2. Floating aquatic macrophytes – *Azolla*

Floating aquatic macrophytes are defined as plants that float on the water surface, usually with submerged roots. Floating species are generally not dependent on soil or water depth.

Azolla spp. are heterosporous free-floating freshwater ferns that live symbiotically with *Anabaena azollae*, a nitrogen-fixing blue-green algae. These plants have been of particular interest to botanists and Asian agronomists because of their association with blue-green algae and their rapid growth in nitrogen deficient habitats (Islam and Haque, 1986). The genus *Azolla* includes six species distributed widely throughout temperate, sub-tropical and tropical regions of the world. It is not clear whether the symbiont is the same in the various *Azolla* species.

Azolla spp. consists of a main stem growing at the surface of the water, with alternate leaves and adventitious roots at regular intervals along the stem. Secondary stems develop at the axil of certain leaves. *Azolla* fronds are triangular or polygonal and float on the water surface individually or in mats. At first glance, their gross appearance is little like what are conventionally thought of as ferns; indeed, one common name for them is duckweed ferns. Plant diameter ranges from 1/3 to 1 inch (1-2.5 cm) for small species like *Azolla pinnata* to 6 inches (15 cm) or more for *A. nilotica* (Ferentinos, Smith and Valenzuela, 2002).

2.1 CLASSIFICATION

The genus *Azolla* belongs to the single genus family Azollaceae. The six recognizable species within the genus are grouped under two subgenera: *Euazolla* and *Rhizosperma*.

The four species under the sub-genus *Euazolla* are *A. filiculoides*, *A. caroliniana*, *A. mexicana* and *A. microphylla*. It is thought that these four species originated from temperate, sub-tropical and tropical regions of North and South America (Van Hove, 1989). However, Zimmerman *et al.* (1991) found three of these species (*A. caroliniana, A. mexicana* and *A. microphylla*) to have close taxonomic affinity and similar responses to phosphorus deficiency and recommended that they be considered as a single species.

The two species under sub-genus *Rhizosperma* are *A. nilotica* and *A. pinnata*. *A. nilotica* is a native of East Africa and can be found from Sudan to Mozambique (Van Hove, 1989). *A. pinnata* has two different varieties that vary in their distribution patterns. *A. pinnata* var. *imbricata* originates from subtropical and tropical Asia while *A. pinnata* var. *pinnata* occurs in Africa and is known as African strain.

2.2 CHARACTERISTICS

2.2.1 Importance

Because *Azolla* has a higher crude protein content (ranging from 19 to 30 percent) than most green forage crops and aquatic macrophytes and a rather favourable essential amino acid (EAA) composition for animal nutrition (rich in lysine), it has also attracted the attention of livestock, poultry and fish farmers (Cagauan and Pullin, 1991). In Asia *Azolla* has been long used as green manure for crop production and a supplement to diets for pigs and poultry. Some strains of *Azolla* can fix as much as 2-3 kg of nitrogen/ha/day. *Azolla* doubles its biomass in 3-10 days, depending on conditions, and easily reaches a standing crop of 8-10 tonnes/ha fresh weight in Asian rice fields; 37.8 tonnes/ha fresh weight (2.78 tonnes/ha dry weight) has been reported for *A. pinnata* in India (Pullin and Almazan, 1983). Recently, Liu *et al*. (2008) have reported the application of *Azolla* as a controlled ecological life support system (CELSS) for its strong photosynthetic oxygen-releasing capacity. *Azolla* provides a protected environment and a fixed source of carbon to the blue-green filamentous algae *Anabaena* spp. (Wagner, 1997).

2.2.2 Environmental requirements

The potential for rearing *Azolla* is restricted by climatic factors, water and inoculum availability, the incidence of pests, phosphorus requirements and the need for labour intensive management (Cagauan and Pullin, 1991). Water is the fundamental requirement for the growth and multiplication of *Azolla*. The plant is extremely sensitive to lack of water. Although *Azolla* can grow on wet mud surfaces or wet pit litters, it prefers growing in a free-floating state (Becking, 1979). A strip of water not more than a few centimetres deep favours growth because it provides good mineral nutrition, with the roots not too far from the soil, and also because it reduces wind effects (Van Hove, 1989). Strong winds can accumulate *Azolla* to one side of the stretch of water, creating an overcrowded condition and thus slowing growth.

Azolla can survive a water pH ranging from 3.5-10, reported optimum growth occurring at pH 4.5-7.0. Watanabe *et al*. (1977) reported that the growth of *Azolla* was optimum at pH 5.5 and FAO (1977) recorded that soils of pH 6 to 7 support the best growth.

Salinity tolerance of *Azolla* species also varies. The growth rate of *A. pinnata* declines as its salinity increases above 380 mg/l (Thuyet and Tuan, 1973). According to Reddy *et al*. (2005) *Azolla* can withstand salinity of up to 10 ppt but Haller, Sutton and Burlowe (1974) reported that the growth of *A. caroliniana* ceases at about 1.3 ppt and higher concentrations result in death. *A. filiculoides* has been reported to be most salt-tolerant (I. Watanabe pers. comm., cited by Cagauan and Pullin, 1991).

Azolla grows in full to partial shade (100-50 percent sunlight) with growth decreasing quickly under heavy shade (Ferentinos *et al*., 2002). Generally, *Azolla* requires 25-50 percent full sunlight for its normal growth; slight shade is of benefit to its growth in field conditions. The biomass production greatly decreases at a light intensity lower than 1 500 lux (Liu *et al*., 2008).

Like all other plants, *Azolla* needs all the macro- and micro-nutrients for its normal growth and vegetative multiplication. All elements are essential; phosphorus is often the most limiting element for its growth. For normal growth, 0.06 mg/l/day is required. This level can be achieved in field conditions by the split application of superphosphate at 10-15 kg/ha (Sherief and James, 1994). 20 mg/l is the optimum concentration (Ferentinos *et al*., 2002). The symptoms of phosphorous deficiency are red-coloured fronds (due the presence of the pigment anthocyanin), decreased growth and curled roots. Macronutrients such as P, K, Ca and Mg and micronutrients such as Fe, Mo and Co have been shown to be essential for the growth and nitrogen fixation of *Azolla* (Khan and Haque, 1991).

The temperature tolerance of *Azolla* varies widely between its species and strains. In general, *Azolla* has low tolerance to high temperature and that restricts its use in tropical agriculture. There are, however, strains that can adapt successfully to high temperature. Cagauan and Pullin (1991) ranked different *Azolla* species from the most to the least heat-tolerant, based on the optimum temperature for good growth: *A. mexicana* > *A. pinnata* var. *pinnata* > *A. microphylla* > *A. pinnata* var. *imbricata*, *A. caroliniana* > *A. filiculoides* (Table 2.1). In general, the optimum temperature for growth of all *Azolla* species is around 25 °C, except that of *A. mexicana*, whose optimum temperature is

TABLE 2.1
Temperature tolerance of five species of *Azolla*

Subgenera	Species	Water temperature (°C)		
		Minimum	Maximum	Optimum for growth
Euazolla	*A. filiculoides*	0-10	38-42	20-25
	A. caroliniana	<0-10	45	20-30
	A. mexicana	-	-	30-33
	A. microphylla	5-8	45	25-30
Rhizosperma	*A. pinnata*			
	A. pinnata var. pinnata	<5	>40	16-33
	A. pinnata var. imbricata	0	45	20-30

Source: modified from Cagauan and Pullin (1991)

~30 °C. According to Sherief and James (1994), the favourable water temperature for rapid multiplication of *Azolla* is generally between 18 and 26 °C.

The optimum relative humidity for *Azolla* growth is between 85-90 percent. *Azolla* becomes dry and fragile at a relative humidity lower than 60 percent.

2.3 PRODUCTION

Multiplication of *Azolla* in nature and in the laboratory is entirely through vegetative reproduction. However, sexual reproduction, which is essential to the survival of the population during temporary adverse conditions also, occurs. When *Azolla* fronds reach a certain size depending on the species and the environment, generally 1 to 2 cm in diameter, the older secondary stems detach themselves from the main stem as a result of the formation of an abscission layer, thus giving rise to new fronds. This is the most usual mode of multiplication.

Sherief and James (1994) have described a simple *Azolla* nursery method for its large-scale multiplication in the field for Indian farmers. The field for an *Azolla* nursery must be thoroughly prepared and levelled uniformly. It is divided into different plots by providing suitable bunds and irrigation channels. Water is manipulated at a depth of 10 cm. Ten kg of fresh cattle dung mixed in 20 L of water is sprinkled in each plot and an *Azolla* inoculum of 8 kg is introduced to each plot. Superphosphate (100 g) is applied in three split doses at intervals of four days as a top dressing fertilizer. For insect control, furadone granules at 100 g/plot are applied seven days after inoculation. Fifteen days after inoculation, *Azolla* is harvested. From one harvest, 40-55 kg of fresh *Azolla* is obtained from each plot. Reddy and DeBusk (1985) reported the yield of *Azolla* (*A. caroliniana*) to be 10.6 t DM/ha/year in nutrient non-limiting waters of central Florida, USA.

According to Ferentinos *et al.* (2002) the nitrogen fixation capacity of *Azolla* was found to vary from 53-1 000 kg/ha with a dry matter production of 39-390 tonnes/ha, in crop cycles of 40-365 days. The linear growth phase is usually between 6 and 21 days and is characterized by low lignin and cell wall fractions. Due to its high lignin content (20 percent), nitrogen is released slowly from the plant initially, with about two-thirds released on the first 6 weeks after application. Under flooded conditions, 40-60 percent of the available N is released after 20 days and 55-90 percent within 40 days after application

Reddy *et al.* (2005) described the production of *Azolla* in earthen raceways (10.0 m x 1.5 m x 0.3 m) in CIFA, Bhubaneswar. 6 kg of *Azolla* was inoculated in each raceway. 50 kg single super phosphate and pesticide (1-2 mg/l) were applied and a water depth of 5-10 cm was maintained. 18-24 kg/raceway/week was produced. About one tonne of *Azolla* could be harvested every week from water spread area of 650 m^2, with a phosphorus input-nitrogen output ratio of 1:4.8.

TABLE 2.2
Chemical analyses of various *Azolla* species

Azolla species	Moisture (percent)	Composition[1] (percent DM[1])					Minerals (percent DM)			Reference
		CP	EE	CF	Ash	CC	Ca	P	K	
A. filiculoides	93.5[2]	25.0-28.5	3.1	n.s.	17.3	4.4-11.5	0.5-1.5	1.0-1.5	6.0	modified from Cagauan and Pullin (1991)
A. caroliniana		20.6-22.6	n.s.	n.s.	n.s.	8.5	0.6	1.3	5.3	modified from Cagauan and Pullin (1991)
A. pinnata var. *imbricate*		26.0	n.s.	n.s.	n.s.	4.1	0.4	1.3	4.5	modified from Cagauan and Pullin (1991)
A. pinnata (tank culture)		18.2	1.3	n.s.	21.7	n.s.	1.6	0.6	n.s.	modified from Cagauan and Pullin (1991)
A. pinnata (field culture)		22.2	2.9	n.s.	18.3	14.7	n.s.	n.s.	n.s.	modified from Cagauan and Pullin (1991)
A. pinnata		21.4	2.7	12.7	16.2					Alalade and Iyayi (2006)
A. microphylla (lab. culture)		21.8	2.9	n.s.	21.6	15.6	n.s.	n.s.	n.s.	modified from Cagauan and Pullin (1991)
A. microphylla (field culture)		20.0-26.0	3.0-3.5	n.s.	14-15	4.0-14.0	0.7	1.6	5.5	modified from Cagauan and Pullin (1991)
A. microphylla hybrid (field culture)		19.0	4.0-4.5	n.s.	16.0-17.0	2.5-3.0	n.s.	n.s.	n.s.	modified from Cagauan and Pullin (1991)
Various Azolla spp.		13.0-30.0	4.4-6.3	n.s.	9.7-23.8	5.6-15.2	0.2-0.7	0.1-1.6	0.3-6.0	Reddy *et al.* (2005)
Azolla sp.		n.s.	n.s.	n.s.	n.s.	n.s.	1.0	0.4	2.5	Ferentinos, Smith and Valenzuela, (2002)

[1] CP = crude protein; EE = ether extract; CC = crude cellulose; Ca = calcium; P = phosphorus; K = potassium
[2] Data obtained from Tacon (1987)

2.4 Chemical composition

The chemical composition of *Azolla* species varies with ecotypes and with the ecological conditions and the phase of growth. The dry matter percentage of different *Azolla* species varies widely and there is little agreement between the published data on this subject: values of 5 to 7 percent can, however, be taken as fair estimates (Van Hove, 1989). A summary of the chemical composition of various *Azolla* species is presented in Table 2.2. Generally, the crude protein content is about 19-30 percent DM basis during the optimum conditions for growth (Peters *et al*., 1979; Becking, 1979). Under natural conditions, values near 20-22 percent are frequent. The protein contents of *Azolla* species are comparable to or higher than that of most other aquatic macrophytes. Like most of the other aquatic macrophytes, *Azolla* have high ash contents, varying between 14-20 percent. No clear interspecific difference in the crude lipid levels of various *Azolla* species occurs; the value is around 3-6 percent on a DM basis.

Amino acid compositions of *Azolla* spp. are presented in Annex 1 Table 2. Generally, these species are low in methionine but high in lysine (except for *A. pinnata*). *A. microphylla* is richest in all EAA except in methionine. The poorest species with respect to most of the EAA is *A. filiculoides* although lysine and methionine contents in this species are moderate. The EAA composition of *Azolla* species is comparable to that of the aquatic plants commonly used as fish feed ingredients. The lysine and methionine contents of most *Azolla* species appear to be higher than some 'conventional' plant protein sources.

2.5 USE AS AQUAFEED

In spite of its attractive nutritional qualities and relative ease of production in ponds and rice-fields, reports on the use of *Azolla* in aquaculture are extremely limited. The value of *Azolla* as a fish feed is still being studied. Available literature on the use of *Azolla* for this purpose has been reviewed as follows under the headings experimental studies and field studies.

2.5.1 Experimental studies

A few studies have been carried out in aquaria to examine the preference for various *Azolla* species by different cichlid species and a carp hybrid. These tests were carried out using fresh *Azolla*; the results are summarized in Table 2.3. These preference tests indicate that *A. caroliniana* (Figure 2.1) is one of the most preferred species for cichlids.

A number of growth studies have been carried out to evaluate *Azolla* as fish feed under laboratory rearing conditions. Most of these studies were conducted on cichlids and little or no attempt was made to use *Azolla* as a feed for grass carp, a predominantly macrophytophagous feeder. In these studies, *Azolla* was fed either in fresh or dried powdered form as a whole feed or by partially replacing fishmeal in pelleted diets.

Almazan *et al.* (1986) conducted a study where *A. pinnata* was fed to Nile tilapia (*Oreochromis niloticus*) fingerlings and adult males. Fingerlings were fed *Azolla* in fresh, powder, and pellet form, replacing the complete control diet mix from 10 percent to 90 percent. The control diet consisted of 40 percent fishmeal, 40 percent rice bran, 10 percent cornstarch, 9 percent corn meal and 1 percent Afsillin (micronutrient premix). Negative

FIGURE 2.1
Azolla/mosquito fern *(Azolla caroliniana)*

Source: www.msrosenthal.com/Ferns/images/Florida_Images/Azolla_caroliniana.jpg

TABLE 2.3
Preference of *Azolla* spp. by different fish species

Fish species	Preferred ***Azolla*** species[1]	Reference
Cichlasoma fenestratum	(1) *A. microphylla*	Antoine *et al.* (1986)
	(2) *A. caroliniana*	
Oreochromis niloticus	(1) *A. filiculoides*	Antoine *et al.* (1986)
	(2) *A. microphylla*	
	(3) A. caroliniana	
O. mossambicus	*A. caroliniana*	Lahser (1967)
O. niloticus	*A. microphylla*	Fiogbé, Micha and Van Hove (2004)
Tilapia rendalli	(1) *A. caroliniana*	Micha *et al.* (1988)
	(2) *A. pinnata var. pinnata*	
	(3) *A. microphylla*	
	(4) *A. filiculoides*	
Hybrid carp (grass carp x bighead carp)	*A. caroliniana*	Cassani (1981)

[1] *Azolla* species are arranged chronologically for each fish species in order of preference i.e. from most preferred to less preferred

or very slow growth was obtained in all *Azolla*-incorporated diets. A lowering of growth performance and FCRs was observed with increasing *Azolla* incorporation. Adult male tilapia were fed dried *Azolla* pellets or fresh *Azolla ad libitum*. Despite feeding to satiation, tilapia suffered weight loss in a 30-day feeding trial. The experiments were carried out in aquaria**.** Similarly, Antoine *et al.* (1986) working with *O. niloticus* and *Cichlasoma melanurum* and Micha *et al.* (1988) with *O. niloticus* and *Tilapia rendalli* reported poor growth and feed utilization when they were fed *A. microphylla*-based diets. Antoine *et al.* (1986) and Micha *et al.* (1988) conducted a 70-day growth trial and fed the target species with three different diets: commercial pellets (34 percent protein); fresh *Azolla* plus 28 percent protein commercial pellets (50:50); and fresh *Azolla* (22 percent protein).

In other studies, El-Sayed (1992; 2008) reported extremely poor performance for *O. niloticus* fingerlings and adults fed diet based on *A. pinnata*. This author incorporated dried *Azolla* powder at 25, 50, 75 and 100 percent replacement of fishmeal protein in a fishmeal-based control diet in a 70-day trial. Fresh *Azolla* as a total diet was also used as a control. Growth and feed utilization efficiency of fish fed with the control diet was significantly higher compared to fish fed with *Azolla*-supplemented diets. The performance of fish was inversely related to the increasing dietary incorporation of *Azolla*. In fish fed with the total *Azolla* (dry/fresh) based diet, the reduction was extremely sharp. Fresh *Azolla*-fed adults started losing weight from the 7th week. Fish fed with fresh plant material had significantly higher moisture content than fish fed with formulated diets. Body protein and lipid levels were negatively correlated with the concentrations of *Azolla* in the diets; ash content showed a positive correlation.

In all the experimental studies examined above (Almazan *et al*., 1986; Antoine *et al.*, 1986; Micha *et al.*, 1988), fish growth was higher in the conventional control diets than in the *Azolla*-based diets. Fish died or negative growth was recorded when fed exclusively with fresh *Azolla*.

In apparent contrast, Santiago *et al.* (1988) found that *O. niloticus* fry fed rations containing up to 42 percent *of A. pinnata* outperformed fish fed a fishmeal-based control diet. Growth and feed utilization of *O. niloticus* fry improved with increased dietary inclusion of *Azolla* and the survival was unaffected. These results differed from the studies of Almazan *et al*. (1986), Antoine *et al.* (1986) and Micha *et al.* (1988) and it must be pointed out that Santiago *et al.* (1988) used a 35 percent protein diet with early fry (11-14 mg). In the other studies, the crude protein level was generally lower and the studies were carried out with advanced fry, fingerling or adults. El-Sayed (2008) noted that young Nile tilapia utilized *Azolla* more efficiently than adults.

Fiogbé, Micha and Van Hove (2004) obtained quite favourable results with *Azolla*-based diets fed to juvenile *Oreochromis niloticus* grown in a recirculating system. Six diets were formulated with almost isonitrogenous levels of protein

(27.25-27.52 percent DM) but different levels of dry *Azolla* meal (0, 15, 20, 30, 40 and 45 percent). All diets with incorporated *Azolla* meal showed weight gain. The *Azolla*-free diet and the diet containing 15 percent *Azolla* produced the same growth performance. The least expensive diet, which contained 45 percent *Azolla*, also showed growth and was thought to be potentially useful as a complementary diet for tilapia raised in fertilized ponds. These authors noted that mixing *Azolla* with some agricultural by-products such as rice bran; the use of fermentable by-products such as yeasts; or the addition of purified enzymes; might improve ingestion and digestibility.

Carcass compositions of fish were reported to be markedly affected by feeding with *Azolla*. Antoine *et al*. (1986) observed that when fed with fresh *Azolla*, both *O. niloticus* and *C. melanurum* had higher moisture and lower lipid concentrations. Similar results and an increase in carcass ash content for *O. niloticus* and *T. rendalli* were reported by Micha *et al*. (1988). El-Sayed (1992) also made similar observations when he fed fresh and dried *A. pinnata* to *O. niloticus*. However, his observation differs from the previous authors to the extent that carcass protein content was negatively correlated with *Azolla* levels in the diets, while the other workers recorded no effect on carcass protein content.

The poor growth of fish fed with diets containing higher levels of *Azolla* may be due to excesses or deficiencies of amino acids, according to Fiogbé, Micha and Van Hove (2004). Cole and Van Lunen (1994) found that inadequate levels of essential amino acids resulted in depression of food intake and growth. Deficiencies of one or more amino acids are known to limit protein synthesis, growth or both.

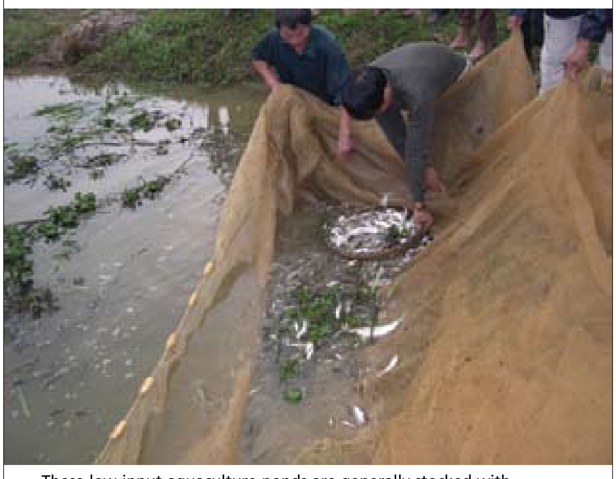
2.5.2 Field studies

Until recently, reports of on-farm utilization of *Azolla* were limited (Cagauan and Pullin, 1991). At that time reports came only from China and Vietnam (Figure 2.2). More recently *Azolla* has increasingly been used as feed and/or fertilizer in studies with rice-fish culture systems in many other Asian countries. Reddy *et al*. (2005) reported that the manuring schedule can be reduced by 30-35 percent through *Anabaena azollae* —*Azolla* biofertilization in aquacultural ponds.

Azolla *in cage culture*

Pantastico, Baldia and Reyes (1986) used fresh whole *A. pinnata* as a supplemental feed for the cage culture of Nile tilapia in Laguna de Bay, Philippines. *Azolla* was propagated in fine mesh net enclosures in the lake and harvested for feeding to tilapia in cages. Four separate experiments were conducted and weight gain was compared with an unfed control. It was assumed that in control cages fish grew by feeding natural food (i.e. plankton) available in the cage. A summary of the results is given in Table 2.4. Although higher weight gain of fish was observed over the unfed control, the difference in mean weight between fish fed fresh *Azolla* and unfed control was about 5-10 g. The results of this cage culture study do not justify fish culture in cages using *Azolla* as the only feed.

FIGURE 2.2
Harvest of fish from a pond (Hoa Binh Province, Viet Nam)



These low-input aquaculture ponds are generally stocked with macrophytophagous fish (primarliy carp species) and fresh *Azolla* (*Azolla pinnata*) are commonly used as supplemental feed.

Courtesy of M.G. Kibria

TABLE 2.4
Cage culture of Nile tilapia using *Azolla* as feed

Initial weight (g)	Stocking density (Numbers/m^3)	Duration (months)	Feeding rate (percent)	Fresh *Azolla* Harvest weight (g)	Unfed control Harvest weight (g)
1.3	25	6	35 and 70	30.3 and 36.3	24.7
1.6	50	4	30	75.0	64.1
6.5	100	5	20	20.2	10.9
13.5	150	3	15	29.3	20.2

*Rice-fish-*Azolla *integration*

One of the most successful uses of *Azolla* is its use as fertilizer and/or feed in an integrated rice-fish-*Azolla* system. This system is based on convenient layout of the fields, which allows the simultaneous development of rice, *Azolla* and different fish with complementary nutritional requirements (Van Hove, 1989). In this ecological agricultural layout, each of the partners contributes to the equilibrium of the system. The fish (a correct mixture of planktophages, macrophytophages and polypages) derive a benefit from *Azolla* - more or less, depending on the species; their waste promotes the proliferation of plankton that is consumed by some of the fish and fertilizes the rice. The polyphagous fish protect the rice and *Azolla* from a number of insects and molluscan pests.

Cagauan and Pullin (1991) reviewed the rice-fish-*Azolla* integrated system and described its physical set-up, which is provided with pits (pond refuse/ main channel) and ditches (trenches). Lateral or peripheral trenches are interconnected with each other and with the main channel. Trenches serve as links for the fish from the main channel to rice field area and also as a growing area for *Azolla* during the paddy cultivation period. Depending on the size of the rice field, trenches may be dug at 15-20 m intervals in single or rib-shaped patterns. In India, a 0.2 ha rice field was provided with 0.5 m deep and 0.5 m wide trenches and a 1.0 m deep and 1.5 m wide main channel (Shanmugasundaram and Ravi, 1992). Cagauan (1994) used 1 m wide and 0.75 m deep pond refuge connected to an outer trench that was 0.3-0.4 m wide and 0.2-0.3 m deep in a 200 m^2 paddy field. The trenches and main channels should occupy about 10-15 percent of the rice field area (Cagauan and Pullin, 1991; Shanmugasundaram and Balusamy, 1993). Inoculation of the rice field with *Azolla* at the rate of 4.5-6.0 tonnes/ha is done 20 days before rice transplanting. Propagated *Azolla* biomass is ploughed under, together with inorganic fertilizer, before rice transplanting. The field is then reflooded to allow the *Azolla* that floated during the incorporation to grow and serve as a fish fodder. In case of insufficiency of *Azolla* in the channels and trenches, additional supplemental feed is given. The fish species cultured in these rice-fish-*Azolla* systems are mainly Nile tilapia. Other species are common carp, Indian major carp, Java barb, etc. Grass carp may not be a suitable species for this system, as they may damage the rice crop by feeding on its leaves.

The use of *Azolla* (*A. microphylla*) as a fertilizer in rice-fish farming was studied by Cagauan and Nerona (1986) and Cagauan (1994). Cagauan and Nerona (1986) used three fertilizer regimes: *Azolla* only; inorganic fertilizers (urea and ammonium phosphate) only; and *Azolla* plus inorganic fertilizers for rice-fish culture with Nile tilapia as the target species. When a combination of *Azolla* and inorganic fertilizers was used, it was possible to reduce the standard rate of inorganic fertilizers by half. Fish yields were the same with *Azolla* or inorganic fertilizers alone, whereas the yields of both fish and rice were higher in the combined *Azolla* and inorganic fertilizer regime (Table 2.5).

Shanmugasundaram and Ravi (1992) reported the use of *Azolla* (*A. microphylla)* as nitrogen fixing fertilizer and feed for Nile tilapia (*O. niloticus*) stocked in a low-lying ricefield (0.2 ha) in the Tanjore Deltaic Zone, Tamil Nadu, India. The ricefield was

TABLE 2.5

Use of *A. microphylla* as fertilizer in rice-fish culture system- fish species *(O. niloticus)*

Initial weight (g)	Fish density (Numbers/ ha)	Duration (days)	Fertilizer regimes	Fertilizer rate (kg/ha)	Quantity of N (kg/ha)	Fish yield (kg/ha)	Rice yield (kg/ha)
8.9-9.4	5 000	75	*Azolla* only	3 750	5.63	45.1	2 567
			Inorganic fertilizer	150	38.5	45.0	3 096
			Azolla	3 750	5.6		
			Inorganic fertilizer	75	19.3	79.0	3 524

Source: Cagauan and Nerona (1986)

provided with trenches and connected to a main channel and the fish were raised in these trenches. The stocking density used was 6 000/ha for fingerlings weighing 19 g. Both fresh and dried *Azolla* were fed. Dried *Azolla* was incorporated in a supplemental fish feed and applied at 5 percent BW/day. The formula of this supplemental feed was stated to be *Azolla* (50 percent), rice bran (15 percent), chicken manure (10 percent), corn meal (5 percent), sorghum meal (5 percent), broken rice (2.5 percent) and groundnut cake (2.5 percent). The provision of water space for the fish lowers rice yields by about 300 kg/ha but the fish harvest compensates. Rice and fish culture yields a net income of US$258/crop/ha, compared to US$207/crop/ha for rice alone.

Furthermore, Shanmugasundaram and Balusamy (1993) reported the use of *Azolla* (*A. microphylla)* as feed to raise Indian major carps (catla, rohu and mrigal) stocked in low-lying wetlands in Bhavanisagar, Tamil Nadu, India. These authors used a 0.25 ha ricefield provided with trenches (1.0 m depth and width) to shelter the fish. Stocking density was 3 000/ha, using a 1:1:1 ratio of catla, rohu and mrigal. *Azolla* was applied twice at 2.0 tonnes/ha. Supplemental feed containing banana pseudostem and cow dung (1:1) was fed along with rice bran at 5 percent BW/ per day. Banana pseudostem and cow dung were incubated overnight before mixing with rice bran. Both rice and fish yields increased, with higher benefit cost ratios (1.88) in rice-fish-*Azolla*

TABLE 2.6

Economics of rice-fish-*Azolla* integration in India

Treatment	Rice yield (kg/ha)	Fish yield (kg/ha)	Gross return (US$/ha)	Net return (US$/ha)	Benefit cost ratio
Rice alone	8 765	-	822	353	1.75
Rice-fish	7 813	98.5	812	297	1.57
Rice-fish-*Azolla*	9 226	154.0	985	463	1.88

Source: Shanmugasundaram and Balusamy (1993)

TABLE 2.7

Results of rice-fish-*Azolla* integration highlighting increase in fish yield

Culture system	Fish species	Average harvest weight (g)		Yield (tonnes/ha)	
		With *Azolla*	Without *Azolla*	With *Azolla*	Without *Azolla*
Monoculture	*Oreochromis niloticus*	150-200	100-150	1.20	0.63
Polyculture	*Cyprinus carpio*	600	350	0.35	0.15
	Ctenopharyngodon idella	150	130	0.17	0.15
	Oreochromis niloticus	125	100	0.54	0.40
	Total			**1.06**	**0.70**

integration than rice-fish cultivation (1.57) (Table 2.6). Similarly, substantial increases in fish yield in rice-fish culture with *Azolla* compared to rice-fish without *Azolla* have been reported by Cagauan and Pullin (1991). Fish yields from rice-*Azolla*-fish culture trials were higher than those for rice-fish culture (Table 2.7). Yields from Nile tilapia in monoculture and from polyculture of common carp, grass carp and Nile tilapia were 1.20 and 1.06 tonnes/ha/year respectively from a rice-fish-*Azolla* system, compared with 0.63 and 0.70 tonnes/ha/year respectively from rice-fish fields without *Azolla*.

In rice farming systems, including rice-fish culture, *Azolla* is best incorporated as a fertilizer during its linear growth phase, when there is maximum productivity, low lignin content and therefore rapid decomposition. The value of *Azolla* as a fish feed is also highest during its linear growth phase. The crude protein content of *Azolla* is generally higher during this phase. The amino acid content of *Azolla* increases during the linear growth phase and falls sharply when the growth slows down with a corresponding increase in its lignin content. Digestibility clearly decreases after the linear growth phase with increasing lignin content (Van Hove *et al*., 1987). It is therefore important to maintain an equilibrium between the population of fish and that of *Azolla*, either by introducing, when necessary, a supplementary biomass of *Azolla* collected elsewhere, or by harvesting the excess biomass in order to keep the *Azolla* population in the linear growth phase.

*Pig-duck-fish-*Azolla *and fish-*Azolla *integration*

Very few reports are available on the use of *Azolla* as fish feed in pond culture; however, there are reports of integrated studies. Majhi, Das and Mandal (2006) fed grass carp (*Ctenopharyngodon idella*) fingerlings with finely chopped *Azolla caroliniana* placed over a feeding basket under pond conditions. *Azolla* was well accepted by grass carp. The final weight gain of *Azolla*-fed fish was significantly higher compared to the control fish. The net profit for production of grass carp was US$0.12/m^2.

Gavina (1994) studied pig-duck-fish-*Azolla* integration. Nile tilapia were stocked in three earthen ponds with a uniform water depth of 50 cm. The ponds were fertilized with a mixture of dry pig and duck manure at the rate of 500 kg/ha. After initial manure application, the water level was increased to 80 cm in all three experimental ponds. The ponds were stocked at three densities: 10 000/ha; 20 000/ha; and 30 000/ha. All treatments were manured (pigs and ducks) with 100 kg fresh material/ha/day and supplemented with fresh *Azolla* at 200 g/m^2/week. The consumption of *Azolla* by fish was not monitored. However, it was observed that the fresh *Azolla* were seeded at a

TABLE 2.8
Weight gain comparisons of *Azolla*-fed fish

Fish	No. of fish	Initial weight (g)	Final weight (g)	Survival (percent)	Culture period (days)	Total weight increase (g)	SGR (percent)	*Azolla* feed coeff.
Grass carp[1]	30	54.7	118.7	100	112	1 920	0.69	49.0
Crucian carp[1]	30	75.0	110.8	100	112	1 074	0.35	31.2
Nile tilapia[1]	30	24.7	163.1	100	100	4 152	1.89	52.2
Silver carp[1]	39	96.8	92.2	76.7	112	-1 017	-0.04	0.0
Nile tilapia (15 percent inclusion)[2]	25	1.67	3.23	56	30	21.84	2.20	
Nile tilapia (45 percent inclusion)[2]	25	1.70	2.28	61.3	30	8.89	0.98	
Grass carp[3]	-	22.7	270.3	100	150		1.65	
Tilapia zillii[4]	15	2.2	4.7	93	91	34.88	0.83	

[1] modified from FAO (1988 cited by Cagauan and Pullin, 1991)
[2] Fiogbé, Micha and Van Hove (2004)
[3] Majhi, Das and Mandal (2006)
[4] Abdel-Tawwab (2008)

rate of 200 g/m^2/week (10 kg/50 m^2) and cleared by fish after 6 or 7 days. It was found that *Azolla* could be a viable source of supplementary feed, considering the high cost of commercial feeds. The study was conducted for a period of three months. Mean net yield varied between 8.22 and 10.97 kg/ha/day (3-4 tonnes/ha/year) at stocking densities ranging between 10-30 000/ha.

Weight gain comparisons of *Azolla*-fed fish were carried out by the Soil and Fertilizer Institute of the Hunan Academy of Agricultural Sciences (FAO, 1988 cited by Cagauan and Pullin, 1991) using grass carp, Nile tilapia, crucian carp (*Carassius auratus*) and silver carp (*Hypopthalmychthys molitrix*) (Table 2.8). The weight gain by *Azolla*-fed grass carp averaged 174 g/fish compared with 134 g/fish for Nile tilapia and 35.8 g/fish for crucian carp. A weight decrease of 4.6 g/fish was observed for silver carp.