Section 1

North and South America Culture

Chapter 1

Halibut aquaculture in North America

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1.1 Life history and biology

The Atlantic halibut is a large pleuronectid flatfish distinguishable from other right-eyed flatfishes by its large mouth, which opens as far back as the anterior half of its lower eye, its concave caudal fin, and the distinctive arched lateral line. Dorsally, the adult fish is more or less uniformly chocolate brown or olive and the blind side is usually white, though in some cases, it may be partially brown (Collette and Klein-Macphee 2002). This species is among the commercially important groundfish of the Gulf of Maine where it has been harvested since the early part of the nineteenth century. The fishery was quickly depleted and has not been of economic importance since the 1940s. Annual catches after 1953 have been less than 100 metric tons on an average. The Atlantic halibut is one of the largest fish in the region. The largest individual caught on record was 280 kg (head on gutted) and was estimated to weigh 318 kg (live weight).

In the western North Atlantic, older juvenile and adult halibut undergo extensive migrations between feeding grounds and spawning areas (McCracken 1958; Cargnelli *et al*. 1999; Kanwit 2007). Coastal shelf areas of Browns Bank and the southwestern Scotian Shelf are thought to be important nursery grounds (Stobo *et al*. 1988; Neilson *et al*. 1993). Atlantic halibut are known to spawn at great depths where temperatures are generally stable and are between 5 and 7°C (Haug 1990; Neilson *et al*. 1993). The Atlantic halibut is a batch spawner, producing several batches of eggs during the spawning season in relatively regular intervals of 3–4 days (Smith 1987; Haug 1990; Holmefjord and Lein 1990; Norberg *et al*. 1991). The clear eggs are quite large for a marine fish (3 mm in diameter) and are bathypelagic during development, floating close to the ocean floor, and are neutrally buoyant at relatively high salinity of around 36 ppt.

After hatching, the larva hangs in a head down position exhibiting very little swimming activity (Pittman *et al*. 1990a). Halibut larvae hatch in a very primitive developmental state and organogenesis proceeds at a slow pace (Lonning *et al*. 1982; Blaxter *et al*. 1983; Pittman *et al*. 1990a). At around 150°C days, the

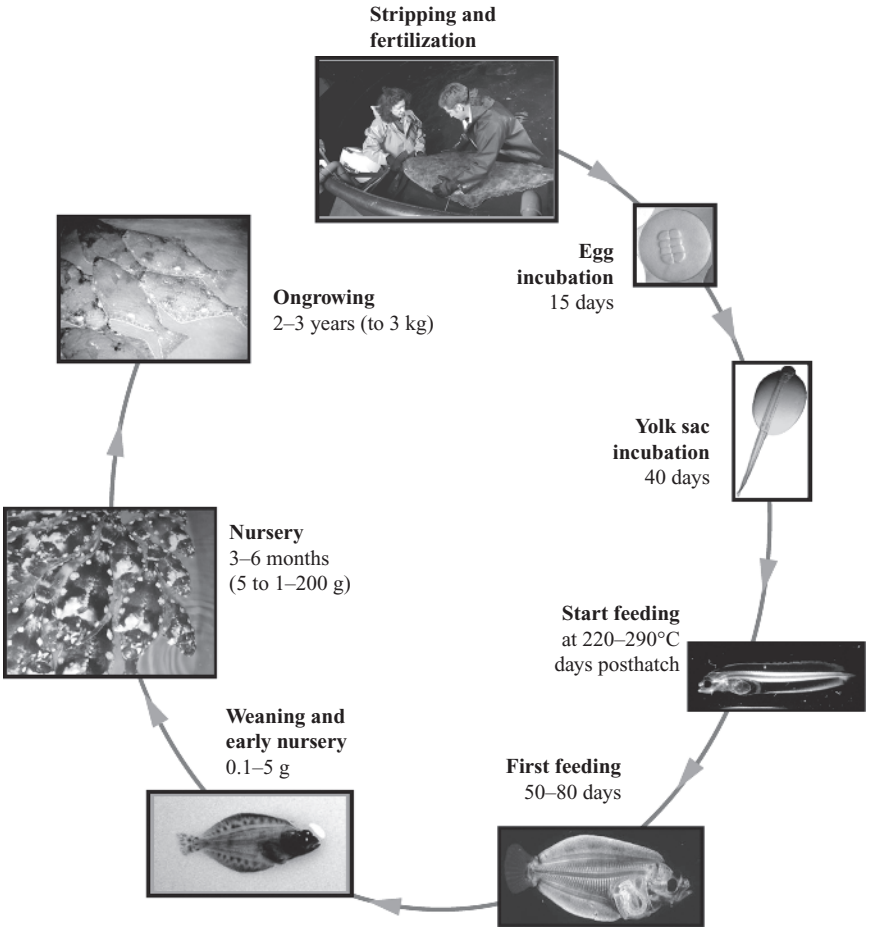

Figure 1.1 Production cycle of the Atlantic halibut.

eyes, mouth, and intestine become functional and the eye takes on pigmentation (Blaxter *et al.* 1983; Pittman *et al.* 1990b; Kvenseth *et al.* 1996).

Exogenous feeding can begin from around 240°C days and metamorphosis occurs around 80 days posthatch. At this point, the stomach is formed, the left eye migrates to the right side of the head, and the fish becomes fully pigmented. For aquaculture purposes, this represents the end of the hatchery phase and coincides with the establishment onto formulated feeds that will continue until harvest.

Capture of early life stages in the wild is very rare, little is known about their distribution and for researchers attempting to close the life cycle (Figure 1.1) in the hatchery, there has been a lot of trial and error.

Apart from the earliest trials (e.g., Rollefsen 1934), research into the techniques for the culture of halibut began in the 1980s and a few juveniles were reared past metamorphosis in the first attempts (Blaxter *et al.* 1983).

The Atlantic halibut has a number of attributes that make it an excellent candidate for aquaculture. These characteristics include firm, white, mild tasting flesh with a good shelf life, a high fillet yield, efficient feed conversion rates, and

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resistance to many common marine diseases. However, challenges with juvenile production and diversion of research resources and investment capital to other marine fish species, such as cod, have resulted in slow growth of this industry.

1.2 Broodstock

1.2.1 Acquisition of broodstock

Captive broodstock populations were first set up in Scotland and Norway in the early 1980s (Blaxter *et al.* 1983; Rabben *et al.* 1986; Smith 1987). Mature wild fish are caught using longlines or "tub trawls." A size 14/0 or larger circle hook is recommended to reduce injuries to the fish (Kanwit 2007). Fish for the University of Maine program, based at the Center for Cooperative Aquaculture Research (CCAR), were caught between 2000 and 2002. These 112 fish ranging in size from 9 to 40 kg were brought into the fishing ports of Jonesport, Stonington, and Steuben by fishermen participating in an experimental tagging program run by the Maine Department of Marine Resources (DMR) (Kanwit 2007). The fish were transferred from holding tanks on the boats to live transport tanks supplied with oxygen and driven by truck overland to the facility. Additional fish from research hatcheries in Canada were recruited to this founding population to result in a total population of 120 mature fish. An additional 150 fish reared at the CCAR hatchery were selected from the 2006 production run for broodstock. Additional wild fish from a DMR tagging study were also added in 2007. All mature hatchery reared (F1) fish have been genotyped using microsatellite markers developed in Canada (Jackson *et al.* 2003) to establish pedigree for future breeding programs.

Halibut may take up to 3 years to acclimate sufficiently to spawn in captivity following capture. Weaning onto a nonliving food item can be improved by using live fish such as mackerel as an intermediate step in the tanks. The use of large tanks, low light levels, good water quality, and temperature regimes that follow the natural environment of the halibut will all help to ensure successful acclimation.

1.3 Biosecurity

Fish recruited to a broodstock population are very valuable animals once weaned onto feed and acclimated to spawn in captivity. They are hard to replace and can give viable gametes for many years. It is therefore essential to use good biosecurity practices to help prevent the introduction of pathogens into a facility holding these fish. Quarantine of new fish from the wild should be done in a separate facility, for up to 6 months, preferably with a higher level of biosecurity in place. For example, there should be thorough disinfection of effluent water from such a facility through appropriate levels of ozonation, ultraviolet sterilization (or both), water pasteurization, or chlorination. Movement of personnel, equipment, water quality test samples, and handheld meters should

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be restricted. Mortalities occurring during quarantine should be quickly tested for pathogens and should be handled separately from other stocks or facilities. A quarantine system at the CCAR was used recently to receive wild halibut that were the subject of a tagging study run by the Department of Marine Resources. The system comprises four tanks that are 4 m in diameter and 2 m in depth. The 5% daily makeup water that leaves this facility is disinfected with a high level of ozone and then ultraviolet sterilization.

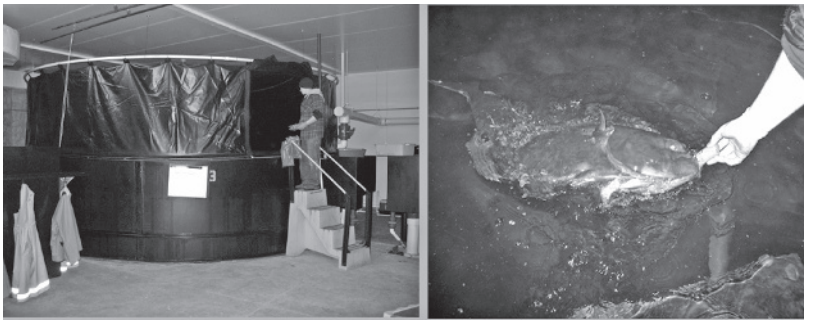
Established broodstock fish should be kept in a separate, designated facility. Water supplies should be filtered and treated with ozone or a UV sterilizer. Feed given to broodstock fish ideally should be in a dry form; although for halibut, the lack of knowledge of the nutritional requirements and suitable replacements for raw or frozen ingredients is an ongoing problem. Effective hygiene barriers should be in place at all entrances to broodstock facilities to ensure staff and visitors clean and sterilize footwear and hands.

Although broodstock facilities, which contain wild fish, should be near the incubation and the larval rearing facilities so that gametes can be conveniently carried over, it is necessary to ensure that effective hygiene barriers exist between broodstock and incubation systems. It is particularly important to disinfect the eggs before incubation.

1.3.1 System design and requirements

Broodstock Atlantic halibut are generally large fish that need to be housed in large tanks between 5 and 15 m in diameter. The broodstock at the CCAR are held in a designated facility, which comprises two recirculation systems, each with three tanks of 6.5 m in diameter and 1.5 m in depth (see Figure 1.2).

The recirculation system includes a moving bed biofilter, an UV sterilizer, a submersible circulating pump, and a drum filter (90 μm screen). The two systems are temperature controlled via titanium heat exchangers connected to oil-fired heating and electrical chillers. The room temperature and humidity are controlled via a dedicated HVAC unit. The optimum water temperature for



(a) (b)

Figure 1.2 One of the six 6.5-m diameter halibut broodstock tanks at the CCAR (a) and hand feeding with sausage diet (b).

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broodstock halibut ranges from around 6°C in the winter to around 10°C in the summer. Water exchange is relatively slow at around 0.5 exchanges per hour. To enable the monitoring of egg releases during the spawning season, egg collectors are installed in the side box outlet where side and bottom drains meet before running to the treatment system.

The recommended stocking density for halibut is around 15 kg/m^2. Tank bottoms should be textured to prevent the formation of papillomas that are common in halibut kept in smooth-bottomed tanks at low densities (Ottesen and Strand 1996; Ottesen *et al*. 2007). An essential piece of equipment for the halibut broodstock facility is a table on which fish can be handled for manual stripping. All facilities have this and there are as many designs as there are broodstock managers. Some tables are power assisted (hydraulic or pulley block) to help lift what can be very large fish out of the water. Most are covered with some sort of soft pad such as neoprene rubber to help prevent injury to the valuable fish. The eyes of broodstock halibut are vulnerable and cataracts, gas bubbles, or other types of eye traumas are seen in some facilities. The cause of these problems is not clear and may be related to handling, in tank injury, gas supersaturation, or nutritional deficiencies.

1.4 Photothermal conditioning

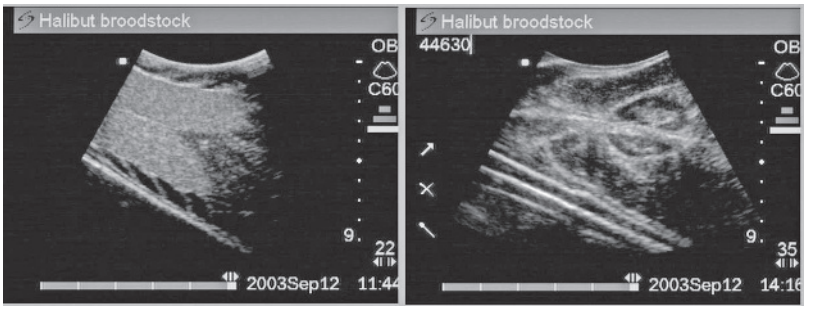
The spawning season occurs between November and April under natural photoperiod (Kjorsvik *et al*. 1987; Haug 1990; Neilson *et al*. 1993). However, year-round egg production is possible using altered photoperiod (Smith *et al*. 1991; Holmefjord *et al*. 1993; Naess *et al*. 1996).

Manipulation of photoperiod is routinely used to influence natural spawning cycles enabling the production of the out-of-season eggs and, when multiple broodstocks are used, year-round production (Smith *et al*. 1991; Holmefjord *et al*. 1993; Naess *et al*. 1996). Delays of up to 6 months can be achieved in a single year. Advancing spawning time is more difficult and more than 3 months per year is not recommended since the fish need to build up reserves over the summer months for the subsequent spawning season. Halibut are sensitive to changes in light levels and good light proofing around holding tanks is necessary to ensure clear photoperiod signals. With photoperiod shifted stocks, attention must be paid to water temperature in out-of-season spawning groups to ensure good egg quality (Brown *et al*. 2006).

In the broodstock facility at the CCAR, the light to each tank is controlled via PLC and can simulate dawn/dusk via programmable dimming. The light source is from a dimmable compact fluorescent lamp suspended above the water in the center of the tank.

1.5 Monitoring gonad development

Captive halibut are generally stripped by hand although natural spawning can occur (Holmefjord and Lein 1990). The natural spawning period in the North



(a) (b)

Figure 1.3 Ultrasound scans of broodstock halibut showing an example of female (a) and male (b).

Atlantic occurs between late December and late March (Kjorsvik *et al.* 1987; Jákupsstovu and Haug 1988; Haug 1990).

Halibut are determinate batch spawners ovulating at intervals of 70–90 hours over the spawning season (Holmefjord 1991; Norberg *et al.* 1991). During the maturation process, batches of oocytes are sequentially hydrated. Adult female halibut have large gonads and are highly fecund. Adult female fish, weighing between 20 and 60 kg, are capable of producing between 6 and 16 batches, each of 10 to 200 $\times$ 10^3 eggs in a spawning season (Haug and Gulliksen 1988; Brown *et al*. 2006).

Egg collectors installed on each tank to intercept egg releases are checked regularly during the spawning season, often many times per day. Fish are usually allowed to spawn in the tank for the first two ovulations to give an indication of spawning interval. A marked reduction in viability can occur if fertilization is delayed longer than 4–6 hours after ovulation (Bromage *et al.* 1994). It has been shown that close observation of individual female ovulatory cycles can help to pinpoint the timing of stripping and improve viability and fertilization rates for halibut (Norberg *et al.* 1991; Holmefjord 1996) though this can be very time-consuming and potentially stressful for the fish. Egg quality can be highly variable in halibut and predicting the correct timing for manual stripping is one of the most difficult challenges remaining for halibut culture.

Ultrasound can be used to sex the fish (see Figure 1.3) and estimate the stage of development of the gonad (Shields *et al*. 1993; Martin-Robichaud and Rommens 2001). Individual fish are marked by PIT tags, FLOY tags, and/or sheep tags. The latter are easiest to use and are rarely lost.

1.5.1 Diet and nutrition

The natural diet of Atlantic halibut caught in various North Atlantic fishing grounds was described by MacIntyre (1953). Prey composition includes a wide variety of fish, mollusks, and crustaceans. The current lack of knowledge of broodstock halibut nutritional requirements means that the practice of feeding raw fish and shellfish is still quite common. This carries serious health risks for the broodstock and resulting eggs, larvae, and juveniles. Diseases found in the wild

components can be transmitted to the captive broodstocks. The feeding of raw fish has been implicated in the transmission of such viral diseases as nodavirus (VNN) and viral hemorrhagic septicemia (VHS) (Dannevig *et al.* 2000).

Atlantic halibut broodstock nutrition studies are very challenging for a number of reasons. Egg quality is highly variable due to many confounding factors such as timing of stripping and replicated studies are hard to set up with such large, valuable fish. It has been shown that broodstock Atlantic halibut can be conservative in the levels of nutrients, in particular essential fatty acids, that they sequester to the eggs (Bruce *et al*. 1993) and despite varying levels in the diet, it may take months or years for deficiencies to emerge.

In two recent studies in Scotland (Mazorra *et al*. 2003; Alorend 2004), it took 3 years for dietary changes in fatty acid composition to have any effect. These studies did indicate that formulated feeds have the potential to replace raw fish components, though survival rates were not particularly high for resulting eggs and larvae. These investigators tested different dietary levels of the fatty acid arachidonic acid (ARA), an essential fatty acid thought to be important in broodstock nutrition due to its role as a precursor for prostaglandins which are involved in egg development and maturation (Bell and Sargent 2003). Mazorra *et al.* (2003) showed an improvement in egg quality when ARA levels were boosted to 1.8% and the authors suggest that the ratio of docosahexanoic acid (DHA) to eicosapentanoic acid (EPA) to ARA should be 8:4:1. The work of Alorend (2004) suggested that dietary levels of >4 mg/g ARA over the long term have a negative impact on egg quality and she suggested an optimum level of 3 mg/g of ARA.

It is important to ensure that broodstock feeds are formulated with the highest quality ingredients and often include components such as squid meal, squid hydrolysate, and krill meal. Broodstock nutrition studies have been ongoing at the CCAR for over 5 years in what is probably the longest running experiment of its kind with this species. Three different diets are under evaluation; two of these are formulated feeds that are compared to the traditional raw fish and squid diet. The formulated diets are mixed as a semi-moist paste and extruded into a 30-mm sausage skin. Given the variable quality of eggs from captive broodstock halibut, varying forms of reproductive dysfunction, and difficulties associated with accurate timing of manual egg collection, it is still unclear whether formulated feeds can match wet fish ingredients.

1.5.2 Controlled spawning

The reproductive endocrinology of this species has been studied in relatively little detail. Methven *et al.* (1992) studied the seasonal changes in vitellogenin and sex steroid levels in captive male and female halibut. They observed the typical pattern of increasing levels of estradiol 17β and testosterone during gonadal recrudescence followed by a drop coinciding with the first release of eggs. Subsequent fluctuating levels of estradiol 17β, testosterone, and vitellogenin were thought to correspond to sequential maturation and release of egg batches. More recently, Kobayashi *et al*. (2008) using advanced molecular techniques has

shed more light on follicular expression of gonadotropic receptors FSH-R and LH-R.

Very few attempts have been made to control spawning using steroid hormones in halibut. Spermiation in male halibut generally starts before the females are ready to spawn and in captive males, spermiation may stop before all female broodstock have completed spawning. Though milt can be cryopreserved (Rana *et al*. 1995) or extended, the application of gonadotropin-releasing hormone agonist (GNRHa) implants has proved useful in synchronizing spermiation (Vermeirssen *et al*. 1999; Martin-Robichaud *et al*. 2000; Vermeirssen *et al*. 2004). The application of GNRHa implants also reduces spermatocrit and the resulting milt is easier to collect and use during artificial fertilization. Induction of spawning in female Atlantic halibut has not been documented and it is likely that this technique may be worth exploring in the future.

1.5.3 Egg collection and incubation

Eggs and milt are collected manually by hand stripping the fish out of the water raised on stripping tables. Fertilization is generally achieved using the wet method whereby milt is mixed into seawater then poured over and mixed gently with the eggs. This should be done quickly as the milt remains motile for only a couple of minutes. The motility of sperm is checked under a low power objective on a microscope prior to fertilization to confirm viability. A typical ratio in this mixture would be 1 mL to 1,000 mL to 1,500 mL (milt:eggs:water). The eggs are left to "water harden" for 20 minutes then rinsed of excess milt and ovarian fluid. After a sample is taken for fertilization checks, which are best done at the 8-cell stage after about 16 hours at 6°C, the eggs are stocked to upwelling incubators. A typical stocking density is up to 300 eggs per liter.

Blastomere morphology is easily examined in this species owing to the peripheral displacement of the large cells during early cell divisions and the lack of opacity of the egg. A strong link between the gross morphology of these blastomeres and egg viability has been demonstrated (Shields *et al*. 1997) which enables the hatchery manager to make decisions about which egg batches are worthwhile.

In general, the eggs of the Atlantic halibut have a relatively high specific gravity owing to their high inorganic content (Riis-Vestergaard 1982) and they will sink at ambient salinities found in most coastal marine hatcheries. To counteract this, the eggs are incubated in upwelling tanks. These are usually cylindroconical tanks of volume between 100 and 1,000 liters. A gentle flow enters through a bottom inlet and leaves via a surface outlet which is often a "banjo filter" with a 1-mm screen. This screen must have a large surface area to reduce velocity at the outlet to prevent collection of eggs at the outlet. Bunching of eggs here will cause high mortality. Room temperature is maintained at 6°C with an air chiller and the room is light proof, all procedures being carried out using low intensity light.

Bacterial contamination of halibut eggs may lead to a reduction in viability and it is common practice to use surface disinfectants, for example, glutaraldehyde (400 ppm, 10 minutes) (Harboe *et al*. 1994a). Increased survival rates

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during first feeding have been attributed to such treatments; however, this practice is not universally adopted. An alternative and less toxic egg disinfectant, peroxyacetic acid (200 ppm, 1 minute) initially tested in the United Kingdom with promising results (Kristjansson 1995) has been adopted by many hatcheries. Outbreaks of nodavirus in Norwegian hatcheries led to the development of ozone disinfection techniques. An exposure to a concentration of 2 mg/L with a contact time of 2 minutes is effective against this pathogen (Grotmol and Totland 2000; Grotmol *et al*. 2003).

Once per day, dead and nonviable eggs are removed from the tanks using the "salt plug technique" developed in Norway (Jelmert and Rabben 1987). The flow is turned off and about 10–20 liters of high salinity (40 ppt) seawater is injected into the bottom of the tank. Live eggs generally float on the resulting halocline and nonviable eggs drop to the bottom where they can be tapped off with the salt plug. The flow is then restored and the volume of dead eggs is recorded. Hatching takes place in the incubators after approximately 75–80°C days postfertilization. Hatched larvae will usually float in the surface layer and can be removed using plastic jugs. Larvae are transferred in jugs to yolk sac incubators in lightproof, insulated containers. Light can delay hatching (Helvik and Walther 1993) and this fact is used in some hatcheries to synchronize hatching of a batch. Eggs can be moved to the yolk sac incubation system just prior to hatching or immediately after hatching, in which case empty egg cases and hatching debris are left behind.

1.6 Larval culture

1.6.1 System design and requirements

The long yolk sac absorption phase in halibut (220–290°C days) necessitates a separate yolk sac incubation system. Usually housed in a light proof, temperature-controlled room set at the temperature between 5 and 6°C, the tanks are similar to egg incubation tanks but much larger (see Figure 1.4).

These cylindroconical tanks range in volume from 700 liters to large silos of 3–13 m^3 favored by Norwegian operators (Harboe *et al.* 1994b; Berg 1997). Incubators at the CCAR have a volume between 700 and 1,000 liters. The Canadian hatchery uses large, Norwegian/Icelandic style silos. Flows are upwelling and the outlet is set close to the top of the tank. A filter with a large surface area prevents entrapment of the larvae. Incubators in use at the CCAR have one inlet for salt water and do not use oxygen or aeration.

Prior to first feeding, larvae are moved to larger volume rearing tanks which are typically 2–10 m^3. These are circular fiberglass or plastic tanks, generally dark in color, with bottom drains, and often with additional side drains. Overhead lighting is provided either by fluorescent or incandescent lighting and the light intensity can be relatively high. Tanks are provided with aeration to create turbulence and prevent crowding of larvae under the light source, particularly at the start of feeding. Many facilities now incorporate self-cleaning equipment in the larval rearing tanks to reduce labor associated with siphoning out settled organic matter (Van der Meeren *et al*. 1998).

Figure 1.4 Yolk sac larvae incubator.

1.6.2 Hatchery protocols

The period from hatching to first feeding, when the endogenous reserves stored in the yolk sac are absorbed, can last up to 50 days depending on the temperature. During this period, larvae are held in upwelling cylindroconical incubators. Reported stocking densities in the larger silos are in the region of 1–20 larvae/liter (Olsen *et al*. 1999). Densities of around 45 larvae/liter are typical in the yolk sac incubation tanks used at the CCAR that compensates somewhat for the smaller volume. In practical terms, this means that larvae from an average single batch of hatched larvae can usually be accommodated in one incubator. Typical survival rates in these incubators range from 50 to 80%, similar to those reported in Norwegian installations (Mangor-Jensen *et al*. 1998).

Strict temperature control is necessary during this phase since suboptimal temperatures can cause developmental abnormalities or high mortality (Bolla and Holmefjord 1988; Lein *et al*. 1997a). Salinity must also be within a narrow range (Lein *et al*. 1997b; Bolla and Ottesen 1998) and maintenance of good water quality is required. The larvae are generally kept in near or complete darkness because they are strongly attracted to a light source at the later stages of this phase. The transition to exogenous feeding can occur between 200 and 290°C days and the duration of the live feed stage is typically 50–70 days (Harboe *et al*. 1990; Lein and Holmefjord 1992). Current practice at CCAR is that at about 240°C days posthatching, the larvae are moved out to covered larval rearing tanks. The larvae are strongly positively phototactic toward the end of the yolk sac period (Naas and Mangor-Jensen 1990) and this fact is used to attract

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the larvae to the surface for collection. Generally, the larvae are transported at high density to larval rearing tanks as quickly as possible and numbers are estimated from sample counts. The larvae are maintained in clear water in the larval rearing tank in complete darkness while the temperature is raised gradually to 10°C. First feeding begins at 290°C days posthatching when viable larvae should initiate feeding within a few hours of the first addition of feed.

Larvae are fed live prey, which in intensive hatcheries means *Artemia*, although rotifers, cultured copepods, wild zooplankton, or a mixture of these have been used (Holmefjord *et al.* 1993; Naess *et al.* 1995). "Green water" is generally used in intensive systems since it has been found to be beneficial for first feeding success (Naas *et al.* 1992; Holmefjord *et al.* 1993; Guldbransen *et al.* 1996).

Mass production of halibut was initially achieved in Norway using semi-intensive techniques and these have been reviewed by Mangor-Jensen *et al.* (1998). Larvae reared in indoor incubators are moved to outdoor bag enclosures prior to first feeding and fed harvested wild zooplankton and *Artemia*. Though this technique can potentially generate large numbers of fry and was the mainstay of production up until the mid-1990s, output from these systems fell drastically in 1995 and it is now accepted that the method has drawbacks. Seasonal variations in wild zooplankton harvests can result in shortages of live prey. There is also a greater risk of exposure to pathogens, for example, nodavirus (VNN) or infectious pancreatic necrosis (IPN), which can cause serious mortalities in halibut (Grotmol *et al.* 1997). Large size variations are also a characteristic of fry reared in these systems and this can cause problems at weaning (Berg 1997). The development of methods for hatchery production in Iceland and the United Kingdom focused on intensive techniques using *Artemia* as the primary live food source. Larvae are reared exclusively in tanks through the entire rearing process (see Figure 1.5).

U.S. and Canadian techniques for halibut culture evolved from technology transfers from commercial hatcheries in Norway and Iceland, and from research institutions in the United Kingdom (Seafish Industry Authority and the Institute of Aquaculture in Stirling). Semi-intensive production methods using wild harvested zooplankton were in use in some Canadian hatcheries up until the late 1990s but this culture methodology is no longer practiced in Canada.

1.6.3 Water quality

Methods currently used in Maine at the CCAR make extensive use of marine recirculation technology. This has resulted in a greater degree of control of water quality and important physical parameters of temperature, gas saturation levels, and salinity. It also has resulted in, as yet, unexplained benefits of consistency in larval survival thought to be associated with biofiltration. The possible probiotic effects of stable bacterial populations in the biofilters, pipes, and tanks could actually limit the impact of opportunistic pathogenic bacteria so commonly implicated in crashes of populations of larvae in the first feeding stage (Verner-Jeffreys *et al.* 2003). Makeup water supplies for egg incubation, yolk sac larval

Figure 1.5 Late pelagic phase halibut larvae feeding on Artemia.

rearing, first feeding, and for live food production are filtered to 1 μm and UV treated to 200 μWsec/cm^2.

Flow rates to the larval rearing tanks should start at a low rate of exchange and increase gradually as feed inputs and biomass increase. At the CCAR, tanks of 2–8.5 m^3 are used, depending on batch size. Water exchange rates start at once per 24 hours and by day 50 post first feeding, reach up to a 6-hour turnover.

Microalgae are commonly used for halibut rearing (Naas *et al*. 1992). The use of algae, or the "green water technique" as it is commonly known, has been in use since early times in the development of marine fish hatchery techniques and the practice is still almost universal. However, the use of algae pastes and algae substitutes is becoming more widespread. Experiences over the last few years at the CCAR with the use of powdered clay suggest that this is a very cost-effective alternative and in terms of juvenile quality, there have been no detrimental effects (Brown, unpublished data).

1.6.4 Food and feeding

Halibut larvae have a relatively large mouth size and can start to feed on *Artemia* nauplii from the outset. It is common practice to begin with freshly hatched nauplii and feed at a density of 1 per mL. Feeding should occur in the majority of the population within 4 hours with a vigorous batch of larvae. Nauplii are given for 2–3 days before switching to a 24-hour enriched *Artemia*. As the larvae grow, larger, ongrown *Artemia* should be given. While some hatcheries will grow *Artemia* for up to 4 days (Olsen *et al*. 1999), this requires a great deal of tank space. Experience at CCAR has demonstrated that a 48-hour enriched *Artemia*

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will supply the energetic and nutritional needs of halibut larvae from around 450°C days posthatch. Good results in terms of survival (average 35%), pigmentation (>95% normal), and eye migration (>95% normal) can be achieved with an *Artemia*-only diet using commercially available enrichment products.

The early trials using *Artemia* as the sole source of food prior to weaning demonstrated that nutritional deficiencies in this prey organism compared to wild copepods resulted in poor rates of normal metamorphosis (Naess *et al*. 1995). U.K. trials, however, indicated that by manipulating the nutritional profile of *Artemia* through enrichment strategies, fry quality could be improved (Gara *et al*. 1998) though rates of normal development were still relatively low. A compromise strategy to achieve good rates of growth, pigmentation, and eye migration was devised whereby *Artemia* were used as the main prey organism but copepods were fed during a critical period which became known as the "copepod window" (Naess *et al*. 1995). Breakthroughs by commercial hatcheries, in particular Fiskey in Iceland, demonstrated that with the correct enrichment regime, well pigmented fry with good eye migration morphology could be produced with *Artemia*-only feeding strategies. Work to develop diets which can mimic the biochemical profile of copepods, based partly on the detailed work of Van der Meeren *et al*. (2008) has resulted in proprietary enrichment products that produce juveniles with acceptable rates of normal morphology.

However, problems with eye migration and pigmentation still remain in some hatcheries (Hamre *et al*. 2007; Hamre and Harboe 2008) and the causes are still the subject of considerable debate. The requirement for essential fatty acids (EFAs) is often the focus of studies to find the cause of these abnormalities and advice on levels EFAs, in particular DHA, EPA, and ARA, is abundant (McEvoy *et al*. 1998; Sargent *et al*. 1999; Hamre and Harboe 2008). Other possible factors include overall energy intake (Gara *et al*. 1998), iodine and thyroid levels (Solbakken *et al*. 2002), and even photoperiod (Solbakken and Pittman 2004).

Multiple feedings help to ensure that *Artemia* presented to the larvae are freshly enriched and that valuable nutrients are not lost or catabolized. It is common practice to feed 3–4 times daily. This also enables the hatchery manager to keep a close track of how much a population of larvae is eating. Automated feeding systems help to reduce the need for staff to feed at night but in commercial hatcheries, night checks are common practice anyhow. Lights are left on for 24 hours and feed should also be available round the clock.

1.6.5 Formulated feeds

Despite many trials testing early weaning of halibut prior to metamorphosis, including work conducted by Brown (1998), lower survivals and poor growth are generally the result of most formulated feeds when this is attempted too early. Once larvae are through metamorphosis, a good batch of fish will wean very quickly, usually within 2–3 days. Protracted cofeeding strategies are not necessary and weaker fish unable to wean rapidly should be removed from the population at this point. The accumulation of uneaten feed at this stage presents a challenge for hatchery staff and self-cleaning tanks are desirable. Fast circular,

self-cleaning flows are possible once fish are settled onto the bottom thus helping particle movement, which in turn helps to attract fish to feed.

1.6.6 Microbial environment

One of the principal reasons for inconsistent output from marine hatcheries is early die offs during larval rearing, often associated with changes in microbial flora. Water for larval rearing is often highly filtered and sterilized. Larvae emerge from incubation systems with guts which are largely uncolonized with bacteria. Added to the larval rearing tanks is a cocktail of bacteria coming from the live feed cultures; microalgae, *Artemia*, or rotifers and these bacteria have often shown up as dominant microbial flora in the larval gut in studies that monitor changes in bacterial flora through the rearing cycle for a number of species, including halibut (Verner-Jeffreys *et al.* 2003). Added to this environment is the build up of organic material in the form of dead larvae, fecal wastes, and dead prey items, which all act as substrate for colonization.

Microbial conditions do tend to be more stable in recirculating hatchery systems and this was demonstrated for Atlantic halibut by Verner-Jeffreys *et al.* (2004). Recirculation systems are used for all stages of hatchery production at the CCAR halibut hatchery.

It is important to control the build up of waste in the larval rearing systems to deprive the microbial food web of substrate. One of the most labor-intensive tasks in a commercial hatchery is the removal of organic wastes, which gets collected in the slow-moving tanks, used for larval rearing. This is often simply done by manually siphoning or with the use of a squeegee. If this is not done carefully, this material can easily be resuspended. The automation of tank cleaning is commonly quoted by hatchery managers as a priority and there are some systems available commercially. A group working on marine fish culture in Austevoll, Norway, at the Institute of Marine Research designed a system that incorporates a rotating squeegee arm to collect debris which is sucked up through outlet holes in the arm. The design was described in Van der Meeren *et al.* (1998) and equipment based on a variation of this design has since been commercialized and is in use in Atlantic halibut hatcheries in Norway.

1.6.7 Harvest

Halibut larvae are robust by the time they reach 150 mg wet weight, close to metamorphosis and can be harvested by net or siphon in the pelagic phase or after settling to demersal habit. At this point, they are moved to weaning tanks with a treatment system of sufficient scale that dry diet can be fed to excess without major disruption of water quality caused by the build up of uneaten feed. Water exchange at this stage should be at least once per hour. Smaller individuals will be targeted by larger, dominant fish and cannibalism is common. The smaller fish at this point are best removed from the population as they will tend to be

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the slower growers throughout the growout stage and will not help the farmer's bottom line.

1.6.8 Hatchery economics

The market price of Atlantic halibut juveniles remains quite high ($5 or more per 5 g fish) and is a barrier to entry for many would-be halibut growers. With relatively few major players and what are still relatively small production runs, there are few hatcheries that benefit from economy of scale. The biology of the halibut results in a long hatchery cycle. This fact cannot be changed and will always mean that a halibut juvenile will cost more than a salmon, cod, or turbot juvenile. However, the market price at harvest is also higher and for some farmers, halibut is already an attractive option for growout.

1.7 Potential for stock enhancement

There are a number of features that make the Atlantic halibut a good candidate for stock enhancement. Most hatcheries are still rearing a significant proportion of their fish from eggs spawned from wild origin fish. This could be seen as a benefit for stock enhancement in terms of genetics. Atlantic halibut juveniles are generally robust and transport at relatively high densities with little mortality. In the hatchery, they spend a long time on live feeds and could be stocked out at the end of this phase without affecting their instinct for predation. One major disadvantage for this species is the cost of rearing which would make any restocking effort expensive. Also, their relatively slow growth, and thus time to legal landing size, would make assessment of their recapture rate and recruitment to the fishery very complicated. Other factors that would need to be considered would be juvenile quality, especially eye migration and pigmentation. Presumably, albino fish would be more susceptible to predation. No restocking efforts for this species have been attempted so far.

1.8 Growout

1.8.1 System design

There are two basic approaches to ongrowing; using land-based tanks or raceways (Adoff *et al.* 1993; Blanquet and Lygren 1997; Brown 2002) or at sea in cages (Martinez-Cordero *et al.* 1994; Brown 2002).

Atlantic halibut juveniles will spend at least part of the growout cycle in a land-based system whether they stay in tanks all the way through to harvest, or move out to net pens for the latter part of the growout cycle. Nursery systems differ from growout system only in scale of tanks. Halibut are grown in a variety of tank types; shallow raceways, large circular tanks, semisquare tanks, and in tanks made of a variety of materials; fiberglass, concrete, polyethylene, glass

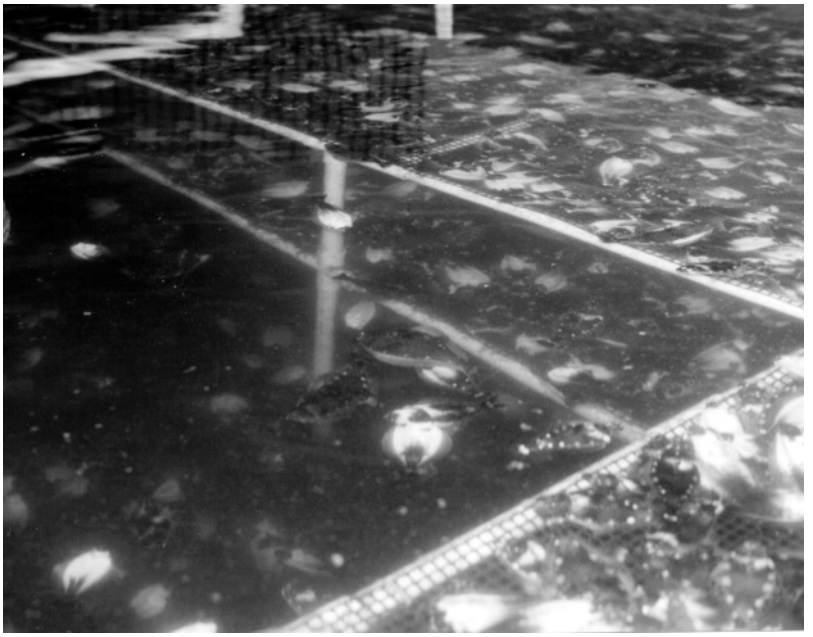

Figure 1.6 Halibut juveniles using shelving.

fused to steel, PVC-formed concrete, etc. This author conducted trials in Canada to test the use of shallow raceways compared to deeper tanks and found that growth during the juvenile stages (25–250 g) was significantly better in deeper tanks. Access to slow-sinking pellets in shallow water is impeded and aggression and collisions during feeding were more frequently observed. Added to this is the problem of deteriorating water quality from one end of the tank to the other and accumulation of uneaten feed. Deep circular tanks (4–10 ft deep) are very effective for halibut and while most flatfish species do not voluntarily occupy shelving if provided, Atlantic halibut will (see Figure 1.6).

The extent to which they do use it will depend on shelf spacing, hydrodynamics, lighting, and overall stocking density. Multiple layers of fish utilizing shelving in deep tanks can make very efficient use of space and tank volume. At the time of writing, there were only three growout operations using land-based facilities in North America (two in Nova Scotia and one in Maine). These facilities all employ recirculation technology of various descriptions and all report good growth and survival in these systems.

Cage designs for flatfish have evolved from round fish-type cage designs and are often simply modified from existing units. An important consideration for flatfish cages is the provision of a rigid base to ensure that when stocked with fish, the net pen will not distort or sag. This can cause aggregation of fish, dead spots with poor water exchange, poor feed distribution, and uneven loads on the net panels and frame. Cage bases are generally net panels tensioned to a rigid frame constructed of steel or plastic. Surface cages are most commonly used and a variety of designs, constructed of steel, plastic, or even wood have been used in Scotland, Norway, and Canada. Cages are generally 3–7 m in

depth and net bases are fabricated from 6 to 15 mm netting and sides can be manufactured from larger mesh (e.g., 22 mm). Use of smaller mesh bases can reduce feed waste since halibut tend to take some feed off the bottom. Predator netting is very important since the fish in the cage spend a large proportion of the time adjacent to the netting, within easy reach of predators that are not excluded by additional barriers. Occasional losses of halibut to predators including seals and otters have been reported in Canada. Surface cages have been in use for small-scale ongrowing halibut for many years in New Brunswick, Canada. Submersible cage designs have been tested successfully for halibut at the University of New Hampshire offshore aquaculture site near the Isles of Shoals, New Hampshire (Howell and Chambers 2005). Early trials in Maine explored the use of a submersible cage for halibut (Duym 1996).

The use of lobster pounds was investigated in New Brunswick between 1999 and 2003. These types of facilities were found suitable for halibut initially but high mortalities were observed during extremely cold winter conditions in these shallow enclosures which are only flushed twice daily by tidal exchange. Following heavy losses due to extremely low temperatures, the use of these pounds was abandoned.

The use of cameras, which are already widely used in surface cages to allow visual observation, particularly during feeding, will be an essential component of submersible cage systems for flatfish as will the development of suitable feed delivery systems.

1.8.2 Environmental conditions

The optimum water temperature for halibut decreases with increasing fish size (see Table 1.1). The upper lethal temperature limit is around 18–20°C depending upon feeding and dissolved oxygen levels and the lower limit is near—1.3°C. Halibut can tolerate a wide range in salinity and in fact growth can be higher at salinities lower than full strength sea water (Imsland *et al*. 2008) and this opens up the possibility to utilize ground water sources or geothermally heated water sources. Recommendations for other water quality parameters are similar to other marine species. Ammonia nitrogen (unionized NH_3–N) should be maintained below 0.05 mg/L, pH range should be 7.2–8.0, dissolved O_2 kept

Table 1.1 Recommended stocking density and water temperature for Atlantic halibut.

Size range (g)	Recommended stocking density (kg/m^2)	Optimum temperature range (°C)
0–10	5	11–14
11–20	10	11–14
21–50	15	11–13
50–150	20	11–13
150–400	30	10–12
400–1,000	40	9–11
1,000+	50	7–11

above 6 mg/L and dissolved CO_2 kept below 20 mg/L. Total gas supersaturation should be avoided.

For halibut in open water pens, high current and wave motion cause increased swimming activity and sheltered sites are required for halibut. Exposure to high levels of UV light in cages (and outdoor tanks) under strong sunlight can cause health problems. Halibut are particularly susceptible and may develop fat cell necrosis, which may eventually lead to high levels of mortality following secondary infection (Bricknell *et al*. 1996). This problem is avoided with the use of shade netting (>80% is recommended) over the cages, particularly for juvenile fish or where shallow nets (<4 m) are used.

1.8.3 Diet and nutrition

Most major commercial feed companies are making diets tailored specifically for halibut farmers. Reported optimal levels of protein range from 45 to 63% (Hjertnes and Opstvedt 1990; Aksnes *et al*. 1996; Hamre *et al*. 2003). Berge *et al*. (1999) demonstrated that a significant fraction of the protein (44% of nitrogen) could come from soy protein concentrate. Increasing dietary lipid can have a protein sparing effect but may also result in elevated carcass fat deposition (Aksnes *et al*. 1996). Lipid utilization was recently examined by Martins *et al*. (2007) and their study revealed that halibut can tolerate up to 25% lipid in the diet but no beneficial effect is gained from levels higher than 14%. Halibut have a limited capacity for digestion and utilization of carbohydrates (Hatlen *et al*. 2005), indicative of their carnivorous feeding habits in the wild. While halibut are chiefly visual feeders, there is good evidence that higher levels of attractants in the diet will stimulate an increase in appetite (Yacoob and Browman 2007). Slow-sinking pellets are preferred, as fast-sinking pellets may remain uneaten in a crowded tank under layers of fish. One cost-saving feature in common with most non-salmonid diets is the fact that the expensive artificial pigments are not needed.

In general, feeding frequency decreases with increase in fish size and decrease in temperature. Hand feeding is still commonly used in halibut farms and automatic feeders are utilized, however, as a method to ensure satiation and during the fry/nursery stage to reduce aggression.

1.8.4 Stocking density and grading

As with all flatfish, surface area rather than volume is the critical factor in determining capacity of a tank or cage, assuming that water exchange is adequate for removal of metabolites and supply of oxygenated water. As the fish grow in size, the thickness of the fish increases and with it, the maximum recommended stocking density would increase. Table 1.1 presents guidelines on stocking density for various size ranges of fish.

Regular grading of halibut is required to reduce size variation, which if left unchecked can result in cannibalism and increased aggression. Aggression has been shown to lead to a high incidence of eye damage in halibut (Greaves and

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Tuene 2001). Grading can be done with hand nets or mesh sorters during the nursery stage and grading tables during growout. Effective mechanical graders have been developed for halibut and are in use in most large facilities. If normal good husbandry practices are employed, halibut generally survive handling extremely well.

1.8.5 Harvesting and processing

Harvest of halibut or any flatfish from a net pen is slightly more challenging than for round fish as they are not easily captured in a seine net. Fish pumps can be used for smaller halibut. Starvation of fish for 24 hours is advisable prior to harvest though the removal of guts immediately postslaughter will negate the effect of a full stomach during processing. Rapid slaughter is desirable both from a welfare standpoint and to avoid rapid onset of rigor, drop in pH, reduced shelf life, and the development of "chalkiness" in the flesh (Kramer and Paust 1985). Halibut can be slaughtered in the same way as other farmed fish by automated percussive, electrical stunning, or anesthesia with CO_2 or ice/water slurry. Manual percussive stunning can be used in the area near the eyes, though this will cause damage and may affect the marketability of smaller fish sold "head on." Bleeding immediately postmortem by incision of a major artery during gutting or removal of gill arches is recommended. The presence of blood veins and spots in the fillet detracts from both appearance and taste. Bleeding is less effective following anesthesia than percussive stunning (Akse and Midling 2001). The fillet yield of halibut can be as high as 60% but is typically around 55%. Fat content in the fillet is around 3–4% and slightly lower near the tail (Nortvedt and Tuene 1998). Fat storage along the fin margins can result in fat contents of close to 45% in this region. A shelf life of up to 3 weeks on ice has been reported in chilled products without a loss in quality (Akse and Midling 2001).

1.8.6 Marketing

The traditional market for halibut has been based on the wild harvest of relatively large fish (>10 kg). Most of what is sold at retail is fresh and in the form of steaks. With the availability of farmed fish to the market, new product forms are emerging. Farmed halibut are usually marketed above 3 kg and fish over 5 kg fetch the best prices. However a small niche market for "plate sized" halibut does exist and these smaller whole halibut can also obtain very good prices. Small volumes (<100 MT/year) are sold into U.S. markets from Norway and Canada currently. The 4-month off-season for the Pacific halibut fishery is generally targeted for much of the farmed halibut sales in North America in order to fetch the highest prices.

1.9 Production economics

Very few studies have attempted to look at the economics of farming marine species in the United States. Efforts at predicting overall production costs for

halibut have been made by a number of industry analysts around the world for the purposes of assessing the potential economic feasibility of halibut farming. Most halibut farms are still in Norway and only the larger scale farms there, which produce 200–300 MT/year are actually profitable with production costs approaching 5–6 €/kg ($3.3–4.0/lb) (Engelsen *et al*. 2004). A report prepared for the State of Alaska (Forster 1999) predicted U.S. production costs of between $3.19/kg ($1.45/lb) and $4.09/kg ($1.89/lb) based on cage growout of large halibut. An analysis of Canadian halibut farming (Penney 1999) found that sea cage farming of halibut would be profitable with an internal rate of return (IRR) of 9% but predicted that a land-based operation, using raceways and flow through would not be profitable. In contrast, McCallum (2000) predicted that at certain economies of scale (≥100 MT per annum), land-based recirculating growout of halibut in Canada was expected to give a good return on investment (IRR of 15%) with a production cost between $7.75/kg ($3.53/lb) at 100 MT/year and $7.19/kg ($3.27/lb) at 300 MT/year. In a study commissioned by the Maine Department of Marine Resources, Gardner Pinfold (2003) modeled IRRs of 9% for land-based recirculating production and 15% for sea-based growout on the basis of an ex-farm price of $4.50/lb.

1.10 Summary: industry constraints and future expectations

Long growout time is one of the main drawbacks for this species. As with many species, the halibut exhibits sexual dimorphism whereby males tend to grow more slowly than females and mature at a smaller size and younger age (Haug 1990). Triploidy has already been successfully induced in halibut using cold shocks (Holmefjord and Refstie 1997) or pressure shock (Brown 1998) and this may be one solution to this problem. Canada has taken the lead with production of all female fish reared from fertilizing eggs with milt from sex reversed males (Hendry *et al*. 2003). This technology is under development in Norway, Scotland, and the United States as well and could help to improve the economics of growout significantly. Breeding programs are under way in Scotland, Canada, Norway, and the United States though most eggs still come from wild caught broodstocks and there is little published information on performance of F1 stocks. Slower growing fish may be marketed at a smaller size and markets for fish under 1 kg are being developed by farms in North America and prices for these fish sold direct to the restaurants are high (>$10/lb).

Pilot halibut farming efforts have been underway elsewhere in North and South America. A facility in Hawaii with access to deep cold water has a small number of fish to growout for local markets (Jim Parsons, personal communication) and hatchery facilities in Punta Arenas, Chile, have been under development for some time (Alvial and Manriquez 1999). This project has been supplied with eggs, broodstock, and juvenile halibut from private and government entities in Canada.

Atlantic halibut has been on the lists of new, promising species for aquaculture for many years. As a result of a long-term research effort in many countries, many of the technical hurdles have been overcome. However, levels of production

remain modest. Farmed halibut is generally well received in the marketplace and larger farming operations, particularly those which are vertically integrated, are likely to remain profitable and grow over the next few years.

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