North American Flounders

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Introduction

Flounder culture in North America is in its infancy. Meaningful research on native flounders only began during the early 1990s. This research has focused on three flat-fish with potential for aquaculture in North America: summer flounder (*Paralichthys dentatus*), southern flounder (*Paralichthys lethostigma*) and winter flounder (*Pseudopleuronectes americanus*) (Daniels *et al.*, 1996; Rivara and Bengtson, 1999; Howell and Litvak, 2000). These fish are found primarily on the Atlantic coast of the USA and have different primary natural ranges that overlap slightly at their extremes (Fig. 9.1). Some fish of each species can be found beyond these ranges but they are found in greatest abundance within the primary natural ranges. Winter flounder are found north of Cape Cod to Labrador, Canada (Buckley, 1989). Summer flounder are found from Cape Cod to Cape Hatteras (Rogers and van den Avyle, 1983). Southern flounder are found from Cape Hatteras along the coast to Florida and along the northern coastline of the Gulf of Mexico (Reagan and Wingo, 1985). Winter flounder are right-eyed flounder belonging to the family Pleuronectidae. Summer and southern flounder are left-eyed flounder belonging to the family Bothidae. In the wild, winter flounder rarely exceed 2.25 kg, while summer and southern flounders grow to a size of about 12–15 kg.

The different natural ranges and the corresponding differences in environmental conditions will probably have a profound effect on the environmental requirements for the culture of these flounders. However, because these fish have only recently been investigated for their aquaculture potential, culture techniques specific to each fish have not yet been established. Instead, much of the technology used during the hatchery and grow-out phases for one fish is also used, with few modifications, for the other two flat-fish. But culture conditions such as light intensity, water depth and stocking density appear to have a

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Fig. 9.1. Primary natural ranges for three North American flounders.

greater effect on the success of flounder culture than for other marine finfish. For example, researchers have commonly observed that flounder stocked at low densities often do not feed actively and may lose weight or eventually die. Therefore, stocking density is a fundamental variable with greater potential importance for the success of a grow-out operation than the nutritional value of the feed. Because culture conditions have such an effect on feeding, and feeding is an integral part of nutrition, a general description of common culture practices is provided to familiarize the reader with flounder culture. As specific culture techniques are developed for each of the North American flounders, future modifications of nutritional requirements may be necessary to accommodate changes in feeding behaviour and improved fish growth brought about by better culture conditions.

Larviculture techniques for flounder are similar to those used for other marine finfish. North American flounders spawn small eggs (0.7–1.1 mm in diameter) during the late autumn to early spring. As with most marine fish, yolk-sac larvae have small mouth openings and require live feed at first feeding. Therefore, appropriately sized live feeds must be cultured and maintained in a flounder hatchery. Hatchery managers prefer rotifers (*Brachionus plicatilis*) as first food for larvae because they are easy to culture, swim slowly and are readily eaten by larval fish. Although nutritionally very poor, rotifers can easily be enriched on emulsified oils (Rainuzzo *et al.*, 1994). Based on the size requirements, rotifers can be filtered to select for the appropriate size for the fish. Southern and summer flounder are capable of consuming rotifers within a size range of 50–150 μm at first feeding. Winter flounder eat slightly smaller-sized rotifers because of their smaller mouths. Larval flounder consume rotifers for the first 2–3 weeks and then begin consuming *Artemia* nauplii. Postmetamorphic flounder are weaned on to dry feeds at around 50–60 days posthatch and remain on these extruded diets throughout the grow-out cycle. Most foodfish are cultured in shallow, indoor or covered tanks with either recirculating or flow-through water maintained at an exchange rate of 100–200% h^{-1}.

Nutrient Requirements

Protein and amino acids

Although the nutritional requirements of other flat-fish, such as Japanese flounder (*Paralichthys olivaceus*) have been extensively investigated, very little is known about the nutritional requirements of North American flat-fish (Bengtson, 1991; Howell and Litvak, 2000). A few studies have been done on southern and summer flounder (Bengtson, 1991; Daniels and Gallagher, 2000) but there is little information available on the nutritional requirements of winter flounder (Hebb *et al.*, 1997).

One of the unique aspects of the flounder nutrition work described here is that several studies have sought to link physiological and biochemical assessments to nutrition in addition to the more traditional measurements of growth and survival. For example, Bisbal and Bengtson (1991) used biochemical methods to assess the overall nutritional status of summer flounder. They reported that under normal conditions the ribonucleic : deoxyribonucleic acid (RNA : DNA) ratio rose as the larval fish advanced from 16 days posthatch to 60 days. But, when the animals were starved, the RNA : DNA ratio declined markedly from the basal level during the starvation period.

Generally, fish have higher protein requirements on a gram protein kJ^{-1} (or $kcal^{-1}$) basis compared with land animals. This phenomenon is due in part to the fact that fish do not have to maintain a constant body temperature and they exert less energy to maintain position and move in water (Lovell, 1989). This effect is apparently exacerbated in flat-fish because of their sedentary nature.

Although studies investigating protein in diets for various species of juvenile flat-fish are variable and often contradictory, it is clear that protein requirements are higher for flat-fish than for other finfish. Guillaume *et al.* (1991) reported that the protein requirement of turbot (*Scophthalmus maximus*), sole (*Solea solea*) and plaice (*Pleuronectes platessa*) was between 57 and 60% of the diet. Aksnes *et al.* (1996) predicted a 74% protein requirement for halibut (*Hippoglossus hippoglossus*). Danielssen and Hjertnes (1991) found that turbot from the same egg batch grew best at protein levels in excess of 45%. However, significant growth differences ($P < 0.001$) of comparable magnitude were noted between egg batches from different parents when the dietary protein was the same, indicating that parentage is at least as important as dietary protein.

Recent work by Daniels and Gallagher (2000) indicated that growth of summer flounder was significantly better with 56% protein in the diet compared with 50, 45, 40 and 35% protein. In addition, red blood-cell counts were significantly higher in fish fed 52% or 56% protein, and haematocrits were highly correlated ($P < 0.01$) with dietary protein. Juvenile southern flounder fed isocaloric diets showed significantly higher growth and lower feed conversion ratios ($P < 0.05$) when fed diets containing at least 48%. These fish grew 65–125% larger than those fed less than 48% protein. Based on these results, a dietary protein level of 48% appears to be sufficient for optimum growth of juvenile southern flounder. Winter flounder also appear to have a minimum protein requirement of 40–45% for optimum growth of juvenile fish (Hebb *et al.*, 1997). In this study, highest specific growth rate, feed conversion and protein efficiency were obtained with a diet containing 45% protein and 10% lipid. Therefore, North American flounders, as a group, have a demonstrated requirement for high levels of dietary protein.

As with many fish, the major source of protein for studies with flat-fish has been fish-meal. Although there are currently no reports on the replacement of fish-meal in diets for southern or summer flounder, Kikuchi and co-workers have demonstrated that Japanese flounder, a congener of the southern and summer flounder, can effectively use soybean meal (Kikuchi, 1999a,b; Kikuchi *et al.*, 1994a), feather meal (Kikuchi *et al.*, 1994b) and meat and bone-meal (Kikuchi *et al.*, 1997) in diets; however, none of these sources can totally replace fish-meal (Kikuchi *et al.*, 1993).

Lipids and fatty acids

Lipid requirements of larval flat-fish have received considerable attention, because of the roles that essential fatty acids play in both early survival and development (Kanazawa, 1997; Venizelos and Benetti, 1999). Poor nutrition during the rotifer-feeding stage has also been linked to incomplete or abnormal pigmentation of juvenile fish. Specifically, insufficient intake of docosahexaenoic acid (DHA), 22:6n-3, during the early larval stages is a major cause of pigmentation problems. Minimum requirements for DHA have not been established for North American flounders. Hatchery managers typically use commercially available enrichment products with a high DHA content to ensure adequate nutrition. Abnormal pigmentation does not appear to affect flesh quality but, instead, is a marketing problem because of the unusual appearance of the whole fish. Because the market value of fillets is considerably lower than that of whole fresh fish, abnormally pigmented flounder represent a loss of potential income for the producer. Optimum larval nutrition reduces abnormal pigmentation to below 10–20% of the population. The residual amount of abnormal pigmentation observed, even with high DHA levels, has led some researchers to conclude that other factors, such as genetics, also contribute to abnormal pigmentation (Kanazawa, 1997). Anecdotal evidence of abnormally pigmented flounder caught from the wild suggests that abnormal pigmentation occurs with some frequency in natural populations. It is likely that these fish are more susceptible to predation because of their inability to adequately camouflage themselves in the sand or mud.

Bisbal and Bengtson (1991) found that fatty acid requirements for larval summer flounder during metamorphosis could be met by brine shrimp (*Artemia*) enriched with eicosapentaenoic acid (EPA), 20:5n-3, and DHA or with EPA alone. Larvae that did not receive either EPA and DHA or EPA alone had a high incidence of albinism and incomplete eye migration. Additionally, Rainuzzo *et al.* (1997) demonstrated that the type of algae on which rotifers were raised could influence the number of flounder larvae completing metamorphosis, but that enrichment with highly unsaturated fatty acids (HUFA) could overcome this problem. These results are similar to those reported for winter flounder fed commercially available diets containing different levels of DHA (Blair *et al.*, 1998).

Alves *et al.* (1999) demonstrated that the addition of algae (*Tetraselmis suecica* and *Isochrysis galbana*) increased survival of summer-flounder larvae. The authors suggested that improved survival was not due to the direct nutritional value of the algae to the larvae, but rather to a number of possible indirect factors, such as maintenance of the nutritional value of rotifers to the larvae, increased feeding due to turbidity or reduction in bacterial load. Devresse *et al.* (1994) reported improved pigmentation in Japanese flounder (*P. olivaceus*) and European turbot (*S. maximus*) larvae fed rotifers and *Artemia* enriched with DHA. These authors reported that the DHA : EPA ratio seemed to be the most important factor in proper pigmentation. McEvoy *et al.* (1998) found that live foods enriched with EPA enhanced normal pigmentation in turbot and halibut (*H. hippoglossus*) but that enrichment with arachidonic acid, 20:4n-6, led to malpigmentation.

Recent work in our laboratory using enriched rotifers and *Artemia* demonstrated that southern-flounder larvae require n-3 HUFA for good growth and survival. Larvae fed rotifers lacking n-3 HUFA at first feeding did not survive to 26 days posthatch and did not develop past the 'B' stage (van Maaren and Daniels, 2000). In addition, EPA appeared to accumulate in the tissues. The addition of n-6 fatty acids from maize oil did not improve growth, nor did the addition of arachidonic acid. However, the role of arachidonic acid in these fish was unclear since unenriched rotifers contained significant amounts of the fatty acid. HUFA are also required for juvenile flounder. Whalen *et al.* (1999) reported that yellowtail flounder (*Pleuronectes ferrugineus*) required approximately 2.5% HUFA in the diet. Deficient diets produced fish with fatty livers and inferior growth.

Practical Diets and Feeding Practices

At present, practical diet formulations for flounder should contain 45–50% protein, 10–15% lipid and less than 10% ash, with a caloric density of no greater than 3.0 kcal g^{-1}, which is somewhat less in this sedentary flat-fish compared with other species (Table 9.1). Information on brood-stock diets does not exist. Most producers and researchers feed live mullet or frozen fish, shrimp and squid to brood-stock and supplement these feeds with pelleted food when possible. Daily feeding rates should be adjusted when using semimoist food or raw fish (Table 9.2). Flounder in a grow-out system are fed several times per day, but brood-stock are usually fed only once daily or on alternate days.

Although Kikuchi and co-workers (Kikuchi *et al.*, 1994a,b, 1997; Kikuchi, 1999a,b) have demonstrated the suitability of replacing fish-meal with various alternative sources of protein (i.e. defatted soybean meal, maize-gluten meal, meat and bone-meal) for Japanese flounder, the ability of these ingredients to meet the needs of southern, summer and winter flounder has not been investigated. Therefore, it is prudent to feed fish-meal-based feeds at this time.

Practical methods for weaning hatchery-reared flounder from a diet of live feed (*Artemia*) on to dry formulated diets have not been well established (Daniels and Hodson, 1999). The weaning success of flat-fish is unpredictable.

Table 9.1. General feed formulation for North American flounder.

Ingredient	% of dry weight
Fish-meal	50–60
Ground winter wheat	20–30
Menhaden oil	5–6
Vitamin mix	1.5
Mineral mix	1.5
Ascorbate phosphate	0.5
Choline chloride	0.2
Proximate composition	
Protein	45–50
Lipid	10–15
Ash	10

Table 9.2. Feeding rates for various-sized flounder fed different feeds.

Fish size		Daily feeding rate (%)*		
Length (cm)	Weight (g)	Raw fish	Moist pellet	Dry pellet
4–5	1–2	60	24	15
10–15	8–32	20	8	5
20–25	90–180	10–15	4–6	2–4
> 25	> 150	3–5	1–4	1

* Weight of feed/weight of fish.

Survival rates during weaning are highly variable and can range from 45% to 90% between batches (Lee and Litvak, 1996a,b). Several factors appear to affect weaning success: fish age, feeding behaviour, duration of the change-over period when both live food and formulated diets are fed and the properties of the formulated diets, such as colour, texture and buoyancy. As fish grow and consume more food, the cost of producing brine shrimp becomes prohibitive. To reduce production costs, fish should be weaned as early as possible.

Artemia are a high-cost food for larval flounder but are necessary for good growth and development. Techniques for weaning premetamorphic fish to dry feeds have met with limited success. As a result, most weaning programmes are started when fish have completed metamorphosis and have reached an age of 50–55 days posthatch. Many commercial weaning programmes employ a gradual reduction of live food until dry diets are the sole feed. This gradual weaning is usually completed in 2–3 weeks. Despite the prolonged weaning period, the mortality of fish that refuse dry feed or from cannibalism between cohorts can reach 20–30%. Frequent grading of fish is often employed to minimize mortality from cannibalism (Ward and Bengtson, 1999), but this practice is stressful to fish and can lead to some mortality from excessive handling.

Once fish are weaned on to dry diets, stocking densities must be maintained at a sufficient level to encourage active feeding (Table 9.3). Young fish should be fed to satiation up to six times per day, while fish larger than 10 g can be fed four times per day. Flounder readily accept dry diets so semimoist feeds are not needed for grow-out. Contrary to popular belief, flounder will come to the surface to feed on floating (extruded) pellets. The use of floating feed offers several advantages to the culturist. Feeding activity can be more easily observed than with sinking feeds and uneaten pellets are quickly removed from the tank either with a net or in the overflow of the tank water. Flounder generally only eat sinking feeds as they are in the process of falling to the bottom. Pellets that reach the tank bottom are normally not consumed and may get trapped under the fish, making it more difficult to remove uneaten feed from the tank. Therefore, flounder diets for

Table 9.3. Example of stocking densities used to maintain active feeding and optimum growth of three North American flounders.*

	Stocking densities	
Body weight (g)	ind. m^{-2}	kg m^{2}
1.5	1600	2.4
10	400	4
50	200	10
100	150	15
250	100	25
500	60	30

* Summer flounder (*Paralichthys dentatus*); southern flounder (*Paralichthys lethostigma*); winter flounder (*Pseudopleuronectes americanus*).

grow-out should be extruded so that they float on the water surface or sink very slowly to the tank bottom.

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