for *semah* and *empurau*, broodstock should be fed to satiation at least once per day (1-2% dry weight feed to wet weight).

Artificial feeds should be stored under cool (<20°C) and dry (<75% humidity) conditions to prevent spoilage. Feeds can become rancid when stored in damp, warm conditions and breakdown of fatty acids in feeds containing insufficient amounts of anti-oxidants can result in the production of harmful toxins. Moulds and fungi growing on the feeds may also produce toxins. Do not use feeds that have a musty or stale smell, are discoloured or appear to be "sweating". Feeds showing any signs of contamination by moulds or fungi should be discarded.

The incidence of nutritional diseases in farmed mahseer can be reduced by maintenance of good husbandry and management practices.

- Use feeds that are specifically formulated for the species and age of the fish.
- Purchase quality feeds from recognised suppliers.
- Use appropriate feed rates for the age and conditions under which they are being reared.
- Ensure the palatability, texture and size of feeds, and frequency/timing of feeding are optimised.
- Feeding strategies may be facilitated by using automatic feeding devices.
- Feeds should be stored according to the manufacturer's instructions.

3.2.5 Biosecurity

Biosecurity management in a hatchery/aquaculture facility, such as the IFRPC, aims to protect the facility against unwanted introductions of animals and infectious diseases, and to prevent the escape of animals and pathogens from the facility to the surrounding environment. Escaped animals may out-compete, displace, prey on, or alter the habits of endemic species, and/or alter the habitat itself. They may also may also interbreed wild indigenous populations of the same species (genetically different strains or populations) or related species (interspecific hybridisation) affecting genetic structure (change in allele frequencies, genetic diversity etc.).

Regardless of the water source, water should be physically screened (preferably less than 0.5 mm) to prevent unwanted animals entering the facility, and ideally sterilized (ozonated, UV irradiated) to eliminate waterborne pathogens.

To ensure that pathogens are not introduced with new stock, all fish being moved onto the facility, regardless of their origin, must be quarantined. The Quarantine facility should be separate from the rest. New stock should be quarantined for at least 2 weeks before introducing to the production facility. During that time, fish should be checked regularly for signs of disease, and if necessary be given therapeutic treatments to kill pathogens.

Reduction of escape from hatcheries/aquaculture facilities is achieved by locating facilities away from flood prone land (ie above 1 in 100 year flood level). Physically screening discharge water will prevent escape of animals to receiving waters. Maintaining hygienic conditions on the facility and having an active health monitoring/management program will reduce the incidence of pathogens on the facility and in discharge water.

3.2.6 Health management

Disease outbreaks have long been recognised as a significant constraint to aquaculture production and economic viability. A wide range of pathogens (viruses, bacteria, parasites etc), environmental factors (water quality, etc) and even husbandry factors have caused heavy losses in aquaculture facilities (Hoole *et al.* 2001, Sarig 1971, Noga 2000, Brown 1993). Often these factors are linked in disease outbreaks. For example, a decline in water quality associated with poor husbandry practices may lead to an increase in the incidence of bacterial infections. Not only can disease outbreaks inflict heavy mortalities of stock, but fish in poor health have considerably lower growths rates which increases the time and cost to grow them to a marketable size.

The major sources of pathogens are the water supply, especially if fish are present in that source water, and new fish stock brought to the facility from other locations. Both these sources must to

be appropriately screened and treated to ensure that introduction of pathogens to facilities is minimised and biosecurity maintained.

A stringent fish health-monitoring program incorporating regular sampling and examination for diseases is required to maintain healthy fish stocks. Recording information about each holding facility (tank or pond) or batch of fish greatly facilitates disease diagnosis and long-term management. These records should include daily mortalities, health checks, water quality and treatments. The type of information that should be recorded and frequency of collection is summarised in Annex 1. Sampling should be frequent enough to ensure diseases are detected in time to reduce their impact on fish health and survival.

Stress is considered as an environmental externality that reduces the ability or capacity of a fish to maintain health and well-being. More importantly, stress can reduce growth and illicit poor fish health. Stressed fish suffer from depressed immune systems and consequently lowered resistance to disease or parasitic infestation. Stressors in fish farming include handling, transport, territorial behaviour, predation pressure, confinement and overstocking, chemicals and water quality and parasitism/disease.

A wide variety of diseases and parasites have been recorded from freshwater fish, including viruses, bacteria, fungi, protozoans, trematodes, nematodes, cestodes and copepods. However, there is little published information on the diseases of mahseers, and none on *semah* and *empurau*. In the absence of such information published literature on other species is required to assist in disease diagnosis and treatment (Hoole *et al.* 2001, Noga 2000, Schlotfield and Alderman 1995, Iwama *et al.* 1997).

Monitoring is an important part of early identification, management of disease problems. A decision support flowchart to assist in the identification of common health problems is presented in (Figure 1).

Fish health management strategies in aquaculture operations aim to maintain the health and well-being of stock, while optimising fish production. Key actions, which are critical to achieving this include:

- Take an active approach to managing the health of stock. Monitor health of stock regularly.
- Maintain hygienic conditions.
- Maintain biosecurity (sterilise inlet water and quarantine all new stock)
- Guard against poor water quality. Monitor water quality regularly
- Minimise unnecessary stress.
- Feed fish an appropriate diet (nutritional composition, size and amount).

3.2.7 Data management

In order to effectively manage the breeding program, data on each broodfish should be maintained in a database which should include information on: source of fish (where collected from in the wild), location of fish on the farm, length and weight data, sex, diet and spawning data. The latter should include dates and times of spawning induction, hormone treatments, volume of gametes (eggs and milt) stripped number eggs stripped, individuals used in meetings, fertilization rates and hatch rates.

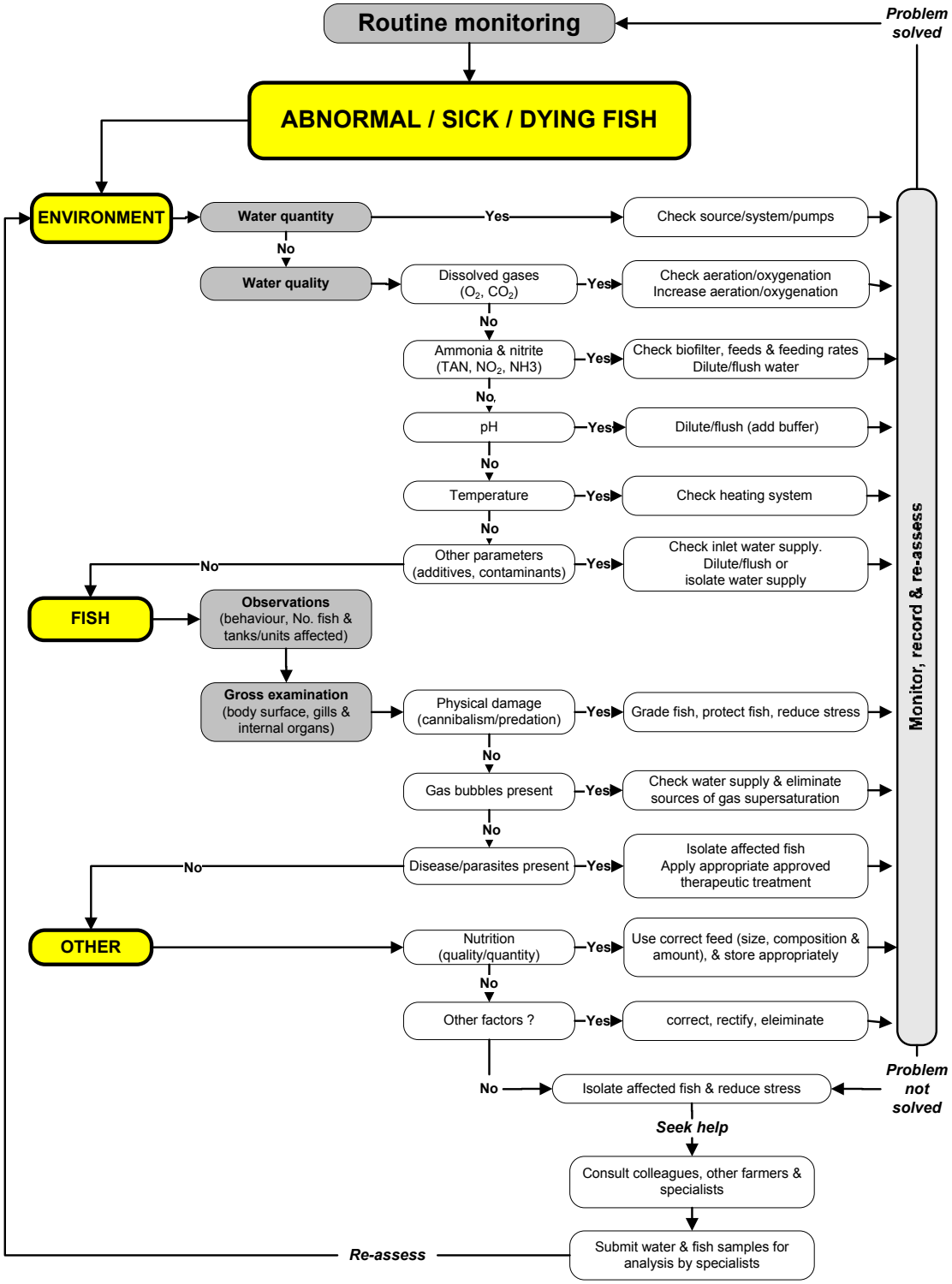

Figure 1. Decision support pathway for fish health management

Maintenance of broodstock:

1. Broodstock can be kept in ponds or concrete tanks. Stocking densities of 0.5 – 1.25 kg/m^3 and 10 kg/m^3 could be used in ponds and tanks, respectively.
2. Handling should be undertaken with care to prevent stress and physical damage to stock. Broodstock must be sedated with an anaesthetic when handling for tagging or hormone injection. The use of anaesthetics in this regard should strictly follow manufacturer instructions, and local rules and regulations.
3. Ensure enough supply of good quality water to maintain broodstock. Water should be stored and treated if necessary to prevent introduction of pathogens.
4. No specific diets are formulated for *semah* and *empurau*. However, commercial diets developed for seabass or tilapia can be used to feed broodstock. Supplement feedings using fruits and vegetables are recommended.
5. Feeds should be stored in cool and dry conditions. Spoiled feeds should not be used.
6. Fish infected with diseases should be isolated to prevent further spread of infectious agents to other stocks. Fish health specialists should be consulted for appropriate diagnosis and treatment protocols.

3.3 Artificial propagation

3.3.1 Spawning in captivity

In the wild, *semah* and *empurau*, being tropical species, are asynchronous or intermittent spawners capable of spawning several times per year, providing that key environmental stimuli are present. Previous research has shown that captive *semah* and *empurau* broodstock can be induced to spawn in most months of the year, and that the breeding performance of both species improves with time in captivity (Ingram *et al.* 2007a). Indeed, some fish can be successfully stripped without the need of a hormone injection. Breeding performance of *semah* and *empurau* may be influenced more by habituation to captivity than environmental (seasonal) factors that influence spawning in the wild. The improvement in breeding of these species are attributed to a number of key factors including improved broodfish maintenance and husbandry techniques specifically suited to the species, improved nutrition and refinement of hormone induction (hypophysiation) techniques.

The lack of any clear seasonal patterns in the breeding performance of captive *semah* and *empurau* broodfish suggest that, once fish have become acclimatized to captivity, and husbandry conditions are appropriate, males will spermiate all-year-round and females may be induced to spawn at any time of the year or season. Indeed, some fish *empurau* broodstock have been stripped up to five times a 12 months period (some as little as 30 days apart) (Ingram *et al.* 2007a). Nevertheless, the influence of monsoonal climate patterns in spawning of these species is evident as indicated by breeding performance in captive *semah* and *empurau* being highest in the NE monsoon period (Ingram *et al.* 2007a).

3.3.2 Hormone induced spawning

Semah and *empurau* were successfully spawned in captivity for the first time in April 2003 at the IFRPC. Since then captive spawning techniques have been further developed through a series of breeding trials conducted over several years (De Silva *et al.* 2004, Ingram *et al.* 2005, 2006, 20007). These trials showed that the proportion of fish induced to spawn, and hatch rates, have improved considerably since the initial breakthrough, highlighting that improved husbandry and breeding techniques combined with acclimatisation of broodfish to captivity have enhanced the breeding potential of *semah* and *empurau* in captivity.

3.3.2.1 Females

Spawning has been induced in captive female *semah* and *empurau* broodstock weighing from 600g and 2,500 g, respectively. Both pond-held and tank-held *semah* and *empurau* can be induced to spawn.

Ovaprim (Syndel Laboratories Ltd., Vancouver, Canada), at a dose rate of 0.5 mL/kg, was the most successful treatment for inducing ovulation in both species. Ovaprim is injected intra-muscularly, adjacent to the second dorsal fin. Use of Ovaplant (Syndel Laboratories Ltd., Vancouver, Canada), may promote final oocyte maturation and greatly improve the success rate of spawning induction using Ovaprim. Ovaplant pellets (75 or 150 μg) are implanted intra-muscularly with an applicator provided by the supplier, 4-5 weeks prior to the intended date of spawning induction. The guide in Table 4 can be used to determine Ovaplant dosages for *semah* and *empurau*. Previous successful spawnings have occurred when Ovaplant dose rates have been 28-68 μg/kg (mean 43 μg/kg). Since the initial breakthrough in hormone-induced spawning some female broodfish of both species have ovulated and been successfully hand-stripped without use of hormones.

Table 4. Guideline for Ovaplant dose rates for *semah* and *empurau*

Fish weight (g)	Ovaplant pellet size (μg/fish)	Ovaplant dose rate (μg/kg)
500 – 2,500	75	30 – 150
2,500 – 4,500	150	33 – 60
4,500 – 7,000	75 + 150	32 – 50

The latent period is defined as the time between injection and stripping of gametes. To determine if ovulation has occurred, female *semah* and *empurau* are anaesthetised and pressure applied to the abdomen to determine whether eggs could be stripped from the fish. Eggs have been hand-stripped from fish treated with hormones 23-53 hours post-injection (at 26-30°C), and some fish were stripped up to three times during this period. However, hatch rates are greatest in eggs stripped 23-26 hours post-injection. After stripping all fish should be treated with 5% salt solution to reduce stress and potential infection.

3.3.2.2 Males

Male *semah* and *empurau* mature at a smaller size than females and are running ripe from 200 g and 350 g, respectively. During breeding trials, males have been injected with either HCG (250 iu/kg)

or Ovaprim (0.2-0.25 ml/kg), but since mature broodstock are typically always running ripe, hormone therapy may not be necessary for spermiation.

3.3.3 Stripping and fertilization

Ovulated eggs and milt are hand-stripped from females and running-ripe males. Fish must be anaesthetised during handling and stripping. A dry fertilization method similar to that described by Joshi *et al.* (2002) is used to fertilize the stripped eggs of mahseer. Ovulated eggs are stripped into a clean dry bowl (Figure 2a). Avoid allowing water and mucus entering the bowl while stripping. Consequently, excess water should be wiped from the broodfish before stripping.

Artificial propagation:

1. Only healthy and diseases-free fish should be used for artificial propagation.
2. For females, Ovaplant may be used to facilitate maturation 4-5 weeks prior intended spawning date.
3. Ovaprim at a dose of 0.5 ml/kg is used to induce spawning in females.
4. Hatch rates are greatest in eggs stripped 23-26 hours post-injection.
5. Mature males are typically running ripe through the year and therefore do not need to be injected with hormones.
6. A dry fertilization method should be used to fertilize eggs and sperms of *semah* and *empurau*. Do not allow water and mucus entering the mixture of eggs and sperms.

Up to 9,970 eggs and 5,400 eggs have been stripped, on any one occasion, from *semah* and *empurau* broodstock, respectively (Figure 2). The mean batch fecundity (number of eggs stripped at any one time) is 860 eggs/kg (max. 2,270 egg/kg) and 1,080 eggs/kg (max. 4,460 eggs/kg) for *semah* and *empurau*, respectively (Ingram *et al.* 2006). Stripped eggs are firm, and pale yellow to golden orange in colour (Figure 4). There is no apparent relationship between hatch rate and egg colour (Ingram *et al.* 2005). Non-viable eggs are white and opaque.

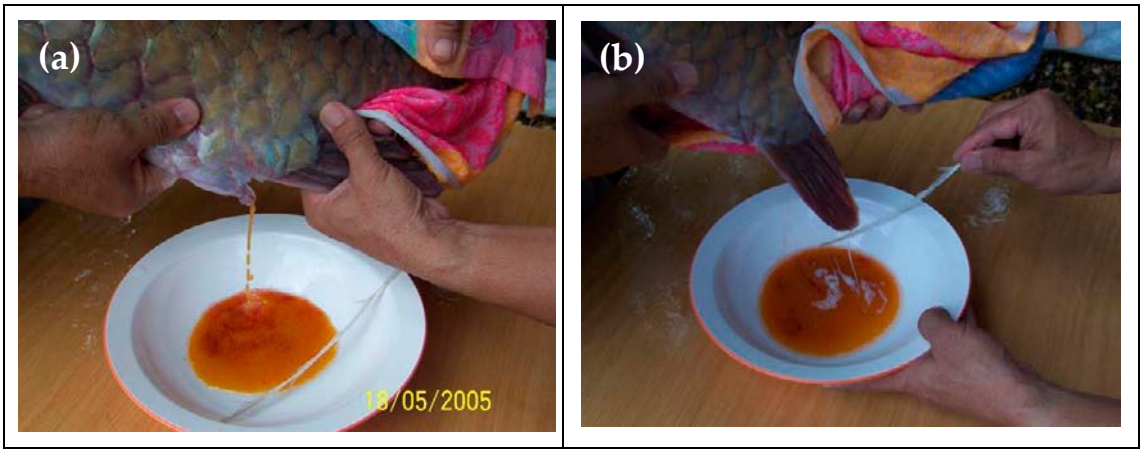

Figure 2. (a) stripping eggs. (b) stripping milt onto eggs

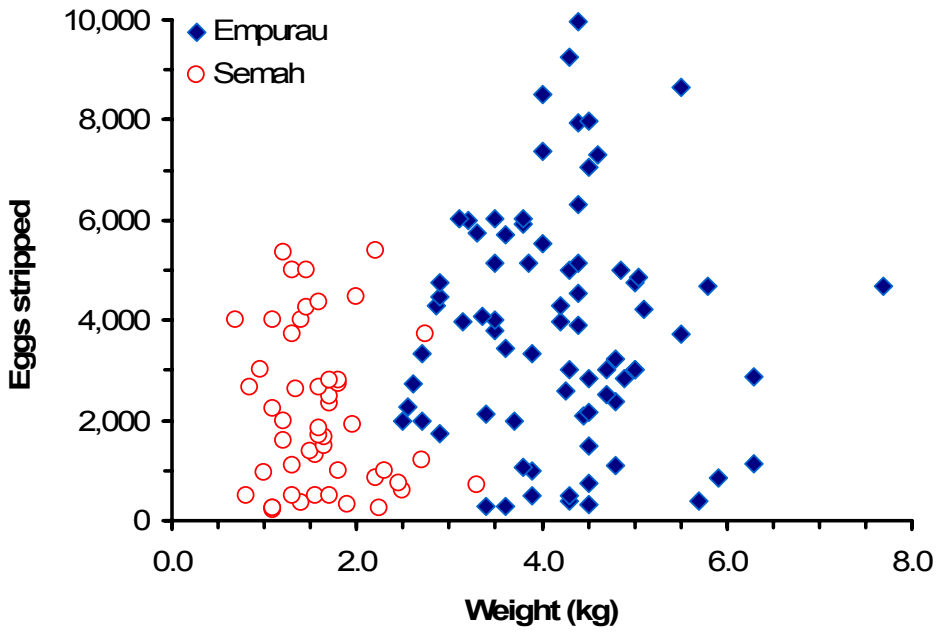

Figure 3. Number of eggs stripped from *semah* and *empurau* broodstock

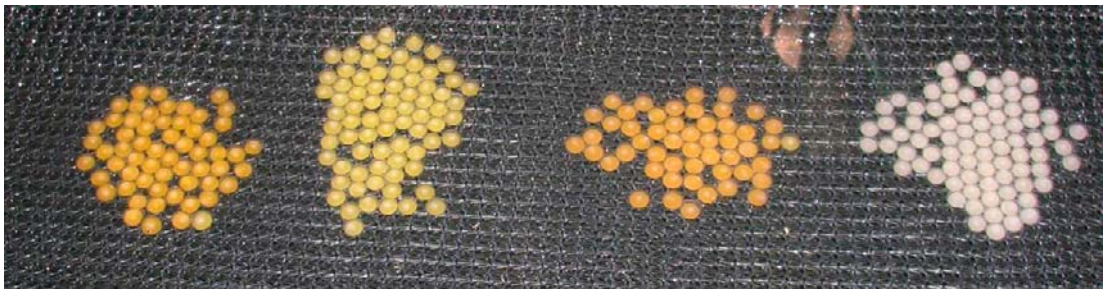

Figure 4. Colour variation in fertilized *empurau* eggs

Milt may be collected into a clean 5 mL syringe (which allows the volume stripped to be measured) before adding to stripped eggs. Alternatively, milt may be stripped directly over stripped eggs (Figure 2b). Sperm activity in both species can be highly variable. Sperm is activated by both freshwater and saline solutions (0.4% and 0.8% NaCl solution), with activity lasting up to 4 minutes in some samples. The optimal ratio of eggs to milt has yet to be determined for mahseer. However, as a rough guide apply 0.25-0.5 mL milt to every 100 mL eggs. Milt from one or two males are used to fertilize eggs from one female.

The eggs and milt are then mixed together by swirling or stirring with a clean dry feather. After allowing up to5 minutes for eggs to be fertilized, the eggs are rinsed with fresh hatchery water to remove superfluous sperm and ovarian fluid. The cleaned eggs are then decanted into clean 50 mL, 100 mL or 250 mL measuring cylinder (depending on volume of eggs stripped) and the settled volume measured before transfer to an incubator. The relationship between stripped egg volume and number of eggs for *semah* and *empurau* is presented in Figure 5.

Eggs from each stripped female should be incubated separately so that the hatch rate for each can be determined.

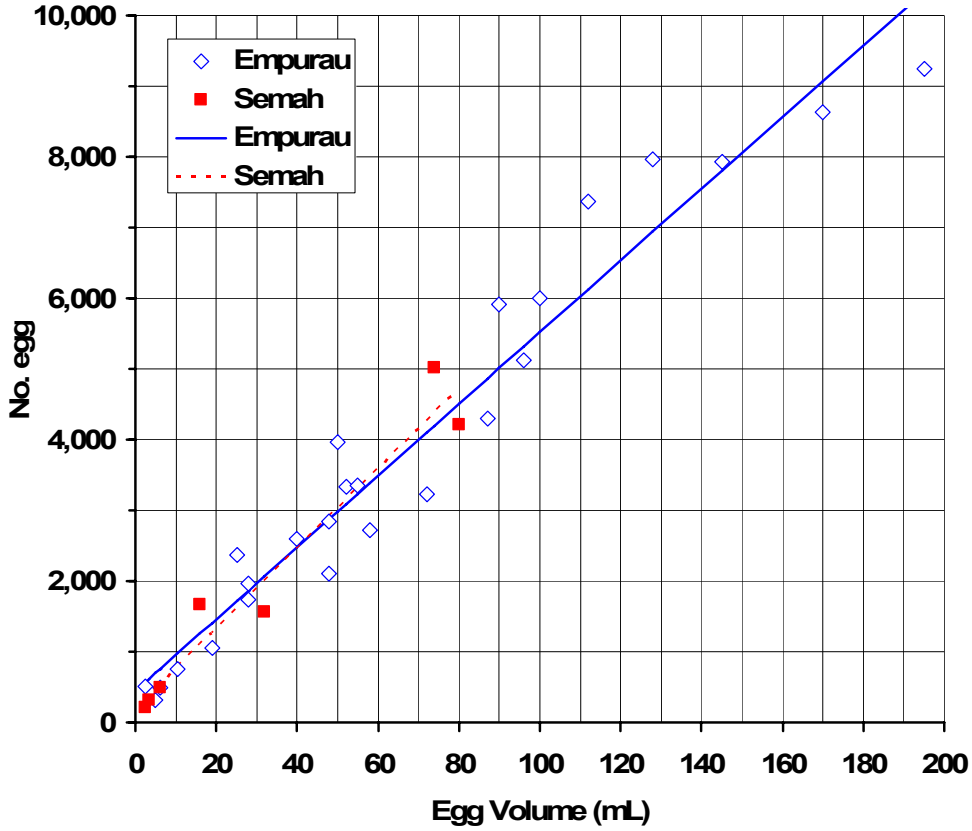

Figure 5. Relationship between stripped egg volume and number of eggs for *empurau* (No eggs = 353 + 51.6·egg vol. [adj R^2 = 0.93]) and *semah* (No eggs = 192 + 56.7·egg vol. [adj R^2 = 0.94])

3.3.4 Egg incubation

Eggs can be incubated in eggs jars (Macdonald or downing) or on flat horizontal mesh trays located in troughs or tanks (Figure 6) which are aerated and supplied with a constant flow and water (1-2.5mL/min.).

Artificial propagation (continued):

7. Fertilized eggs can be incubated in MacDonald jars or on flat horizontal mess trays in troughs or tanks. The latter should be aerated with constant flow water.
8. Eggs should be treated daily with formalin (1 ml conc. formalin per L for 20 min.) 24 hr and 48 hr after fertilization to prevent fungus infection. Ensure aeration during formalin treatment.
9. Unfertilized and infected eggs should be removed daily.
10. Hatching commences 69-90 hours and 68-82 hours (26-30°C) post-fertilization for *semah* and *empurau*, respectively, and is generally completed within 48 hours.

Figure 6. Egg incubation. MacDonald jar (left) and flat trays in flow-though system(right)

Eggs are prone to fungal attack during incubation. However, daily removal of unfertilized or dead eggs, and daily treatment with either formalin (1 ml conc. formal per L for 20 min.) 24 hr and 48 hr after fertilization, assists in preventing fungal infection. Ensure the water is well aerated during treatment as formalin reduces dissolved oxygen concentrations.

Egg development and hatching are described in Ingram *et al.* (2005). *Semah* eggs are slightly smaller than *empurau* eggs; mean diameter of stripped eggs was 2.69 mm and 2.48 mm for *semah* and *empurau*, respectively. Following fertilization eggs swell by up to 20% in diameter within 15 minutes. Water-hardened eggs are spherical, demersal, non-sticky and translucent. The surface of the eggs is slightly crenulated. Development rates are similar for *semah* and *empurau*.

By 1 hour post-fertilization (PF) early development of a blastodisc is apparent. At this stage the yolk cell appears granular with dark inclusions throughout, which persist for the duration of development. Some eggs also possess small oil globules within the yolk cell. By four hours PF, a large and prominent blastodisc is visible, sitting high upon the yolk cell (Figure 7b). At 24 hours PF, the early embryo stage is reached with the trunk of the embryo developed approximately half way around the embryo (Figure 7c). Formation of somites along the trunk and development of the embryonic shield are evident. In addition to the dark inclusions within the yolk sac, small dark particles of material are also present within the perivitelline space. By 48 hours PF, the otic vesicle and a beating heart are visible, the margin of the eye has become pigmented and the tail bud is free of the yolk sac and flicking every 4-10 seconds (Figure 7d). At this stage, a second smaller yolk sac had developed immediately posterior to the considerably larger primary yolk sac. At 72 hours PF, embryos were very active within the chorion. The smaller secondary yolk sac had become more elongate and noticeably darker in colour than the larger primary yolk sac (Figure 7e). Some embryos possess pigmented blood.

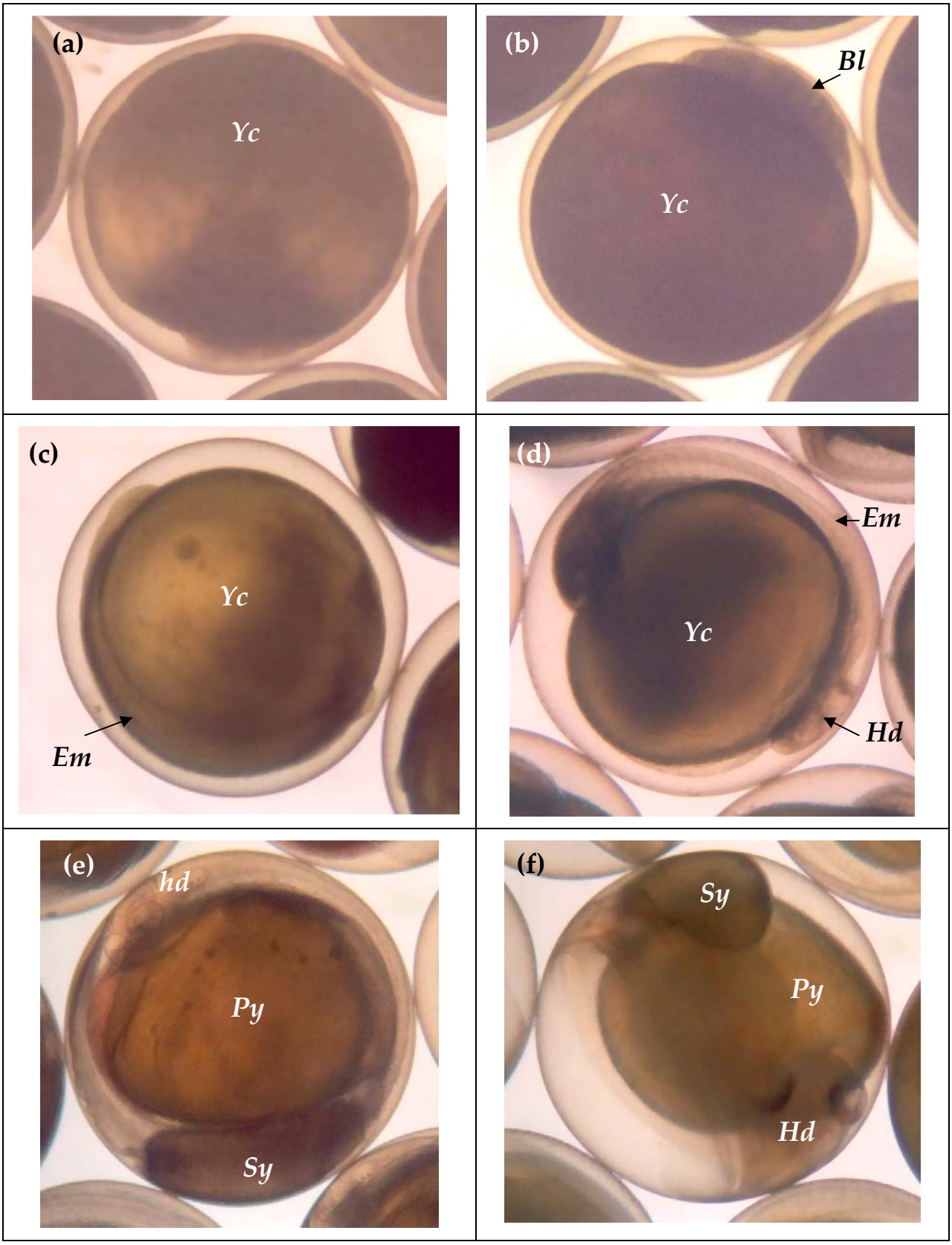

Figure 7. Egg development of *semah* and *empurau*. (a) 1.7 hrs post fertilization (PF), cleavage period (*semah*, dia = 2.98 mm). (b) 3.7 hrs PF, morula stage, late cleavage (*empurau*, dia = 3.13 mm). (c) 25.2 hrs PF, gastrula period, early embryo (*semah*, dia = 3.02 mm). (d) 47.4 hrs PF (*semah*, dia = 2.97 mm). (e) 72 hrs PF (*empurau*, dia =3.29 mm). (e) 92 hrs PF (*semah*, dia = 2.96 mm). Bl = Blastodisc. Em = Embryo. Py = Primary yolk sac. Hd = Head. Sy = Secondary yolk sac. Yc = Yolk cell.

Prior to hatching embryos exhibit vigorous bouts of activity. Hatching commences approximately 69-90 hours and 68-82 hours (26-30°C) post-fertilization for *semah* and *empurau*, respectively, and is generally completed within 48 hours.

Light exposure (incubation in ambient light against incubation in the dark) did not affect egg survival and the incidence of larval deformities. Differences between incubation under turbulent (active treatment) conditions and still conditions are inconclusive. However, use of formalin treatments to prevent fungal infections greatly improved hatch rates in still incubation conditions (mean hatch rates for formalin treated eggs: 26-44%, mean hatch rates for non-formalin treated eggs: 13%).

3.4 Larviculture

At hatch *empurau* larvae are slightly larger than *semah*, with total length being 7.4-10.2 mm (mean 9.1 mm) and 7.3-9.4 mm (mean 8.8 mm), respectively (Figure 8). At hatch, pectoral fin buds and undifferentiated dorsal and anal finfolds are present, yet the mouth is not open. The yolk sac in both species is relatively large and elongate, being 0.52- 0.72 (mean 0.60) the length of the total length of the larvae. There is a noticeable constriction between the primary and secondary yolk sac in most larvae, and in some larvae these two sections of the yolk sac are completely separate. The primary yolk sac is approximately twice the size, in both lateral and dorsal view, of the secondary yolk sac. Margins of both yolk sacs are pigmented. Many larvae lay on their sides on the substrate, though exhibited accessional bouts of swimming.

By 96 hrs PF, one day post-hatch (PH) development of gill arches and rudiments of gill filaments is apparent. Some melanophores are present internally in the head region, along the dorsal surface of the notochord. Blood flow is obvious across the yolk sac and vein running ventral to the notochord. Most larvae are now sitting upright on the substrate and exhibiting negative phototaxis.

By two days PH, the mouth is open, the eyes fully pigmented, the dorsal finfold is becoming differentiated and melanophores are proliferating along the dorsal surface of the notochord and across the dorsal surface of the yolk sac.

By three days PH, flexion has commenced and melanophores are spreading across the surface of the head and along the bases of the dorsal and anal fins.

By four days PH hypural plates and rays of the caudal fin and internal organs including the liver, gall bladder are evident in some larvae. During the yolk sac absorption period, larvae grow in length and the yolk sac declines in size.

By five day PH, some larvae have inflated swim bladders (Figure 8) and commence feeding with *Artemia* nauplii clearly visible in the gut and intestine. At this stage melanophores had become denser and were spreading throughout the myomeres, along the notochord and laterally across the yolk sac, and a dense patch of melanophores had developed over the ventral hypural plate.

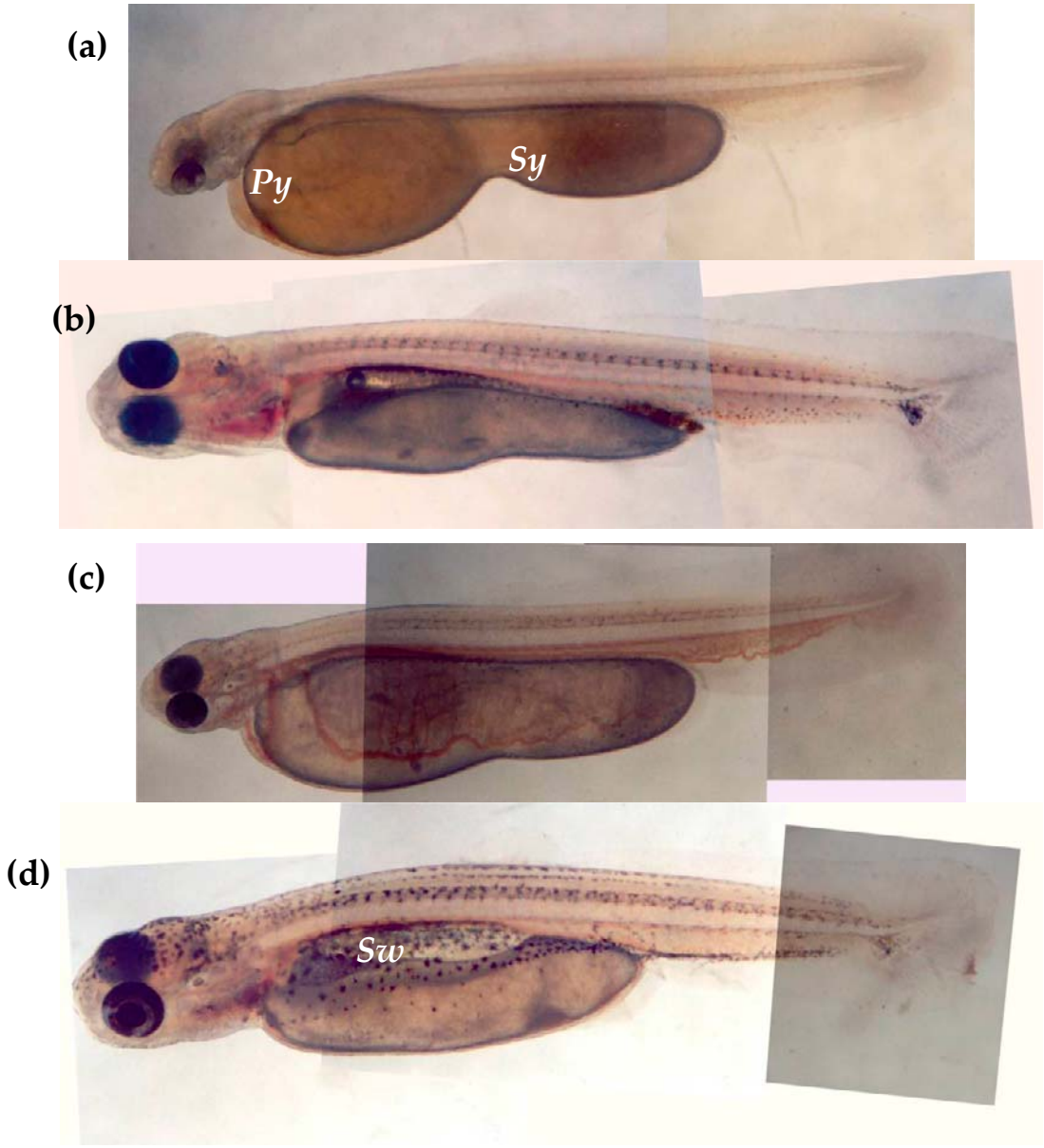

Figure 8. *Semah* and *empurau* larvae. (a) *Empurau* (94 hrs PF, day 1 post-hatch (PH)) (TL = 9.82 mm). (b) *Empurau* (day 5 PH) (TL= 12.24 mm). (b) *Semah* (day 1 PH) (TL=8.91 mm). (d) *Semah* (day 5 PH) (TL = 11.2 mm). Py = Primary yolk sac. Sw = Swim bladder. Sy = Secondary yolk sac.

At commencement of feeding *semah* and *empurau* fry readily consume newly hatched *Artemia* nauplii. As the larvae grow, energy reserves in the yolk sac will diminish and their appetite will increase. There are no larval feeds rates published for mahseer, however, feed rates presented in Table 5 are suggested as a guide to feeding *Artemia* nauplii to *semah* and *empurau* larvae in the first few weeks of exogenous feeding.

Larviculture

1. Hatched larvae can be moved to troughs for rearing.
2. Newly hatched *Artemia* nauplii are recommended as suitable feed for *semah* and *empurau* larvae.

Table 5. Suggested *Artemia* nauplii feeding rates for larval *semah* and *empurau* (figures based on 1,000 larvae)

Days feeding	Feed level	*Artemia* volume (mL) per 1,000 larvae	No. Feeds per day	Rate (mL *Artemia*/feed)
1-4	Low	2.5	4	0.63
5-9	Medium	7.5	6	1.25
10-25	High	12.5	6	2.08
>25	High-High	20.0	6	3.33

3.5 Fry rearing (nursery phase)

The nursery phase is the period where larvae undergo metamorphosis and are grown through to a fingerling stage (typically 1 – 5 g weight). During this phase fish may be also weaned from live food to artificial diets, which is a critical stage of production. The growth and survival of fry during the nursery phase are greatly affected by the culture conditions and husbandry techniques. Consequently, a number of preliminary experiments were conducted on *semah* and *empurau* fry to identify and evaluate various factors that may affect growth and survival during this phase.

3.5.1 Tank culture

Stocking density can affect the growth of fish reared in tanks and ponds. In one experiment, the growth of *semah* fry (initial TL = 25.6 mm, Wt=0.13g) stocked at 0.6 fish/L was significantly greater than for fish stocked at higher densities (Figure 9).

The colour of culture tanks can affect the growth and survival of larval and juvenile fish (Tamazouzt *et al.* 2000). However, in one experiment, there was no significant difference between *empurau* reared in dark blue and light blue coloured tanks for most parameters measured. However, due to changes in growth in the latter weeks of the experiment, final weight and TUGC were significantly greater in *empurau* reared in the light blue - coloured tanks.

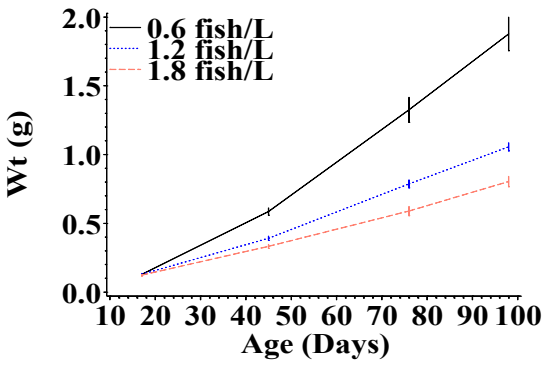

Figure 9. Growth of semah fry reared at different densities

Initial feeding and weaning of *empurau* (initial TL=12.4 mm) and *semah* (initial TL=9.6mm) post-larvae was studied in an experiment in which fish at the onset of exogenous feeding were fed either newly hatched *Artemia* nauplii for one month and then weaned onto a Cargill starter diet (Control), or one of three commercial fish fry diets (Gemma (Skretting), NRD (Inve) or Uni-President) without weaning. During the experiment fish were fed to satiation up to 6 times each day. After 19 weeks, both *semah* and *empurau* fed on a quality diets high in protein (eg. Gemma) had significantly higher growth rates than fish fed diets low in protein content,

and grew faster than fish fed *Artemia* before weaning (Figure 10). Fish fed Gemma were 2x (*semah*) to 3x (*empurau*) heavier than the control fish at the end of the experiment. Survival rates, which ranged from 32-96% (64%), were also significantly higher in fish fed high quality diets. This experiment also suggested that stocking density may have reduced growth rates in the latter weeks; in one tank of fish fed Gemma the density reached 2.5kg/m^3 (5.8 fish/L) on the final day.

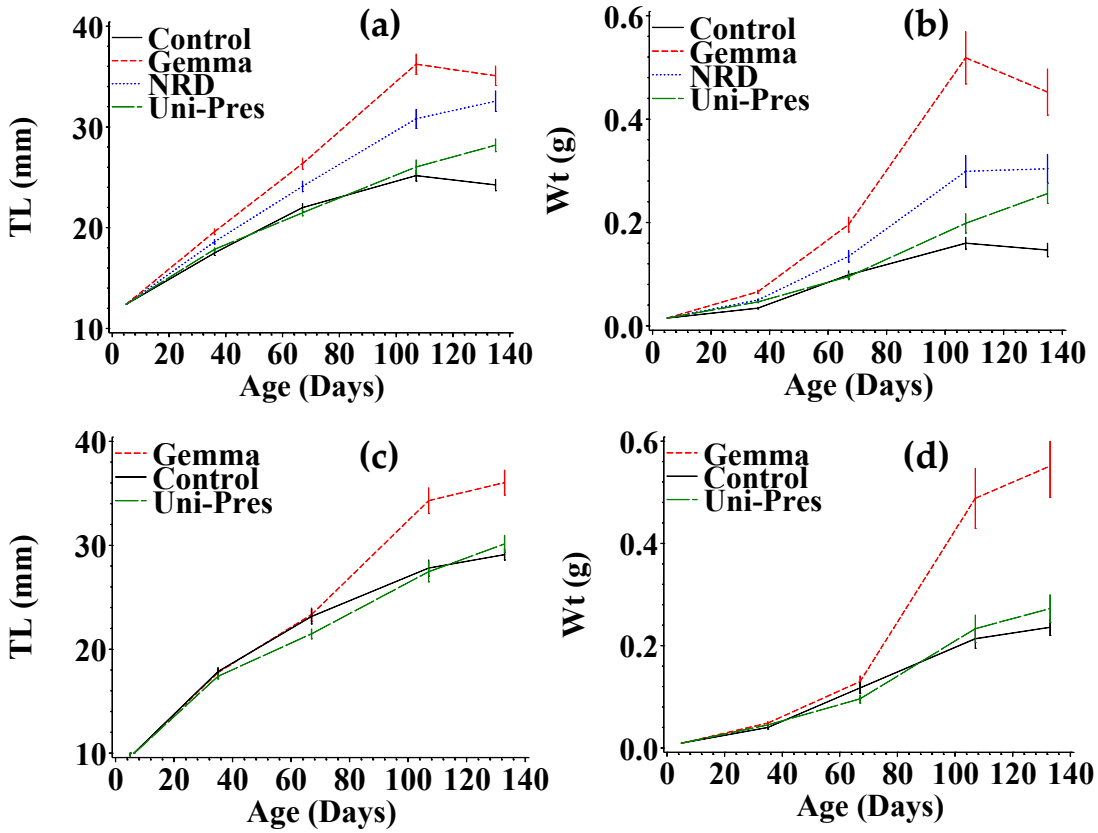

Figure 10. Growth of *empurau* (a & b) and *semah* (c & d) fed different diets during weaning (values = mean ± standard error)

These results indicate that use of high quality diets will improve growth rates of tank-reared *semah* and *empurau* fry, and the need for feeding live food such as *Artemia* nauplii prior to weaning is less critical. Feed rates for *semah* and *empurau* fry reared in tanks have not been well defined to date, however it is recommended that fish be fed to satiation at least 4-6 per day during the nursery phase. Stocking density affects the growth of fish in tanks and therefore it is recommended that densities be maintained below 2.5 kg/m^3 during this phase.

Tank maintenance should involve daily cleaning to remove uneaten food and dead fish, to reduce failing of the water, and regular checks of water quality, growth of fish and health checks (see Annex 1). Tanks should be well aerated and be provided with a constant flow of clean water.

3.5.2 Pond culture

Growing juvenile fish in earthen ponds that have been fertilized to encourage the development of plankton blooms on which the fish feed, is a widely used and cost effective means of mass rearing juvenile fish species in aquaculture worldwide (e.g. Jana and Chakrabarti 1993, Feldlite and Milstein 2000, Opuszynski and Shireman 1993). This technique is called greenwater pond culture. Studies on other *Tor* species, especially *T. putitora*, have shown excellent growth rates when feed live zooplankton (Bista *et al.* 2002, Rahman *et al.* 2006). *T. putitora* fry (initial weight 0.012 g, stocked at 60, 80 or 100 fish/m^2) reached a final mean weight of 1.34-2.73 g after 8 weeks (SGR 8.43-9.7%/day) in fertilized nursery ponds (Rahman *et al.* 2006).

One experiment has been conducted to evaluate greenwater pond culture of *semah* and *empurau*. Dry earthen ponds were filled, limed and fertilized with diammonium phosphate (20 kg/ha every 1-4 weeks). Once plankton blooms developed, *empurau* (initial TL=14.7 mm) and *semah* (initial TL=13.2 mm) post-larvae were stocked into ponds at different densities (10-30 fish/m^2). Over the 11 weeks of the experiment, growth rates were greatest in ponds stocked at the lowest density (Figure 11), but survival rates ranged from 2-68% (mean 34%), were not significantly different between densities. In ponds, growth rates of *semah* and *empurau* are comparable (Figure 11).

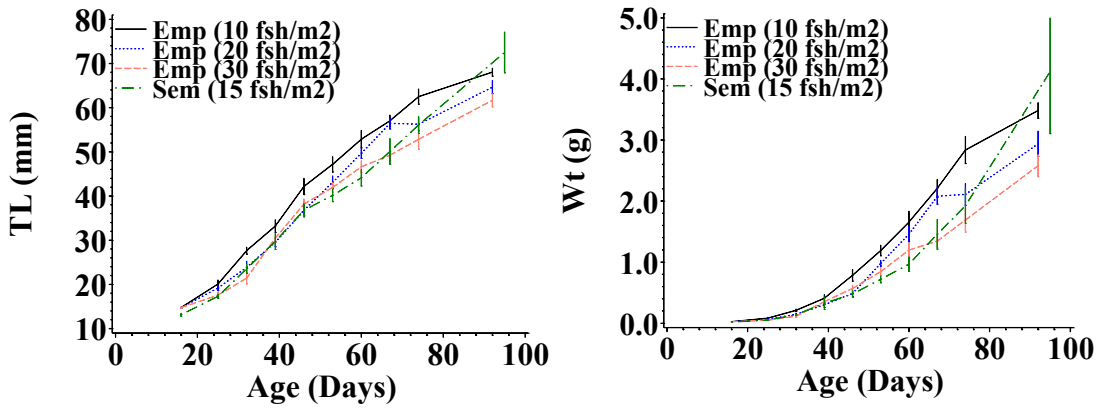

Figure 11. Growth of *empurau* (Emp) and *semah* (Sem) in fertilized earthen ponds at different stocking densities (values = mean ± standard error)

This preliminary experiment demonstrates that the growth rates of *semah* and *empurau* reared in fertilized earthen are greater (6-fold) than for tank-reared fish, but survival rates are considerably lower (2-fold). Further research is required to improve pond management techniques to enhance survival, in particular refinement of fertilizer regimes to enhance plankton production and introduction of supplementary feeding. Reduced growth at higher stocking densities in ponds may be due in part to reduced food availability as a result of over-grazing. Rahman *et al.* (2006) found that the growth of *T. putitora* fry was also affected by stocking density in nursery ponds, with highest growth rates being observed for the lowest stocking density (60 fish/m^2), a greater density than used in the present study. Supplementary feeding may improve growth rates in ponds stocked at higher densities.

The principal objective of rearing juvenile fish in fertilized fry ponds is to provide an environment in which the fish survive and grow rapidly. This relies on the maintenance of an appropriate size and abundance of food organisms (zooplankton and macroinvertebrates), while simultaneously

maintaining water quality suitable for survival and growth. It is important to time preparation and filling of ponds to coincide with fish spawnings to ensure that when juvenile fish are ready to be stocked into the ponds, there has been sufficient time for the ponds to develop suitable blooms of plankton. ***Semah* and *empurau* post-larvae should not be stocked into ponds until plankton densities and composition are suitable and preferred prey is present.**

Monitoring of water chemistry should include measurement of Nitrogen and phosphorus as these will provide indicators of nutrient availability for phytoplankton growth. Water quality should be monitored at least weekly (see Annex 1). Due to diurnal variations in some parameters, a monitoring program should ensure that samples are routinely collected at the same time each day. Additional samples should be collected at times when critical levels in key water quality parameters occur, such as early morning (low dissolved oxygen) and late afternoon (high pH). Supplementary aeration (such as by paddlewheels) is mandatory to aerate and circulate the water, to maintain dissolved oxygen concentrations and prevent pond stratification. Ponds should be flushed regularly (at least weekly) with fresh water to replace water lost through evaporation and seepage, and to reduce the effects of poor water quality events such as high pH or high ammonia. Regular (weekly) sampling of plankton concentrations will provide a guide to pond productivity and food availability. Fish should be sampled weekly to monitor their growth and condition, and to check for diseases (see Annex 1).

Fry rearing (nursery phase):

1. During the nursery phase, *empurau* and *semah* fry can be reared in either tanks or ponds.
2. Fry can be weaned onto artificial diets immediately after commencement of exogenous feeding.
3. In tanks, fish should be fed to satiation at least 4-6 per day using a high quality diet, and stocking density should be maintained below 2.5 kg/m^3 during this phase.
4. Tanks should be well aerated, provided with a constant flow of clean water, and cleaned daily.
5. Fertilize fry ponds to encourage plankton growth. Stocking occurs once plankton density is high and an abundance of preferred prey is present.
6. Monitor water chemistry and flush ponds regularly to ensure water quality. Fish should be sampled weekly to monitor their condition and to check for diseases.

3.6 Grow-out

Since the development of captive breeding techniques for *Tor* spp. (mahseers), interest in the culture of these species for human consumption, and to produce juveniles for stock enhancement and replenishment programs, has grown considerably. To date much of the focus has been on the trans-Himalayan group of mahseer, especially *T. khudree* (Sykes), *T. putitora* (Hamilton) and to a lesser extent *T. tor* (Hamilton) (Islam and Tanaka 2004, Bista *et al.* 2002, Ogale 2002, Sehgal 1999, Rai *et al.* 2006). The value and aquaculture potential of the mahseers has been recognised and several species have been translocated to other countries for culture and fisheries development (eg. Coates 1997). Recently there has been increasing interest in the farming of *semah* and *empurau*, and

in Peninsula Malaysia, at least one commercial farm has commenced growing "kelah" for human consumption and the aquarium trade (Anon. 2005).

Between 2003 and 2006 a series trials were conducted on *semah* and *empurau* to obtain preliminary information on their performance (growth, survival, food conversion efficiencies etc) under various pond and cage culture conditions, and to evaluate their commercial aquaculture potential. These trials were performed on captive bred (F1) *semah* and *empurau* juveniles produced at the IFRPC. Fish were fed on commercial available artificial diets (Cargill or Dinding: 25-45% protein, 5-10% fat). Feed rates for fish in ponds and cages ranged from 2-10%/day (dry weight feed to weight of fish) (subject to age), which were adjusted following each weight check. Fish were fed by hand 1-2 times/ day. Further information on these trials is provided in Ingram *et al.* (2007b). In addition, some *empurau* produced at the IFRPC were also transferred to grow-out ponds at several commercial farms, (ie. PM Aquaculture, Lundu and Tr. Japar, Kemalih). Some information from these facilities was included in data synthesis.

3.6.1 Pond culture

Pond culture trials have been conducted at the IFRPC, Tartat and the Integrated Agriculture Station (IAS), Layar, Betong. Ponds were 180-550 m^2, 0.7-1.75 m in depth, mostly lined with high density polyethylene, and aerated. A small constant flow of water was provided to each pond to maintain water level. Each pond was stocked with 120-7,000 fish (0.44-20 fish/m^2, 0.03-389 g initial mean weight) and monitored for up to 138 weeks (Table 6).

Growth rates of *empurau* were considerable greater in ponds than for *semah* (Figure 12). In two ponds, *empurau* reached a mean weight of 800 g (range 380-1,250 g) by 33 months of age. In contrast, at 22 months of age, *semah* had reached a mean weight of just 71 g (range 45-110 g). Growth rates of fish from different spawning trials were considerably different from each other. This was especially evident in *empurau* where fish from the April 2003 trials were more than double the weight of fish from the November 2003 trials after 20 months (Figure 12). *Empurau* fry stocked into two ponds at the IAS reached a mean weight of 147 g (range 110-250 g) after 65 weeks. Growth rates of these fish were slightly greater than for *empurau* reared in ponds at the IFRPC (Figure 13a).

Information on survival at different stages of grow-out is limited as many ponds had yet to be harvested at the time of publication. However, survival of both species cultured in ponds was generally good. Survival of *empurau* fry (initial mean wt = 0.04-2.2 g) reared in three ponds was 67%, 75%, and 77% after 55, 121 and 107 weeks, respectively. Survival of *semah* fry (initial wt = 0.04 g) reared in two ponds after 45 and 64 weeks was 86% and 94%, respectively. However, on two occasions, mass mortality occurred in two ponds stocked with *empurau*, which were associated with hot weather, heavy rainfall overnight and highly eutrophic pond conditions.

Food conversion ratios (FCR's) (see Annex 2 for formula) in pond-reared fish were highly variable but similar for both species. Mean values were 1.27 $\pm$ 0.08 s.e (Max. 3.67) and 1.17 $\pm$ 0.21 s.e (Max. 3.04) for *semah* and *empurau*, respectively. However, fish pellets provided to the fish in the ponds is not the sole food consumed. Gut contents analysis of eight pond-reared *empurau* (55 g to 285 g wt) indicated that their diet, along with fish pellets, also included plant matter such as pieces of unidentified plant tissue, algal cells and grass seeds, and animal matter, including cyclopoid copepods, chydorid cladocerans, chironomid larvae and rotifers.

Table 6. Summary of stocking details for *Semah* and *empurau* reared in ponds at the IFRPC and the IAS

Species/ Location	Spawning trial	No. of ponds stocked	Age at stocking (days)	Size at stocking (g)	Stocking density (fish/m²)	Duration (weeks)
Empurau						
IFRPC	Apr 03	2	68	2.0-2.24	2.52-2.68	121-138
	Apr 03	2	816-915	382-389	0.37-0.71	15
	Nov 03	1	464	37	0.44	22
	Nov 03	3	22-24	0.044	5.56-6.15	94-95
	Apr 04	4	16-35	0.03-0.05	1.4-20	15-72
	Apr 05	1	197	10.3	0.94	17
IAS	Sep 04	2	62	0.07	0.71	65
Semah						
IFRPC	Nov 03	1	473	44.5	0.78	31
	Dec 03	1	20	0.04	1.12	93
	Jan 04	1	18	0.04	2.6	29
	Jul 04	1	204	34.3	0.96	31

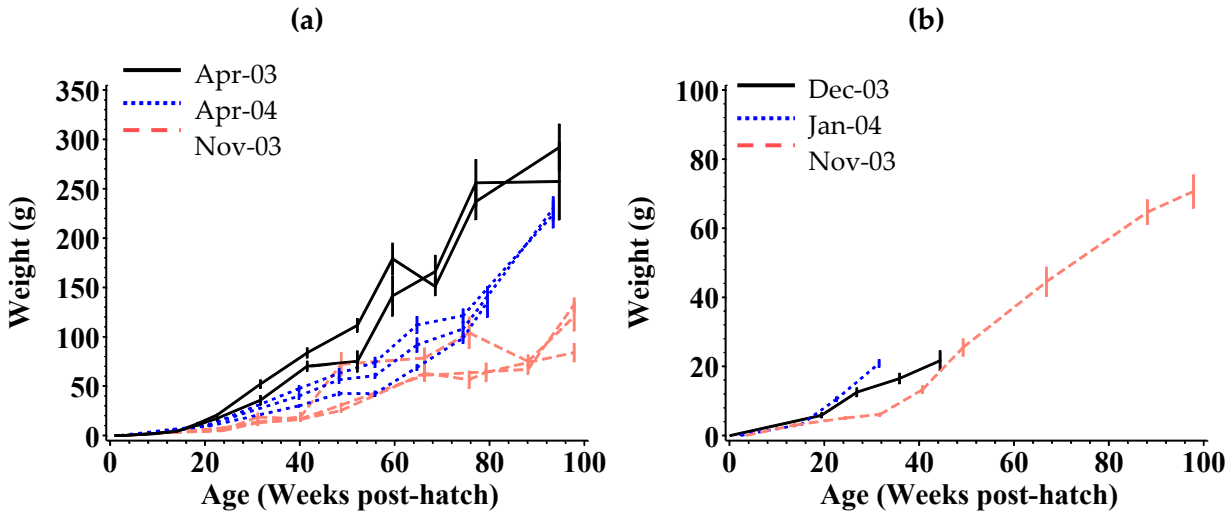

Figure 12. Growth (mean weight ± s.e.) of (a) *Empurau* and (b) *Semah* derived from different spawning trails

Sexual maturity in pond-reared fish was first observed in *empurau* with males running ripe from 24 months of age and 195 g wt. One female *empurau*, which was 33 months of age and weighing 1.25 kg, had oocyte diameters up to 1.55 mm and a gonadosomatic index value of 2.5%. Signs of sexual maturation have yet to be observed in pond-reared *semah*.

3.6.2 Cage culture

Cage culture trials were conducted at Batang Ai Reservoir where six floating cages (3m x 3m x 3m) suspended from a pontoon, were each stocked with *semah* and *empurau* derived from spawning trials conducted at the IFRPC. Each cage was stocked with 120-161 fish (0.71-5.8 fish/m^3, 0.07-24.4 g initial mean weight) and monitored up to 80 weeks (Table 7).

Table 7. Summary of stocking details for *Semah* and *empurau* reared in cages at Batang Ai

Species	Spawning trial	No. of cages stocked	Age at stocking (days)	Size at stocking (g)	Stocking density (fish/m^3)	Duration (weeks)
Empurau	Apr 04	2	121-177	0.56-1.51	5.7-5.8 fish/m^3	80
	Sep 04	2	62	0.07	0.71 fish/m^2	65
Semah	Dec 03	2	318	24.4	4.0 fish/m^3	70

Empurau reared in two cages grew from 0.6 g to 175 g (range 100-320g) in 80 weeks whereas *semah* grew from 24.4 g to 97 g (range 45-210 g) in two cages over a 70 week period. Growth rates of *empurau* in cages was generally lower than for fish reared in ponds at the IFRPC, but there was a noticeable increase in growth in the latter weeks of the culture period (Figure 12a). FCR for fish reared in cages (mean 1.87 $\pm$ 0.09 s.e.), especially *semah*, were generally higher than for fish reared in ponds at the IFRPC (mean 1.26 $\pm$ 0.08 s.e.). Survival in cages was considerably greater for *empurau* than for *semah*. Survival of *empurau* fry (initial wt = 0.56-1.51g) reared in two cages was 91% and 98% after 79 and 71 weeks, respectively, whereas survival of *semah* fry (initial wt = 17.8 g) reared in two cages after 70 weeks was 42% and 56%.

3.6.3 Data synthesis

The length-weight and age-weight relationships, and the relationship between median fish weight (median weight between Wt_1 and Wt_2) and specific growth rates (SGR's) (see Annex 2 for formula), for *semah* and *empurau* reared in ponds and cages were determined by linear regression following log transformation. The relationships are represented by the following regression equations:

Empurau: Log Wt (g) = -5.36 + 3.19 Log TL (mm) (Adj R^2=0.91)

Log Wt (g) = -4.23 + 2.29 Log Age (days post hatch) (Adj R^2=0.83)

Log SGR = 0.667 – 0.527 Log median wt (g) (Adj R^2=0.49)

Semah: Log Wt (g) = -5.07 + 3.08 Log TL (mm) (Adj R^2=0.97)

Log Wt (g) = -2.93 + 1.69 Log Age (days post hatch) (Adj R^2=0.89)

Log SGR = 0.789 – 0.790 Log median wt (g) (Adj R^2=0.59)

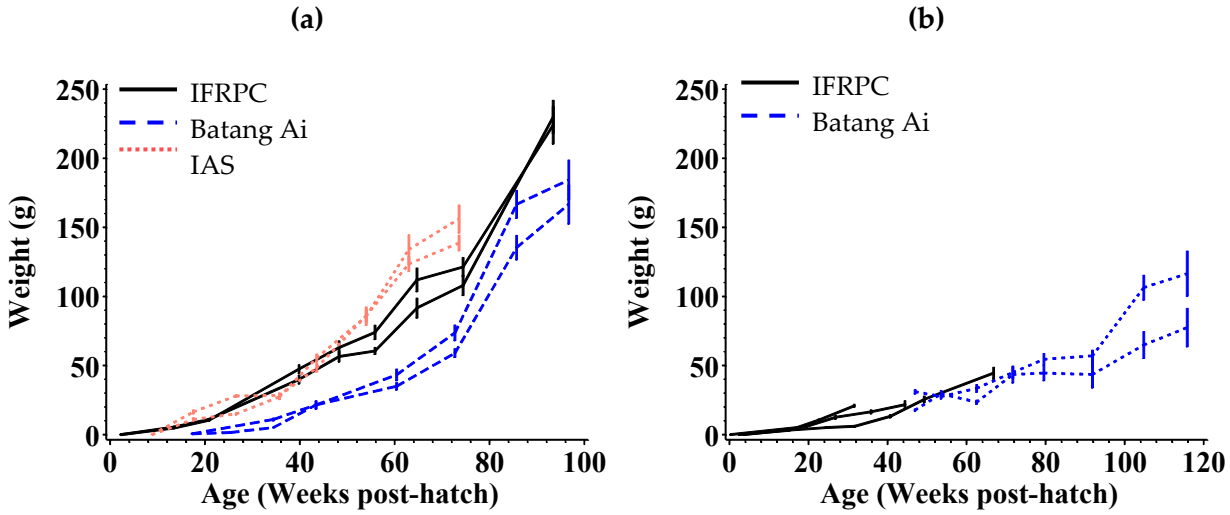

Figure 13. Growth (mean weight ± s.e.) of (a) *Empurau* in ponds at the IFRPC and IAS and cages at Batang Ai, and (b) *Semah* in ponds at the IFRPC and cages at Batang Ai

Length-weight equations for the two species were almost identical (Figure 14), which is not surprising given that both species are very similar in appearance and body shape. However, age-weight equations were very different with *empurau* being considerably heavier in weight for a given age than *semah* (Figure 15). The relationships between median fish weight (weight between Wt_1 and Wt_2) and SGR for each species are presented in Figure 16.

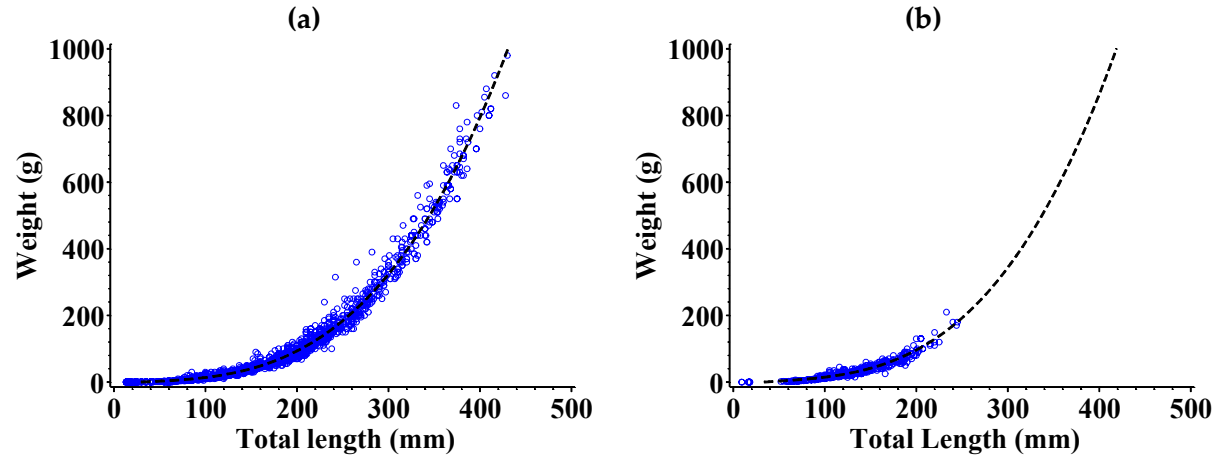

Figure 14. Length-weight relationships for (a) *empurau* and (b) *semah*, reared in ponds and cages. (see text for equations).

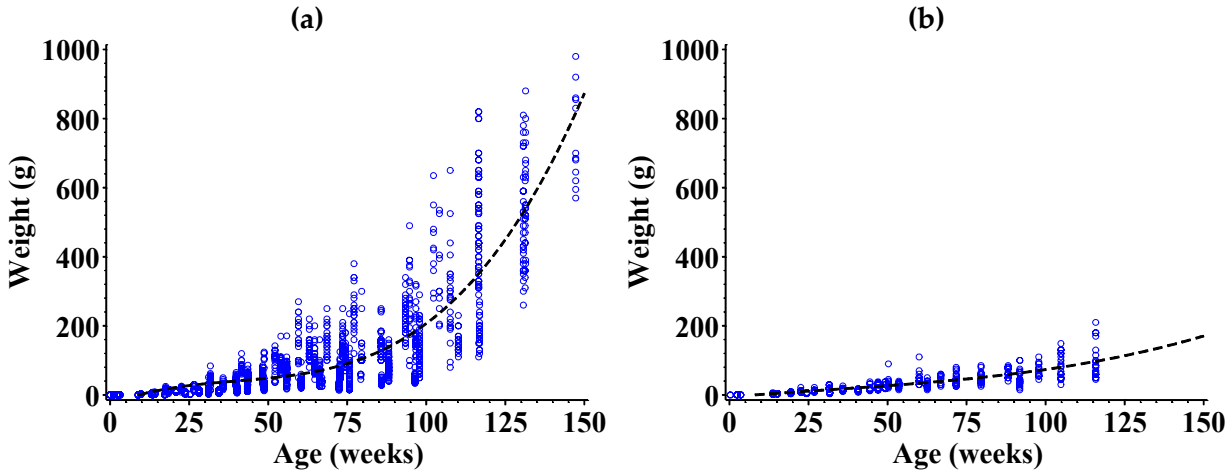

Figure 15. Age-weight relationships for (a) *Empurau* and (b) *Semah*, reared in ponds and cages. (see text for equations).

Trials have show that *semah* and *empurau* can be reared under a range of culture conditions. Growth rates are similar between pond-reared and cage-reared fish, suggesting that both methods are suitable for growing these species. However, the growth rates of *empurau* are considerably greater than *semah* in both ponds and cages (Figure 12, Figure 13, and Figure 16). SGR's recorded during trials ranged from –0.38–13.4 %/day and -0.75-7.0 %/day (Table 8) for *semah* and *empurau*, respectively (at 23-35°C), and as expected, growth rates declined with increasing age (Figure 16). These results suggest that *empurau* has more aquaculture potential than *semah*, although the growth rates observed suggests that these species are also relatively slow growing in captivity compared to other cultured finfish species in the tropics. However, *empurau* being reared in ponds at PM Aquaculture, Lundu, are growing substantially faster than fish reared in ponds at the IFRPC, Tarat (Figure 17). At 60 weeks of age these fish were more than double the size of fish reared at the IFRPC. Growth rates of *empurau* at PM Aquaculture, Lundu were 0.37-3.84 %/day (mean 1.34 %/day) (fish mean weight from 10g to 350g).

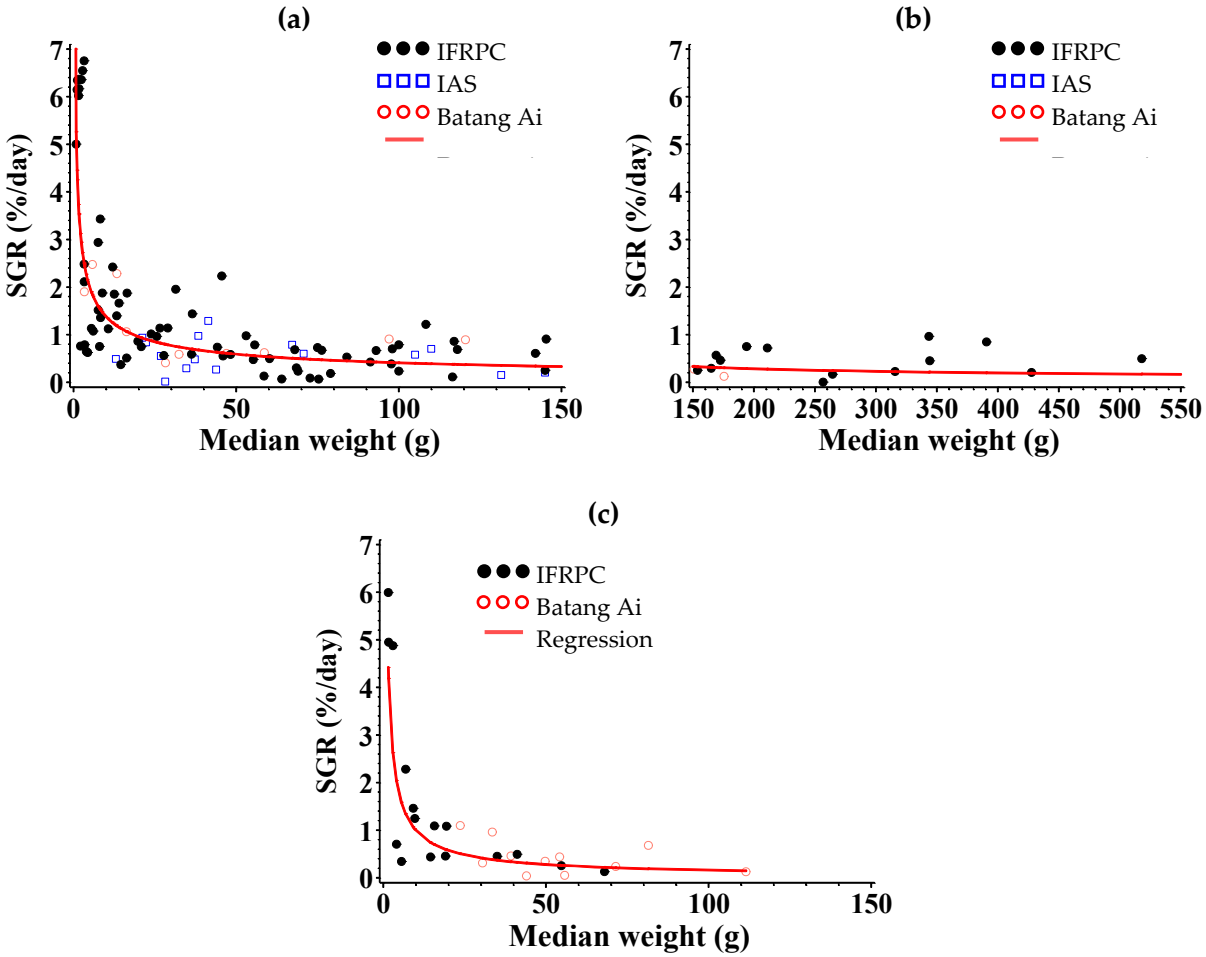

Figure 16. SGR's for (a) *Empurau* for median weight up to 150g, (b) *Empurau* for median weight over 150 g, and (c) *Semah* for median weight up to 150 g. (see text for line equations).

Table 8. Summary of information from grow-out trials conducted on *semah* and *empurau* reared in ponds and cages at different locations

Species	**Median fish weight* (g) (values = mean and range)**					
Parameter	<5	5-50	50-150	150-250	250-500	>500
Empurau						
SGR (%/day)	3.0 (-1.4-12.6)	1.4 (-0.17-13.4)	0.56 (-0.38-1.52)	0.32 (-0.27-1.38)	0.20 (-1.01-0.97)	0.11 (-0.19-0.5)
Survival (%)	87 (10-100)	-	50 (23-76)	83 (3-100)	100	-
Feed rate (%/day)	4.4 (3-10)	3.4 (3-10)	2.8 (2-5)	2.7 (2-3)	2.6 (2-3)	2
FCR	0.72 (0.1-2.3)	1.2 (0.4-2.2)	1.6(0.7-3.7)	1.9 (1.3-2.6)	1.6 (0.6-3.7)	1.7 (1.2-2.0)
Density (kg/m^3)	0.5 (max. 4.0)	0.1 (max.0.7)	0.4 (max. 1.0)	0.9 (max. 1.5)	0.9(max. 1.5)	0.24 (max. 0.4)
Semah						
SGR (%/day)	3.0 (-0.1-7.0)	063 (-0. 36-2.3)	0.24 (-0.75-1.4)	0.21 (-0.27-0.71)	0.14 (-0.5-0.83)	0.1 (-0.4-0.7)
Survival (%)	89 (2-100)	-	95 (83-100)	98 (95-100)	98 (87-100)	98 (96-100)
Feed rate (%/day)	4.0 (3-10)	3	3 (2.5-3)	3.0 (2.5-3)	2.5 (2-3)	2.3 (2-3)
FCR	1.15 (0.1-2.2)	1.4 (0.5-2.6)	2.0 (0.9-3.5)	2.2 (1.5-4.4)	2.1 (1.4-4)	2.1
Density (kg/m^3)	0.2 (max. 1.9)	0.12 (max. 0.14)	0.3 (max. 0.6)	0.6 (max. 0.9)	1.0 (max. 1.7)	1.9 (max. 2.6)

Median fish weight = median weight between Wt_1 and Wt_2 (see SGR formula, Annex 2)

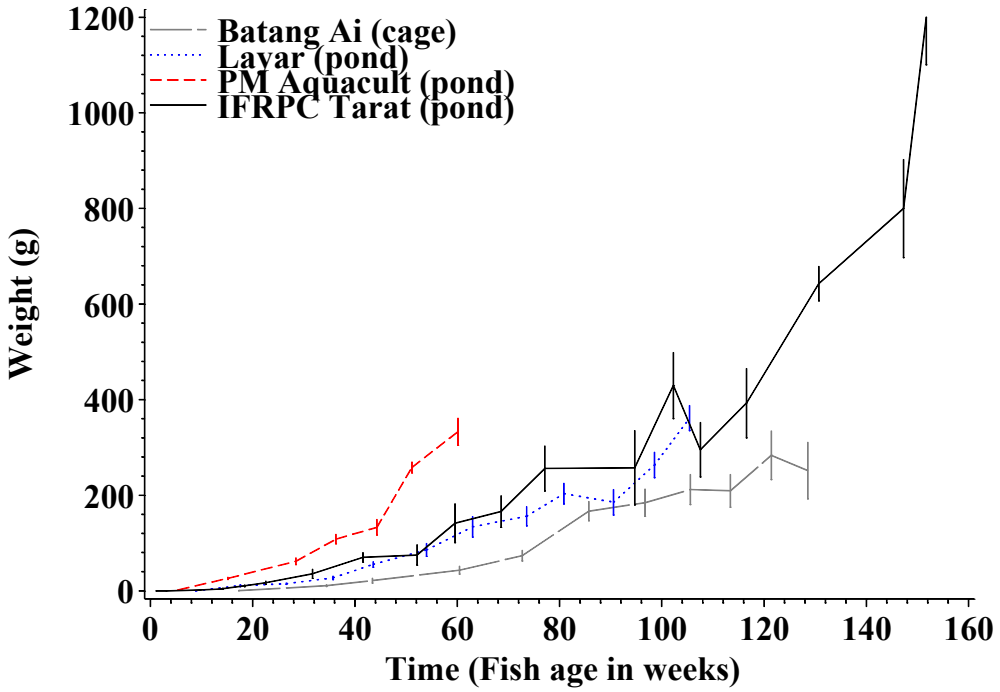

Figure 17. Growth of *empurau* (mean weight ± standard error) at different locations and culture conditions

Diets used in the present study were not specifically formulated for *semah* and *empurau* and so may have constrained growth. Islam (2002) suggested that the low amounts of protein and the number or type of plant materials used in formulated diets may have contributed to the lower growth rates in pond-reared *T. putitora.* Currently there are no commercial diets specifically formulated for *semah* and *empurau*. However, development of diets specifically formulated for *Tor* spp. is likely to improve the growth rates of these species. A number of nutritional studies have been undertaken on other *Tor* species to identify dietary requirements (Bazaz and Keshavanath 1993, Sunder *et al.* 1998, Joshi *et al.* 1989, Bista *et al.* 2002). These have suggested that crude protein requirement is between 35% and 45%, though it is likely that the protein levels of around 45% will be optimal for maximum growth (Islam and Tanaka 2004, Sunder *et al.* 1998).

FCR's in the present study were highly variable, with values from <1 to 4.4 observed (Table 8). Gut contents analysis indicate that *empurau* are omnivorous, being both planktivorous filter-feeders and scrapers, which suggests that this species will supplement it diet by foraging on naturally occurring food. Keshavanath *et al.* (2002) found that provision of artificial substrates to promote the growth of periphyton improved net production of *T. khudree*. Provision of artificial substrates may also improve growth in *semah* and *empurau*.

Current information on the grow-out of *semah* and *empurau*, as presented here, is still cursory in nature. Culture conditions and husbandry techniques applied during these trials may not be optimal to maximising growth of these species. The effects of numerous factors, such as diet composition, stocking density, culture conditions, water quality, etc, on growth have yet to be fully explored. Future R and D into the growout of *semah* and *empurau* should aim to improve and refine culture techniques to enhance production performance of these species, including general husbandry techniques (e.g. stocking densities and culture systems), water quality requirements, nutritional requirements, feeding strategies and development of specifically formulated diets. In addition, studies into polyculture of these species with other species may also have potential to enhance overall production and productivity. Selective breeding will also provide for improved growth rates in future generations.

Grow-out:

1. Empurau and semah can be on-grown under a range of conditions in ponds, tanks and cages.
2. Fish can be feed by hand 1-2 times/ day with good quality artificial diets (approx. 45% protein, 5-10% fat) at rates of 2-10%/day (dry weight feed to weight of fish) (subject to age/ weight of fish).
3. Tanks should be well aerated, provided with a constant flow of clean water, and cleaned daily.
4. Ponds should be well aerated. Monitor water chemistry and flush ponds regularly to ensure water quality. Fish should be sampled regularly to monitor their condition and to check for diseases

3.7 Genetic consideration

Breeding of *semah* and *empurau* in the current context is to serve two different purposes, (1) to stock in to natural waters to replenish the depleted populations and (2) to provide seed for aquaculture. It is important to realize that stock enhancement and aquaculture have different goals and therefore genetic management aspects of in these two activities are also different.

Management of wild fisheries of the two species should not allow mixing of stocks from different river systems. Translocations and mixing stocks could pose a potential threat to genetic diversity and integrity of natural populations, especially in the case of semah where significant population genetic structure was identified.

Please refer to the "Guidelines for genetic management" for more details on this aspect.

4 Future prospects for mahseer aquaculture

Mahseer are an important group of fish to the Asian region, both culturally and as a food source, in particular to certain ethnic communities. Its very wide distribution and species diversity is of interests to science, and the successes in artificial propagation of some of the constituent species have also made the group important aquaculturally. The successes in artificial propagation of certain species of mahseer have also permitted the possibility to replenish depleted wild populations, and around such populations develop successful eco-tourism ventures.

The importance of this group of fish to the region was aptly demonstrated by the Kuala Lumpur Declaration (*Mahseer 2006:Biology, Culture and Conservation, the International Symposium on Mahseer)* of March 2005, and the major recommendation made at this international gathering to set-up a "Mahseer Research and Development Center" to further research and aquaculture development of selected species of this group of fish and to encourage scientific endeavor.

In Asia there is an increasing trend and a growing perception that dependence of aquaculture on alien/ exotic species need to be gradually reduced, and the use of indigenous species encouraged. In this regard some of the mahseer species in particular species such as *T. douronensis*, *T. tambroides*, *T. puttitora* and the like are ideal candidates for aquaculture in the region. Mahseer

species in general are relatively slow growing group of fish compared to most species used in aquaculture in freshwaters in the region. However, this relative slow rate of growth is more than compensated for by the potential higher price these species would command.

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Annex 1. Monitoring guidelines

Parameter	Frequency of sampling	Comments
General (all data sheets)		
Pond/tank number	Every sample period	
Date and time	Every sample period	
Primary water quality parameters		
Temperature	Daily	May fluctuate diurnally
Dissolved oxygen	1-2 daily (tanks) 1-3 weekly (ponds)	Marked diurnal fluctuations can occur in ponds
pH	1-2 daily (tanks) 1-3 weekly (ponds)	Marked diurnal fluctuations can occur in ponds
Ammonia	Daily (tanks) Weekly (ponds)	Amount of ammonia in toxic form influenced by temperature and pH
Nitrite	Daily (tanks) Weekly (ponds)	
Nitrate	Daily (tanks) Weekly (ponds)	
Other water quality parameters		
Alkalinity	Weekly (ponds)	Where water source is low in alkalinity
Phosphorus or phosphate	Weekly (ponds)	
Secchi disk depth	Daily to weekly (ponds)	Indicator of plankton density
Turbidity	As required	
Suspended solids	As required	
Salinity or conductivity	As required	
Fish health		
Sample collection	1-2 weekly	
Behaviour	Each sampling	Feeding activity, swimming behaviour etc.
Appearance	Each sampling	Skin, eyes, fins, gills, colouration, markings, fin condition, shape of body etc.
No. fish affected	Each sampling	No. of moribund fish, No. of dead fish, etc.
No. fish sampled	Each sampling	
Length of fish	Each sampling	
Weight of fish	Each sampling	
Parasite species observed	Each sampling	If cannot be identified, photograph and/or draw and describe size, appearance and behaviour.
Location of parasites on/in fish	Each sampling	body surface, gills, fins, internal organs
No. of parasites present	Each sampling	No. observed per field of view
Action taken	Each sampling	What action was taken following inspection of fish (ie, treatments applied et.)

Parameter	Frequency of sampling	Comments
Additional useful information		
Tank/pond volume, date filled, date stocked		
Source/Species/strain of fish stocked		
Length, weight and age at stocking, and Number fish stocked		
Feeding information (Feed type and feed rate etc)		
Chemicals added (type, amount and reason for addition)		

Annex 2. Useful conversions and formulae

Volumes

1 m^3 = 1,000 L

1,000 mL = 1 L (litre)

1,000,000 L = 1 ML

Weight

1,000 ug = 1 mg

1,000 mg = 1 g

1,000 g = 1 kg

1,000 kg = 1 tonne

Conversions

1 ppm = 1 mg/L

1 ppm = 1 L in 1,000,000 L

1 ppm = 1 mL in 1,000 L

1ppt = 1 g/L = 0.1%

1 ppt = 1,000 ppm

1% = 10 ppt = 10 g/L

1 mg/L = 1 g in 1,000 L

Specific growth rate (SGR), is expressed as the percentage increase in body weight per day (%/day) and is determined by using the formula:

$$\text{Specific Growth Rate (SGR)} = \frac{(\ln Wt_2 - \ln Wt_1)}{(t_2 - t_1)} \times 100\,\%$$

where: t = time in days.
$\ln Wt_2$ = natural logarithm of the average weight at time t_2.
$\ln Wt_1$ = natural logarithm of the average weight at time t_1.

Food Conversion Ratio (FCR) is determined by the formula:

$$\text{Food Conversion ratio (FCR)} = \frac{\text{Food consumed (g dry } Wt\text{) between } t_1 \text{ and } t_2}{\text{Increase in fish biomass (g wet } Wt\text{) between } t_1 \text{ and } t_2}$$

where: t_1 = Initial time.
t_2 = Final time.
Wt_2 = Final fish weight (g) (at time t_2).
Wt_1 = Initial fish weight (g) (at time t_1).