1. Algae

Algae have been used in animal and human diets since very early times. Filamentous algae are usually considered as 'macrophytes' since they often form floating masses that can be easily harvested, although many consist of microscopic, individual filaments of algal cells. Algae also form a component of periphyton, which not only provides natural food for fish and other aquatic animals but is actively promoted by fishers and aquaculturists as a means of increasing productivity. This topic is not dealt with in this section, since periphyton is not solely comprised of algae and certainly cannot be regarded as macroalgae. However, some ancillary information on this topic is provided in Annex 2 to assist with further reading. Marine 'seaweeds' are macro-algae that have defined and characteristic structures.

Microalgal biotechnology only really began to develop in the middle of the last century but it has numerous commercial applications. Algal products can be used to enhance the nutritional value of food and animal feed owing to their chemical composition; they play a crucial role in aquaculture. Macroscopic marine algae (seaweeds) for human consumption, especially *nori* (*Porphyra* spp.), *wakame* (*Undaria pinnatifida*), and *kombu (Laminaria japonica)*, are widely cultivated algal crops. The most widespread application of microalgal culture has been in artificial food chains supporting the husbandry of marine animals, including finfish, crustaceans, and molluscs.

The culture of seaweed is a growing worldwide industry, producing 14.5 million tonnes (wet weight) worth US$7.54 billion in 2007 (FAO, 2009). The use of aquatic macrophytes in treating sewage effluents has also shown potential. In recent years, macroalgae have been increasingly used as animal fodder supplements and for the production of alginate, which is used as a binder in feeds for farm animals. Laboratory investigations have also been carried out to evaluate both algae and macroalgae as possible alternative protein sources for farmed fish because of their high protein content and productivity.

Microalgae and macroalgae are also used as components in polyculture systems and in remediation; although these topics are not covered in this paper, information on bioremediation is contained in many publications, including Msuya and Neori (2002), Zhou *et al*. (2006) and Marinho-Soriano (2007). Red seaweed (*Gracilaria* spp.) and green seaweed (*Ulva* spp.) have been found to suitable species for bioremediation. The use of algae in integrated aquaculture has also been recently reviewed by Turan (2009).

1.1 CLASSIFICATION

The classification of algae is complex and somewhat controversial, especially concerning the blue-green algae (Cyanobacteria), which are sometimes known as blue-green bacteria or Cyanophyta and sometimes included in the Chlorophyta. These topics are not covered in detail this document. However, the following provides a taxonomical outline of algae.

Archaeplastida
- Chlorophyta (green algae)
- Rhodophyta (red algae)
- Glaucophyta

Rhizaria, Excavata
- Chlorarachniophytes

- Euglenids

Chromista, Alveolata

- Heterokonts
  - Bacillariophyceae (diatoms)
  - Axodine
  - Bolidomonas
  - Eustigmatophyceae
  - Phaeophyceae (brown algae)
  - Chrysophyceae (golden algae)
  - Raphidophyceae
  - Synurophyceae
  - Xanthophyceae (yellow-green algae)
- Cryptophyta
- Dinoflagellates
- Haptophyta

The following sections discuss the characteristics and use of both 'true' algae and the Cyanophyta, hereinafter referred to as 'blue-green algae').

1.2 CHARACTERISTICS

Filamentous algae and seaweeds have an extremely wide panorama of environmental requirements, which vary according to species and location. Ecologically, algae are the most widespread of the photosynthetic plants, constituting the bulk of carbon assimilation through microscopic cells in marine and freshwater.

The environmental requirements of algae are not discussed in detail in this document. However, the most important parameters regulating algal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. Macronutrients (nitrate, phosphate and silicate) and micronutrients (various trace metals and the vitamins thiamine (B_1), cyanocobalamin (B_{12}) and biotin) are required for algal growth (Reddy *et al.*, 2005). Light intensity plays an important role, but the requirements greatly vary with the depth and density of the algal culture. The pH range for most cultured algal species is between 7 and 9; the optimum range is 8.2–8.7. Marine phytoplankton are extremely tolerant to changes in salinity. In artificial culture, most grow best at a salinity that is lower than that of their native habitat. Salinities of 20–24 ppt are found to be optimal. Lapointe and Connell (1989) suggested that the growth of the green filamentous alga *Cladophora* was limited by both nitrogen and phosphorus, but the former was the primary factor. Hall and Payne (1997) found that another green filamentous alga, *Hydrodictyon reticulatum*, had a relatively low requirement for dissolved inorganic nitrogen in comparison with other species. Rafiqul, Jalal and Alam (2005) found that the optimum environment for *Spirulina platensis* under laboratory conditions was 32 °C, 2 500 lux and pH 9.0. Further information on the environmental requirements of algae cultured for use in aquaculture hatcheries is contained in Lavens and Sorgeloos (1996). The environmental requirements of cultured seaweeds are discussed by McHugh (2002, 2003).

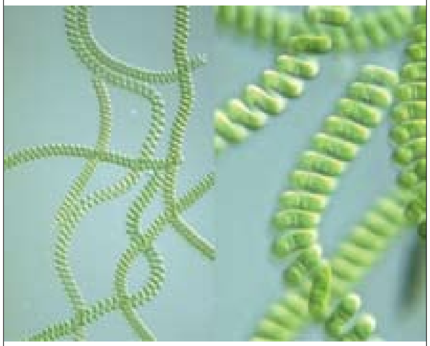
A brief description of some of the filamentous algae and seaweeds that have been used for feeding fish, as listed in Tables 1.1–1.3, is provided in the following subsections.

1.2.1 Filamentous algae

Filamentous algae are commonly referred to as 'pond scum' or 'pond moss' and form greenish mats upon the water surface. These stringy, fast-growing algae can cover a pond with slimy, lime-green clumps or mats in a short period of time, usually beginning their growth along the edges or bottom of the pond and 'mushrooming' to the surface. Individual filaments are a series of cells joined end to end which give the thread-like appearance. They also form fur-like growths on submerged logs, rocks and even on animals. Some forms of filamentous algae are commonly referred to as 'frog spittle' or 'water net'.

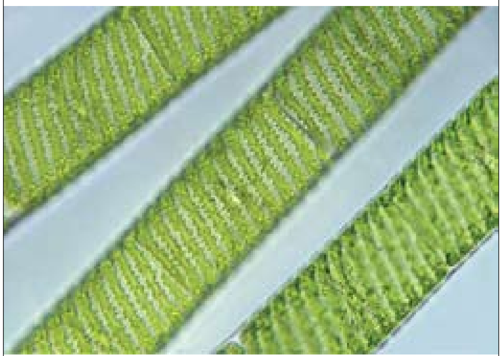
Spirulina, which is a genus of cyanobacteria that is also considered to be a filamentous blue-green algae, is cultivated around the world and used as a human dietary supplement, as well as a whole food. It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries (Figure 1.1).

FIGURE 1.1
***Spirulina* sp.**



Source: scienceblogs.com/energy/2008/08/big_energy_fr...

FIGURE 1.2
***Spirogyra* sp.**

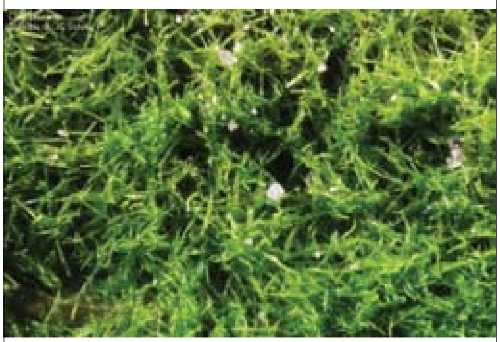


Source: Wim van Egmont©

Spirogyra, one of the commonest green filamentous algae (Figure 1.2), is named because of the helical or spiral arrangement of the chloroplasts. There are more than 400 species of *Spirogyra* in the world. This genus is photosynthetic, with long bright grass-green filaments having spiral-shaped chloroplasts. It is bright green in the spring, when it is most abundant, but deteriorates to yellow. In nature, *Spirogyra* grows in running streams of cool freshwater, and secretes a coating of mucous that makes it feel slippery. This freshwater alga is found in shallow ponds, ditches and amongst vegetation at the edges of large lakes. Under favourable conditions, *Spirogyra* forms dense mats that float on or just beneath the surface of the water. Blooms cause a grassy odour and clog filters, especially at water treatment facilities.

Cladophora (Figure 1.3) is a green filamentous algae that is a member of the Ulvophyceae and is thus related to the sea lettuce (*Ulva* spp.). The genus *Cladophora* has one of the largest number of species within the macroscopic green algae and is also among the most difficult to classify taxonomically. This is mainly due to the great variations in appearance, which are significantly affected by habitat, age and environmental conditions. These algae, unlike *Spirogyra*, do not conjugate (form bridges between cells) but simply branch.

FIGURE 1.3
***Cladophora* sp.**



Source: Biopix.dk©

FIGURE 1.4
Water net (*Hydrodictyon* sp.)

Source: silicasecchidisk.conncoll.edu

Another green filamentous alga, *Hydrodictyon*, commonly known as 'water net', belongs to the family Hydrodictyaceae and prefers clean, eutrophic water. Its name refers to its shape, which looks like a netlike hollow sack (Figure 1.4) and can grow up to several decimetres.

1.2.2 Seaweeds

Ulva are thin flat green algae growing from a discoid holdfast that may reach 18 cm or more in length, though generally much less, and up to 30 cm across. The membrane is two cells thick, soft and translucent and grows attached (without a stipe) to rocks by a small disc-shaped holdfast. The water lettuce (*Ulva lactuca*) is green to dark green in colour (Figure 1.5). There are other species of *Ulva* that are similar and difficult to differentiate.

FIGURE 1.5
Sea lettuce (*Ulva lactuca*)

Source: Mandy Lindeberg (www.seaweedsofalaska.com)

FIGURE 1.6
Eucheuma cottonii

Source: www.actsinc.biz/seaweed.html

It is important to recognize that the genera *Eucheuma* and *Kappaphycus* are normally grouped together; their taxonomical classification is contentious. These are red seaweeds and are often very large macroalgae that grow rapidly. The systematics and taxonomy of *Kappaphycus* and *Eucheuma* (Figure 1.6) is confused and difficult, due to morphological plasticity, lack of adequate characters to identify species and the use of commercial names of convenience. These taxa are geographically widely dispersed through cultivation (Zuccarello *et al*., 2006). These red seaweeds are widely cultivated, particularly to provide a source of carageenan, which is used in the manufacture of food, both for humans and other animals.

Gracilaria is another genus of red algae (Figure 1.7), most well-known for its economic importance as a source of agar, as well as its use as a food for humans.

FIGURE 1.7
***Gracilaria* sp.**

Source: Eric Moody© (Wikipedia)

FIGURE 1.8
Porphyra tenera

Source: http://www.fao.org/fishery/species/search/en

The red seaweed *Porphyra* (Figure 1.8) is known by many local names, such as laver or *nori*, and there are about 100 species. This genus has been cultivated extensively in many Asian countries and is used to wrap the rice and fish that compose the Japanese food *sushi*, and the Korean food *gimbap*. It is also used to make the traditional Welsh delicacy, laverbread.

1.3 PRODUCTION

As in the case of their environmental conditions, the methods for culturing filamentous algae and seaweeds vary widely, according to species and location. This topic is not covered in this review but there are many publications available on algal culture generally, such as the FAO manual on the production of live food for aquaculture by Lavens and Sorgeloos (1996). Concerning seaweed culture, the following summary of the techniques used has been has been extracted from another FAO publication (McHugh, 2003) and further reading on seaweed culture can also be found in McHugh (2002).

> Some seaweeds can be cultivated vegetatively, others only by going through a separate reproductive cycle, involving alternation of generations.
>
> In vegetative cultivation, small pieces of seaweed are taken and placed in an environment that will sustain their growth. When they have grown to a suitable size they are harvested, either by removing the entire plant or by removing most of it but leaving a small piece that will grow again. When the whole plant is removed, small pieces are cut from it and used as seedstock for further cultivation. The suitable environment varies among species, but must meet requirements for salinity of the water, nutrients, water movement, water temperature and light. The seaweed can be held in this environment in several ways: pieces of seaweed may be tied to long ropes suspended in the water between wooden stakes, or tied to ropes on a floating wooden framework (a raft); sometimes netting is used instead of ropes; in some cases the seaweed is simply placed on the bottom of a pond and not fixed in any way; in more open waters, one kind of seaweed is either forced into the soft sediment on the sea bottom with a fork-like tool, or held in place on a sandy bottom by attaching it to sand-filled plastic tubes.
>
> Cultivation involving a reproductive cycle, with alternation of generations, is necessary for many seaweeds; for these, new plants cannot be grown by taking cuttings from mature ones. This is typical for many of the brown seaweeds, and *Laminaria* species are a good example; their life cycle involves alternation between a large sporophyte and a microscopic gametophyte-two generations with quite different forms. The sporophyte is what is harvested as seaweed, and to grow a new sporophyte it is necessary to go through a sexual phase involving the gametophytes. The mature sporophyte releases spores that germinate and grow into microscopic gametophytes. The gametophytes become fertile, release sperm and eggs that join to form embryonic sporophytes. These slowly develop into the large sporophytes that we harvest. The principal difficulties in this kind of cultivation lie in the management of the transitions from spore to gametophyte to embryonic sporophyte; these transitions are usually carried out in land-based facilities with careful control of water temperature, nutrients and light. The high costs involved in this can be absorbed if the seaweed is sold as food, but the cost is normally too high for production of raw material for alginate production.
>
> Where cultivation is used to produce seaweeds for the hydrocolloid industry (agar and carrageenan), the vegetative method is mostly used, while the principal seaweeds used as food must be taken through the alternation of generations for their cultivation.

1.4 CHEMICAL COMPOSITION

A summary of the chemical composition of various filamentous algae and seaweeds is presented in Table 1.1. Algae are receiving increasing attention as possible alternative protein sources for farmed fish, particularly in tropical developing countries, because of their high protein content (especially the filamentous blue-green algae).

The dry matter basis (DM) analyses reviewed in Table 1.1 show that the protein levels of filamentous blue green algae ranged from 60–74 percent. Those for filamentous green algae were much lower (16–32 percent). The protein contents of green and red seaweeds were quite variable, ranging from 6–26 percent and 3–29 percent respectively. The levels reported for *Eucheuma/ Kappaphycus* were very low, ranging from 3–10 percent, but the results for *Gracilaria*, with one exception, were much higher (16–20 percent). The one analysis for *Porphyra* indicated that it had a protein level (29 percent) comparable to filamentous green algae. Some information on the amino acid content of various aquatic macrophytes is contained in Annex 1.

The lipid levels reported for *Spirulina* (Table 1.1), with one exception (Olvera-Novoa *et al*. (1998), were between and 4 and 7 percent. Those for filamentous green algae varied more widely (2–7 percent). The lipid contents of both green (0.3–3.2 percent) and red seaweeds (0.1–1.8 percent) were generally much lower than those of filamentous algae. The ash content of filamentous blue-green algae ranged from 3–11 percent but those of filamentous green algae were generally much higher, ranging from just under 12 percent to one sample of *Cladophora* that had over 44 percent. The ash contents of green seaweeds ranged from 12–31 percent. Red seaweeds had an extremely wide range of ash contents (4 to nearly 47 percent) and generally had higher levels than the other algae shown in Table 1.1.

1.5 USE AS AQUAFEED

Several feeding trials have been carried out to evaluate algae as fish feed. Algae have been used fresh as a whole diet and dried algal meal has been used as a partial or complete replacement of fishmeal protein in pelleted diets.

1.5.1 Algae as major dietary ingredients

A summary of the results of selected growth studies on the use of fresh algae or dry algae meals as major dietary ingredients for various fish species and one marine shrimp is presented in Table 1.2. Dietary inclusion levels in these studies varied from 5 to 100 percent. Fishmeal-based dry pellets or moist diets were used as control diets.

The results of the earlier growth studies showed that the performances of fish fed diets containing 10–20 percent algae or seaweed meal were similar to those fed fishmeal based standard control diet. The responses were apparently similar for most of the fish species tested. These inclusion levels effectively supplied only about 3–5 percent protein of the control diet. In most cases, these control diets contained about 26–47 percent crude protein. This shows that only about 10–15 percent of dietary protein requirement can be met by algae without compromising growth and food utilization. There was a progressive decrease in fish performance when dietary incorporation of algal meal rose above 15–20 percent. However, although reduced growth responses were recorded with increasing inclusion of algae in the diet, the results of feeding trials with filamentous green algae for *O. niloticus* and *T. zillii* indicated that SGR of 60–80 percent of the control diet could be achieved with dietary inclusion levels as high as 50–70 percent.

Recent work by Kalla *et al*. (2008) appears to indicate that the addition of *Porphyra* spheroplasts to a semi-purified red seabream diet improved SGR. In addition, Valente *et al*. (2006) recorded improvements in SGR when dried *Gracilaria busra-pastonis* replaced 5 or 10 percent of a fish protein hydrolysate diet for European seabass.

TABLE 1.1
Chemical analyses of some common algae and seaweeds

Algae/ seaweed	Moisture (percent)	Proximate composition[1] (percent DM)					Minerals[1] (percent DM)		Reference
		CP	EE	Ash	CF	NFE	Ca	P	
Filamentous blue-green algae									
Spirulina maxima, spray dried powder	6.0	63.8	5.3	9.6	n.s.		n.s.	n.s.	Henson (1990)
Spirulina, commercial dry powder	3-6	60.0	5.0	7.0	7.0	21.0	n.s.	n.s.	Habib *et al.* (2008)
Spirulina spp., dry powder	n.s.	55-70	4-7	3-11	3-7		n.s.	n.s.	Habib *et al.* (2008)
Spirulina maxima, dry powder, Mexico	10.2	73.7	0.7	10.5	2.1	13.0	n.s.	n.s.	Olvera-Nova *et al.* (1998)
Filamentous green algae									
Spirogyra spp., fresh, USA	95.2	17.1	1.8	11.7	10.0[2]		n.s.	n.s.	Boyd (1968)
Cladophora glomerata, meal, Scotland	1.6	31.6	5.2	23.6	11.2	28.4	n.s.	n.s.	Appler and Jauncey (1983)
Cladophora sp., fresh, USA[3]	90.5	15.8	2.1	44.3	13.3	24.5[4]	n.s.	n.s.	Pine, Anderson and Hung (1989)
Hydrodictyon reticulatum, fresh, USA	96.1	22.8	7.1	11.9	18.1[2]		n.s.	n.s.	Boyd (1968)
Hydrodictyon reticulatum, meal, Belgium	5.7	27.7	1.9	32.6	14.9	22.9	n.s.	n.s.	Appler (1985)
Green seaweeds									
Ulva reticulata, fresh, Tanzania	n.s.	25.7	n.s.	18.3	38.5		n.s.	0.1	Msuya and Neori (2002)
Ulvaria oxysperma, dried, Brazil	16-20	6-10	0.5-3.2	17-31	3-12		n.s.	n.s.	Pádua, Fontoura and Mathias (2004)
Ulva lactuca, dried, Brazil	15-18	15-18	1.2-1.8	12-13	9-12		n.s.	n.s.	Pádua, Fontoura and Mathias (2004)
Ulva fascita, dried, Brazil	18-20	13-16	0.3-1.9	17-20	9-11		n.s.	n.s.	Pádua, Fontoura and Mathias (2004)
Red seaweeds									
Eucheuma cottonii, fresh, Indonesia	91.3	4.9	0.4	43.5	8.4	42.8[4]	0.5	0.2	Tacon *et al.* (1990)
Eucheuma cottonii, dry powder, Malaysia	10.6	9.8	1.1	46.2	5.9	37.0[4]	0.3	n.s.	Matanjun *et al.* (2009)
Eucheuma denticulatum, fresh, Tanzania	n.s.	7.6	n.s.	46.6	22.3		n.s.	<0.1	Msuya and Neori (2002)
Kappaphycus alvarezü, oven dried meal, Philippines	10.1	3.2	0.6	18.1	5.9	72.2[4]	n.s.	n.s.	Peñaflorida and Golez (1996)
Gracilaria heteroclada, oven dried meal, Philippines	9.3	17.3	1.8	21.7	4.6	54.6	n.s.	n.s.	Peñaflorida and Golez (1996)
Gracilaria lichenoides, fresh, Indonesia	88.1	15.6	1.2	36.7	6.6	39.9[4]	0.8	0.3	Tacon *et al.* (1990)
Gracilaria sp., sun-dried meal, Thailand	7.2	19.9	0.1	31.4	4.9	43.7	n.s.	n.s.	Briggs and Funge-Smith (1996)
Gracilaria crassa, fresh, Tanzania		13.2	n.s.	15.0	38.7		n.s.	<0.1	Msuya and Neori (2002)
Porphyra purpurea, meal, England	4.7	28.7	0.4	4.1	6.7	60.1[4]	n.s.	n.s.	Davies, Brown and Camilleri (1997)

[1] DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fibre; NFE = nitrogen free extract; Ca = calcium; P = phosphorus
[2] Cellulose
[3] Mean of proximate composition values of algae collected from flowing and static water
[4] Adjusted or calculated; not as cited in original publication

TABLE 1.2

Performance of various fish species fed fresh algae or dried algal meal

Algae/ fish species	Rearing system	Rearing days	Control diet	Composition of test diet	Inclusion level (percent)	Fish size (g)	SGR (percent)	SGR as percent of control	FCR	References
Filamentous green algae										
Cladophora glomerata/ Nile tilapia *(Oreochromis niloticus)*	Laboratory recirculatory system	56	Fish meal based pellet (30 percent protein)	5, 10, 15 and 20 percent protein of control feed replaced by algal meal and one diet prepared by algal meal as the only sources of protein (25 percent protein)	16.1	1.88-2.09	3.11	97.5	1.21	Appler and Jauncey (1983)
					32.3		2.80	87.8	1.42	
					48.4		2.77	86.8	1.51	
					64.5		2.06	64.6	2.09	
					82.5		1.85	58.0	2.33	
Hydrodictyon reticulatum/ Nile tilapia *(Oreochromis niloticus)*	Laboratory recirculatory system	50	Fish meal based pellet (30 percent protein)	5, 10, 15 and 20 percent protein of control feed replaced by algal meal and one diet prepared by algal meal as the only sources of protein (25 percent protein)	19.2	0.92-1.04	2.22	91.7	1.83	Appler (1985)
					38.3		1.85	76.4	2.18	
					57.5		1.48	61.2	2.49	
					70.6		1.52	62.8	2.63	
					98.5		1.07	44.2	3.60	
Hydrodictyon reticulatum/ Redbelly tilapia *(Tilapia zillii)*	Laboratory recirculatory system	50	Fishmeal based pellet (30 percent protein)	5, 10, 15 and 20 percent protein of control feed replaced by algal meal and one diet prepared by algal meal as the only sources of protein (25 percent protein)	19.2	0.91-1.16	2.04	107.9	2.09	Appler (1985)
					38.3		1.73	91.5		
					57.5		1.45	76.7		
					70.6		1.44	76.2		
					98.5		1.05	55.6		
Filamentous blue-green algae										
Spirulina/ Java tilapia *(Oreochromis mossambicus)*	Indoor static tank	25	Fishmeal based moist diet (26 percent protein)	11 percent fishmeal replaced by *Spirulina* meal	11.0	7.4-8.3	1.96	101.0	-	Chow and Woo (1996)
Seaweeds										
Porphyra purpurea/ thick-lipped grey mullet *(Chelon labrosus)*	Flow-through system	70	Fishmeal based pellet (47 percent protein)	4.5 and 9.0 percent protein of control feed replaced by seaweed meal	16.5	1.15	2.65	88.6	2.06	Davies, Brown and Camilleri (1997)
					33.0	1.15	2.47	82.6	2.28	

TABLE 1.2 (cont.)

Performance of various fish species fed fresh algae or dried algal meal

Algae/ fish species	Rearing system	Rearing days	Control diet	Composition of test diet	Inclusion level (percent)	Fish size (g)	SGR (percent)	SGR as percent of control	FCR	References
Porphyra sp./ Red seabream *(Pagrus major)*	Flow-through system	42	Fishmeal based semi-purified diet (51 percent protein)	5 percent *Porphyra* spheroplasts added to diet	5.0	15.4	3.47	111.6	1.52	Kalla *et al.* (2008)
Ulva rigida/ European seabass *(Dicentrarchus labrax)*	Recirculation system	70	Fish protein hydrolysate based diet (60.8 percent protein)	5 and 10 percent fish protein hydrolysate replaced by dried seaweed	5.0 10.0	4.7 4.7	2.63 2.54	89.8 86.7	1.68 1.80	Valente *et al.* (2006)
Gracilaria cornea/ European seabass *(Dicentrarchus labrax)*	Recirculation system	70	Fish protein hydrolysate based diet (60.8 percent protein)	5 and 10 percent fish protein hydrolysate replaced by dried seaweed	5.0 10.0	4.7 4.7	2.63 1.78	89.8 60.8	1.74 2.31	Valente *et al.* (2006)
Gracilaria busra-pastonis/ European seabass *(Dicentrarchus labrax)*	Recirculating system	70	Fish protein hydrolysate based diet (60.8 percent protein)	5 and 10 percent fish protein hydrolysate replaced by dried seaweed	5.0 10.0	4.7 4.7	2.98 3.37	101.7 115.0	1.56 1.48	Valente *et al.* (2006)
Gracilaria lichenoides/ rabbitfish *(Siganus canaliculatus)*	Floating net cages	100	Carp starter pellet (27 percent protein)	Fresh live seaweed was fed as sole diet	100.0	50.1	Negative growth displayed. SGR of control 0.63 percent			Tacon *et al.* (1990)
Eucheuma cottonii/ rabbitfish *(Siganus canaliculatus)*	Floating net cages	100	Carp starter pellet (27 percent protein)	Fresh live seaweed was fed as sole diet	100.0	48.8	Negative growth displayed. SGR of control 0.63 percent			Tacon *et al.* (1990)
Gracilaria sp./ Giant tiger prawns *(Penaeus monodon)*	Brackishwater recirculatory system	60	Soybean and fish meal based diet (35 percent protein)	1, 2, 3 and 6 percent protein of control feed replaced by seaweed meal. Seaweed meal incorporated by replacing soybean meal and wheat flour	5.0 10.0 15.0 30.0	0.024	7.88 8.03 7.88 7.33	98.3 100.1 98.3 91.4	3.33 3.35 3.50 4.14	Briggs and Funge-Smith (1996)

TABLE 1.3

Results of investigations on the use of algae as additives in fish feed

Algae[1]	Inclusion level (percent)	Fish species	Effect	References
Blue-green algae				
Spirulina	2.0	Red sea bream	Improved carcass quality through modification of muscle lipids	Mustafa *et al.* (1994a)
	2.0	Red sea bream	Improved muscle quality; increased firmness and robustness of raw meat; and improved growth and protein synthetic activity	Mustafa, Umino and Nakagawa (1994)
	5.0	Red sea bream	Elevated growth rates; improved feed conversion, protein efficiency and muscle protein deposition	Mustafa *et al.* (1994b)
	5.0	Nibbler	Improved growth	Nakazoe *et al.* (1986)
	…	Striped jack	Improved flesh texture and taste	Liao *et al.* (1990)
	2.5	Cherry salmon	Elevated growth rates, bright skin colour and fin appearance; improved flavour and firm flesh	Hensen (1990)
	0.5	Yellowtail	Increased survivability and improved weight gain	Hensen (1990)
Spirulina maxima	20.9 [40.0 replacement of fish meal]	Mozambique tilapia	Final body weight, daily weight gain, SGR, feed intake, PER and apparent nitrogen utilization showed no significant differences with control diet	Olvera-Novoa *et al.* (1998)
Brown algae				
Ascophyllum nodosum	5.0 & 10.0	Red sea bream	Improved growth and feed efficiency at 5 percent inclusion level	Yone, Furuichi and Urano (1986a)
	5.0	Red sea bream	Delayed absorption of dietary carbohydrate and protein. The dietary nutrients are utilized effectively by this delaying effect of the seaweed; thus the growth and feed efficiency of red sea bream are improved	Yone, Furuichi and Urano (1986b)
	5.0	Red sea bream	Elevated growth rates; improved feed conversion, protein efficiency and muscle protein deposition	Mustafa *et al.* (1994b)
	5.0	Red sea bream	Increased growth, feed efficiency and protein deposition. Elevated liver glycogen and triglyceride accumulation in muscle	Mustafa *et al.* (1995)
	0.5	Yellowtail	Prevented a nutritional disease that causes retardation of growth and high mortality	Nakagawa *et al.* (1986)
Undaria pinnatifida	5.0	Rockfish	Showed prominent physiological effects on haematocrit value and red blood cell number	Yi and Chang (1994)
	5.0 & 10.0	Red sea bream	Improved growth and feed efficiency, and higher muscle lipid deposition at 5 percent level of inclusion	Yone, Furuichi and Urano (1986a)

TABLE 1.3 (cont.)

Results of investigations on the use of algae as additives in fish feed

Algae[1]	Inclusion level (percent)	Fish species	Effect	References
Red algae				
Porphyra yezoensis	5.0	Red sea bream	Increased growth, feed efficiency and protein deposition. Elevated liver glycogen and triglyceride accumulation in muscle	Mustafa *et al*. (1995)
Porphyra yezoensis	2.0	Yellowtail	Improved flesh quality	Morioka *et al*. (2008)
Porphyra spheroplasts	5.0	Red sea bream	Survival, growth and nutrient retention significantly higher than control	Kalla *et al*. (2008)
Green algae				
Ulva conglobata	5.0	Nibbler	Improved growth	Nakazoe *et al*. (1986)
Ulva pertusa	2.5, 5.0, 10.0 & 15.0	Black sea bream	Ulva meal diets repressed lipid accumulation in intraperitoneal body fat without loss of growth and feed efficiency. Fish fed 2.5, 5 and 10 percent Ulva meal did not show significant body weight loss during wintering. During starvation, lipid reserves were preferentially mobilized for energy	Nakagawa *et al*. (1993)
Ulva pertusa extract	10.0	Black sea bream	Improved tolerance to hypoxia	Nakagawa *et al*. (1984)
Ulva pertusa	5.0	Red sea bream	Activated lipid mobilization and suppressed protein breakdown observed during starvation for fish fed *Ulva* meal supplemented diet before starvation. Preferential use of glycogen observed	Nakagawa and Kasahara (1986)
	5.0	Red sea bream	Demonstrated a decrease in susceptibility to *Pasteurella piscicida,* an elevation of phagocytosis and spontaneous haemolytic and bactericidal activity	Satoh, Nakagawa and Kasahara (1987)
	5.0	Red sea bream	Increased growth, feed efficiency and protein deposition. Elevated liver glycogen and triglyceride accumulation in muscle	Mustafa *et al*. (1995)

[1] Algae were added as dried meal in all diets except otherwise stated

However, the conclusions of the latter authors are confused by the fact that the test diets were not iso-nitrogenous with the control diet; in fact test diets had a lower protein level.

Total replacement of fishmeal by algal meal showed very poor growth responses for *O. niloticus* (Appler and Jauncey, 1983; Appler, 1985) and *T. zillii* (Appler, 1985). Appler and Jauncey (1983) recorded a SGR of 58 percent of control diet when the filamentous green alga (*Cladophora glomerata*) meal was used as the sole source of protein for Nile tilapia. Similarly, Appler (1985) recorded SGRs of 44 percent and 56 percent of control diets when the filamentous green alga (*Hydrodictyon reticulatum*) meal was used as the sole source of protein for *O. niloticus* and *T. zillii*.

Tacon *et al.* (1990) used fresh live seaweeds (*Gracilaria lichenoides* and *Eucheuma cottonii*) as the total diet for rabbitfish in net cages. In both cases negative growth was displayed, although the daily feed intake was more than the control diet. On a dry matter basis, the daily feed intake was 1.99 and 1.98 g/fish/day respectively for *E. cottonii* and *G. lichenoides*, while the feed intake for carp pellets (control diet) was 1.80 g/fish/day. Apparently, a good feeding response was observed for both the seaweeds but very poor feed efficiency was displayed. Apart from commonly observed impaired growth, the use of algae as the sole source of protein in fish feed can also result in malformation (Meske and Pfeffer, 1978).

The apparently poor performance of fish fed diets containing higher inclusion levels of algae may be attributable to several factors. Appler (1985) observed that most of the aquatic plants including algae contain 40 percent or more of carbohydrate, of which only a small fraction consists of mono- and di-saccharides. Low digestibility of plant materials has been attributed to a preponderance of complex and structural carbohydrates. The poor digestibility and the subsequent poor levels of utilization obtained for both tilapia species with increased dietary algal levels may thus be attributable in part to the presence of indigestible algal materials. Pantastico, Baldia and Reyes (1985) reported that newly hatched Nile tilapia fry (mean weight 0.7 mg) did not survive at all when unialgal cultures of *Euglena elongata* and *Chlorella ellipsoidea* were fed to them. These authors concluded that the mortality of tilapia fry might be due to factors such as toxicity and cell-wall composition of the algae fed. This phenomenon might also be attributed to poor digestion of plant material by the less developed digestive system of newly hatched larva. In contrast, Chow and Woo (1990) recorded significantly higher gut cellulase activity in *O. mossambicus* fed *Spirulina*, indicating the ability of this tilapia species to digest cellulose, the main constituent of plant cell walls. Ayyappan *et al*. (1991) conducted a *Spirulina* feeding experiment with carp species. The fry stage of catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), silver carp (*Hypophthalmicthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) were fed with an experimental diet in which 10 percent dried *Spirulina* powder was added to a 45:45 mixture of rice bran and groundnut oil cake. A 50:50 bran-groundnut oil cake control diet was used. The mean specific growth rates of fish fed on the two diets were: catla 0.17, 0.27; rohu 0.19, 0.63; mrigal 0.54, 0.73; grass carp 0.02, 0.40; and common carp 0.15, 0.20; with significant differences between the treatments ($F_{1,4} = 8.88$; $P < 0.05$) and fish species ($F_{4,4} = 5.03$; $P < 0.10$). Rohu and mrigal showed significantly ($P < 0.05$) higher SGRs than catla and common carp. These results clearly demonstrated the beneficial effect of the *Spirulina* diet on the yield and quality of carp fry.

Dietary supplementation of *Chlorella ellipsoidea* powder at 2 percent on a dry-weight basis showed higher weight gain and improved feed efficiency and protein efficiency ratios in juvenile Japanese flounders (*Paralichthys olivaceus*); the addition of *Chlorella* had positive effects as it significantly reduced serum cholesterol and body fat levels and also led to improved lipid metabolism (Kim *et al*., 2002).

Clearly, no definite conclusions can be arrived at this stage about the value of using macroalgae as major dietary ingredients or protein sources in aquafeeds. Moderate growth responses and good food utilization (FCR 1.5–2.0) were generally recorded when dried algal meal were used as a partial replacement of fishmeal protein. However, the collection, drying and pelletization of algae require considerable time and effort. Furthermore, cultivation costs would have to be taken into consideration. Therefore, further cost-benefit on-farm trials that take these costs into consideration are needed before any definite conclusions on the future application of algae as fish feed can be drawn.

1.5.2 Algae as feed additives

The main applications of microalgae for aquaculture are associated with nutrition, being used fresh (as sole component or as food additive to basic nutrients) for colouring the flesh of salmonids and for inducing other biological activities (Muller-Feuga, 2004). Several investigations have been carried out on the use of algae as additives in fish feed. Feeding trials were carried out with many fish species, most commonly red sea bream (*Pagrus major*), ayu (*Plecoglossus altivelis*), nibbler (*Girella punctata*), striped jack (*Pseudoceranx dentex*), cherry salmon (*Oncorhynchus masou*), yellowtail (*Seriola quinqueradiata*), black sea bream (*Acanthopagrus schlegeli*), rainbow trout (*Oncorhynchus mykiss*), rockfish (*Sebastes schlegeli*) and Japanese flounder (*Paralichthys olivaceus*). Various types of algae were used; the most extensively studied ones have been the blue-green algae *Spirulina* and *Chlorella*; the brown algae *Ascophyllum*, *Laminaria* and *Undaria*; the red alga *Porphyra*; and the green alga *Ulva*. Fagbenro (1990) predicted that the incidence of cellulase activity could be responsible for the capacity of the catfish *Clarias isherencies* to digest large quantities of Cyanophyceae.

A summary of the results of selected feeding trials with algae as feed additives is presented in Table 1.3. Most of these research studies were conducted in Japan with Japanese fish species, although the results may well be applicable to other species and in other countries.

Table 1.3 shows that dried algal meals or their extracts have been added to test fish diets at levels up to 21 percent level. The responses of test fish fed algae supplemented diets were compared with fish fed standard control diets. Although various types of algae and fish species were used in these evaluations, not all algae were evaluated as feed additives for every different species. As the main biochemical constituents and digestibility are different among algae, the effect of dietary algae varies with the algae and fish species (Mustafa and Nakagawa, 1995). While studying the effect of two seaweeds (*Undaria pinnatifida* and *Ascophyllum nodosum*) at different supplementation levels for red sea bream, Yone, Furuichi and Urano (1986a) observed best growth and feed efficiency from a diet containing 5 percent *U. pinnatifida* followed by a diet containing 5 percent *A. nodosum*. Similarly, Mustafa *et al.* (1994b) observed more pronounced effects on growth and feed utilization of red sea bream by feeding a diet containing *Spirulina* compared to one containing *Ascophyllum*. In another study, Mustafa *et al.* (1995) studied the comparative efficacy of three different algae (*Ascophyllum nodosum*, *Porphyra yezoensis* and *Ulva pertusa*) for red sea bream and noted that feeding *Porphyra* showed the most pronounced effects on growth and energy accumulation, followed by *Ascophyllum* and *Ulva.* However, research results obtained so far do not specifically identify any specific algae as the most suitable as feed additives for any particular fish species.

Nevertheless, the results of various research studies show that algae as dietary additives contribute to an increase in growth and feed utilization of cultured fish due to efficacious assimilation of dietary protein, improvement in physiological activity, stress response, starvation tolerance, disease resistance and carcass quality. In fish fed algae-supplemented diets, accumulation of lipid reserves was generally well controlled and the reserved lipids were mobilized to energy prior to muscle protein degradation in response to energy requirements. In complete pelleted diets, algal supplementation of 5 percent or less was found to be adequate.

Spirulina are widely used as feed additives in the Japanese fish farming industry. Henson (1990) reported that *Spirulina* improved the performances of ayu, cherry salmon, sea bream, mackerel, yellowtail and koi carp. The levels of supplementation used by Japanese farmers are 0.5-2.5 percent. Henson (1990) further reported that Japanese fish farmers used about US$2.5 million worth of *Spirulina* in 1989. Five important benefits reported by using a feed containing this alga were improved growth rates; improved carcass quality and colouration; higher survival rates; reduced requirement for medication; and reduced wastes in effluents. However, the high cost of most of these algae may limit their use to the commercial production of high value fish only.