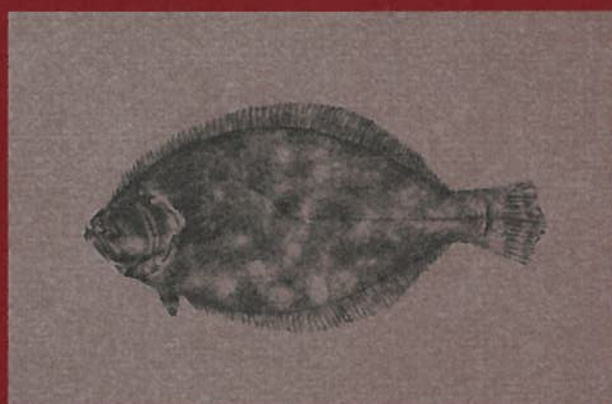
Annotated Bibliography of Flatfish Aquaculture

Robert R. Stickney



Sea Grant
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FOREWORD

This bibliography contains citations and abstracts of publications on flatfish culture produced between the late 1970s and 1997. Also included are references to an earlier bibliography (White and Stickney 1973) that traces the early years of flatfish culture and a more recent one on halibut culture (Stickney and Seawright 1993). Some of the citations that appear below may also be listed in the earlier halibut bibliography although no attempt has been made to reproduce all the citations in that work. Halibut citations subsequent to 1993 are included in the following pages. Also included are references to California halibut (actually a species of *Paralichthys*, not *Hippoglossus*).

Titles for papers published in a language other than English are given in both the published language and in English, with the English title placed between brackets. A few of the references presented herein are not specifically related to culture of the fish but were included because the information may be of use and interest to aquaculturists.

The species that dominates the modern flatfish culture literature today is *Paralichthys olivaceus*, most commonly known as the Japanese flounder but also as the olive flounder, bastard, and bastard halibut. Throughout the summaries, the common name Japanese flounder is used (sometimes parenthetically with another common name). No common names have been altered in the literature citations.

Aksnes, A., T. Hjertnes and J. Opstvedt. 1996. Effect of dietary protein level on growth and carcass compositionin Atlantic halibut (*Hippoglossus hippoglossus* L). Aquaculture, 145:225-233.

The effects of replacing protein with carbohydrate or lipid in diets for Atlantic halibut were studied in three long-term experiments. The fish were fed nine dry pelleted diets with ranging in protein, carbohydrate and lipid from 410 to 719, 14 to 269 and 126 to 325 g/kg diet, respectively. The experiments started with 5 to 7 g fish and lasted until the fish reached 400 to 600 g (14 to 17 months). All groups showed good growth and feed efficiencies during the experimental periods. Feed conversion efficiencies (fish weight gain/weight of feed offered) ranged from 0.99 to 1.50. At 250 g/kg dietary lipid, a significant increase in growth and feed efficiency was observed when dietary carbohydrate was decreased from 269 to 31 g/kg diet with concomitant increases in dietary protein from 410 to 618 g/kg. At high dietary protein levels, increasing the level of lipid in the diet from 127 to 325 g/kg resulted in higher carcass lipid levels ranging from 72.5 to 125 g/kg body weight. No difference in growth or feed efficiency was found with increased dietary lipid levels.

Alvial, A., and A. Trujillo. 1991. Current status of finfish hatcheries in Chile. pp. 117-132, *In*: C-S Lee, M.S. Su, and I. Liao (Eds.). Proceedings of Finfish Hatchery in Asia, 1991. TML Conf. Proc. No. 3, Keelung, Taiwan.

In an attempt to diversify finfish aquaculture, experimental programs have been initiated to develop flatfish aquaculture in Chile. Recently, turbot (*Scophthalmus maximus*) was introduced and juveniles are being produced in a hatchery. Commercial production is anticipated to develop over a period of the next several years. Chilean universities are developing the ability to culture Chilean flounder (*Paralichthys* spp.).

Andreasen, T.V., B. Gulliksen, T. Haug, E.M. Nilssen and E. Ringoe. 1993. Protein content and amino acid composition of young Atlantic halibut (*Hippoglossus hippoglossus* L.) captured in the autumn in north Norway. ICES J. Mar. Sci. 50:93-100.

Samples of four tissue-types from immature Atlantic halibut (*Hippoglossus hippoglossus* L.), captured in the autumn, were analyzed for total protein content and for free and protein-bound amino acids. There was a relatively high total protein content in the muscle tissues, whereas the liver and fin base notch had a lower total protein content. The data suggest a possible test diet for young Atlantic halibut and will serve as a good basis for comparison with captive halibut reared on prepared feeds.

Anonymous. 1995. Havbruksrapporten 1995. [Annual report on aquaculture 1995]. Fisken Havet, Suppl. 3. 55 p.

The Norwegian production of Atlantic salmon exceeded 200,000 tons in 1994. The projection for 1997 was that farmed Atlantic halibut production will exceed that for wild caught halibut in Norway.

Arnold, C.R., W.H. Bailey, T.D. Williams, A.Johnson and J.L. Lasswell. 1977. Laboratory spawning and larval rearing of red drum and southern flounder. Proc. Annu. Conf. Southeast. Assoc. Fish Wildl. Agencies, 31, 437-440.

This paper reports on spawning and larval rearing studies conducted with red drum (*Sciaenops ocellata*) and southern flounder (*Paralichthys lethostigma*) from 1974 to 1977. Adults were placed in spawning tanks equipped with biological filters. Photoperiod and temperature were regulated to simulate seasonal variations. Southern flounder spawned 13 times producing 1.2×10^5 eggs. Eggs were collected and incubated, and larvae were reared to fingerling size.

Baker, B.I., J.F. Wilson and T.J. Bowley. 1984. Changes in putuitary and plasma levels of MSH in teleosts during physiological colour change. Gen. Comp. Endocrinol. 55:142-149.

Immunoreactive MSH was measured in the plasma of eels, trout and flounder, following different periods of adaptation to illuminated white or black backgrounds. In eels and trout, plasma hormone titres changed rapidly in response to the background color. In flounder, the pituitary melanotrophs appeared cytologically unresponsive to background change and plasma hormone levels remained similar for fish from black or white tanks following a three week adaptation period. A difference became apparent after nearly seven weeks adaptation. Flounder pituitary levels were unaltered by background color. MSH appears to be involved in physiological color change in eels and trout, but probably not in flounders.

Bang, I.C., Y. Kim, K-K Kim and J-K Lee. 1995. Studies on the production of all-female populations of olive flounder, *Paralichthys olivaceus.* 2. Hormonal sex reversal. Bull. Natl. Fish. Res. Dev. Agency, 49:49-57.

The effects of various concentrations and treatment methods with estradiol and methyltestosterone on sex reversal, survival and growth of Japanese flounder were investigated. Juvenile flounders received the hormone for 60 days beginning 30 days after hatching either in the diet or added to the water. At 150 days after hatching, fish were sampled to examine sex ratio, survival and growth. Feed treated with 1, 10 and 100 ppm of estradiol had female percentages of 24.3, 67.4 and 96.3%, respectively. Feed treated with 1 and 10 ppm of methyltestosterone produced 100% males. Feed treated with 100 ppm methyltestosterone produced 84.1% males. Immersion treatment in the rearing water with 1ppb of estradiol produced 48.7% females, while 10 and 100 ppb produced 100% females. The survival and growth of each experimental groups were not different from the controls. Among the immersion groups, only treatment with 10 ppb methyltestosterone produced all-male populations.

Benetti, D.D., A. Venizelos and C. Acosta. 1994. Finfish aquaculture development in Ecuador. World Aquacult. 25(2):18-25.

Efforts to diversify Ecuador's shrimp aquaculture and fisheries industries into finfish aquaculture have included flounder (*Paralichthys* spp.). The major challenge is disease control.

Bengtson, D. and G. Nardi. 1995. Summer flounder culture: An update on research and the development of an industry. J. Shellfish. Res. 14:239.

Research on summer flounder (*Paralichthys* dentatus) led by the New England Fisheries Development Association, with participation by the Universities of Rhode Island and New Hampshire and two private companies, GreatBay Aquafarms and Northeast Organics involves studies on pigmentation abnormalities, cannibalism, and weaning at the time of metamorphosis, maximum stocking density for grow-out in recirculation systems, and economics. Another project at North Carolina State University is primarily concerned with the reproductive biology of summer flounder. The University of Connecticut is developing a summer flounder research program in conjunction with the Caribbean Marine Research Center. The National Marine Fisheries Service, through its Northeast Fishing Industry Grants Program, is contributing to the development of the industry. A grant to AquaFuture, Inc. will bring about both a hatchery industry (joint venture of AquaFuture and GreatBay Aquafarms) and a grow-out industry. Mariculture Technologies, Inc. is independently establishing a summer flounder facility in eastern Long Island.

Bengtson, D.A., G. Bisbal, H. Iken and R.P. Athanas. 1994. Culture of summer flounder *Paralichthys dentatus*: Research on hatchery and growout phases. J. Shellfish. Res. 13:312.

Three years of research on summer flounders has answered several questions about their culture and identified some remaining problem areas.

Berge, G.M., A. Krogdahl, Ø. Stroemsnes, A. Groenseth, P. Myhre and E. Austreng. 1991. Digestibility determination in Atlantic halibut (*Hippoglossus hippoglossus*). Fiskeridir. Skr. (Ernaering), 4:117-125.

Experiments were carried out with Atlantic halibut to adapt the digestibility assay with chromic oxide as an indigestible indicator for digestibility studies. Manual stripping proved to be the best method for feces collection. An experiment was carried out to assess optimal time for stripping related to last feeding. Faces were obtained at times evaluated (24, 28, 32, and 36 hours), but the best times for collection were 28 and 36 hours after feeding. Digestibility coefficients ranged from 75 to 88% for protein, from 78 to 87% for fat, and from 0 to 23% for carbohydrates.

Bergh, Ø. 1993. Bacteria with a pathogen-inhibitory activity associated with early life stages of Atlantic halibut (*Hippoglossus hippoglossus* L.). ICES Mar. Sci. Symp. 201:191.

Bacteria associated with the surface of eggs or intestinal bacteria from yolksac larvae and fry of Atlantic halibut were isolated and screened for the ability of inhibiting growth of *Vibrio* sp. A large fraction of the intestinal bacteria isolated from start-feeding larvae and fry, but none of the bacteria isolated eggs or yolksac larvae were shown to possess this pathogen-inhibiting ability. It was concluded that the composition of the intestinal flora of the larvae from the time of first-feeding plays an important part in the defense against colonization and growth of opportunistic pathogens.

Bergh, Ø. 1995. Bacteria associated with early life stages of halibut, *Hippoglossus hippoglossus* L., inhibit growth of a pathogenic *Vibrio* sp. J. Fish Dis. 18:31-40.

Bacteria capable of inhibiting growth of a pathogenic *Vibrio* sp. were isolated from the gastrointestinal tract of cultured halibut larvae during the first feeding and weaning stages. *Vibrio* inhibiting bacteria were not found among isolates from the surface of eggs or the gastrointestinal tract of yolk sac larvae. The fraction of pathogen-inhibitors among the total number of isolates ranged between 0 to 100% (first feeding) and 0 to 66% (weaning). The isolates possessed the characteristics of the *Vibrio*/*Aeromonas*-group, but only 19% were sensitive to a vibriostatic agent. Pathogen inhibition may be an important mutualistic role of the intestinal flora of early life stages of halibut.

Bergh, Ø. and A. Jelmert. 1996. Iodophor disinfection of eggs of Atlantic halibut. J. Aquat. Anim. Health, 8:135-145.

Eggs of Atlantic halibut were treated for 10 min. with various concentrations of an iodophor at various developmental stages from four hours to 12 days after fertilization. Survival of the yolk-sac larvae was recorded at 37 day after hatching; survivors were then examined for developmental abnormalities. Survival and development were affected by both the developmental stage at which eggs were treated and the concentration of disinfectant. The highest number of normal larvae were obtained when eggs were disinfected with 1% Buffodine nine days after fertilization. Disinfection increased egg and larval survival and reduced the prevalence of opaque tissue and occurrence of damaged rostral mouth.

Bergh, Ø., G.H. Hansen and R.E. Taxt. 1992. Experimental infection of eggs and yolk sac larvae of halibut,*Hippoglossus hippoglossus* L. J. Fish Dis. 15:379-391.

Scanning electron micrographs of halibut eggs with an epiflora dominated by *Flexibacter* sp., showed ulcerations colonized by large numbers of bacteria. Studies showed that expo-

sure to these bacteria caused high mortalities in the late egg, hatching, and the early yolk sac stages. Eggs exposed to three different *Vibrio* spp. showed different mortality patterns. Mortality in unifected controls was low throughout the experiment. Transmission electron microscopy showed bacteria present in the gill, heart and frontal yolk sac regions of *Vibrio*-infected larvae.

Bergh, Ø., K.E. Naas and T. Harboe. 1994. Shift in the intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding. Can. J. Fish. Aquat. Sci. 51:1899-1903.

Isolates of aerobic intestinal bacterial flora from unfed Atlantic halibut larvae revealed a population dominated by rods of *the Cytophaga-Flexibacter-Flavobacterium* group. Following the onset of feeding, the flora gradually changed toward a flora dominated by the *Vibrio-Aeromonas* group. The transition was more rapid in fish reared in water containing phytoplankton as compared with larvae reared in filtered water. No major differences in the bacterial flora of the tank water were observed.

Bergh, Ø., A.B. Skiftesvik and O.M. Roedseth. 1994. Host specificity of *Vibrio* infections in marine fish larvae. p. W-5.2, *In*: International Symposium on Aquatic Animal Health. University of California School of Veterinary Medicine, Davis, California. Inst. Mar. Res., Austevoll Aquacult. Res. Stn., Storeboee, Norway

Eggs of Atlantic halibut (*Hippoglossus hippoglossus*), turbot, (*Scophthalmus maximus*) and cod (*Gadus morhua*) were experimentally infected with three different strains of *Vibrio anguillarum* and with *V. salmonicida and V. splendidus*. Large interspecific variations in the susceptibility of the yolk sac larvae to infections by the different bacterial strains were found.

Bergh, Ø., A.B. Skiftesvik, B. Hjeltnes and O.M. Roedseth. 1994. Pathogen-host relations between bacteria and marine fish eggs and larvae. p. 92, In: 3rd International Marine Biotechnology Conference. Tromsø University, Tromsø, Norway.

Eggs of Atlantic halibut (*Hippoglossus hippoglossus*), turbot (*Scophthalmus maximus*) and cod (*Gadus morhua*) were experimentally infected with different strains of *Vibrio* spp., *Flexibacter ovolyticus and Aeromonas salmonicida salmonicida*. There were large interspecific variations in the susceptibility of eggs and larvae to infections by the different bacterial strains. Infecting turbot and halibut larvae with *A. salmonicida salmonicida* led to mortality in both species, but, in contrast to turbot, it was not possible to detect the bacterium in immunohistochemical preparations from halibut larvae.

Bergh, Ø., G.H. Hansen, A. Jelmert, A.B. Skiftesvik, R.E. Taxt, P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Bacterial diseases of eggs and yolk sac larvae of halibut (*Hippoglossus hippoglossus* L.). pp. 389-391, In: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium

In aquaculture, Atlantic halibut (*Hippoglossus hippoglossus*) experience high mortality during the early life stages. Infections by bacteria are believed to be a major cause. This study considered experimental infection, treatment, and characterization of the morphological, ultrastructural and behavioral consequences of infections at early life stages.

Berlinsky, D.L., W.V. King, T.I.J. Smith, R.D. Hamilton II., J. Holloway, Jr. and C.V. Sullivan. 1996. Induced ovulation of southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. J. World Aquacult. Soc. 27:143-152.

Implanted pellets that provide a sustained release of gonadotropin releasing hormone (GnRHa) were used to induce maturation and ovulation in southern flounder, *Paralichthys lethostigma*. Eleven of 12 females in which the ovaries contained follicles with a maximum diameter ≥500 µm, 11 ovulated for the first time within 90 h of hormone implantation. Only

1 fish with a maximum follicle diameter <500 µm ovulated within two weeks after implantation. The percentage of fertile eggs obtained ranged from 7 to 95%. It was concluded that GnRHa implants can be used to induce repeated ovulation in southern flounder.

Bertram, D.F., R.C. Chambers and W.C. Leggett. 1993. Negative correlations between larval and juvenile growth rates in winter flounder: implications of compensatory growth for variation in size-at-age. Mar. Ecol. Prog. Ser. 96:209-215.

This research addresses the hypothesis that fish that grow rapidly as larvae also grow rapidly as juveniles, using winter flounder, *Pleuronectes americanus,* reared from hatching to two months of age as a model. Successive cohorts of fish which metamorphosed on the same day were pooled and measured at weekly intervals during the early juvenile period. Growth rates of larvae and juveniles were inversely related. This resulted in compensation in size-at-age by juveniles that grew relatively slowly as larvae.

Bianchini, A., M.H.S. Santos and L.H. Poersch. 1997. Tolerance of juvenile flatfish *Paralichthys orbignyanus* to acid stress. J. World Aquacult. Soc. 28:202-204.

Tolerance of acid stress was determined for the Brazilian flatfish, *Paralichthys orbignyanus* by exposing the fish to pH levels from 4.0 to 8.0 at 23±0.8°C, 30 ppt salinity and 12L:12D photoperiod. Total mortality was observed at a pH of 4.0 but no mortality occurred within 96 hours at a pH of 5.2, though ventilation rate was higher than in controls (pH=7.8-8.2). When pH was above 6.0, there was no mortality and ventilation rates did not differ from controls. It was concluded that the species is relatively tolerant to acid stress.

Biering, E, F. Nilsen, O.M. Roedseth and J. Glette. 1994. Challenge of Atlantic halibut with infectious pancreatic necrosisvirus (IPNV). p. P-3, *In*: International Symposium on Aquatic Animal Health. University of California School of Veterinary Medicine, Davis.

Bacterial infections, in addition to infections with infectious pancreatic necrosis virus (IPNV), are believed to be a major cause of early life mortality in cultured Atlantic halibut. In Norway, IPNV has been isolated regularly, often in connection to acute mortalities during weaning. In this study, halibut fry challenged at 15°C experienced significantly higher cumulative mortality than unchallenged fry. Moribund and diseased fry showed clinical signs such as distended stomach and uncoordinated swimming. Pathological findings included necrosis of the liver, kidney, and intestine. Immunohistochemistry revealed a strong positive reaction to IPNV in the liver of challenged individuals.

Biering, E, F. Nilsen, O.M. Rodseth and J. Glette. 1994. Susceptibility of Atlantic halibut *Hippoglossus hippoglossus* to infectious pancreatic necrosis virus. Dis. Aquat. Org. 20:183-190.

Infectious pancreatic necrosis virus (IPNV), isolated from Atlantic halibut was used to challenge various sized fry of the same species at 12 and 15°C. Fry challenged at 15°C experienced significantly higher cumulative mortality than unchallenged fry. Mortality was significantly higher in the smallest fry (mean weight 0.1 g) compared with the controls when infected at 12°C. Medium-sized fry (mean weight 1.0 g) did not display any mortality when infected at 12°C. The largest fry (mean weight 3.5 g) were only challenged at 15°C, at which temperature they showed 30% cumulative mortality. All fry infected at 15°C and the small fry infected at 12°C remained IPNV positive during the entire experimental period. In contrast, medium-sized fry infected at the lower temperature seemed able to clear the infection after three weeks. Moribund and diseased fry showed clinical signs such as distended stomachs and uncoordinated swimming. Necrosis of the liver, kidney and intestine were found, but the pancreatic tissue was unaffected. Immunohistochemistry revealed strong positive reactions to IPNV in the livers of challenged individuals.

Billard, R., J. Cosson and L.W. Crim. 1993. Motility of fresh and aged halibut sperm. Aquat. Living Resour. 6:67-75.

Studies of freshly collected Atlantic halibut (*Hippoglossus hippoglossus*) sperm by light microscopy indicated that sperm motility was optimized under osmotic pressures ranging between 400 and 1,100 mOsmol/kg and pH from 6.5 to 8.5. When stored on ice, sperm quality deteriorated rapidly within a few hours. Atlantic halibut sperm could be successfully preserved frozen when diluted with 3 parts extender (sucrose: 150 mM, $CaCl_2$: 1.7 mM, $MgSO_4$: 7 mM, glycine: 86 mM, and Tris: 30 mM at pH: 8), 10% of 1-2 propanediol and one part sperm (3:1).

Bisbal, GA. and D.A. Bengtson. 1993. Reversed asymmetry in laboratory-reared summer flounder. Prog. Fish-Cult. 55:106-108.

Eggs from laboratory-spawned summer flounder (*Paralichthys dentatus*) were hatched, and larvae were reared through metamorphosis in captivity. A high incidence of reversed asymmetry (4.4% of metamorphosed fish) was observed (only two cases of reversal in this species had been reported previously).

Bisbal, G.A. and D.A. Bengtson. 1995. Description of the starving condition in summer flounder, *Paralichthys dentatus*, early life history stages. Fish. Bull. 93:217-230.

The nutritional status of laboratory-reared summer flounder, *Paralichthys dentatus*, larvae and early juveniles after food deprivation was imposed on 6, 16, and 33-day-old larvae as well as on 60-day-old juveniles. In general, tolerance to starvation increased with age: 60 hours in 6- day-old larvae, 72 hours in 16-day-old larvae, 8 days in 33-day-old larvae, and 10 days in 60-day-old juveniles. The results demonstrates that morphological criteria are either not good indicators of nutritional status (eye:head ratio), good only for larvae (pectoral angle), or require extensive calibration (standard length and dry weight). It was also demonstarted that biochemical criteria are either not good indicators (protein content) or are sensitive to starvation only in juveniles (RNA:DNA ratio). Among the histological criteria, thickness of the posterior intestinal mucosa was the most sensitive and consistent indicator of starvation in summer flounder larvae and early juveniles.

Bisbal, G.A. and D.A. Bengtson. 1995. Effects of delayed feeding on survival and growth of summer flounder *Paralichthys dentatus* larvae. Mar. Ecol. Prog. Ser. 121:301-306.

Survival, growth and starvation times were studied in summer flounder *Paralichthys dentatus* larvae hatched in the laboratory at 12.5 and 21°C and maintained from hatching throughout the period of feeding on rotifers. Survival and growth were strongly dependent on water temperature and delay of the initial feeding. At either temperature, the percentage of summer flounder larvae surviving beyond the rotifer phase increased if food was made available at the time of mouth opening. At 12.5°C, hatching started 85 hours after fertilization. Feeding delays resulted in two separate periods of mortality. The point of no return ranged from 11 to 12 days after hatching. Larvae fed at mouth opening showed a maximum survival of 40%. At 21°C, hatching started 60 h after fertilization. Larvae fed at mouth opening showed 90% survival and significant growth in 10 days. A delay of 48 h in initial feeding led to a final survival of 36%, though significant growth was observed. Time to the point of no return was 6 to 7 days from hatching.

Bisbal, G.A., D.A. Bengtson, P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Characterization of starved versus fed summer flounder, *Paralichthys dentatus*, larvae and juveniles. p. 216-218, In: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium

This study was conducted to characterize summer flounder larvae and juveniles subjected to conditions of starvation or normal feeding. Methods of characterization included biochemical, morphometric, and histological criteria. The biochemical results were reported in this presentation.

Bisbal, G.A., D.A. Bengtson, P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Effect of dietary (n-3) HUFA enrichment on survival and growth of summer flounder, *Paralichthys dentatus*, larvae. pp. 56-57, In: LARVI '91, Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

Three experimental emulsions containing low, medium and high levels of highly unsaturated fatty acids (HUFAs) were fed to *Paralichthys dentatus* larvae for 45 days. Growth and survival of the fish in each treatment were determined.

Bjœrnsson, B. 1993. Optimal temperature of immature halibut (*Hippoglossus hippoglossus* L.): Effects of size. ICES Council Meeting Papers, Copenhagen, Denmark. 16 p.

In one experiment six groups of Atlantic halibut (initial mean weight 8 g) were reared at 7.3, 10.0 and 12.8°C, two groups at each temperature. In a second experiment six groups of 140 g initial mean weight were reared for 126 days at 5.0, 7.4, 9.0, 11.1, 13.0 and 14.9°C. A third experiment, lasting 189 days, exposed six groups averaging 2.9 kg to 2.4, 4.6, 7.0, 9.8, 12.6 and 15.1°C. Dry feed was used in the first two experiments and capelin (*Mallotus villosus*) and chopped herring (*Clupea harengus*) in the third. The optimal temperature for growth decreased with fish size. It was about 13°C for 26 g fish, 11.4°C for 280 g fish and 9.7°C for 3.4 kg fish. Optimal temperature for feed conversion decreased with fish size. It was about 13°C for 26 g fish, 10.6°C for 280 g fish and 5.5°C for 3.4 kg fish.

Bjœrnsson, B. 1994. Effects of stocking density on growth rate of halibut (*Hippoglossus hippoglossus* L.) reared in large circular tanks for three years. Aquaculture, 123:259-270.

To estimate optimal stocking density for halibut, two size-classes of halibut (initial mean weight 1.8 and 3.2 kg) were stocked at three different densities, 11, 22 and 33 kg/m^2, and reared in six 8 m circular tanks for three years at 7°C. The fish were fed frozen fish capelin and herring. Average stocking densities for the two size-classes were 18, 43 and 63 kg/m^2 corresponding to 50, 100 and 160% coverage of the tank bottom by fish. The maximum observed stocking density, 95 kg/m^2, corresponded to 215% coverage. There was no significant difference in growth rate between the two size-classes. Growth rate was significantly lower at the highest stocking density, but there was no significant difference between the groups at intermediate and low density. It was concluded that stocking density affects growth rate of halibut when >100% of the tank bottom is covered with fish. The results indicate that the optimal stocking density is somewhere between one and two layers of fish on the tank bottom.

Bjœrnsson, B. 1995. The growth pattern and sexual maturation of Atlantic halibut (*Hippoglossus hippoglossus* L.) reared in large tanks for 3 years. Aquaculture, 138:281-290.

The weight gain of 180 Atlantic halibut (88 females and 92 males) was followed for three years at 7°C. Two size-classes of young halibut (mean weight 1.8 and 3.2 kg) were stocked at two densities (11 and 22 kg/m^2) and fed frozen capelin and herring to satiation 6 days a week. The males became sexually mature at an average weight of 3.2 kg and the females at an average weight of 12.7 kg. In general, mean weight of both sexes increased linearly with time. Deviations from linear growth were related to the onset of sexual maturation.

Bjœrnsson, B. and S.V. Tryggvadottir. 1996. Effects of size on optimal temperature

for growth and growth efficiency of immature Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture, 142:33-42.

A study to determine the optimal temperature for growth of different size classes of immature Atlantic halibut (*Hippoglossus hippoglossus*) was conducted. A 99-day experiment employed fish initially 8 g reared at 7.3, 10.0 and 12.8°C; a 216 day study with 140 g fish reared at 5.0, 7.4, 9.0, 11.1, 13.0 and 14.9° C; a 189 day study with 2.9 kg fish maintained at 2.4, 4.6, 7.0, 9.8, 12.6 and 15.1°C. The halibut of the smaller two sizes were fed dry feed while the largest group were fed capelin and herring. In all three experiments growth rate and growth efficiency peaked at intermediate temperatures. Optimal temperature for growth decreased with increasing fish size. It was about 14°C for 10 to 60 g fish, 11.4°C for 100 to 500 g fish and 9.7°C for 3 to 5 kg fish. Optimal temperature for growth efficiency also decreased with increasing fish size. It was being approximately 14°C, 10.6°C and 5.5°C for the three groups.

Bjœrnsson, B., G. Sigurthorsson, Ø. Lie and G-I Hemre. 1991. Growth rate and food conversion of young halibut (*Hippolossus hippoglossus* L.) fed six different diets. ICES Council Meeting papers, ICES, Copenhagen, Denmark. 20 p.

Six groups of Atlantic halibut (*Hippoglossus hippoglossus*) of initial weight 2.5 kg were fed either lean capelin (*Mallotus villosus*), fat capelin, lean and fat capelin (3:3 days per week), moist feed from capelin silage, dry salmon feed (12 mm extruded feed), or moist feed from ground capelin. Over the 570-day experiment, the fish were weighed every three months. Most of the males became mature in the fall at a mean of ~4 kg. Between 19 September and 31 May, there was virtually no weight gain of the mature males. For the first three months of the experiment the growth rate of the groups on the capelin silage diet and the dry salmon diet were substantially lower than those on the other diets. Later on those two groups were able to compensate to some extent for the initially slow growth.

Bondad-Reantaso, M.G., K. Ogawa, T. Yoshinaga and H. Wakabayashi. 1994. Benedenild (Monogenea: Capsalidae) infection of Japanese marine fish. p. P-51, *In*: International Symposium on Aquatic Animal Health: Program and Abstracts. Univ. California School of Veterinaryu Medicine, Davis.

Benedenia epinepheli, an indigenous parasite, has been found to occur in mixed infection *with Neobenedenia girellae* and in 25 host fish species, including Japanese flounders. *N. girellae* was introduced to Japanese fish through infected amberjack fry imported from Hong Kong and China. Protection of the Japanese flounder *Paralichthys olivaceus* to challenge infections with *N. girellae* was observed.

Bouza, C., L. Sanchez and P. Martinez. 1997. Gene diversity analysis in natural populations and cultured stocks of turbot (*Scophthalmus maximus* L.). Anim. Gen. 28:28-36.

The genetic variability of eight cultured and three natural populations of turbot was studied by electrophoretic analysis of 35 enzymatic loci. The results showed low genetic variability in natural populations of turbot in comparison with other flatfish species. The high level of genetic similarity among the natural populations studied indicates high rates of gene flow. The hatchery stocks showed less genetic variation than the wild populations analyzed, which suggests genetic drift phenomena involved in the foundation and management of broodstocks. That fact and the heterozygosity differences detected among the hatchery stocks supports the existence of inbreeding depression in turbot culture.

Bricknell, I.R., D.W. Bruno, T.J. Bowden and P. Smith. 1996. Fat cell necrosis syndrome in Atlantic halibut, *Hippoglossus hippoglossus* L. Aquaculture, 144:65-69.

This paper reports on a progressive degenerative condition of the dorsal subdermal fat deposits in farmed Atlantic halibut. The problem has been termed fat cell necrosis syndrome. The disease may be related to an imbalance between dietary oxidants and antioxidants combined with an exposure to sunlight. It does not appear to have an infectious or malignant etiology.

Brock, J.A., B.R. LeaMaster and C-S Lee. 1993. An overview of pathogens and diseases in marine finfish hatcheries in Hawaii with comments on strategies for health management and disease prevention. pp. 211-238, *In*: C-S Lee, S.M. Su, and I. Liao (Eds.). Proceedings of Finfish Hatchery in Asia '91. Tungkang Marine Laboratory, TFRI, Keelung, Taiwan.

Japanese flounder (*Paralichthys olivaceus*) is one of at least eight marine fish species being developed for aquaculture in Hawaii. Infectious diseases or syndromes include bacterial, protozoal and monogenetic trematodes as etiologic agents. Bacteria identified from moribund fry and juveniles include *Pasteurella* sp., *Vibrio* spp., *Aeromonas* sp., *Chromobacterium* sp., *Flavobacterium* sp., *Pseudomonas* sp. and an uncharacterized myxobacteria-like agent. Ectoparasitic protozoa affecting fry through the juvenile stages include *Cryptpcaryon* sp., *Ichthyobodo* sp., *Amyloodinium* sp. and *Scyphidia* sp. Viruses or mycotic agents have yet to be identified from disease epizootics.

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Bromage, N., R. Shields, M. Gillespie and R. Johnstone. 1994. U.K. mariculture: Experiences and prospects. pp. 30-32, *In*: T. Nosho and K. Freeman (Eds.). Proceedings, Marine Fish Culture & Enhancement Conference. Washington Sea Grant Program, Seattle, Washington.

Development in the culture of such non-salmonid marine species as Atlantic halibut has been constrained by a lack of information about broodstock management, difficulties associated with captive spawning and stripping, and much poorer survival the resultant eggs and larvae compared with Atlantic salmon. Many questions relating to optimum conditions and facilities required for egg incubation, larval rearing and growout to market size need to be addressed. Water temperature is an important factor.Turbot and Dover sole requiring much higher ambient temperatures for optimum growth than do halibut.

Bromage, N., M. Bruce, N. Basavaraja, K. Rana, R. Shields, R, C. Young, J.Dye, P. Smith, M. Gillespie and J. Gamble. 1991. Egg quality determinants in finfish: The role of overripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. J. World Aquacult. Soc. 25:13-21.

For the Atlantic halibut, *Hippoglossus hippoglossus*, fertilization rate and assessments of cell symmetry at early cleavage stages provide reasonable indicators of egg quality. Regardless of assessment method, it is strongly recommended that performance data from all batches of each broodfish be examined when surveying the overall quality of a stock.

Brown, N.P., N.R. Bromage and R.J. Shields. 1995. The effect of spawning temperature on egg viability in the Atlantic halibut, (*Hippoglossus hippoglosus*). p.181, *In*: F.W. Goetz and P. Thomas (Eds.). Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish. University of Texas at Austin, Austin, Texas.

A study was conducted to determine whether temperature control through water chilling would be effective in improving Atlantic halibut egg viability.

Bruce, M.P., R.J. Shields, M.V. Bell and N.R. Bromage. 1993. Lipid class and fatty acid composition of eggs of Atlantic halibut, *Hippoglossus hippoglossus* (L.), in relation to egg quality in captive broodstock. Aquacult. Fish. Manage. 24:417-422.

Lipid class and fatty acid analyses were carried out on eight batches of pre-fertilized eggs of Atlantic halibut. The egg batches were classified as "viable" or "non-viable" according to fertilization rate, blastomere symmetry and incubation success. The lipid class and fatty acid compositions were very similar between egg categories with the exception of cholesterol, which was shown to be significantly higher in the "non-viable" eggs. Polar lipid classes predominated (67.1% and 67.0% of the total lipid for "viable" and "non-viable" egg batches), with phosphatidylcholine being the major class (43.2% and 43.5%) and triacylglycerol the main neutral lipid class (18.8% and 17.8%). There were no significant differences in fatty acid composition between the egg groups. In viable egg batches there were five major fatty acids 16:0 (17.3%), 18:0 (4.8%), 18:1n-9 (9%), 20:5n-3 (11.9%) and 22:6n-3 (25.9%).

Buckley, L.J., A.S. Smigielski, T.A. Halavik, E.M. Caldarone, B.R. Burns and G.C. Laurence. 1991. Winter flounder *Pseudopleuronectes americanus* reproductive success. 1. Among-location variability in size and survival of larvae reared in the laboratory. Mar. Ecol. Prog. Ser. 74:117-124.

In 1987, the average size of winter flounder (*Pseudopleuronectes americanus*) yolk-sac larvae collected from various sampling sites in Long Island Sound, New York and Narragansett Bay, Rhode Island, varied widely among locations. A direct correlation was found between size of yolk-sac larvae and survival for the first month of life. Fish from Naragansett Bay produced the smallest larvae and exhibited the lowest survival rate. One of the Long Island Sound sites produced the largest yolk-sac larvae with the highest survival rate. Dry weight and RNA content were the best predictors of survival potential among the variables protein, DNA, or lipid content; and RNA/DNA ratio.

Buckley, L.J., A.S. Smigielski, T.A. Halavik, E.M. Caldarone, B.R. Burns and G.C. Laurence. 1991. Winter flounder *Pseudopleuronectes americanus* reproductive success. 2. Effects of spawning time and female size on size, composition and viability of eggs and larvae. Mar. Ecol. Prog. Ser. 74:125-135.

Studies on embryo and larval viability conducted with winter flounder *Pseudopleuronectes americanus* spawning in Narragansett Bay, Rhode Island. Larvae produced from eggs obtained by stripping adults collected at various times during the spawning season were reared through the first month of life. Female size affected including both absolute and relative measures of total reproductive output, egg size, fecundity, and viability. Spawning time affected egg size, fecundity, and viability, but not reproductive rate or gonadosomatic index. Egg size increased with increasing female size and decreased as the spawning season progressed.

Burton, D. and B.A. Everard. 1991. A comparison of the effect of captivity on the epidermis of prespawning and postspawned winter flounder, *Pseudopleuronectes americanus*. J. Zool. 223:1-7.

Near the end of the prespawning period, captive male winter flounder had a higher gonadosomatic index than wild winter flounder, and the seasonal epidermal sexual dimorphism in captive flounder was more pronounced. A thick epidermis was observed in flounder maintained in the laboratory during the postspawning period. Those fish also showed increased mucigenesis in late summer, while wild flounders collected inshore displayed seasonally low values for those characteristics.

Burton, D. and B.A. Everard. 1991. The effect of androgen treatment on the epidermis of post-spawned winter flounder, *Pseudopleuronectes americanus* (Walbaum). J. Fish Biol. 38:73-80.

This study examined the effect of androgen treatment on the epidermis of post-spawned winter flounder, *Pseudopleuronectes americanus* collected from nature and held in captivity. Control flounders displayed epidermal thickening and increased goblet cell frequencies associated with captivity. Males treated with 11-ketotestosterone developed significantly more differential thickening of the blind-side epidermis compared with controls. Testosterone treatment did not induce additional thickening of male epidermis, but did increase differential thickening on the blind side of females.

Burton, M.P.M. 1991. Induction and reversal of the non-reproductive state in winter flounder, *Pseudopleuronectes americanus* Walbaum, by manipulating food availability. Fish Biol. 39:909-910.

Reversal of the non-reproductive state of winter flounders was achieved by improved feeding.

Burton, M.P. and D.R. Idler. 1984. The reproductive cycle in winter flounder, *Pseudopleuronectes americanus* (Walbaum). Can. J. Zool. 62:2563-2567.

Pseudopleuronectes americanus spawn in May and June, but sperm can be activated in January, and male gonadosomatic indices in November were found to be approximately twice those recorded immediately before spawning. Some post-spawning females do not enter the vitellogenic stage but overwinter with two year classes of immature oocytes. This nonreproductive phase in females in related to condition rather than age.

Caddell, S.M., D.M. Gadomski and L.R. Abbott. 1989. Induced spawning of the California halibut, *Paralichthys californicus*, (Pisces: Paralichthydae) under artificial and natural conditions. Fish Bull. Calif. Dept. Fish Game, 174:175-198.

Broodstock of California halibut, *Paralichthys californicus*, were placed under natural (outdoor) and artificially simulated (indoor) environmental conditions. Fish in natural situations produced 27 spawns from February through May 1986; five spawns from March through April 1987; and 64 spawns from late March through September 1988. Since tanks held multiple females, the number of fish participating per spawning incident was unknown. Diel estimates of fertilization time were made from comparisons with egg development studies that have been previously conducted.

Chambers, R.C. and W.C. Leggett. 1987. Size and age at metamorphosis in marine fishes: An analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can. J. Fish. Aquat. Sci. 44:1936-1947.

Offspring of adult winter flounder (*Pseudopleuronectes americanus*) collected from nature in Newfoundland were reared from fertilization to metamorphosis. Length at metamorphosis was significantly less variable than age at metamorphosis.

Chambers, R.C. and W.C. Leggett. 1989. Parental influence on early life history traits of winter flounder (*Pseudopleuronectes americanus*). p. 477, *In*: J.H.S. Blaxter, J.C. Gamble, and H. vonWesternhagen (Eds.). The early life history of fish. Rapp. P-V. Reun. Ciem. 191.

Winter flounder (*Pseudopleuronectes americanus*) were reared in the laboratory from fertilization through metamorphosis. Forty-five percent of the variation in yolk diameter was attributable to differences among adult females. The influence of parentage on length-at-age of progeny during the larval period decreased with the age of progeny. Length at metamorphosis was significantly affected by parentage, though age at metamorphosis was not. Variation in developmental rate to metamorphosis appeared to be largely under environmental control. Both age at hatching and age at starvation appeared to be related to male parentage. One experiment showed a significant male-by-temperature interaction on age at hatching, though the contribution of temperature to variation in age at hatching was far greater than male effects.

Chang, Y.J. and S.K. Yoo. 1989. Rearing density of flounder, *Paralichthys olivaceus* juveniles in a closed recirculating sea water system — possibility of high-density rearing. Cont. Inst. Mar. Sci. Natl. Fish. Univ. Pusan, 21:27-38.

Japanese flounder, *Paralichthys olivaceus*, juveniles (2.53± 0.24 cm total length and

1.12± 0.12 cm body height) were reared at densities of 10, 20, 30, and 40 individuals per 137.75 cm^2 of bottom area.

Chang, Y.J., S.H. Kim and H.S. Yang. 1995. Culture of the olive flounder (*Paralichthys olivaceus*) in a semi-closed recirculating seawater system. J. Korean Fish. Soc. 28:457-468.

The conclusion reached in this study was that commercial culture of Japanese flounder is more effective in a the semi-closed recirculating seawater system than in a flow-through tank system with respect to fish productivity and protection of the marine environment.

Cho, K.C., J.H. Kim, C.S. Go, Y. Kim and K-K Kim. 1995. A study on seedling production of the spotted flounder, *Verasper variegatus*. Bull. Natl. Fish. Res. Dev. Agency, 50:41-57.

Eggs of the spotted flounder, *Verasper variegatus,*, averaged 1.97 mm in diameter and floated. Thy hatched about 91 hours after fertilization. Newly hatched larvae averaged 4.4 mm in total length and possessed a yolk sac that was completely absorbed the sixth day after hatching. The larvae reached a mean length of 15.5 mm 32 days after hatching at which time metamorphosis was complete. Growth and survival rate of larvae cultured at 15.7°C were better than at 13.2°C. Larvae reared in sea water containing $5x10^5$ cells/ml of *Chlorella* and with a rotifer concentration of 20/ml had the best growth and survival compared to larvae reared with the other concentrations of *Chlorella* and rotifers. Juveniles maintained for 8 months after hatching averaged 14.0 cm total length and weighed an average of 42.5 g.

Cho, Y.C. and S.G. Yang. 1991. Study on the early spawning inducement of bastard halibut, *Paralichthys olivaceus*. Bull. Natl. Fish. Res. Dev. Agency, 45:183-195.

Bastard halibut, or Japanese flounder (*Paralichthys olivaceus*) to determine if early spawning could be developed in two and three year old adults reared in tanks from April 1989 to April 1990. The sea water temperature range was 12.7 to 25.7°C during the experiment. Light was controlled with an incandesent bulb (60W) that provided 30 lux at the center of rearing tank. The fish matured after exposure to a daily light cycle 11.5:12.5 until the middle of October and 13:11 until November 10, and began spawning in a light cycle of 13.5:10.5 on November 13, 1989. The total number of eggs deposited by two year old fish was 50,905,000 over 143 days. For three-year-old fish the number of eggs obtained was 123,400,000 over 149 days.

Choe, J-Y. and T. Yagi. 1993. Nichi multiplied by Kan hirame yoshoku keiei no hikaku. (A comparative study of Japanese flounder culture business between Korea and Japan.). Bull. Fac. Fish. Nagasaki Univ. 73:27-37.

This study shows that Japanese flounder production represents a significant portion of Korea's total aquaculture production, while the contribution of Japanese flounder to total aquaculture production in Japan is relatively low. The paper examines the major factors that affect the profitability of Japanese flounder culture business in Korea.

Choi, H.S. 1991. Study on *Edwardsiella tarda* isolated from cultured bastard halibut (*Paralichthys olivaceus*). Bull. Natl. Fish. Res. Dev. Agency, 45:197-205.

From August to September 1988, an outbreak of bacterial disease occurred among Japanese flounder, *Paralichthys olivaceus* in Tolsan, Yochon, Korea. The diseased fish showed abdominal inflamation, accumulation of hemorrhagic ascites, and greyish white spots in the liver. *Edwardsiella tarda* was isolated from infected fish. Pathogenicity of the isolate to flounder, yellowtail and red seabream was demonstrated after intraperitoneally injected. The bacterial strains examined were sensitive to tetracycline, oxytetracycline, oxolinic acid and doxycycline but resistant to chloramphenicol, amphicillin and erythromycin.

Clearwater, S.J. and L.W. Crim. 1995. Milt quality and quantity produced by yellowtail flounder (*Pleuronectes ferrugineus*) following GnRH-analogue treatment by microspheres or pellet. p. 113, *In*: F.W. Goetz and P. Thomas (Eds.). Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, Texas.

GnRH-a treatment of yellowtail flounder (*Pleuronectes ferrugineus*) males results in a stimulation of milt volume without a negative effect on sperm quality. Both microspheres and 100% cholesterol pellets increase sperm volume, with a trend toward a longer term response in the males treated with a higher dosage pellet (197 mu g/kg).

Crawford, C.M. 1984. Preliminary results of experiments on the rearing of Tasmanian flounders, *Rhombosolea tapirina* and *Ammotretis rostratus*. Aquaculture, 42:75-81.

Two Tasmanian species of flounder were successfully cultured following hormone-induced ovulation, stripping and egg fertilization. Survival rates of larvae fed rotifers followed by *Artemia* nauplii were as high as 94-98% for *R. tapirina* and 65% for *A. rostratus* larvae from first-feeding to metamorphosis.

Daniel, E.S., C.C. Parrish, D.C. Somerton and J.A. Brown. 1993. Lipids in eggs from first-time and repeat spawning Atlantic halibut, *Hippoglossus hippoglossus* (L.). Aquacult. Fish. Manage. 24:187-191.

The lipid contents of unfertilized eggs from first-time and repeat spawning Atlantic halibut females were compared. Egg lipid content for a first-time spawner ranged from 0.18 to 0.41 mg/egg, while the range for the repeat spawner was 0.27 to 0.38 mg/egg. Mean total lipid content values per egg were similar for both fish at ~0.31 mg/egg. The repeat spawner produced more eggs than the first-time spawner, thus allocating more lipid to egg production. In both cases, between 62 and 84% of the lipids was phospholipid.

Daniels, H.V., D.L. Berlinsky, R.G. Hodson and C.V. Sullivan. 1996. Effects of stocking density, salinity, and light intensity on growth and survival of southern flounder *Paralichthys lethostigma* larvae. J. World Aquacult. Soc. 27:153-159.

Experiments were conducted with southern flounder, *Paralichthys lethostigma*, larvae during first feeding and metamorphosis to determine the effects of stocking density, salinity and light intensity on growth and survival. In one study, stocking densities of 10, 20, 40, and 80 fish/l were evaluated during first feeding. Growth and survival (overall 6.9%) were not significantly different for stocking rates up to 80/l. In a study comparing the growth and survival of larvae stocked at 20 and 33 ppt salinity, survival through first feeding was similar. A third experiment evaluated stocking larvae at densities of 1/l and 3/l under two different light intensities (1,600 lux vs 340 lux) during metamorphosis. Light intensity had no effect on growth or survival, but fish stocked at 3/l had significantly lower survival than fish at 1/l. The fourth experiment tested the effects of different salinities (0, 10, 20 and 30 ppt) on larval growth and survival during metamorphosis. Complete mortality occurred at 0 ppt. Growth and survival past metamorphosis were not significantly different at 10, 20, and 30 ppt, but unmetamorphosed fish did not survive to day 60 at 10 ppt. Larviculture of southern flounder may require a two-step process with high stocking rates (80 fish/l) through first feeding and lower densities (1/l) through metamorphosis. Fingerling production in fertilized nursery ponds might be possible at salinities as low as 20 ppt.

Davenport, J., E. Kjoersvik and T. Haug. 1990. Appetite, gut transit, oxygen uptake and nitrogen excretion in captive Atlantic halibut, *Hippoglossus hippoglossus* L., and lemon sole, *Microstomus kitt* (Walbaum). Aquaculture, 90:267-277.

Atlantic halibut, *Hippoglossus hippoglossus*, eat larger satiation meals (mean 11.7% body weight) than lemon sole, *Microstomus kitt* (2.6% body weight). Total gut clearance time was about 120 h for halibut and 72 h for lemon sole. Halibut feed in midwater and require several body lengths of approach swimming before taking large items of food, while lemon sole eat only off the bottom. The two species can be stocked in the same tank without exhibiting aggressiveness. A duoculture system holding small numbers of lemon sole with the more valuable halibut is recommended as a means of minimizing food waste and tank fouling. Oxygen uptakes of 0.07 to 0.11 ml O_2/g/h (depending on nutritional state) were recorded for the two species. Starved halibut excreted 2.32 µg N/g/h and fed animals excreted 5.08 µg N/g/h. The corresponding values for lemon sole were 3.26 µg N/g/h and 6.37 µg N/g/h, respectively.

Dedi, J., T. Takeuchi, T. Seikai and T. Watanabe. 1995. Hypervitaminosis and safe levels of vitamin A for larval flounder (*Paralichthys olivaceus*) fed *Artemia* nauplii. Aquaculture, 133:135-146.

Ten-day-old larval Japanese flounder fed the Tien-tsin strain of *Artemia* enriched in a medium containing 0, 20, 40, 60, , or 100 mg vitamin A palmitate (1 µg vitamin A palmitate = 1 IU of the vitamin). The enriched *Artemia* were offered to flounder larvae thorugh metamorphosis, after which the fish were fed an artificial diet containing 25 IU vitamin A/g. Feeding *Artemia* enriched with more than 40 mg vitamin A palmitate produced a negative effect on growth of fish accompanied by a high incidence of bone deformity. Fish survival was not affected by elevated vitamin A levels in *Artemia*. Nearly all the fish showed normal body coloration. The safe level of vitamin A in *Artemia* nauplii was estimated to be less than 50 IU/g on a dry weight basis (20 mg vitamin A palmitate in a 10-liter medium).

Devresse, B., Ph. Leger, P. Sorgeloos, O. Murata, T. Nasu, S. Ikeda, J.R. Rainuzzo, K.I. Reitan, E. Kjorsvik and Y. Olsen. 1994. Improvement of flat fish pigmentation through the use of DHA-enriched rotifers and *Artemia*. Aquaculture 124:298-288.

Two separate experiments were carried out on Japanese flounder (*Paralichthys olivaceus*) and European turbot (*Scophthalmus maximus*). First-feeding larvae were fed enriched *Brachionus plicatilis* and *Artemia* nauplii. Various experimental or commercial emulsions (with different n-3 highly unsaturated fatty acid (n-3 HUFA) contents and docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA ratios) were used: (1) control (no enrichment), (2) emulsion prepared with lipids extracted from halibut (*Hipoglossus hippoglossus*) roe, (3) emulsion prepared with lipids extracted from the copepod *Acartia tonsa*, (4) marine and super *Artemia* (Higashimaru Inc., Japan)., (5) Super Selco, and (6) High DHA Super Selco (*Artemia* Systems, Belgium). Treatments were evaluated over 50 days with Japanese flounder. Treatment 6 produced highest survival (21.5%), followed by 12.3% for treatment 4, and 1.8% for the control (treatment 1). No differences were observed in growth (length in mm) but pigmentation at day 40 was affected by treatment (92.6% pigmented larvae for treatment 6 and 76.2% for treatment 4). Turbot larvae showed no difference in survival after 27 days when reared with treatments 2, 3, 5 and 6. However, pigmentation rate was increased to 68.2% fully pigmented fish in conjunction with treatment 6. Pigmentation rates for the other treatments were 57.1, 38.8 and 30.5% for treatments 2, 3 and 5, respectively. Pigmentation does not seem to be related to the n-3 HUFA content, but rather to the DHA/EPA ratio.

Dojiri, M. and R.A. Brantley. 1991. *Lepeophtheirus spatha*, a new species of copepod (Siphonostomatoida: Caligidae) parasitic on the California halibut from Santa Monica Bay, California. Proc. Biol. Soc. Wash. 104:727-735.

Lepeophtheirus spatha, a new species, is described from the external body surfaces of the California halibut *Paralichthys californicus* collected in Santa Monica Bay, California. The parasite appears to be most closely related to *L. paralichthydis*, which was collected from the body surfaces of *Paralichthys olivaceus* from Japan.

Dushkina, L.A., M.V. Pereladov and L.V. Spektorova. 1987. Marikul'tura v sotsialisticheskikh stranakh. [Marine aquaculture in socialist countries.] Rybn. Khoz. 8:47-49.

Discusses aquaculture development in Bulgaria, the German Democratic Republic, Romania and the USSR (this was published before German reunification and the dismantling of the USSR). Mariculture in Bulgaria was based on the rearing of mussels (*Mytilus* spp.), grey mullet (*Mugil cephalus*), and "Black Sea flounder" (Pleuronectiformes). Flatfish culture was not mentioned with regard to the other nations covered in the paper.

Evans, R.P., C.C. Parrish, J.A. Brown and P.J. Davis. 1996. Biochemical composition of eggs from repeat and first-time spawning captive Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture, 139:139-149.

Eggs from a repeat and two first-time spawning Atlantic halibut (*Hippoglossus hippoglossus*) were compared for biochemical content. The results were then related to fertilization success and larval survival. Eggs from the repeat spawner averaged 81% fertilization compared with 56% for the first-time spawners. Egg dry weight decreased in all cases with subsequent batches and was significantly lower in the first-time spawners' eggs. Eggs from the first-time spawners were significantly lower in total lipid, triacylglycerol, and sterol. Those eggs also had lower levels of docosahexaenoic acid and arachidonic acid. The results indicate that repeat spawning Atlantic halibut produce biochemically different eggs from first-time spawners, which may relate to the better quality of eggs obtained from repeat spawners.

Finn, R.N. and H.J. Fyhn. 1993. Metabolic O:N ratios of developing halibut eggs (*Hippoglossus hippoglossus* L.). pp. 338-340, *In*: B.T. Walther and H.J. Fyhn, (Eds.). Physiology and Biochemical Aspects of Fish Develpoment. University of Bergen, Bergen, Norway.

Rates of O_2 uptake and NH_3 excretion followed exponential patterns in developing eggs of Atlantic halibut (*Hippoglossus hippoglossus*). No disproportional increase was observed in the O_2 uptake at hatching, but NH_3 excretion was high during the first day after hatching. Calculated daily metabolic O:N ratios decreased from after fertilization until the first day after hatching. The data suggest that developing halibut embryos utilize lipids or carbohydrates during the mid-egg stage, but convert to amino acids as the time of hatching approaches.

Finn, R.N., I. Ronnestad and H.J. Fyhn. 1995. Respiration, nitrogen and energy metabolism of developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.). Comp. Biochem. Physiol. 111A:647-671.

The present investigation quantifies the partitioning of yolk enthalpy into production of biomass, dissipation due to metabolism and losses associated with excretion of nitrogenous end products during the endogenous nutrition of developing yolk-sac larvae of Atlantic halibut.

Flores, H.,G. Ortiz and A. Silva. 1994. Water stability of moist feeds for Chilean flounder. Aquaculture, 124:287.

This study was designed to evaluate the optimum binder level of moist feeds for two species of Chilean flounders (*Paralichthys microps* and *P. adspersus*). Five moist diets were prepared with fresh fish, fish meal, fish oil and wheat flour. The binder percentages were 0, 0.5, 1.0, 1.5 and 2.0%. Two commercial salmonid diets were also used; one prepared like the other moist feeds with 2% binder, the other dry with 0.3% binder. The water content of ranged from 43.9 to 53.0% in the moist diets and was 8.3% in the dry diet. A binder product from Hoechst Laboratories, which is a water-soluble cellulose ether derivative, was dissolved in 100 ml seawater and added to the feeds. Water stability tests indicated that binder percentages more than 1.5% provide more stable moist diets for flounders and other slow-feeding fishes. Stability of these moist diets was strongly influenced by the type and quantity of fresh fish. Experience reveals that diets will be more stable if they include *Isacia conceptionis* (Pomacentridae) rather than *Trachurus symmetricus* (Carangidae). The meat of *I. conceptionis* has a lower oil content.

Fujii, T. and M. Noguchi. 1993. Interactions between released and wild Japanese flounder (*Paralichthys olivaceus*) on a nursery ground. pp. 57-65, *In*: M.R. Collie, and J.P. McVey (Eds.). Interactions between cultured species and naturally occurring species in the environment. US-Japan Aquaculture Panel Symposium 22, Homer, Alaska.

Overfishing of the Japanese flounder, *Paralichthys olivaceus*, has caused a reduction in the stock size. To enhance the stock, cultured Japanese flounder have been released. This paper describes the interactions between released and wild flounder off Igarashi-Hama on the northwestern coast of Japan from 1990 to 1992. Wild flounder growth rate varied annually depending on the abundance of mysids; the most important food for flounder on the nursery ground. When mysids were less abundant, cultured flounder dispersed rapidly from the release site, ingested small amounts of food, and grew slowly. When mysids were not abundant, released flounder consumed gammarids which the wild flounder did not eat.

Fukuda, Y., S. Matsuoka, Y Mizuno and K. Narita. 1996. *Pasteurella piscicida* infection in cultured juvenile Japanese flounder. Fish Pathol. 31:33-38.

Since 1991, mortalities have occurred in 0-year class Japanese flounder, *Paralichthys olivaceus*, (2 to 22 g) cultured in Oita and Ehime Prefectures when the water temperature ranged from 17 to 24°C. Diseased fish showed dark coloration and swam inactively. Daily and cumulative mortalities were 0.01 to 0.6% and 0.6 to 4.8% in the two Prefectures. A bacterium identified as *Pasteurella piscicida* was isolated from the kidney of diseased fish. The isolates of the bacterium were highly sensitive to most antibacterial agents tested. Oral administration of oxytetracycline hydrochloride was effective to control the epizootics.

Fukusho, K., T. Yamamoto, T. and T. Seikai. 1986. Hirame hakka kotai shutsugen ni oyobosu shiiku chu no tsukiryo no eikyo. [Influence of various amounts of aeration during larval development of hatchery-reared flounder *Paralichthys olivaceus* on the appearance of abnormal coloration.] Bull. Natl. Res. Inst. Aquacult. 10:53-56.

Juvenile Japanese flounder, *Paralichthys olivaceus*, were reared from 6.9 mm to 21-23 mm total length, in 100 l tanks fed. The fish were fed a combination of *Artemia* nauplii and rotifers, with various degrees of aeration (50 to 1000 ml/min). Differences in water agitation were apparent among treatments, but there were negligible differences in dissolved oxygen levels. No relationship was found between the occurrence of abnormal pigmentation and amount of aeration during larval flounder development.

Fukusho, K., M. Okauchi, H. Tanaka, S.I. Wahyuni, P. Kraisingdecha and T. Watanabe. Tetoraserumisu de baiyoshita shiomizutsubowamushi no hirame shigyo ni taisuru jiryokachi. [Food value of a rotifer *Brachionus plicatilis*, cultured with *Tetraselmis tetrathele* for larvae of a flounder *Paralichthys olivaceus*.] Bull. Natl. Res. Inst. Aquacult. 7:29-36.

Larval rearing of Japanese flounder (*Paralichthys olivaceus*) was conducted to evaluate the food value of a rotifer (*Brachionus plicatilis*) cultured with the alga *Tetraselmis tetrathele*. The food value of the *Tetraselmis*-fed rotifers was evaluated as high and the requirement of larval flounder for n-3 highly unsaturated fatty acids (HUFA) was estimated to be less than that of red seabream (*Pagrus major*). The fed rotifers contained about 0.3% n-3 HUFA, which may be sufficient for larval flounder. However, combination culture with *Chlorella* or secondary culture with *Chlorella* would be required to increase survival rate of fry.

Fukusho, K., H. Nanba, T. Yamamoto, Y. Yamasaki, M.T. Lee, T. Seikai and T. Wantanabe. 1987. Hirame hakka boujo no tameno madai ran no kokateki kyuujiho. [Reduction of albinism in juvenile flounder *Paralichthys olivaceus* hatchery-reared on fertilized eggs of red sea bream *Pagrus major*, and its critical stage for the effective feeding.] Bull. Natl. Res. Inst. Aquacult. 12:1-7.

The critical and effective stage of feeding to reduce albinism was investigated in Japanese flounder (*Paralichthys olivaceus*) larvae changing the initial feeding time of fertilized red sea bream larvae (*Pagrus major*) eggs. The effective period for feeding of eggs is when larvae are from 7 to 10 mm in total length. Larval flounder at about 8 mm preferred newly hatched red sea bream yolk sac to eggs. Flounder larvae began to feed on the eggs at sizes of 8.5 to 9.0 mm. The critical stage determining the appearance of albinism in hatchery-reared juvenile flounder was estimated to be the initial stage of metamorphosis (8.0-8.5 mm).

Furuta, S. 1994. Predation on juvenile Japanese flounder (*Paralichthys olivaceus*) by diurnal piscivorous fish: Field observations and laboratory experiments. pp. 295-296, *In*: Y. Watanabe, Y. Yamashita, and Y. Oozeki. (Eds.). Proceedings of an International Workshop: Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema Publ., Brookfield, Vt.

However, many fundamental problems, inhibiting further success, remain. A major biological problem with stock enhancement programs involving the mass release of hatchery-reared Japanese flounder seems to be predation on flounder juveniles soon after their release. Stomach content analysis of piscivorous fish inhabiting release areas revealed that bartail flathead (*Platycephalus indicus*) and age 1 and 2 Japanese flounder are the most significant predators on released flounder juveniles. Laboratory experiments demonstrated that hatchery-reared flounder are more vulnerable to predation than wild juveniles. Hatchery-reared juveniles exhibit longer periods of feeding in the water column than do wild fish which may make the cultured fish more susceptible to predation. Starvation of released juvenile flounders may also contribute to increased susceptibility predation.

Furuta, T., T. Iida, K. Trongvanichnam, I. Sakaguchi and H. Wakabayashi. 1995. Indirect enzyme-linked immunosorbent assay (ELISA) for the detection of antibody in serum of Japanese flounder. Fish. Sci. 61:663-667.

An indirect enzyme-linked immunosorbent assay (ELISA) was developed to detect antibody in serum of Japanese flounder against *Edwardsiella tarda*. Sensitivity of the ELISA was more than six times higher than that of formerly used agglutination methods. Flounders were vaccinated by three different methods: intraperitoneal injection and two types of immersion. The titers of two types of vaccinated groups were significantly higher than that of a control group but there were no significant differences in the survival rates between vaccinated and control groups following a challenge test.

Gadomski, D.M. and S.M. Caddell. 1991. Effects of temperature on early-life-history stages of California halibut *Paralichthys californicus* Fish. Bull. 89:567-576.

Laboratory experiments were conducted to determine how growth and survival of early life history stages of California halibut *Paralichthys californicus* are influenced by temperature, and how optimal temperature ranges may change with ontogeny. Highest survival of developing embryos occurred at increasingly higher temperature ranges. Growth and development rates of all early life history stages were proportional to temperature. Eggs hatched successfully at 12, 16 and 20°C. Death occurred prior to embryo formation at 8 and 24°C. Survival 17 days after hatching ranged from 23 to 46% at 16, 20 and 24°C, but almost all larvae died at 12°C after an initial period of high survival. Survival of 3-month-old juvenile halibut was significantly greater at 20, 24 and 28°C (57 to 76%) than at 16°C (31%). When the temperature of 1-month-old larvae was raised from 16°C to 20°C, settlement time was advanced by about one week.

Gadomski, D.M. and S.M. Caddell. 1996. Effects of temperature on the development and survival of eggs of four coastal California fishes. Fish. Bull. 94:41-48.

Eggs of California halibut, *Paralichthys californicus*, had a temperature tolerance range of 12 to 20°C. Time to hatching is inversely related to temperature, and the relationship was approximately exponential.

Gadomski, D.M., S.M. Caddell, L.R. Abbott and T.C. Caro. 1989. Growth and development of larval and juvenile California halibut, *Paralichthys californicus*, reared in the laboratory. Fish Bull. Calif. Dept. Fish Game, 174:85-98.

California halibut, *Paralichthys californicus*, were reared for two months after hatching in 300 l tanks with flow-through seawater systems. Larvae were subjected to temperatures of 16 and 20°C and low and high rotifer densities. Growth was significantly greater at the higher temperature and the high food density. Development was also faster at 20°C than 16°C, resulting in metamorphosis occurring sooner.

Garcia-Riera, M.P. and G-I Hemre. 1996. Organ responses to ^{14}C-glucose injection in Atlantic halibut, *Hippoglossus hippoglossus* (L.), acclimated to diet of varying carbohydrate content. Aquacult. Res. 27:565-571.

After adapting Atlantic halibut, *Hippoglossus hippoglossus* (L.), to three different levels of available dietary carbohydrate (22, 47 and 82 g/kg), the uptake of intraperitoneally injected glucose, both labeled and unlabeled, by the liver, white muscle, plasma, gills, heart, kidney and gastrointestinal wall, as well as concentrations of glucose in plasma and glycogen in organs, were determined. Even 16 hours after glucose injection, halibut showed hyperglycemia. There was a negative correlation between plasma glucose and liver glycogen concentrations, hepatosomatic index, and liver weight.

Gatesoupe, F-J., T. Arakawa and T. Watanabe. 1989. The effect of bacterial additives on the production rate and dietary value of rotifers as food for Japanese flounder, *Paralichthys olivaceus*. Aquaculture, 83:39-44.

Two food additives containing live lactic bacteria were provided to rotifers fed on live algae and baker's yeast. One additive improved rotifer production rate (59 rotifers/ml/day) over the control group (48 rotifers/ml/day). The second additive did not improve rotifer production but improved rotifer dietary value as demonstrated by significantly more rapid growth at 18 days than control larvae. The total amount of aerobic bacteria in rotifers was also affected by this additive.

Grace de Jesus, E., Y. Inui and T. Hirano. 1990. Cortisol enhances the stimulating action of thyroid hormones on dorsal fin-ray resorption of flounder larvae *in vitro*. Gen. Comp. Endcocrinol. 79:167-173.

While thyroid hormones and cortisol stimulated fin-ray resorption in larval flounder *in vitro*, there was no clear synergistic effect of cortisol in *an in vivo* experiment. The study concludes that a sufficient quantity of cortisol may be produced by the larvae interrenally.

Gulbrandsen, J. 1991. Functional response of Atlantic halibut larvae related to prey density and distribution. Aquaculture, 94:89-98.

The relationship between prey density and feeding success of Atlantic halibut larvae was examined in this study. One experiment was designed for the purpose of controlling zooplankton swarming, which otherwise would have complicated the interpretation of a response. Another investigated the response in numbers of prey eaten by each larva. It was concluded that rotifers (*Brachionus plicatilis*) can be prevented from horizontal patching simply by providing diffused light; hunger motivation is seemingly without effect. Brine shrimp (*Artemia* sp.), on the other hand, appear to be less well controlled. Atlantic halibut seemed to have an optimal feeding response at approximately 12 rotifers/ml.

Gulbrandsen, J. 1996. Effects of spatial distribution of light on prey ingestion of Atlantic halibut larvae. J. Fish. Biol. 48:478-483.

Low intensity concentrated light was found to produce swarms of *Artemia* sp., but did not affect the distribution of *Brachionus plicatilis*. Low intensity diffused light, however, did not create swarms with either prey. As a consequence, Atlantic halibut larvae ingested more *Brachionus* than *Artemia* in concentrated light. That result was not observed in diffused light.

Gulbrandsen, J., I. Lein and I. Holmefjord. 1996. Effects of light administration and algae on first feeding of Atlantic halibut larvae, *Hippoglossus hippoglossus* (L.). Aquacult. Res. 27:101-106.

The effects of two light regimes (low-intensity overhead light and submerged light at intermediate intensities) that independently had shown positive effects on feeding and growth of Atlantic halibut larvae were studied. An alleged beneficial effect of algae was also investigated. Four experimental regimes were established: low-intensity overhead light with and without algae (*Tetraselmis* sp.), and submerged light with and without algae. Submerged light was superior to overhead light with respect to larval growth, survival and feeding. The presence of algae improved larval growth and survival, but no effect was shown on feeding incidence. There was no interaction between the effects of algae and those of the light regime.

Ha, B-S., D-S Kang, J-H Kim, O-S Choi and H-Y Ryu. 1993. Metabolism of dietary carotenoids and effects to improve the body color of cultured flounder and red sea bream. Bull. Korean Fish. Soc. 26:91-101.

Japanese flounders, *Paralichithys olivaceus*, and sea bream were fed diets containing ß-carotene, lutein ester, astaxanthin, astaxanthin monoester, astaxanthin diester or ß-apo-8'-carotenal for eight weeks. In flounders, carotenoid deposition and pigmentation were higher in fish fed diets containing ß-carotene and lutein ester. The main carotenoids of flounders were zeaxanthin and lutein. Aifference in lutein and ß-carotene content was observed between wild and cultured flounders.

Hallaraker, H., A. Folkvord and S.O. Stefansson. 1995. Growth of juvenile halibut (*Hippoglossus hippoglossus*) related to temperature, day length and feeding regime. Neth. J. Sea Res. 34:139-147.

Extending the feeding period and/or day length did not significandy increase growth rates of juvenile Atlantic halibut, *Hippoglossus hippoglossus*, of 5 to 20 g. Growth was affected by temperature, with highest growth rates occurring at 13°C followed by 10, 16 and 7°C for juveniles of 20 to 90 g. Juvenile halibut grew approximately isometrically from 20 to 90 g. Weight-specific oxygen consumptions of 80 g juveniles were similar to rates measured for other flatfish species.

Hallaraker, H., A. Folkvord, K. Pittman and S.O. Stefansson. 1995. Growth of *Hippoglossus hippoglossus* L. related to temperature, light period, and feeding regime. ICES Mar. Sci. Symp. 201:196.

The growth potential at 7, 10, 13 and 16°C and the effects of continuous light versus natural photoperiod on juvenile Atlantic halibut were investigated. The halibut grew best at 13°C and slowest at 7°C. They also grew better at 10°C than at 16°C. Daily growth rates over 3% were observed in the 13°C group. No significant differences in growth of survival were found in the light period or feeding regime experiments.

Hansen, G.H., Ø. Bergh, J. Michaelsen and D. Knappskog. 1992. *Flexibacter ovolyticus* sp. nov., a pathogen of eggs and larvae of Atlantic halibut, *Hippoglossus hippoglossus* L. Int. J. Syst. Bacteriol. 42:451-458.

Flexibacter ovolyticus sp. nov., was isolated from the adherent bacterial epiflora of

Atlantic halibut (*Hippoglossus hippoglossus* L.) eggs and was shown to be an opportunistic pathogen on halibut eggs and larvae. A total of 35 isolates were characterized as rod shaped, gram negative, Kovacs oxidase positive, pale yellow, and exhibited gliding motility. They did not produce acid from any of the wide range of carbohydrates tested. The strains were catalase and nitrate reductase positive, did not produce H_2S, and did not grow under anaerobic conditions. *F. ovolyticus* resembles *Flexibacter maritimus*, but differs from the latter species in several biochemical and physiological characteristics.

Harboe, T. and I. Huse. 1993. Diurnal variations in feeding incidence of halibut larvae (*Hippoglossus hippoglossus*). ICES Mar. Sci. Symp. 201:196-197.

Halibut (*Hippoglossus hippoglossus*) larvae at 0, 44 and 77 degree days after first-feeding were examined for feeding incidence every two hours over a 32-hour period. The larvae, which were reared in an outdoor system (natural light conditions) consisting of 120 l black plastic bags suspended in water baths, had maximum feeding incidence at sunset and at sunrise. Feeding incidence was low during daylight hours.

Harboe, T., Huse, I. and G. Oie. 1994. Effects of egg disinfection on yolk sac and first feeding stages of halibut (*Hippoglossus hippoglossus* L.) larvae. Aquaculture, 119:157-165.

Halibut eggs were treated with the disinfectant glutaric dialdehyde at regimes, 400 ppm for 10 min or 800 ppm for 2.5 minutes and treatment effects evaluated. To elucidate the effects of disinfection on the first feeding stage, both *Artemia* and wild zooplankton were fed. No significant differences in survival or percentage of deformed larvae were found between the larval groups during the yolk-sac period. Differences in survival appeared during start feeding where eggs exposed to 400 ppm glutaric dialdehyde showed significantly higher survival either the higher treatment level or the control group on both food types. The 400 ppm group also showed higher growth, which was most pronounced in the wild zooplankton-fed group.

Harboe, T., S. Tuene, A. Mangor-Jensen, H. Rabben and I. Huse. 1994. Design and operation of an incubator for yolk-sac larvae of Atlantic halibut. Prog. Fish-Cult. 56:188-193.

System designs and operational procedures for mass rearing Atlantic halibut yolk-sac larvae were developed from 1988 to 1992 at the Austevoll Aquaculture Research Station, Norway. Tanks with a cylindrical upper part and a conical lower part have been developed. Water inflow at the bottom and exit through overflow sieves provides a continuous exchange. Survival of larvae through the yolk-sac stage has improved each year. Survival rates from 60 to 80% were achieved in 1992.

Harmin, S.A. and L.W. Crim. 1989. Induced spawning of winter flounder using GnRH-A pellet implantation and its effects on egg/larval quality. Bull. Aquacult. Assoc. Can. 89-3:46.

Female winter flounder (*Pseudopleuronectes americanus*), were either sham implanted or received implant gonadotropic hormone releasing hormone (GnRH-A) in May, which is early in the breeding season. The hormone was administered by a quick-release cholesterol-cellulose (50:50) implant containing 40 µg of hormone. The objectives were to determine the ovulatory and spawning response and the resulting egg and larval survival. Spermiating males spawned naturally. The data indicate that GnRH-A can be used successfully to induce spawning in female winter flounder and that good quality eggs and larvae can be produced.

Harmin, S.A. and L.W. Crim. 1992. Gonadotropic hormone-releasing hormone analog (GnRH-A) induced ovulation and spawning in female winter flounder, *Pseudopleuronectes americanus* (Walbaum). Aquaculture, 104:375-390.

Winter flounder females generally fail to ovulate spontaneously in the laboratory at winter water temperatures ($\leq$0°C) or after exposure to 5°C. Treatment of flounder with gonadotropic hormone releasing hormone (GnRH-A) resulted in ovulation of a few females at 0° C, but accelerated ovulation and spawning reliably in prespawning female flounder maintained at 5°C., GnRH-A was capable of inducing spawning of some females in February, approximately three months prior to the normal spawning season. Good quality offspring (based on rates of fertilization, hatching, and larval survival) can be produced after accelerated spawning by GnRH-A treatment.

Harmin, SA. and L.W. Crim. 1993. Influence of gonadotropic hormone-releasing hormone analog (GnRH-A) on plasma sex steroid profiles and milt production in male winter flounder, *Pseudopleuronectes americanus* (Walbaum). Fish Physiol. Biochem. 10:399-407.

The effects of gonadotropic hormone-releasing hormone analog (GnRH-A) treatment on the onset and duration of increases in plasma sex steroids and milt production (milt volume and number of spermatozoa) were investigated in prespawning male winter flounder. Maturing males were treated during the winter with a single injection of either 20 or 200 μg/kg of GnRH-A, resulting in increases in plasma levels of testosterone and 11-ketotestosterone within 12 hours. Steroid hormone levels remained elevated for several days. The androgenic steroid response of males was delayed after the administration of 2 μg/kg GnRH-A. While a single GnRH-A injection in December or January advanced the onset of spermiation in some males, only small amounts ($<$ 50 μl) of milt could be collected. By March, all males were in spermiating condition following GnRH-A treatment. Significant increases in sperm production, particularly increases in milt volume, occurred only in fish twice treated with GnRH-A.

Hart, PR. and G.J. Purser. 1995. Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina* Gunther, 1862). Aquaculture, 136:221-230.

Experiments were conducted with greenback flounder (*Rhombosolea tapirina*) to determine the optimal salinity for fertilization and egg buoyancy, the optimal temperature and salinity combination for egg incubation, and the optimal temperature for yolk absorption. Optimal fertilization rates occurred at salinities of 35 to 45 ppt. Eggs were buoyant at salinities above 28 ppt. Optimal egg incubation temperature was approximately 12°C. Salinity had no effect on optimum incubation temperature if maintained between 15 and 45 ppt. Yolk absorption appeared to be most efficient at 15°C. The completion of yolk sac absorption coincided with first feeding, at which time the oil droplet had not been absorbed.

Hart, P.R. and G.J. Purser. 1996. Weaning of hatchery-reared greenback flounder (*Rhombosolea tapirina* Gunther) from live to artificial diets: Effects of age and duration of the changeover period. Aquaculture, 145:171-181.

The greenback flounder *Rhombosolea tapirina* is considered to have aquaculture potential in Australia, but development has been hampered by poor survival during weaning from live *Artemia* to artificial diets. In the studies reported in this paper, fish weaned for 10 days from Day 50 post-hatch or later had higher mortality than fish weaned earlier, but no differences in final weights or lengths were observed between fish weaned on different days post-hatch. Weaning from *Artemia* to artificial diets was shown to be possible from Day 23 post-hatch. When a 10-day overlap in *Artemia* and prepared feeds was used, a survival rate of 82.2% was achieved. A five-day overlap resulted in a reduction in final length compared with overlaps of 10 or 20 days, but survival was not affected. Overlaps of 20 days resulted in significantly heigher final weights than 5 or 10 days. Best growth and highest survival were reported from Day 23 post-hatch with a 20-day overlap, but a 10-day overlap may result in reduced *Artemia* cost with minimal effect on performance.

Hart, P.R., W.G. Hutchinson and G.J. Purser. 1996. Effects of photoperiod, temperature and salinity on hatchery-reared larvae of the greenback flounder (*Rhombosolea tapirina* Gunther, 1862). Aquaculture, 144:303-311.

The greenback flounder is a potential aquaculture candidate in southern Australia. Experiments were conducted with larvae to determine the optimal photoperiod, temperature and salinity for rearing of larvae through metamorphosis. The optimum photoperiod was 18 to 24 hours light, which increased the growth rate but had no effect on survival. Total darkness resulted in 100% mortality by the twentieth day following hatch. The optimum larval rearing temperature for was not accurately determined, though, 19 to 20°C produced better growth than lower temperatures. Survival was significantly lower at 16°C than at 17 or 19°C. Survival was lower at 15 ppt salinity than either 25 or 35 ppt.

Hashimoto, S., T. Hiraishi, Tomonori; K. Suzuki, K. Yamamoto and K. Nashimoto. 1996. Hirame no ami-ikesu nai ni okeru yuei noryoku ni tsuite. [Swimming ability of bastard halibut *Paralichthys olivaceus* at the bottom of net cage]. Bull. Jpn. Soc. Sci. Fish. 62:12-16.

Swimming speed-fatigue tests were conducted to understand the performance ability of bastard halibut (Japanese flounder) *Paralichthys olivaceus*. A polyethylene net was set on a slant in a circular tank and the fish was put onto the net with its blind side facing a water current. Fish of 4.6 to 36.3 cm were classified into 5 size groups and fatigue time was measured at five water temperatures. Fatigue time was measured as a function of current velocity. Fatigue time was longer at higher temperatures with smaller fish performing better at the same temperature.

Hatae, K., K.H. Lee, T. Tsuchiya and A. Shimada. 1989. Yoshokugyo to tennengyo no tekusucha tokusei ni tsuite. [Textural properties of cultured and wild fish meat.] Bull. Jpn. Soc. Sci. Fish. 55:363-368.

Textural differences between cultured and wild fish meat of red sea bream, yellowtail and flounder could be quantitatively demonstrated by discriminant analysis of six or seven measured physical properties. In the case of raw fish meat, the difference in physical properties was the greatest in yellowtail, followed by red sea bream, and finally flounder. In the case of cooked meat, which the difference in physical properties became smaller in yellowtail and red sea bream and larger in flounder.

Haug, T. 1990. Biology of the Atlantic halibut, *Hippoglossus hippoglossus* (L., 1758). Adv. Mar. Biol. 26:1-70.

This paper reviews the taxonomy of halibut and takes the view that the Atlantic and Pacific halibut are separate species. The paper then discusses the biology, fishery, and aquaculture potential of the Atlantic species.

Haug, T., E. Ringo and G.W. Pettersen. 1988. Total lipid and fatty acid composition of polar and neutral lipids in different tissues of Atlantic halibut, *Hippoglossus hippoglossus* (L.) Sarsia, 73:163-168.

Total lipid contents, neutral and polar lipids, and their fatty acid compositions were analyzed on various tissues of spawning Atlantic halibut, *Hippoglossus hippoglossus*. Liver had the highest total lipid content (>50 % of the dry weight of the tissue). All tissue lipid fractions were dominated by neutral lipids (~75 % of the lipid fraction). Triacylglycerols were the dominant neutral lipid. It contained high concentrations of 18:1, while the polar lipid fraction had high levels of 20:5n-3 and 22:6n-3.

Hayashi, M. 1995. Growth, survival and vitality of red sea bream, *Pagrus major*, and

Japanese flounder, ***Paralichthys olivaceus*, fry fed microbound diets. Bamidgeh, 47:119-128.**

Rearing experiments were conducted to replace live food (rotifers and brine shrimp) by microbound diets for mass-produced red sea bream, *Pagrus major*, and Japanese flounder, *Paralichthys olivaceus* fry. Four test diets were used in each experiment, replacing 67 to 88% of the live foods. All test diets promoted good survival rates after 30 days of rearing in both species. Fry growth on test diets was comparable to that of fry fed solely live foods.

Hjelmeland, K, I. Ugelstad and I. Lein. 1994. Synthesis of the digestive enzyme trypsin in halibut (*Hippoglossus hippoglossus* L.) larvae and juveniles. p. 62, *In*: Abstracts and List of Participants, 3rd International Marine Biotechnology conference, Tromsø, Norway.

Results from studies from 1991 to 1993 indicate that significant synthesis of trypsin in halibut larvae starts around 150 degree days and that the level of trypsin in yolk-sac larvae is at a maximum at around 260 degree days after hatching.

Helvik, J.V., and B.T. Walther. 1993. Development of hatchability in halibut (*Hippoglossus hippoglossus*) embryos. Int. J. Dev. Biol. 37:487-490.

Atlantic halibut (*Hippoglossus hippoglossus*) eggs raised in darkness hatched between days 14.5 and 16 after fertilization. Eggs incubated in white light remained unhatched though embryonic growth continued. Transfer of photo-arrested eggs to darkness induced rapid and synchronous hatching. Hatching was not observed prior to day 14. Nonsynchronous hatching over three days was seen when eggs were induced on day 14 + 1 h, or on day 14 + 9 h. However, darkness-induction on day 14 + 22 h produced synchronous hatching within 140 min. The high rate of inducibility persisted until day 18, before declining slowly. Hatching-induction was not observed beyond day 22.

Helvik, J.V. and B.T. Walther. 1993. Environmental parameters affecting induction of hatching in halibut (*Hippoglossus hippoglossus*) embryos. Mar. Biol. 116:39-45.

The influence of light, oxygen, and turbulence on hatching of Atlantic halibut (*Hippoglossus hippoglossus*) was examined. Light arrested hatching of halibut eggs, and transfer of such eggs to darkness resulted in rapid and synchronous hatching. Oxygen level seemed to have a minor role in the regulation of hatching in halibut, though induction of hatching was delayed under hypoxic conditions compared to higher oxygen levels. Turbulence delayed hatching for 1.5 days both in eggs incubated in turbulence, and in eggs subjected to turbulence at the time of hatching. Turbulence had an immediate inhibitory effect on hatching, but the process was reversible. If turbulence was discontinued, hatching resumed after 150 to 250 min.

Hiraishi, T., K. Nashimoto, T. Takagi, K. Suzuki and K. Motomatsu. 1995. Ami ikesu ani ni okeru hirame tosunagarei no nagare ni taisuru teii noryoku. [Stationary ability of bastard halibut *Paralichthys olivaceus* and longsnout flounder *Limanda punctatissima* to water current at the bottom of a net cage]. Bull. Jpn. Soc. Sci. Fish. 61:363-368.

To estimate and control the water current velocity at the bottom of a net cage for aquaculture of benthic fishes, it is necessary to know the stationary-holding ability of the fish in the net. In this experiment, bastard halibut (Japanese flounder), *Paralichthys olivaceus*, and longsnout flounder, *Limanda punctatissima*, were used. The fish was carefully placed inside a net that was laid horizontally inside a small flume tank such that its body axis was parallel to the stream line and its head was at the upper stream side. Each Japanese flounder was allowed to stay about five minutes in the tank. A slow water current was applied and increased gradually. The current velocity at the time when the fish slipped and could not hold the same position inside, was recorded and termed as the slipping speed.

Hirota, Y., Y. Koshiishi and N. Naganuma. 1990. Hirame chigyo ga sesshusita ami no ookisa to setsuji nisshukisei. [Size of mysids eaten by juvenile flounder *Paralichthys olivaceus* and diurnal change of its feeding activity.] Bull. Jpn. Soc. Sci. Fish. 56:201-206.

Juvenile Japanese flounder, *Paralichthys olivaceus,* in the Sea of Japan feed primarily on mysids. Five mysid species were identified in the 197 flounder stomachs examined. *Acanthomysis robusta* was most numerous (93.5% of 1,673 mysids recovered). The percentage of mysid weight in stomachs to body weight peaked (4.9%) in the evening and dropped to 0.0% after midnight in flounders <30 mm. Larger juveniles showed a similar feeding pattern.

Hjertnes, T. and J. Opstvedt, J. 1990. Effects of dietary protein levels on growth in juvenile halibut (*Hippoglossus hippoglossus* L.). pp. 189-193, *In*: M. Takeda, and T. Watanabe (Eds.). The current status of fish nutrition in aquaculture. Proceedings of the Third International Symposium on Feeding and Nutrition in Fish, Toba, Japan.

A study was conducted with Atlantic halibut to evaluate the effects of different levels of dietary protein in dry feed. Juveniles were found to grow on diets with 58% protein, which is similar to or higher than the requirement for salmon of the same size held at a similar water temperature.

Hjertnes, T., K.E. Gulbrandsen, F. Johnsen and J. Opstvedt. 1993. Effect of dietary protein, carbohydrate and fat levels in dry feed for juvenile halibut (*Hippoglossus hippoglossus* L.). Agronomique, Paris Colloq. Inra, 61:493-496.

Results of a feeding experiment designed to test the effects of different dietary protein, carbohydrate and fat levels in dry feed on juvenile Atlantic halibut are presented.

Hobbs, R.C., L.W. Botsford and R.G. Kope. 1990. Bioeconomic evaluation of the culture/stocking concept forCalifornia halibut. Fish Bull. Calif. Fish Game, 174:417-450.

A bioeconomics model used to evaluate the culture for enhancement stocking of the California halibut, *Paralichthys californicus,* is described. The model provides guidance for future research and preliminary estimates of feasibility. The model mimics growth of a cohort in culture from postlarvae to release, then in the ocean from release to mortality from natural or fishing mortality. Costs of culture and the benefits of cultured fish to the fishery are calculated. The cost of postlarval fish is shown to be a substantial part of culture costs. A graphic method is used to determine the release time that minimizes cost per recruit (about 300 days). The cost per released fish caught could be near $5 if natural growth rates could be achieved in culture and the culture period could be extended to 300 d.

Hole, G. and K. Pittman. 1995. Effects of light and temperature on growth in juvenile halibut (*Hippoglossus hippoglossus* L.). ICES Mar. Sci. Symp. 201:197.

The growth and histological development of red and white muscle fibres were investigated in juvenile halibut (*Hippoglossus hippoglossus* L.) reared at temperatures of either 11 or 14°C and under a 12-h photoperiod of 1, 10, or 500 lux. Approximately 600 fish averaging 1.8g were randomly distributed among twelve 1 m^2 tanks. The fish were fed a pelleted ration every six minutes during the daylight phase. Best growth with fewest mortalities and fewest wounds was obtained at the lowest light intensities (1 and 10 lux). Significantly better growth was obtained at 14°C than at the lower temperature. Initially, the red and white muscle fibres were distributed in alternating bands within the fillet. By the end of the experiment a more normal distribution had been achieved.

Holm, J.C. 1993. Production of juveniles with emphasis on Atlantic halibut. pp 24-25, *In*: T. Nosho and K. Freeman (Eds.). Marine Fish Culture and Enhancement. Washington Sea Grant Program, Seattle, Washington.

Substantial effort is being invested by the Norwegian Research Council to promote the commercial success of culturing Atlantic halibut (*Hippoglossus hippoglossus*). Other candidate species include the turbot (*Scophthalmus* [*Psetta*] *maximus*) also are subjects for Norwegian scientific projects.

Holmefjord, I., P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Timing of stripping relative to spawning rhythms of individual females of Atlantic halibut (*Hippoglossus hippoglossus* L.). pp. 203-204, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

An experiment was conducted to examine the effects of timing of stripping relative to the spawning rhythms of individual Atlantic halibut females.

Holmefjord, I., Y. Olsen, P. Lavens, P. Sorgeloos, E, Jaspers and F. Ollevier. 1991. An intensive approach to Atlantic halibut fry production. pp. 331-334, In: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

Results of three years of research on the intensive production of Atlantic halibut fry are reported. Halibut eggs were obtained from captive broodstock by stripping or after natural spawning. Light manipulation was used to control the time of spawning and increase the total spawning period. Live feed research documented that both rotifers (*Brachionus* sp.) and *Artemia* can be enriched with very high levels of highly unsaturated (n-3) fatty acids. Yolk sac larvae can be produced both in small stagnant units (down to 30 ml) and in large flow-through systems (up to 15,000 l). A major requirement in both systems is to avoid any stress on the larvae, e.g. high temperature, mechanical stress, high microbial load, and high ammonia levels. Algal addition to the first feeding-tanks has enhanced both survival and growth of halibut larvae. Highest growth rates have been recorded with collected zooplankton and addition of algae.

Holmefjord, I. J. Gulbrandsen, I. Lein, T. Refstie, P. Léger, T. Harboe, I. Huse, P. Sorgeloos, S. Bolla, Y. Olsen, K.I. Reitan, O. Vadstein, G. Øie and A. Danielsberg. 1993. An intensive approach to Atlantic halibut fry production. J. World Aquacult. Soc. 24:275-284.

Yolk sac larvae of Atlantic Halibut are presently produced either in small stagnant units or in large flow through systems. A major consideration is to avoid stress of the larvae, caused by mechanical disturbances, high bacterial loads, or high ammonia levels. Halibut larvae begin to ingest algae earlier than rotifers (*Brachionus* sp.). Supplementation of algae to first feeding tanks resulted in enhanced survival and growth. Both rotifers and *Artemia* can be enriched with high levels of highly unsaturated n-3 fatty acids. Enriched live feed enhances survival and growth of halibut larvae. High growth rates have been obtained with wild zooplankton and the addition of algae, but enriched cultivated feed combined with algae resulted in growth of the same magnitude as with wild zooplankton.

Honda, H. and K. Kikuchi. 1997. Management of a seawater recirculation fish culture system for Japanese flounder. pp. 165-171, *In*: B.J. Keller, P.K. Park, and J.P. McVey (Eds.). Interactions between cultured species and naturally occuring species in the environment. Proceedings of the 24th U.S.-Japan Aquaculture Panel Symposium. Texas A&M Sea University Sea Grant College Program, College Station. TAMU-SG-97-102.

Japanese flounders were grown from 3.5 g to 480 g in 330 days in a recirculating water system. Rearing density reached 38.4 kg/m^3 in the rearing tanks. Ammonia and nitrite were maintained at <1 mg/l during the first 100 days, after which ammonia fluctuated between 0.5 and 7.8 mg/l and nitrite between 1.0 and 4.0 mg/l. Nitrate concentration reached 359 mg/l on the 190th day then decreased due to denitrification in some anaerobic areas that formed in the system.

Honma, A. 1981. Present status of aquaculture techniques in Japan. J. Assoc. Underwat. Technol. Lond. 7:5-12.

Since 1960, research has led to the development of standard techniques of larvae production for a number of marine species, including flounders. Methods of aquaculture, feeding patterns, water use, aquaculture management, propagation techniques are outlined. The paper also includes tables of larva production.

Hoornbeek, F.K. and G. Klein-Macphee. 1987. Intergeneric flounder hybridization. pp. 79-85, *In*: K. Tiews (Ed.). Selection, hybridization and genetic engineering in aquaculture. Schr. Bundesforschungsanst. Fisch., vol. 18-19.

This paper reports on the production of reciprocal crosses between the smooth flounder (*Liopsetta putnami*) and winter flounder (*Pseudopleuronectes americanus*). The karyotype of the hybrid revealed 48 acrocentric chromosomes. Chromosomes of the parental species, each with $2n = 48$ chromosomes, could not be distinguished in the hybrid. True hybridization was confirmed between winter flounder females x smooth flounder males by isoelectric focusing of sarcoplasmic proteins. Average fin ray counts were intermediate between parental counts. Pharyngeal tooth features were intermediate between those of the parental species. Features of both parental species could be found in scale ctenii. Hybrids had not reproduced by age 4, whereas laboratory-hatched smooth flounders spawn at age 2.

Hoornbeek, F.K., P.J. Sawyer and E.S. Sawyer. 1982. Growth of winter flounder (*Pseudopleuronectes americanus*) and smooth flounder (*Liopsetta putnami*) in heated and unheated water. Aquaculture, 28:363-373

Young of the first year (age 0) and second year (age 1) winter flounder (*Pseudopleuronectes americanus*) and smooth flounder (*Liopsetta putnami*) were reared at the Jackson Estuarine Laboratory, Durham, New Hampshire between November 1975 and June 1976. Rearing in heated water led to weight gains that were approximately three times more rapid than in unheated water. Fish were fed a moist diet at 10% of body daily. Food conversions based on dry weight of food/wet weight of fish ranged from 1:1 for age 0 winter flounder in heated water to 27:1 for age 1 smooth flounder in unheated water. Diseases, including *Vibrio anguillarum* , myxobacteria, and the protozoan parasites *Kudoa* sp. and *Nosema* sp. produced losses.

Hosoya, K. and K. Kawamura. 1997. Osteological evaluation in artificial seedlings of *Paralichthys olivaceus* (Temminck and Schlegel). pp. 107-114, in: B.J. Keller, P.K. Park, and J.P. McVey (Eds.). Interactions between cultured species and naturally occuring species in the environment. Proceedings of the 24th U.S.-Japan Aquaculture Panel Symposium. Texas A&M Sea University Sea Grant College Program, College Station. TAMU-SG-97-102.

Developmental anomalies are common in cultured flounder. This paper examined the developmental changes in the trunk and outlined the morphological characteristics of young Japanese flounders.

Iida, Y., K. Masumura, T. Nakai, M. Sorimachi and H. Matsuda. 1989. A viral disease in larvae and juveniles of the Japanese flounder *Paralichthys olivaceus*. J. Aquat. Anim. Health, 1:7-12.

From 1985 to 1987, disease outbreaks in Japanese hatcheries produced mass mortality occurred in larvae and juveniles of the Japanese flounder *Paralichthys olivaceus*. Fish from 10 to 30 days old and reared at about 18-20°C were affected. Mortality usually reached 80-90% in a few weeks. Affected fish had opaque fins and a hyperplastic epidermis on the fins and skin. Hexagonal virus particles in the nucleus and cytoplasm of cells were revealed through electron microscopy. Isolation of the causative agent was not successful. The disease was transmitted to healthy larval flounder by exposing them to a 0.45 μm filtrate of diseased fish homogenate. The agent is thought to be a herpesvirus.

Inui, Y., M. Tagawa, S. Miwa and T. Hirano. 1989. Effects of bovine TSH on the tissue thyroxine level and metamorphosis in prometamorphic flounder larvae. Gen. Comp. Endocrinol. 74:406-410.

Bovine thyroid-stimulating hormone (TSH) was microinjected into prometamorphic flounder larvae to examine the effects on metamorphosis as well as the tissue thyroxine (T_4) and triiodothyronine (T_3) levels. The results suggest that increased secretion of TSH from the pituitary stimulates the thyroid, resulting in a surge of the tissue T_4 concentration.

Ishida, N. 1990. Tansuigyo oyobi kaisuigyo ni okeru okisorinsan no soshikinai nodo no hikaku. [Comparison of tissue level of oxolinic acid in fresh and sea water fishes after the oral administration.] Bull. Jpn. Soc. Sci. Fish. 56: 281-286

Tissue levels of oxolinic acid, an antibacterial agent, were determined after 3, 6, 12, 24, 48 and 72 hours after an oral dose was given to serveral species of fishes, including flounders. Oxolinic acid was considered to reside in a higher concentration and for longer time in the freshwater fishes than in the seawater fishes.

Ishida, N. 1990. 7 Shu kokotsugyo ni okeru okisorinsan no tanju chu taishabutsu no hikaku. [Comparison of biliary metabolites of oxolinic acid in seven species of teleosts.] Bull. Jpn. Soc. Sci. Fish. 56:55-59.

Biliary metabolites of oxolinic acid, after oral dose were compared in seven species fishes including flounder.

Iwata, N., H. Honda and M. Kiyono. 1993. Effects of temperature on growth of Japanese flounder (*Paralichthys olivaceus*). p. 391, *In*: M. Carrillo, L. Dahle, J. Morales, P. Sorgeloos, N. Svennevig, and J. Wyban (Eds.). From Discovery to Commercialization. Spec. Publ. 19, European Aquacult. Soc., Gent, Belgium.

Two rearing experiments were conducted to examine the effects of temperature on growth of Japanese flounder (*Paralichthys olivaceus*). The first was run at 10, 15, 20, 25 and 30°C and the second at 20, 23, 25, 27 and 30°C. Fish in both studies were fed commercial pelleted food to satiation twice a day for 20 days. In the first experiment, conducted with four weight groups of fish, the growth of animals in each weight group increased with temperature up to 20 or 25°C, but decreased at 30°C. The daily feeding rate increased at temperatures up to 25°C, but decreased at 30°C. The maximum food conversion efficiency was obtained at 20°C in each weight group. Experiment 2 was carried out with fish initially weighing 12 g. The optimum temperature for growth of Japanese flounder within the weight range examined is considered to be between 20 and 25°C.

Iwata, N., K. Kikuchi and H. Kurokura. 1995. Growth of the Japanese flounder *Paralichthys olivaceus* at different temperatures. Bamidgeh, 47:178-184.

Japanese flounders were reared at 10, 15, 20, 25 and 30°C with fish in initial body weight to determine the effects of temperature of growth of fish initially weighing about 4, 16, 88 and 176 g. It was concluded that rearing temperature should be maintained between 20 and 25°C for high growth rate. Extrapolation of the data suggested that the flounders might grow faster at 15°C than 20 or 25°C when over 700 g.

Iwata, N., K. Kikuchi, H. Honda, M. Kiyono and H. Kurokura. 1994. Effects of temperature on the growth of Japanese flounder. Fish. Sci. 60:527-531.

Japanese flounder of about 4, 16, 88 and 176 g initial body weight were reared at temperatures of 10, 15, 20, 25 and 30°C. Best growth occurred at 20 and 25°C. Best feed conversion efficiency occurred at 25°C. The results indicate that the optimum temperature for rearing Japanese flounder is between 20 and 25°C

Izquierdo, M.S., T. Arakawa, T. Takeuchi, R. Haroun and T. Watanabe. 1992. Effect of n-3 HUFA levels in *Artemia* on growth of larval Japanese flounder (*Paralichthys olivaceus*). Aquaculture, 105:73-82

Nine groups of *Artemia* containing different levels of n-3 HUFA were prepared by feeding them various lipid emulsions. Japanese flounder of 11.4 mm total length were divided into 18 groups and fed with one of the 9 types of *Artemia* for 34 days, in duplicate. Moisture, lipid conten, and fatty acid composition of *Artemia* and fish were determined. Larval flounder were able to survive for long periods on *Artemia* with a low n-3 HUFA content (1.8% on a dry weight basis). However, total length and body weight of fish increased significantly with increased *Artemia* n-3 HUFA level up to 3.5%. A considerable amount of 22:5n-3 was found in both the neutral and polar lipid fractions of the fish, suggesting that the fatty acid has an important role in lipid metabolism.

Jelmert, A. 1995. Effects of temperature on eggs and larvae of Atlantic halibut(*Hippoglossus hippoglossus* L.). ICES Mar. Sci. Symp. 201:198.

Eggs of the Atlantic halibut (*Hippoglossus hippoglossus*) were disinfected with 150 ppm iodine, divided into four experimental groups, and incubated at 4, 6, 8, and 10°C. One day after hatching the part of the eggs in each group were divided into subgroups and transferred to higher and lower temperatures. Hatching percentage was optimum (94.4%) at 6°C and minimum (2.8%) at 10°C. Total mortality occurred at 10°C, and 8°C resulted in higher cumulative mortality than 6°C and 4°C. Transfer to a lower temperature in the yolk-sac stage increased mortality, but had no significant effect on deformities. Increasing the temperature to as high as 10°C after hatching had no influence on mortality or deformities.

Jenkins, G.P. 1987. Age and growth of co-occurring larvae of two flounder species *Rhombosolea tapirina* and *Ammotretis rostratus*. Mar. Biol. 95:157-166.

Daily growth increments on otoliths were used to age larval *Rhombosolea tapirina* and *Ammotretis rostratus* collected from Port Phillip Bay, Victoria. Australia. Growth in the field was faster than that recorded for the same species in the laboratory at higher water temperatures and prey abundances.

Jenkins, W.E. and T.I.J. Smith. 1997. Production of southern flounder *Paralichthys lethostigma* juveniles in an outdoor nursery pond. J. World Aquacult. Soc. 202:211-214.

Spawning of southern flounder, *Paralichthys lethostigma*, was delayed until March through temperature and photoperiod control. Nearly 30,000 larvae averaging 2.0±0.1 mm were produced and stocked into a fertilized, polyethylene-lined pond, the bottom of which was covered with 20 cm of soil. The fish were provided with prepared feeds and sampled monthly. At harvest in October, the fish averaged 96 mm TL. All 1,746 juveniles captured were normally pigmented. Growth was rapid during the first three months but was slow or ceased from July to October, possibly because of a limited supply of proper food. The study demonstrated that southern flounder are hardy and tolerant of a range of environmental conditions.

Jensen, J.O.T., W.C. Clarke, J.N.C. Whyte and W. Damon. 1992. Incubation and larval rearing of sablefish (*Anoplopoma fimbria*) and Pacific halibut (*Hippoglossus stenolepis*). Bull. Aquacult. Assoc. Can. 92-3:49-51.

Recent studies at the Pacific Biological Station (Nanaimo, British Columbia, Canada) focused on developing culture techniques for the bathypelagic eggs and larvae of sablefish (*Anoplopoma fimbria*) and Pacific halibut (*Hippoglossus stenolepis*). Techniques for gamete collection, storage, and fertilization have been developed, embryo and larval development rates have been determined, as have temperature and salinity tolerance, and the effect of changes in salinity on neutral buoyancy. Conical upwelling incubators for egg incubation and yolksac larval development have been utilized. A computer-based control and monitoring system maintained was put in place to control water temperatures and flows as well as alerting staff via telephone when female broodstock have ovulated.

Jeon, I.G., K.S. Min, J.M. Lee, K.S. Kim and M.H. Son. 1992. A study on the bottom of floating netcage for olive flounder, *Paralichthys olivaceus*, culturing. Bull. Natl. Fish. Res. Dev. Agency, 46:91-108.

Six types of net-pen bottoms were evaluated in conjunction with rearing trials with olive (Japanese) flounder (*Paralichthys olivaceus*) in Korea to accommodate the flounder's benthic nature. Best performance over the rearing period was associated with a polyethylene net bottom, though a PVC (polyvinyl chloride) board bottom was associated with best fish growth and surivival during an 20 to 35 day acclimation period. Suitable initial acclimatization was seen as being critical to successful culture of Japanese flounder in net-pens.

Jeon, I. G., K.S. Min, S. L. Kwang, J.M. Lee and K.S. Kim, and M.H. Son. 1993. Optimal stocking density for olive flounder, *Paralichthys olivaceus*, rearing in tanks. Bull. Natl. Fish. Res. Dev. Agency, 48:57-70.

The effect of stocking density on the performance of olive (Japanese) flounders was investigated at initial densities of 1/3:1, 1/2:1, 1:1, 2:1 and 3:1 in ratio of body surface to tank bottom area. Daily feeding rate increased in proportion to the increment of stocking density. Food conversion efficiency and daily growth rate were highest at the ratio of 2:1. It was concluded that in order to maximize tank culture productivity in land-based farms, Japanese flounders should be stocked at 2:1 in the surface ratio of body to bottom.

Jeon, J-K., P.K. Kim, Y-J Park and H-T Huh. 1995. Study of serum constituents in several species of cultured fish. J. Korean Fish. Soc. 28:123-130.

Data on the serum components of several marine fish species commonly cultured in Korea, including olive (Japanese) flounder,*Paralichthys olivaceus*, were collected. The concentration of triglycerides was high, but the cholesterol concentration was low in Japanese flounder, the sum of the two components being 600 mg/dl. Lipase activity was very low in Japanese flounder. Among the five species examined, the alanine aminotransferase level was highest in flounder.

Joergensen, L., H. Grasdalen, R. Billard and N. de Pauw. 1989. Phosphate metabolites in larvae of halibut (*Hippoglossus hippoglossus*) studied by *in vivo* NMR. p. 303, IN: Aquaculture Europe '89. Spec. Publ. 10, European Aquacult. Soc., Gent, Belgium.

In vivo ^{31}P-NMR was used to characterize phosphate metabolites in yolk-sac Atlantic halibut larvae at three stages of development. The major contributors to the NMR spectrum were inorganic phosphate, phospholipids, sugar phosphates, phosphocreatine, and ATP. From day 10 to day 50 after hatching the relative amount of sugar phosphates, phosphocreatine and ATP increased by 450, 445 and 242%, respectively, while that of NMR-visible phospholipids decreased by 62%. The marked increase in phosphocreatine coincided with the increased larval activity.

Jung, S-H. and D-S Sim. 1992. The hematological and hemochemical characteristics of the cultured flounder, *Paralichthys olivaceus* naturally infected with pathogen bacteria. Bull. Natl. Fish. Res. Dev. Agency, 46:151-160.

Changes in various hematological and hemochemical characteristics were examined in cultured Japanese flounder, *Paralichthys olivaceus* naturally infected with *Vibrio* sp., *Edwardsiella tarda* or *Streptococcus* sp. were studied. Elevated red blood cell counts, hemoglobin and hematocrit were observed in fish showing signs of values and vibriosis. Fish with vibriosis and edwardsiellosis showed hypochromic normocytic anemia, and fish with streptococcicosis showed hypochromic microcytic anemia. Changes in hemochemical characteristics were also observed.

Kanamaki, S. 1997. Monitoring systems useful in mass production of larvae for Japanese fish culture. pp. 149-155, *In*: B.J. Keller, P.K. Park, and J.P. McVey (Eds.). Interactions between cultured species and naturally occuring species in the environment. Proceedings of the 24th U.S.-Japan Aquaculture Panel Symposium. Texas A&M Sea University Sea Grant College Program, College Station. TAMU-SG-97-102.

Monitoring systems used for mass culture of Japanese flounders, with special emphasis on two water quality regulating systems; one for for temperature, pH, and dissolved oxygen; the other for phytoplankton, are described.

Kanazawa, A. 1992. New directions in microdiet research in Japan. Badmidgeh, 44:123.

Mass production of Japanese flounder (*Paralichthys olivaceus*) was successfully carried out by using automatic feeders for dispensing a microparticulate diet. A combination of the microparticulate diet and rotifers plus *Artemia* led to the production of two million Japanese flounder of 30 mm average total length and 83% survival.

Kanazawa, A. 1993. Essential phospholipids of fish and crustaceans. pp. 519-530, *In*: S.J. Kanshik and P. Luquet, P (Eds.). Fish Nutrition in Practice. Institut National de la Recherche Agronomique, Paris.

Larval fish (including Japanese flounder) showed high growth and survival rates when fed diets with soybean lecithin including 1.0 to 2.0% as phosphatidylcholine + phosphatidylinositol. Phosphatidylethanolamine slightly improved growth and survival. Soybean lecithin was not only effective in enhancing growth and survival of the larvae, but also promoted enhanced growth of older fish. at the juvenile and young stages. Research showed that phospholipids are required for the transport of dietary lipids, particularly cholesterol and triglycerides in the body.

Kanazawa, A., S. Koshio and S-I Teshima. 1989. Growth and survival of larval red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* fed microbound diets. J. World Aquacult. Soc. 20:31-37.

To establish the practical use of microbound diets for the mass rearing of larval fish, rearing experiments were conducted with larval red sea bream, *Pagrus major*, and Japanese flounder, *Paralichthys olivaceus*. A mixture of various protein sources were used, and dietary amino acid patterns approximated those of larval whole body protein. Substitution of artificial feeds for live foods was found possible for larval fish production, although improvements in microbound diets may be necessary before they are adequate for use on a large scale.

Kanai, K., S. Tawaki and Y. Uchida. 1988. An ecological study of *Edwardsiella tarda* in flounder farm. Fish. Pathol. 23:41-47.

The distribution of *Edwardsiella tarda* on a flounder farm in Nagasaki city was examined in 1985 and 1986. *E. tarda* was rarely detected from sea water or fouling materials on net-pens except during an epizootics, but was isolated from the intestines of 10 to 50% of apparently healthy flounders. During an epizootic the incidence of *E. tarda* in the intestines rose to 60 to 100% of the fish sampled. When the number of *E. tarda* in the intestines rose above 10^3 CFU/g, the bacterium was often isolated from the kidney.

Kashiwakura, M., A. Seto and K. Hasegawa, K. 1994. Heterotrophic microalgal production of docosahexaenoic acid (DHA) and its use for aquaculture of marine juvenile fish. p. 61, *In*: 3rd International Marine Biotechnology Conference: Program,

Abstracts and List of Participants. Tromsø University, Tromsø, Norway.

A significant increase in survival rate and larvae activity of larval marine fishes, including flounder, were observed through feeding rotifers and brine shrimp nauplii enriched with a marine algae containing high levels of docosahexenoic acid (DHA). The increase in DHA was achieved by heterotrophic fermentation of marine algae (*Crypthecodinium cohnii*).

Kawamura, G., H. Mori and A. Kuwahara. 1989. Comparison of sense organ development in wild and reared flounder *Paralichthys olivaceus* larvae. Bull. Jpn. Soc. Sci. Fish. 55:2079-2083.

The sense organ morphology of wild Japanese flounder, *Paralichthys olivaceus*, larvae was compared with that of cultured flounder larvae. Mechanoreceptors developed earlier in cultured larvae, whereas the eyes and taste buds developed earlier in wild larvae. The formation of rod and twin-cone photoreceptors coincided with the initiation of shoreward migration and the change from pelagic to benthic habit of the larvae. After taste bud formation, wild larvae exhibited selective feeding on the zooplankton *Paracalanus parvus*.

Keefe, M. and K.W. Able. 1993. Patterns of metamorphosis in summer flounder, *Paralichthys dentatus*. J. Fish. Biol. 42:713-728.

In the laboratory, mortality of summer flounder during metamorphosis ranged from 17 to 83%, and was significantly greater in flounder maintained at approximately 4° C relative to those maintained at an average temperature of 10.1°C. Laboratory-reared summer flounder averaged 24.5 to complete metamorphosis at ambient spring temperatures (16.6°C). The time to completion of metamorphosis in wild-caught flounder maintained in the laboratory was temperature dependent.

Kent, D.B. 1994. Evaluating the use of hatchery-reared juveniles to enhance depleted marine fisheries in southern California. pp. 27-28, *In*: T. Nosho and K. Freeman (Eds.). Proceedings, Marine Culture & Enhancement Conference. Washington Sea Grant Program, Seattle, Washington.

Since 1984 the California Department of Fish and Game has been involved in a program to determine the economic feasibility of culturing and releasing juvenile marine fish into wild habitats along the Southern California coastline. Initial goals were to capture and maintain broodstock, develop techniques for the artificial control of spawning requirements of juvenile fish, develop protocols for hatching and rearing larvae and small juveniles, evaluate the economic feasibility of enhancing marine fish populations, assess the pattern of mortality during the first year of life to determine the optimum age and size for release, define release sites to realize maximum survival, develop techniques to differentiate genetically differing stocks and assess the impact of released fish on wild stocks, and determine and evaluate pertinent population characteristics and habitat. These goals have been applied to both white sea bass (*Atractoscion nobilis*) and California halibut (*Paralichthys californicus*).

Khan, R.A. 1985. Pathogenesis of *Trypanosoma murmanensis* in marine fish of the northwestern Atlantic following experimental transmission. Can. J. Zool. 63:2141-2144.

The susceptibility of four species of marine fish, including *Pseudopleuronectes americanus*, to leech-transmitted *Trypanosoma murmanensis* was assessed 49 to 60 days after infection by comparing condition factor, organ somatic indices, parasitological, hematological and histological, with control animals. The fish were maintained at temperatures that simulated the environment where transmission occurs naturally. High mortality occurred in juvenile winter flounder, but deaths decreased with increasing fish size. The most common pathological sign observed in young fish at necropsy was anemia, but that was not always correlated with the level of parasitism. Condition factor and somatic indices of liver, spleen and heart were altered in some infected fish groups.

Kikuchi, K. 1995. Nitrogen excretion rate of Japanese flounder — a criterion for designing closed recirculating culture systems. Bamidgeh, 47:112-118.

The nitrogen excretion rate of 1.6 to 6.5 g, 15 to 56 g and 163 to 575 g Japanese flounder was measured. The ammonia and urea excretion rates of starved flounder decreased with growth and the ammonia excretion rates of small fish varied more and were much higher than those of larger fish. Rates of ammonia excretion of fed fish were two to three times higher immediately after feeding than those of starved fish. Peak excretion occurred 3 to 6 hours after feeding, and then declined. The urea excretion rate peaked 6 to 12 or 12 to 24 hours after feeding. Daily rates of ammonia, urea, and feces nitrogen excretion per unit weight of fed fish were much higher in smaller fish.

Kikuchi, K., W.T. Yang and H. Honda. 1993. The state of the art of flatfish culture in the Far East. p. 293, *In*: M. Carrillo, L. Dahle, J. Morales, P. Sorgeloos, N. Svennevig and J. Wyban (Eds.). From Discovery to Commercialization. Spec. Publ. 19, European Aquacult. Soc., Gent, Belgium.

Only the Japanese flounder *Paralichthys olivaceus* has been successfully cultured commercially. Culture of the speceis began around 1975 in Japan. Most spawners are from the cultured stock. Photoperiod and/or temperature are controlled to induce maturation. Usually two spawning seasons are scheduled: autumn and spring. The nutritional value of *Artemia* as larval food is improved through the use of commercially available highly unsaturated fatty acid products and the diatom *Phaeodactylum*. Juveniles are stocked in culture tanks at about 5 cm. Larger sizes are stocked in cages. Cages are usually 5 x 5 x 2.5 m to 5 meter deep. A hard stable flat cage bottom and placement in well protected areas are necessary. Enhancement and recovery studies with flounder have been conducted by government agencies in Japan and Korea.

Kikuchi, K., H. Honda and M. Kiyono. 1993. Effect of dietary protein source on growth and nitrogen excretion of Japanese flounder (*Paralichthys olivaceus*). p. 400, *In*: M. Carrillo, L. Dahle, J. Morales, P. Sorgeloos, N. Svennevig and J. Wyban (Eds.). From Discovery to Commercialization. Spec. Publ. 19, European Aquacult. Soc., Gent, Belgium.

Rearing experiments employing diets containing white fish meal, cuttlefish meal, soybean meal, feather meal, brewers yeast and egg white albumin as sole sources of protein were conducted to examine the effect of dietary protein source on growth of Japanese flounder. Dietary crude protein levels ranged from 41 to 51%. Rearing trials were conducted for eight weeks with 2 g and five weeks for 25 g fish (all at 20°C). Effects of protein source on nitrogen excretion were evaluated in 7 to 21 g fish. The results suggested that a large proportion of the fish meal used in Japanese flounder diets can be replaced with soybean meal.

Kikuchi, K., T. Furuta and H. Honda. 1994. Utilization of feather meal as a protein source in the diet of juvenile Japanese flounder. Fish. Sci. 60:203-206.

Feeding experiments were conducted at 20°C on Japanaes flounder fed diets containing 0, 12, 25, 37 and 50% feather meal to examine the potential of that protein as a substitute for fish meal. Juvenile fish, about 3 g initial body weight received the diets for eight weeks. Weight gain of fish fed the diets containing 12 and 25% feather meal did not differ from the control fish or those receiving a diet containing 80% white fish meal, however, weight gain was lower in fish fed the 37 and 50% feather meal diets. Feed and protein conversion efficiencies of fish fed the 12% feather meal diet were similar to those of the control group, but decreased as the level of dietary feather meal increased. Supplementing crystalline amino acids in the feather meal diets slightly improved performance. The research demonstrated that 12 to 25% feather meal is an appropriate substitute for fish meal in the diet of juvenile Japanese flounder.

Kim, Y., S.B. Hur and H.S. Choi. 1993. Control of spawning periods of olive flounder, *Paralichthys olivaceus*, in indoor culture. Bull. Natl. Fish. Res. Dev. Agency, 48:71-79.

The effects of temperature and photoperiod on induction of spawning in olive (Japanese) flounders were studied in 3 experiments. The first experiment was carried out using only photoperiod control, the second with control of temperature heating and the control of photoperiod, and the third with control of the temperature by heating and cooling along with photoperiod control. The first spawning day of the third experiment was 6 months earlier than that of natural stock. The spawning periods and the number of eggs obtained in the three experiments were 117 days, 11,185,000 eggs; 110 days, 12,438,000 eggs; and 94 days, 24,378,000 eggs, respectively. The first spawning of the fish in the third experiment occurred at 13° C and continued to 15°C. Photoperiod was 14L:10D. On the other hand, the manipulation of temperature and photoperiod until the first spawning needed 76 days, and that until the end of the spawning needed 170 days in minimum.

Kim, Y., S.K. Yang and J.M. Baik. 1990. Feeding effects for flounder (*Paralichthys olivaceus*) larvae culture in the seedling production. Bull. Natl. Fish. Res. Dev. Agency, 44:95-101.

Survival and growth rates of Japanese flounder, *Paralichthys olivaceus* larvae fed various diets were examined. Larval growth at 5 days after hatching did not vary as a function of diet. However, at 10 days, larvae fed on rotifers cultured with artificial feed showed the best growth at 10 days after hatching. Daily growth rate of larvae fed on rotifers cultured with artificial feed was similar to that on rotifers fed baker's yeast. Flounders fed strictly on artificial feed showed the poorest daily growth rate. At 40 days after hatching, larvae fed on rotifers cultured with *Chlorella* showed the best survival (74%) and those fed on rotifers cultured with artificial feed and yeast had 69% and 44% survival. Total mortality of larvae fed solely on artificial feed occurred by 30 days after hatching.

Kimura, T. and M. Yoshimizu. 1986. Isolation of a new rhabdovirus from cultured hirame (Japanese flounder, *Paralichthys olivaceus*) in Japan. pp. 323-326, *In*: J.L. Maclean, L.B. Dizon and L.V. Hosillos (Eds.). Proc. First Asian Fisheries Forum, Manila, Philippines, 26-31 May 1986.

A new rhabodovirus was isolated from moribund cultured Japanese flounder, *Paralichthys olivaceus*, in 1984 and again in 1985. At temperatures between 5 and 20°C, the virus replicated and induced cytopathic effects, with eventual cytolysis in susceptible cell lines. The virus replicated optimally at 15 to 20°C. From the evidence obtained thus far, this virus, named HRV (hirame rhabdovirus), is a new pathogenic virus of fish, and is considered to be an important pathogen of cultured flounders and salmonids in Japan.

Kimura, T. and M. Yoshimizu. 1991. Viral diseases of fish in Japan. Ann. Rev. Fish Dis. 1:67-82.

Viral infections found in Japan include rhabdovirus infection and epidermal hyperplasia or necrosis of Japanese flounder. The importance to the aquaculture industry in Japan is reviewed.

Kitada, S., H. Kishino and Y. Taga. 1993. Nidan chusutsu no shijo-chosa ni yoru shubyo-horyu koka no hantei. [Estimates of stocking effectiveness evaluated by a two-stage sampling survey of commercial landings]. Bull. Jpn. Soc. Sci. Fish. 59:67-73.

The effectiveness of fish stock enhancement programs was evaluated using a two-stage random sampling survey of commercial landings. A conservative estimate of 36,982 recoveries from 246,300 hatchery-reared fish over 3 years after release was obtained for the flounder fisheries off Fukushima Prefecture. The ratio of the recovery rate to the number of fish released was 0.15. Total income and benefit were estimated at 32.6 and 7.9 million yen. The conclusion was that the stock enhancement program was economically profitable.

Kitajima, C., Y. Yamane and S. Matsui. 1994. Hirame shichigyo no hatsuiku ni tomonau hiju no henka. [Developmental changes in specific gravity in the early stages of the Japanese flounder *Paralichthys olivaceus*.]. Bull. Jpn. Soc. Sci. Fish. 60:617-623.

The specific gravity of the Japanese flounder *Paralichthys olivaceus* increased gradually from 1.022 to 1.028 in the egg and prelarval stages and markedly from 1.028 to 1.060 in the postlarval period. Specific gravity remained nearly constant after metamorphosis; however, in the early postlarval period there was a daily pattern of increasing specific gravity during the day decreasing specific gravity at night. It is suggested that ontogenetic changes in the buoyancy of larvae and juveniles of the flounder control their habitat transition. It also seems necessary that the larvae have negative buoyancy, even in the pelagic phase in order to maintain position in the water column and migrate vertically.

Kjoersvik, E. and A.L. Reiersen. 1992. Histomorphology of the early yolk-sac larvae of the Atlantic halibut (*Hippoglossus hippoglossus* L.)—An indication of the timing of functionality. J. Fish Biol. 41:1-19.

This paper describes the histomorphological development of organs of the yolk-sac larvae at 6°C. Kidneys appeared to be functional 16 days after hatching and primitive lamellae on the gill arches were beginning to form at that time. The liver was segmented into lobes between 20 and 23 days after hatching, and the gall bladder seemed functional from day 23. The hindgut became extensively folded from day 26, and branchial capillaries were first observed at that time. The larvae were able to capture food particles 24 days after hatching. Digestion of food appeared to begin between 24 and 26 days after hatching.

Klein-MacPhee, G., W.H. Howell and A.D. Beck. 1980. International study on *Artemia*. 7. Nutritional value of five geographical strains of *Artemia* to winter flounder *Pseudopleuronectes americanus* larvae. pp. 305-312, *In*: G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds.). The brine shrimp *Artemia*, Vol. 3, ecology, culturing, use in aquaculture. Proc. Internat. Symp. on the brine shrimp *Artemia salina*, Corpus Christi, Texas, August 20-23.

Artemia (brine shrimp) nauplii from 5 geographical locations (San Pablo Bay, USA: Great Salt Lake, Utah, USA; Shark Bay, Australia; Macau, Brazil; and Margherita di Savoia, Italy) were compared as food sources for late stage winter flounder larvae. Results showed survival of fish fed *Artemia* strains from Australia (93.9%), Italy (89.1%), and Brazil (88,4%) was significantly better in fish fed Utah (46.1%) and San Pablo Bay (38.8%) nauplii. There was no significant difference among fish survival percentages within the two groups (Australia, Italy, and Brazil vs. Utah and San Pablo Bay). Total fish length was significantly greater in fish fed Australia and Italy strains than in those fed the other three strains. There was no significant difference between fish fed Australia and Italy or among those fed the other three strains. Dry weights of fish fed Italy and Australia strains were significantly greater than those fed Utah, Brazil, or San Pablo Bay.

Kodama, H., T. Murai, Y. Nakanishi, F. Yamamoto, T. Mikami and H. Izawa. 1987. Bacterial infection which produces high mortality in cultured Japanese flounder (*Paralichthys olivaceus*) in Hokkaido. Jpn.J. Vet. Res. 35:227-234.

In August, 1985, an infectious disease occurred in two groups of young Japanese flounder (*Paralichthys olivaceus*) reared on a commercial farm in Hokkaido. As a result, over 80% of the fish died within two weeks. Signs of infection included ulcerative lesions and loss of the skin with exposure of the underlying muscle. Hemorrhage and loss of fins, protrusion of the rectum, and swelling of the spleen were also observed. *Edwardsiella tarda* was isolated from various tissues and organs.

Kosutarak, P., A. Kanazawa, S-I Teshima and S. Koshio. 1995. Interactions of L-ascorbyl-2-phophate-Mg and n-3 highly unsaturated fatty acids on Japanese flounder juveniles. Fish. Sci. 61:860-866.

An experiment was conducted Japanese flounder, *Paralichthys olivaceus* juveniles (initial body weight 0.35 g), using supplemental L-ascorbyl-2-phosphate-Mg at 0, 10, and 1,000 mg/100 g (APM) of diet and n-3 highly unsaturated fatty acids (n-3 HUFA) at 0, 1, and 2% of diet on). Best growth over a 50-day feeding period was observed in fish receiving the APM-supplemented groups with 1 and 2% n-3 HUFA. Analysis of variance on weight gain, feed conversion efficiency and concentration of ascorbic acid in the liver showed significant interactions between APM and n-3 HUFA levels. The fatty acid composition of total lipid in whole-body, neutral, and polar fractions in the liver reflected those of the dietary lipids.

Koya, Y., T. Matsubara and T. Nakagawa. 1994. Efficient artificial fertilization method based on the ovulation cycle in barfin flounder *Verasper moseri*. Fish. Sci. 60:537-540.

Ovulation cycles, time course of a decline of fertilization rate due to over-ripening, and effect of semen quality on the fertilization rate were investigated to establish an efficient technique of artificial fertilization in barfin flounder *Verasper moseri*. The ovulation cycle is 3 to 4 days at 6°C. The fertilization rate was ~90% immediately after ovulation and decreased with time after ovulation, falling to 45% two days after ovulation. Differences in spermatocrit values had little influence on the fertilization rate. It is important to use eggs obtained soon after ovulation.

Kulikova, N.I. 1981. Razrabotka fiziologicheskikh osnov iskustvennogo vosproizvodstva kambalovykh i kefalevykh ryb Azovo-Chernomorskogo bassejna. [Development of physiological foundations for artificial reproduction of flounders and mullets in the Azov-Black Sea basin.] pp. 6-20, *In*: N.E. Sal'nikov (Ed.). Ecologo-Physiological Foundations of Aquaculture in the Black Sea.

The turbot *Scophthalmus maeoticus* and the flounder *Platichthys flesus luscus* are characterized by asynchronous oocyte growth and batch spawning. There are no sharp boundaries between successive stages of ovicells. The gonadotropic function of the pituitary gland becomes more intensive during growth and gonad maturation. The gonadotropic activity of the pituitary is much higher in males than in females at all stages of the sexual cycle. The seasonal dynamics of the level of corticosteroid hormones in the blood serum of the turbot is similar to that of gonadotropic pituitary activity.

Kurokawa, T. and T. Suzuki. 1996. Formation of the diffuse pancreas and the development of digestive enzyme synthesis in larvae of the Japanese flounder *Paralichthys olivaceus*. Aquaculture, 141:267-276.

The results of this study suggest that the larval Japanese flounder pancreas begins to synthesize digestive enzymes at 2 days post-hatch, and that enzymes are secreted from 3 days post-hatch onwards. Further, it was concluded that the digestive system of the flounder assumes the adult form in the early juvenile stage following metamorphosis.

Kurokura, H., T. Matsumoto, K. Namba and S. Aoki. 1995. Oxygen consumption of larval flounder *Paralichthys olivaceus* measured by an improved water bottle method. Fish. Sci. 61:7-10.

Oxygen consumption in the larvae of the olive (Japanese) flounder, *Paralichthys olivaceus*, was measured by an improved water bottle method. Early morning measurements taken at 10 day intervals revealed significant differences in mass specific oxygen consumption. The formula $M = aW^b$ was adopted, where M and W were metabolic rate and body mass and b was the metabolic exponent.

Kusakari, M. and S. Urawa. 1990. Histopathology of the skin of yearling Japanese flounder *Paralichthys olivaceus* infected with the flagellate *Ichthyobodo* sp. Fish. Pathol. 25:59-68.

Cultured Japanese flounder, *Paralichthys olivaceus*, along the coasts of Hokkaido, Japan are frequently attacked by the ectoparasitic flagellate, *Ichthyobodo* sp., which attaches to the skin and gills. The external sign, restricted to the dorsal skin, is characterized by increased secretion of mucus followed by extensive epidermal erosion and ulceration along with an increasing number of parasites. Heavy infections cause hyperplasia of malphigian cells and depletion of mucous cells. There is also epidermal spongiosis due to the intercellular edema.

Kusuda, R. 1992. Bacterial fish diseases in mariculture in Japan with special emphasis on streptococcicosis. Bamidgeh, 44:140.

Increased infectious disease incidence in Japanese aquaculture has been associated with eutrophication, overcrowded farms, and the transport of the fish. About 25% of total mariculture production has been lost to disease in recent years. Flounders have been affected by edwardsiellosis (*Edwardsiella tarda*), and gliding bacterial infection (*Flexibacter maritimus*).

Kusuda, R. and F. Salati. 1993. Major bacterial diseases affecting mariculture in Japan. Ann. Rev. Fish. Dis. 3:69-85.

The most important bacterial diseases affecting culture of yellowtail, sea bream, and flounder in Japan are caused by *Enterococcus seriolicida*, *Pasteurella piscicida*, *Vibrio anguillarum*, *Nocardia kampachi*, and *Edwardsiella tarda*.

Kvenseth, A.M., K. Pittman and J.V. Helvik. 1996. Eye development in Atlantic halibut (*Hippoglossus hippoglossus*): Differentiation and development of the retina from early yolk sac stages through metamorphosis. Can. J. Fish. Aquat. Sci. 53:2524-2532.

At hatching, the eyes of Atlantic halibut were transparent and the retina undifferentiated. The retina differentiated slowly in sectors, and appeared functional around 150 degree days posthatching (about 50% yolk absorption). This timing coincided with the development of functionality in other organs and the ability to feed. At the 70% yolk absorption, when feed is first offered by culturists, the eyes were fully pigmented. Eye migration started at approximately 80 days posthatching, concurrent with the reorganization of the cones in the outer retina from single rows into a square-type cone mosaic. At 130 days, when metamorphosis was complete, the retina contained groups of rods and appeared to be mature, but until this stage, cones were the primary photoreceptor. The observed characteristics pointed to increased visual sensitivity at the time of settling. The results suggest than in culture, larvae can see to feed after 150 degree days, and at settling, they can be successfully fed under dim light.

Lee, G.W.Y. and M.K. Litvak. 1996. Weaning of metamorphosed winter flounder (*Pleuronectes americanus*) reared in the laboratory: Comparison of two commercial artificial diets on growth, survival and conversion efficiency. Aquaculture, 144:251-263.

The effects of two dry diets on growth, survival and conversion efficiency of laboratory-reared juvenile winter flounder (*Pleuronectes americanus*) were evaluated. Weaning onto the two diets was successful with approximately 70% survival by the end of the experiment (37 days). Food conversion efficiency ranged from 0.52 to 17.50% depending on the sampling period. Specific growth rates (length) over the experimental period were similar for juveniles fed the two starter diets (1.32%/day for a salmonid feed and 1.36%/day for a non-salmonid diet). Specific growth rates (wet weight) were also similar for the two feeds

(3.11%/day for salmonid diet and 2.65%/day for non-salmonid diet). There was no significant difference between the diets with respect to fish growth and survival .

Lee, G.W.Y. and M.K. Litvak, M.K. 1996. Weaning of wild young-of-the-year winter flounder *Pleuronectes americanus* (Walbaum) on a dry diet: Effects on growth, survival, and feed efficiency ratios. J. World Aquacult. Soc. 27:30-39.

Weaning winter flounder from a live diet to formulated feed is a critical stage in its culture. Wild young-of-the-year fish were used to determine whether juvenile winter flounder could be weaned from live, *Artemia* onto dry feed. The dry feed formula used was originally developed for turbot and cod by BP Nutrition, Stavanger, Norway. The performance of live feed and dry pellets was compared on the basis of survival, growth, and feed conversion efficiency over three months. It was possible to wean wild juvenile winter flounder onto dry feed after one week. The data supported the conclusion that the prepared diet was a better food source than *Artemia*. Survival was not influenced by food type.

Lein, I. and S. Tvelte. 1995. Effects of different salinities on Atlantic halibut yolk-sac larvae. ICES Mar. Sci. Symp. 201:200.

Experiments were conducted to examine the effect of salinity on survival and development of Atlantic halibut larvae. Larval density was approximately 70 larvae/l. Eleven salinities from 20 to 40 ppt were tested. Larvae kept at lower salinities showed high survival rates, but the frequencies of jaw deformation was highest in those groups. Among larvae kept in high salinity water, the survival rates decreased with increasing salinity, but the frequency of normal larvae was high. The highest number of surviving normal larvae was found when they were kept in salinities between 30 and 34 ppt.

Lein, I., P. Lavens, P.Sorgeloos, E. Jaspers and F. Ollevier. 1991. Viability of Atlantic halibut (*Hippoglossus hippoglossus* L.) eggs exposed to seawater before fertilization. pp. 205-206, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

If the eggs of Atlantic halibut are stripped too early, the hatching rate is reduced. Each female fish has individual intervals between egg releases, and must be allowed to release at least three batches before an approximately correct time for stripping can be estimated, resulting in a large loss of unfertilized eggs.

Lincoln, R.F. 1981. The growth of female diploid and triploid plaice (*Pleuronectes platessa*) x flounder (*Platichthys flesus*) hybrids over one spawning season. Aquaculture, 25:259-268.

A 14-month comparative growth experiment was carried out using female diploid and triploid plaice x flounder hybrids to examine the effects of sterility on growth over the spawning season. Sterile triploid hybrids exhibited continuous growth throughout the experimental period while diploids stopped growing four months prior to spawning. Diploids began losing weight during the spawning period. When the fish were stripped, rapid growth began immediately and resulted in compensatory growth. Data indicated that for the purposes of fish farming, triploidy would not confer any particular growth advantage except that triploids had significantly higher fillet weights.

Liu, H.W., R.R. Stickney and W.W. Dickhoff. 1991. Changes in plasma concentration of sex steroids in adult Pacific halibut, *Hippoglossus stenolepis*. J. World Aquacult. Soc. 22:30-35.

Blood samples were collected from captive Pacific halibut, *Hippoglossus stenolepis* , at intervals of about six weeks from early December 1986 to late November 1987. Concentrations of plasma androgen and estradiol-17B were determined by radioimmunoassay. The plasma concentrations of steroid were highest during autumn and winter in halibut that matured during late winter. The concentrations of steroids in samples collected in December were above 2 ng/ml (estradiol) or 1 ng/ml (androgen) in maturing females and below 0.5 ng/ml for both steroids in non-maturing females. The levels of steroids decreased rapidly about one month before spawning. In a mature male, androgen began to rise in August and November, and reached a peak of 7 ng/ml in early December. One month before spawning, the androgen concentration fell to 0.16 ng/ml. Estradiol concentrations were detectable in the male and varied little during the year. The results suggest that the concentrations of estradiol or androgen measured in blood samples taken during December may be used to determine the sex and state of maturation of Pacific halibut.

Liu, H.W., R.R. Stickney and S.D. Smith. 1991. A note on the artificial spawning of Pacific halibut. Prog. Fish-Cult. 53:189-192.

Adult Pacific halibut (*Hippoglossus stenolepis*) taken from the wild were held in captivity beginning in 1986. The first successful captive spawning with subsequent development of Pacific halibut larvae in the USA was conducted during February and March 1988. One of the eight larvae produced survived for 6 days, by which time eye pigment was visible.

Liu, H.W., R.R. Stickney, W.W. Dickhoff and D.A. McCaughran. 1993. Early larval growth of Pacific halibut *Hippoglossus stenolepis*. J. World Aquacult. Soc. 24:482-485.

Growth of Pacific halibut *Hippoglossus stenolepis* larvae was studied in the laboratory during 1989 and 1991. Larvae increased in length from 6.3 mm at hatching to 9.9 mm 20 d post-hatch. The average daily length increment was 0.17 mm. Dry weight of the larvae increased from an average of 210 mu g at hatching to 570 mu g on day 20, providing a specific growth rate of 4.99. During the same period, mean yolk sac weight decreased from 1,390 mu g to 646 mu g, resulting in a yolk to body conversion efficiency of 48.5%. At hatching, the larval body made up only 13% of total dry weight. On day 20, the larval body made up 46.9% of the total weight. Larvae started feeding at a length of 12 mm after about 90% of their yolk sac had been absorbed.

Liu, H.W., R.R. Stickney, W.W. Dickhoff and D.A. McCaughran. 1994. Effects of environmental factors on egg development and hatchingof Pacific halibut *Hippoglossus stenolepis*. J. World Aquacult. Soc. 25:317-321.

Eggs of Pacific halibut (*Hippoglossus stenolepis*) were incubated under various environmental conditions. Optimum hatching occurred over a temperature range from 6°C to 8°C, whereas temperatures of 3, 10 and 11°C were lethal. Development time from fertilization to 50% hatching varied from 250 h (9°C) to 320 h (6.5°C). Salinity effects on hatching were not as critical as temperature, as long as eggs were floating during the incubation period. Light intensity between 5 and 15 lux did not affect hatching success, but high light intensity (15 lux) and red and blue light (5 lux) produced high levels of larval abnormality. Simulated transport of unfertilized eggs indicated that the eggs can be safely moved and that fertilization rate is acceptable during the first 12 hours after collection.

Liu, H.W., R.R. Stickney, W.W. Dickhoff and D.A. McCaughran. 1993. Neutral buoyancy salinity of Pacific halibut *Hippoglossus stenolepis* eggs and larvae. J. World Aquacult. Soc. 24:486-492.

Samples of halibut eggs in nature have led to theories that development occurs near the sea bed and, alternatively, well up in the water column. Resolution of the conflicting theories and information which should assist culturists in providing the proper environmental conditions for egg development and hatching were the subjects of this study. The neutral buoyancy salinity (NBS) of Pacific halibut eggs and larvae ranged between 29.8-34 ppt.

Eggs and larvae with higher NBS (> 35 ppt) were usually abnormal or stressed. Thus, eggs found near the seabed may be nonviable.

Lopez-Alvarado, J. and A. Kanazawa. 1993. Utilization of crystalline amino acids by Japanese flounder larvae, *Paralichthys olivaceus* (Temminck and Schlegel), fed on zein-microbound diets. p. 410, *In*: M. Carrillo, L. Dahle, J. Morales, P. Sorgeloos, N. Svennevig and J. Wyban (Eds.). From Discovery to Commercialization. Spec. Publ. 19, European Aquacultue Society, Gent, Belgium.

This study was carried out to assess the utilization of crystalline amino acids included in microbound diets fed to Japanese flounder (*Paralichthys olivaceus*) larvae. The larvae were reared on rotifers from first feeding, and were fed microbound diets from day 10 after hatching. Five diets were used in duplicate. Diets differed in the levels of casein and crystalline amino acids as sources of protein. A diet containing fish meal and krill meal was used as a control. Leaching of crystalline amino acids into the rearing water was estimated by analyzing a sample of water in which the diet was leached for five minutes. Significantly better larval growth and higher survival occurred in fish fed the control diet as compared with those fed the experimental diets. Larvae fed diets with 30 and 50 g100 g diet of crystalline amino acids had very slow growth, and experienced complete mortality after 17 days of culture. Leaching of diets with more than 10 g /100 g diet of crystalline amino acids was very high. Leaching occurred at levels of from 60 to 76% in 5 min in all the experimental diets. Larvae fed diets with 50 and 60 g casein/100 g diet had similar performance. Since the level of total amino acids in the diet with 10 g/100 g crystalline amino acids was acceptable, and since larval performance was similar to that of larvae fed the casein diet, that level of crystalline amino acids in experimental diets is considered appropriate for nutritional requirement studies in which the amino acid profiles of the diets have to be manipulated. Whole body amino acid compositions of all the experimental groups were similar.

Lopez-Alvarado, J., C.J. Langdon, S-I Teshima and A. Kanazawa. 1994. Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. Aquaculture, 122:335-346.

Microbound, microcoated, and microencapsulated diets were fed to marine fish in an attempt to improve retention of added amino acids after immersion in water. Binding crystalline amino acids to alginate, carrageenan or zein microparticles gave poor results, with leaching of 80-90% of the crystalline amino acids within minutes after immersion. Microencapsulation of crystalline amino acids within protein-walled capsules gave better results, with capsules retaining up to 60% of some crystalline amino acids after two minutes in water. The best results were obtained by encapsulating crystalline amino acids within lipid-walled capsules. Retention was further improved by including 2% Span 85, a surface-active agent, in the lipid wall of the capsules. With this technique, leaching was reduced to 1.4% over two minutes. Feeding studies with olive (Japanese) flounder (*Paralichthys olivaceus*) indicated that larvae older than 20 days could break tripalmitin-walled capsules.

Malmstroem, T. R. Salte, H.M. Gjoeen and A. Linseth. 1993. A practical evaluation of metomidate and MS-222 as anaesthetics for Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture, 113:331-338.

Metomidate and MS-222 were tested as anaesthetics for Atlantic halibut (*Hippoglossus hippoglossus* L.) at temperatures of 9.5±0.3°C and 10.5 ±0.3°C. The lowest effective concentration of metomidate was 10 mg/l, while MS-222, concentrations of 250 mg/l were required. Doses should be kept below 60 mg metomidate/l or 480 mg MS-222/l. It was concluded that metomidate gives a broader safe anaesthetic range with a lower effective dose than MS-222.

Mangor-Jensen, A and K.E. Naas. 1993. Phototaxis of halibut larvae (*Hippoglossus hippoglossus* L.). pp. 132-138, *In*: B.T. Walther and H.J. Fyhn (Eds.). Physiology and Biochemical Aspects of Fish Development. University of Bergen, Bergen, Norway.

Atlantic halibut larvae (*Hippoglossus hippoglossus*) were reared at 7.3°C in constant darkness during the period between hatching and first feeding, and at 11.0°C in outdoor tanks during feeding. Phototaxis was measured as horizontal movement in a two-compartment activity chamber with the light source located at one end. Light intensities between 0.01-10.000 lux were applied. The larvae did not respond phototactily before day 23 after hatching, at which time they displayed negative phototaxis to light levels lower than approximately 1 lux, and responded positively to light levels higher than approximately 10 lux. This trend continued throughout the yolk-sac stage, and was also pronounced even after the larvae were conditioned to high light intensities after first feeding.

Mangor-Jensen, A. and K.G. Waiwood. 1995. The effect of light exposure on buoyancy of halibut eggs. J. Fish Biol. 47:18-25.

Egg buoyancy, yolk sac osmolality and perivitelline space in light and dark-exposed eggs of Atlantic halibut were followed from three to 12 days after fertilization. In light-exposed eggs, density increased to a maximum at day 6 while the density of dark exposed eggs significantly decreased between day 4 and 10. There was no significant difference between treatments at day 12. The pattern of yolk osmolality reflected the changes in density. Embryonic volume, calculated from estimates of total volume and perivitelline space, decreased rapidly at days 3 to 4 after fertilization in the light exposed group, whereas the control group showed no change. Subsequently, the embryonic volume showed a parallel decrease in both groups. Increased egg density is caused by both the loss of water from the embryonic compartment and by increased yolk osmolality.

Mangor-Jensen, A., K.E. Naas, T. Harboe and J.C. Holm, 1990. Beware of benthic algae in green water larviculture World Aquacult. 21(4):95.

Recent first feeding experiments on Atlantic halibut larvae (*Hippoglossus hippoglossus*) indicate that the use of a green water technique improves the onset of start feeding. In a first feeding experiment, a 7m^2 tank was continuously supplied with an algal suspension. The algal suspension consisted of natural phytoplankton grown with the addition of a commercial fertilizer in a separate tank receiving natural daylight. At the start of the feeding experiment larval Atlantic halibut were transferred from a yolksac production unit to the first feeding unit and fed both natural zooplankton and *Artemia*. After 12 days the experiment was terminated and the larvae were collected for weaning. A massive growth of benthic algae was observed on the bottom and walls of the tank when the larvae were sampled. Large numbers of dead and moribund larvae were found attached to the benthic algae. Of the 1000 larvae originally stocked in the tank, 210 were harvested and approximately the same number were dead or moribund. The benthic algae were identified as *Ulothrix pseudoflacca*.

Martinez Cordero, F.J., McM. Beveridge, J.F. Muir, D. Mitchell and M. Gillespie. 1994. A note on the behaviour of adult Atlantic halibut, *Hippoglossus hippoglossus* (L.), in cages. Aquacult. Fish. Manage. 25:475-481.

Underwater photographic and video recording equipment were employed to observe the behavior of adult Atlantic halibut, *Hippoglossus hippoglossus*, in cages. Significantly more fish were found on the cage bottom than in the water column. Fish tended to congregate around the outside rather than in the center of the cage. During rough weather, the cage bottom heaved violently and proportionately fewer fish remained on the bottom. Fish that did remain on the bottom adopted an arched body posture with heads and tails not in contact with the substrate. Around 25% of the caged fish were active at any time. Most of the active fish remained near the cage surface or bottom. Fish were observed to swim in a circular pattern close to the cage walls and maintained that behavior for periods of up to five minutes with little change in swimming speed.

Masumura, K., Y. Iida, T. Nakai and T. Mekuchi. 1989. The effects of water temperature and fish age on a herpesvirus infection of Japanese flounder larvae, *Paralichthys olivaceus.* Fish Pathol. 24:111-114.

A previous investigation revealed that a new disease in Japanese flounder larvae in Hiroshima Prefecture, Japan was a herpesvirus infection. In this study, the effects of water temperature and fish age on experimental infection of flounder larvae with the causative virus are described. When exposed to the 0.45 μm-filtrate of diseased fish homogenate and kept at 20 or 25°C, flounder larvae showed high mortalities (90-94%) within 9 days. Mortality was considerably delayed at 15°C. Larvae younger than 20 days (< 9.5 mm TL) were highly susceptible but 23 days and older (>11.0 mm TL) showed an abrupt decrease in susceptibility.

Masumura, K., H. Yasunobu, N. Okada and K. Muroga. 1989. Isolation of a *Vibrio* sp., the causative bacterium of intestinal necrosis of Japanese flounder larvae. Fish Pathol. 24:135-141.

From 1986 through 1988, a bacterial disease characterized by opaque intestine or intestinal necrosis with high mortality occurred in larval Japanese flounder (*Paralichthys olivaceus*) reared at private hatcheries and the Prefectural Fisheries Experimental Station in Hiroshima Prefecture. From the intestine of the diseased larvae, a bacteria was isolated and tentatively named *Vibrio* sp. INFL (Intestinal necrosis of flounder larvae) group. The disease condition was reproduced by oral administration of the isolate incorporated into live rotifers and brine shrimp.

Methven, D.A., L.W. Crim, B. Norberg, J.A. Brown, G.P. Goff and I. Huse, 1992. Seasonal reproduction and plasma levels of sex steroids and vitellogenin in Atlantic halibut (*Hippoglossus hippoglossus*). Can. J. Fish. Aquat. Sci. 49:754-759.

Atlantic halibut (*Hippoglossus hippoglossus*) collected off Newfoundland first mature at about. Captive Newfoundland Atlantic halibut (80 cm fork length for males and 115-120 cm for females) did not release milt or eggs or have detectable levels of estradiol-17β or 11-ketotestosterone until exceeding those sizes. Estradiol-17β and testosterone increased to their highest levels in females during gonadal recrudescence before spawning. Lower levels were observed in spawning fish. Individual maturing halibut can be sexed by rising levels of estradiol-17β in females and 11-ketotestosterone in males during late fall and early winter.

Miller, D.C., D.E. Body, J.C. Sinnet, S. Poucher and J. Sewall. 1988. Design and performance of a saltwater low dissolved oxygen test system. J. Shellfish. Res. 7:573.

Acute toxicity tests with winter flounder (*Pseudopleuronectes americanus*) resulted in a mean LC_{50} of 1.9 mg/l dissolved oxygen level for late embryo to hatch stage, 1.5 mg/l for four-day-old larvae, and 1.4 mg/l for juveniles of 2 cm total length.

Miwa, S., M. Tagawa, Y. Inui and T. Hirano. 1988. Thyroxine surge in metamorphosing flounder larvae. Gen. Comp. Endocrinol. 70:158-163.

This research indicated that the metamorphic climax in larval Japanese flounder (*Paralichthys olivaceus*) is induced by a surge of thyroid hormone, and that thyroid hormone may also regulate development before and after the metamorphosis.

Miyazaki, T. 1982. Pathological study on streptococcicoses histopathology of infected fishes. Fish Pathol. 17:39-47.

Various streptococcal β-hemolytic bacterial infections were evaluated in cultured Japanese flounder (*Paralichthys olivaceus*) and a variety of other species. The bacteria frequently infected the eyes. ß-hemolytic bacteria caused extensive suppurative inflammation in the eyes of *P. olivaceus*.

Morrison, C.M. and C.A. MacDonald. 1995. Normal and abnormal jaw development of the yolk-sac larva of Atlantic halibut *Hippoglossus hippoglossus*. Dis. Aquat. Org. 22:173-184.

Normal and abnormal jaw development was studied in laboratory-reared Atlantic halibut larvae. Abrasion of the epidermis of the tail and head with invasion by bacteria and other foreign organisms was common. Mouth and jaw development was often delayed in larvae with eroded tails. Many larvae had gaping jaws, a condition which causes major problems in rearing, since affected fish cannot close their jaws to feed. The jaws of those larvae were not disarticulated, but the anterior parts of the ethmoid and Meckel's cartilage were bent apart. The condition appeared to be associated with abrasion of the head, and invasion by foreign organisms.

Muroga, K., T. Nakai and T. Nishizawa. 1994. Viral disease in Japanese seawater pen culture. p. V-2, *In*: International Symposium on Auatic Animal Health: Program and Abstractes. Univ. California School of Veterinary Medicine, Davis.

Viral diseases in Japanese mariculture include viral epidermal hyperplasia in flounder caused by a herpesvirus, a birnavirus (YAV) and a rhabdovirus (HRV) in flounder.

Muroga, K., H. Yasunobu, N. Okada and K. Masumura. 1990. Bacterial enteritis of cultured flounder *Paralichthys olivaceus* larvae. Dis. Aquat. Org. 9:121-125.

Bacterial enteritis is a devastating disease of larval Japanese flounder (*Paralichthys olivaceus*) caused by a bacterium tentatively named *Vibrio* sp. The disease causes intestinal necrosis in flounder larvae. It was reproduced in 16 to 27day-old flounder larvae by oral challenge with *Vibrio* sp. Histopathological and electron microscopic examinations revealed that pathogen multiplication and resultant pathological changes occurred only in the intestine.

Naas, K.E. and A. Mangor-Jensen. 1990. Positive phototaxis during late yolksac-stage of Atlantic halibut larvae *Hippoglossus hippoglossus* (L.). Sarsia, 75:243-246.

Larvae of Atlantic halibut were reared in large tanks in total darkness. Prior to expected first feeding (day 45 after hatching, at 4°C) their responses to light stimuli were tested. In one experiment exposure time was varied, and positive phototactic response was significant after seven minutes at 300 lux. In a second experiment exposure time was constant (10 minutes) and the larvae were exposed to different light intensities. Positive phototactic response was achieved at levels as low as 0.5 lux. These results emphasize the need to control the application of light during the late yolksac-stage. The positive phototactic response can be used to attract and gather larvae for transfer to first-feeding units.

Naas, K.E., T. Næss, T. Harboe, P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Enhanced first feeding of halibut larvae *Hippoglossus hippoglossus* L.) in green water. pp. 335-338, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

The "green water" technique involves providing an algal suspension in tanks for first feeding marine fish larvae. Experimental studies on the effects of algae are scarce and the algae have either been identified as directly providing nutrition or as contributing to the maintenance of good water quality. This study involved first-feeding Atlantic halibut larvae (*Hippoglossus hippoglossus*) and was performed in nine 1.5 m diameter outdoor tanks. Feeding incidence, growth and survival were evaluated over 21 days from first feeding. Green water was compared with a filtrated water. The larvae were fed non-enriched *Artemia* instar II. Feeding incidence at day 3 was 47% in green water and 0% in filtrated water.

Growth and survival were higher in green water than in filtrated water. Of a total of ~2,250 halibut larvae in the green water tanks, 684 larvae were found alive at the end of the experiment (day 21). Corresponding numbers for the filtrated water tanks were 57 out of 4,500. There was no indicating that the algae were used directly as food or that the algae modified water quality, yet the halibut larvae showed substantially improved survival and growth compared to larvae in filtrated water. The role of algae is not yet fully understood. However, a nutritional effect of algae seems to be of minor importance compared to changing environmental parameters (e.g. light regime) influencing the food uptake.

Næss, T., M. Germain-Henry, and K.E. Naas. 1995. *Artemia* or wild zooplankton as first-feed for halibut larvae (*Hippoglossus hippoglossus*)—implications for abnormal pigmentation. ICES Mar. Sci. Symp. 201:201.

Atlantic halibut (*Hippoglossus hippoglossus*) larvae were offered *Artem*ia and wild zooplankton for different time intervals at first-feeding. The study was conducted in 150-l black plastic bags floating in temperature-controlled water outdoors and filled with "green water" (water containing algae). The experimental feeds were wild zooplankton, wild zooplankton for seven days followed by unenriched, and Super Selco™ enriched. After 19 days best growth and survival associated with the *Artemia* groups, suggesting that wild zooplankton was not necessary as initial food for the species. Beyond metamorphosis (day 57) high percentages of albinic juveniles were found in groups fed exclusively on *Artemia*, but Super Selco™ enrichment significantly decreased the percentage of albinic specimens (70.6% vs. 95.7%). Larvae that had been fed wild zooplankton for the whole period and larvae transferred from an unenriched *Artemia* diet to a diet of wild zooplankton from day 19 had normal pigmentation on the ocular side. Thus, *Artemia* can be used as a first-feed for Atlantic halibut without notable negative effects on growth, survival, or pigmentation.

Næss, T., M. Germain-Henry and K.E. Naas. 1995. First feeding of Atlantic halibut (*Hippoglossus hippoglossus*) using different combinations of Artemia and wild zooplankton. Aquaculture, 130:235-250.

Larvae of Atlantic halibut (*Hippoglossus hippoglossus*) were offered different combinations of *Artemia* and wild zooplankton during first feeding to examine the effects on feeding incidence, growth, survival and pigmentation. Super Selco™ enriched *Artemia* increased the concentration of free amino acids and the lipid content of *Artemia* to approximately the levels found in wild zooplankton, though with a much lower n-3 polyunsaturated fatty acid level. Larvae fed 19 days on enriched *Artemia* were significantly larger than larvae fed wild zooplankton, wild zooplankton for seven days then unenriched *Artemia*, or unenriched *Artemia*. The enriched *Artemia* group also showed the best survival at day 19. The fatty acid composition of larvae fed *Artemia* reflected the composition of their feeds, while the fatty acid composition in the larvae fed wild zooplankton remained relatively similar to that of prefed larvae. After 57 days of feeding, high frequencies of malpigmented juveniles were observed in the *Artemia* and enriched *Artemia* groups (95.7 and 70.6%) as well as the group switched from wild zooplankton to unenriched Artemia after seven days. All larvae fed wild zooplankton or larvae transferred from an unenriched Artemia diet to a diet of wild zooplankton after day 19 became normally pigmented. It was concluded that use of *Artemia* could be successful in providing growth and survival in the early larval stage of exogenous feeding in Atlantic halibut, but had negative effects on pigmentation. Introduction of wild zooplankton prior to 19 days of feeding avoided the pigmentation problem.

Næss, T., T. Harboe, A. Mangor-Jensen, K.E. Naas and B.Norberg. 1996. Successful first feeding of Atlantic halibut larvae from photoperiod-manipulated broodstock. Prog. Fish-Cult. 58:212-214.

Food intake, growth and survival were determined for larval offspring from a broodstock of Atlantic halibut maintained on a 6-month delayed photoperiod. Feeding incidence of larvae fed enhanced *Artemia* in an indoor tank increased from 29.0% at 24 hours to 91.4% after 4 days. There was no acute mortality that could be attributed to the transfer from yolk sac incubators, although a 20% mortality was observed between days 3 and 6. In all, 69% of the larvae survived the 15 day experiment. Food intake, survival, and growth potential during the first-feeding stages appeared to be unaffected by the fact that the larvae were produced off-season. The results indicate that year-round production of juvenile Atlantic halibut is feasible.

Nakajima, K. and M. Sorimachi. 1996. Monoclonal antibodies against viral deformity virus. Fish Pathol. 31:87-92.

A total of 27 clones of hybridoma cells secreting monoclonal antibodies (MABs) against VDV, the causative agent of viral deformity in yellowtail *Seriola quinqueradiata*, were generated. Six isolates of birnaviruses from marine cultured fishes were studied for reactivities of the MABs by immunofluorescence test. The MABs showed same reaction patterns to an isolate from Japanese flounder *Paralichthys olivaceus* as those of VDV by immunofluorescence test. However, some MABs did not react to the other four birnavirus isolates from Japanese flounder.

Nakajima, K., A. Uchida and Y. Ishida. 1989. Gyorui no setsuji-yuin busshitsu, jimechiru-ß-puropiotechin no kingyo no seicho ni oyobosu seika. [Effect of supplemental dietary feeding attractant, dimethyl-ß-propiothetin, on growth of goldfish.] Bull. Jpn. Soc. Sci. Fish. 55:1291.

The thrust of this paper is on the stimulation of growth in goldfish by supplementing feed with the attractant dimethy-ß-propiothetin. Growth augmentation is also reported for flounder during a short rearing period.

Nakajima, K., A. Uchida and Y. Ishida. 1990. Kaisangyo no seicho ni oyobosu shinsetsujiyuinbusshitsu DMPT no tenka koka. [Effect of a feeding attractant, dimethyl-ß-propiothetin, on growth of marine fish.] Bull. Jpn. Soc. Sci. Fish. 56:1151-1154.

The effect of a dietary supplemented sulfonium compound, dimethyl-ß-propiothetin on the growth of various marine fish, including flounder, was examined. Dietary administration of optimum concentrations of the compound elicited a 1.3-fold increase in 13th weeks with treated flounders as compared with a control group.

Nakanishi, M. and S. Fujita. 1986. Arutemia to tennen dobutsu purankuton no heiyo kyuji oyobi yosei arutemia kyuji ni yoru hirame chigyo no hakka kotai shutsugenritsu no gensho. [Reduction in frequency of albinism in a flounder juvenile, *Paralichthys olivaceus*, fed on *Artemia* with wild zooplankton and pre-fed *Artemia*.] Bull. Kyoto Inst. Ocean. Fish. Sci. 10:25-33.

A study was conducted to clarify the relationship between the ratio of wild zooplankton to *Artemia* in the diet of larval Japanese flounder (*Paralichthys olivaceus*) and the frequency of albinism on the ocular side of flounder juveniles. The frequency of the normal-colored juveniles was 23.7%, and survival 25.2% when half the *Artemia* was substituted for by wild zooplankton. On the other hand, when pre-fed *Artemia* were fed to larval flounder, the normal-colored juveniles appeared at a high rate of 46.4%, and survival rate increased to 84.5%. It was concluded that the frequency of albinism in flounder juveniles was effected by algae fed on by zooplankton, including *Artemia*.

Nakano, T., K. Ono and M. Takeuchi. 1992. Levels of zinc, iron, and copper in the skin of abnormally pigmented Japanese flounder. Bull. Jpn. Soc. Sci. Fish. 58:2207.

Zinc, iron and copper levels were determined in abnormally pigmented one-year-old Japanese flounders, *Paralichthys olivaceus* (total length, 35.1± 2.5 cm.). The level of zinc in the pigmented part of the skin was two or three times higher than that in the white part. The iron level in the pigmented part of the skin was also higher than that in the white part. There was no difference in the level of copper between pigmented and white parts of skins.

Nakatsugawa, T. 1983. *Edwardsiella tarda* isolated from cultured young flounder. Fish Pathol. 18:99-101.

Bacteria isolated from cultured young Japanese flounder, *Paralichthys olivaceus* , in the Kurida Bay, Kyoto Prefecture, Honshu that exhibited a disease in October 1982 were identified as *Edwardsiella tarda*. The diseased fish showed abdominal inflation and accumulation of ascites. Pathogenicity of the isolate for flounder was proved positive by intraperitoneal and/or intramuscular injections.

Nardi, G. 1996. Culture of summer flounder, *Paralichthys dentatus*, at Greatbay Aquafarms. J. Shellfish. Res. 15:458.

GreatBay Aquafarms, Inc. was established in 1995 for the culture of marine fish, principally summer flounder (*Paralichthys dentatus*). The initial objective is the production of 5 to 10 gram juveniles from its Portsmouth, NH hatchery which began production in January of 1996. The facility includes a laboratory, phytoplankton and zooplankton culture rooms, and three recirculating systems for the broodstock, weaning, and nursery stages. The egg and early larval stages are flow-through water systems. At capacity, the hatchery is expected to produce between 300,000 and 400,000 juveniles per year. Five stocks of broodfish are maintained. They will be induced to spawn through photoperiod and temperature manipulation. In addition to the culture of rotifers and *Artemia*, copepods will be cultured for use as an early larval food.

Nguyen, H.D., T. Mekuchi, K. Imura, T. Nakai, T. Nishizawa and K. Muroga. 1994. Occurrence of viral nervous necrosis (VNN) in hatchery-reared juvenile Japanese flounder *Paralichthys olivaceus*. Fish. Sci. 60:551-554.

A disease of hatchery-reared juvenile Japanese flounder, *Paralichthys olivaceus*, occured in fish of 17 to 18 mm in total length and results in heavy mortalities when the fish reached about 25 mm. Total losses resulted in two weeks. The disease was characterized by vacuolation in the retinal and brain tissues. A nodavirus was identified as the causative agent of the disease. In addition to the nodavirus, another virus (YAV-like birnavirus) was isolated from diseased fish. However, the latter virus is not thought to play an important role in this infection.

Nilsen, F. 1995. Description of *Trichodina hippoglossis* n. sp. from farmed Atlantic halibut larvae *Hippoglossus hippoglossus*. Dis. Aquat. Org. 21:209-214.

A new species of Trichodina, *T. hippoglossis* n. sp., collected from skin and fins of farmed Atlantic halibut Hippoglossus hippoglossus (L.), is described. Heavy infections were first evident after a temperature rise from 12 to 18°C. Heavily infected specimens showed increased mucus production, which gave the larvae a greyish skin color. The parasite is 78.5±6.9 μm in size.

Nilsen, F. and G.A. Bristow. 1994. Microsporidosis in farmed Atlantic halibut larvae (*Hippoglossus hippoglossus* L.) due to *Pleistophora* sp. p. P-46. *In*: International Symposium on Aquatic Animal Health. University of California School of Veterinary Medicine, Davis.

During the spring and summer 1990, 550 Atlantic halibut larvae, approximately 2 to 4 months of age, were obtained from a commercial hatchery and examined for parasites. Of these a total of 10% were infected with a microsporidian, and exhibited visible as pseudocysts in the abdominal cavity and cysts in the skeletal muscles. Histological examination of infected specimens revealed spores in several internal organs, such as pancreas, liver and kidney, in addition to skeletal muscle. Heavy infections resulted in the severe degeneration of skeletal muscle fibers. Infections with the parasite are assumed to be lethal. The species involved was identified as a member of the genus *Pleistophora* Gurley, 1893.

Nilsen, F., Ness, A. and A. Nylund. 1995. Observation on an intranuclear microsporidian in lymphoblasts from farmed Atlantic halibut larvae (*Hippoglossus hippoglossus* L.). J. Eukaryotic Microbiol. 42:131-135.

An intranuclear microsporidian was observed in lymphoblasts from the kidney of farmed Atlantic halibut larvae (*Hippoglossus hippoglossus*). In addition to spores, intranuclear pre-spore stages were observed. The developmental stages observed are similar to those of the family Enterocytozoonidae, genus *Enterocytozoon*.

Norberg, B. V. Valkner, J. Huse, I. Karlsen and G. Grung. 1991. Ovulatory rhythms and egg viability in the Atlantic halibut (*Hippoglossus hippoglossus*) Aquaculture, 97:365-371..

In 1989, , the ovulatory rhythm of stripped individual female Atlantic halibut (*Hippoglossus hippoglossus*) was monitored to optimize the yield of viable eggs from each fish. Careful monitoring resulted in a 120% increase in total egg yield and a 220% increase in the number of eggs ≥ 80% fertilization rates. Handling stress had no apparent effect on the egg yield.

Oh, K-S, H-J Lee, D-W Sung and E-H Lee. 1988. Comparison of nitrogenous extractives, amino acids in wild and cultured bastard. Coll. Repr. Tong-Yeong Fish. Jr. Coll. 3:307-311.

Nitrogenous extracts and amino acids from the dorsal muscle of wild and cultured bastard (Japanese flounder, *Paralichthys olivaceus*) were examined. Cultured fish muscle had higher moisture content and lower crude lipid and protein wild fish of the same species. Free amino acid profiles were similar in all samples, although there were relative differences in glycine, threonine and proline contents. Wild fish contained higher trimethylamine oxide and total creatinine than cultured bastard. The major amino acids found were glutamic acid, aspartic acid, leucine and isoleucine.

Oh, K-S., R-H. Ro, J-G. Kim and E-H. Lee. 1989. Comparison of lipid components in wild and cultured bastard. Collect. Repr. Tong-Yeong Fish. Jr. Coll. 3:301-305.

The lipid components of the cultured bastard (Japanese flounder, *Paralichthys olivaceus*) were compared with those of wild *P. olivaceus* and flounder (*Pleuronichthys cornutus*). Total lipid contents in dorsal muscle of the three types of fish were 2.0%, 1.6% and 1.9%, respectively. Wild *P. olivaceus* and *P. cornutus* were higher in neutral lipid and glycolipid and lower in phospholipid than cultured *P. olivaceus*. The neutral lipids cosistend primarily of triglycerides (70.7 to 72.6%),. The primary phospholipids were phosphatidylcholine (53.6 to 58.3%) and phosphatidylethanolamine (25.9 to 29.5%). Cultured *P. olivaceus* showed higher levels of polyenes such as docosahexaenoic acid and low levels of monoenes such as palmitoleic and oleic acid than wild fish. *P. cornutus* had a higher percentage of arachidonic and eicosapentaenoic acid compared than did *P. olivaceus*. The major fatty acids in all types of fish were palmitic, docosahexaenoic, oleic, eicosapentaenoic and palmitoleic acid. The fatty acid composition of neutral lipids, glycolipids and phospholipids did not differ significantly among wild and cultured *P. olivaceus*.

Okimasu, E., M. Matsumoto, Y. Yoshida and A. Amemura. 1992. The effect of pigments of *Rhodobacter capsulatus* on free radicals and application of the bacterium as feed to fish larvae. Bull. Jpn. Soc. Sci. Fish. 58:1498-1491.

The photosynthetic bacteria *Rhodobacter capsulatus* was used as a secondary feed for

Japanese flounder larvae and juveniles to elucidate the possible role of carotenoids as free radical scavengers during development of fish larvae. These findings suggest that some pigments inhibit the initiation and progression of lipid peroxidation.

Olney, C.E., P.S. Schauer, S. McLean, Y. Lu and K.L. Simpson. 1980. International study on *Artemia*. 8. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory reared marine fish. pp. 343-352, *In*: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Eds.). The brine shrimp *Artemia*, Vol. 3, ecology, culturing, use in aquaculture. Proc. Internat. Symp. on the brine shrimp *Artemia salina*, Corpus Christi, Texas, August 20-23.

Newly hatched *Artemia* nauplii from Brazil, Australia, Italy and the United States (Utah and San Pablo Bay, California) were analyzed for chlorinated hydrocarbons. Nauplii from Brazil and Australia contained very low levels of PCB and chlorinated insecticides, while Italian nauplii contained the highest levels of HCB, BHCs and DDTs, and San Pablo nauplii were highest in chlordanes, dieldrin and PCBs. Attempts to correlate nauplii residue levels with the survival of laboratory-reared *Pseudopleuronectes americanus*), another fish species, and a crab showed no obvious compounds that could totally account for the poorer performance of the Utah and San Pablo strains. Satisfactory performance of fish fed the Italian strain seems to eliminate HCB, BHCs and DDTs as agents responsible for poor performance. Twelve metals, were measured, but no particularly high concentrations were observed.

Olsen, Y. 1993. Commercial flatfish farming in Europe: Halibut and turbot. p. 292, *In*: M. Carrillo, L. Dahle, J. Morales, P. Sorgeloos, N. Svennevig and J. Wyban (Eds.). From discovery to commercialization. Spec. Publ. 19, European Aquacult. Soc., Gent, Belgium.

The availability of juvenile turbot (*Scophthalmus maximus*) has been the main obstacle to successful aquaculture of the species in Europe. There are still problems related to pigmentation, a high frequency of deformities, and slow growth of juveniles. The production of turbot juveniles was about 1.9 million in 1992. Approximately 30 farms were involved in turbot production in 1992, with a production capacity of about 3,000 tons. Spain is the leading producer with 2,000 tons produced on 20 farms. Over the previous year the price for farmed turbot has been as low as $7 to $9 US/kg. With respect to Atlantic halibut (*Hippoglossus hippoglossus*) the long duration of the yolk sac stage (>30 d) has been a major challenge to culturists as has been establishment of an adequate feeding regime with rotifers and *Artemia*. Advances in technology have addressed both problems and the aquaculture. The first significant quantities of cultured halibut were available from the biggest Norwegian producer at the time of publication. There are still problems with inadequate fry quality (deformations, abnormal pigmentation, slow growth, poor survival) during the first feeding. Most juveniles have been produced with fairly extensive methods using harvested wild zooplankton. The numbers of weaned juveniles produced in Norway increased from two in 1985 to 200,000 in 1991. Halibut have been grown from 3.7 to 7.2 kg in 3 to 4 years.

Opstad, I. and Ø. Bergh. 1993. Culture parameters, growth and mortality of halibut (*Hippoglossus hippoglossus* L.) yolk sac larvae in upwelling incubators. Aquaculture, 109:1-11.

Rate of Atlantic halibut yolk sac larvae mortality increased with increasing rate of flow in upwelling incubators. However, in a stagnant incubator there were no surviving larvae beyond Day 12 after hatching. Larval dry weight decreased with increasing rate of flow, whereas yolk sac dry weight did not differ significantly. Yolk sac utilization efficiency was, therefore, higher with lower flows. The number of bacteria in the stagnant incubator increased rapidly from Day 3. In the incubators that received flowing water, the accumulation of bacteria was delayed, and did not exceed 10^6/ml before Day 16.

Opstad, I., A.B. Skiftesvik and Ø. Bergh. 1995. Effects of light on large-scale rearing of halibut yolk-sac larvae (*Hippoglossus hippoglossus* L.). ICES Mar. Sci. Symp. 201:202.

First-feeding halibut (*Hippoglossus hippoglossus* L.) larvae were influenced by the light regime they were exposed to during the yolksac period. The light regime also affected activity level, growth, and survival of larvae before first-feeding. Halibut yolksac larvae were maintained at light intensities of 1000, 100, 10, 1, and 0 lux from day 1 to day 16 post-hatch. Larvae kept at 1000 lux had the highest activity. Generally, the larvae kept at 10 lux had relatively low activity, and the remaining groups had varying activity during the experimental period. In a second experiment, halibut yolksac larvae were raised in continuous darkness, continuous 10 lux, or a regime of one week with 100 lux followed by two weeks with darkness and 1 week with 10 lux. Larvae reared with continuous 10 lux showed significantly higher growth and survival and also survival upon transfer to normal light for first-feeding.

Oseko, N., M. Yoshimizu and T. Kimura. 1990. Pathogenicity of *Rhabdovirus olivaceus* (Hirame rhabdovirus., HRV) to hirame and salmonid fish. pp. 639-642, *In*: R. Hirano and I. Hanyu (Eds.). Proceedings of the Second Asian Fisheries Forum, Tokyo, Japan, 17-22 April 1989.

Pathogenicity of the *Rhabdovirus olivaceus* (hirame rhabdovirus., HRV) to hirame (Japanese flounder, *Paralichthys olivaceus*) and salmonids was studied. Cumulative mortalities of HRV-infected hirame reared at 5, 10, 15 and 20°C were 40, 60, 20 and 0%, respectively. Highest virus titer in mixed samples of kidney and spleen was obtained from fish reared at 5°C. In both types of fish, kidneys exhibited necrotic changes such as nuclear degeneration of hematopoietic cells and hemorrhage in the interstitial tissue. Spleen tissue showed signs of necrosis and hemorrhage.

Oseko, N., M. Yoshimizu, S. Gorie and T. Kimura. 1988. Histopathological study on diseased hirame (Japanese flounder., *Paralichthys olivaceus*) infected with *Rhabdovirus olivaceus* (hirame rhabdovirus, HRV). Fish Pathol. 23:117-123.

Signs of infection of Japanese flounder with *Rhabdovirus olivaceus* were congestion of the gonads, focal hemorrhage of skeletal muscle and fins, and accumulation of ascitic fluid. The kidney showed necrotic changes by nuclear degeneration of hematopoietic cells and hemorrhage in the interstitial tissue. The spleen showed necrosis and hemorrhage in the pulp, and skeletal muscle revealed hyperemia and hemorrhage of capillary vessels. Hyperemia and hemorrhage were observed in the interstitial tissue of the seminiferous duct, ovarian lamella, and in the connective tissues around the seminal duct and oviduct of the testis and ovary. Mucosa of alimentary tract showed hyperemia and hemorrhage. No liver pathology was noted.

Ototake, M. and T. Matsusato. 1986. Hirame *Paralichthys olivaceus* chigyo no skuutica senmochu (Makuko rui). [Notes on *Scuticociliitida* infection of cultured juvenile flounder *Paralichthys olivaceus.*] Bull. Natl. Res. Inst. Aquacult. 9:65-68.

This paper reports on a ciliate (*Scuticociliatida*) infection that occurred during 1985 on several *Paralichthys olivaceus* farms in Japan. Disease signs included kidney hemorrhaging and collapse of the connective tissue and muscle along with skin discoloration. Ciliates were found on the body surface and in such places as the skin, fins, and brain.

Parrish, C.C., J.A. Brown, E.S. Daniel and D.C. Somerton. 1993. Lipid composition of Atlantic halibut (*Hippoglossus hippoglossus*) eggs over the spawning season. Bull. Aquacult. Assoc. Can. 93-4:35-37.

The lipid content of unfertilized and fertilized eggs from first-time and repeat spawning

Atlantic halibut was compared. There was no significant difference in the total lipid content nor in the lipid class composition between unfertilized and fertilized eggs. The lipids consisted primarily (> 62%) of phospholipids and there was little (< 11%) storage triacylglycerol. The major fatty acid was docosahexaenoic acid, with earlier batches containing higher proportions of that fatty acid than later batches.

Parrish, C.C., J.D. Castell, J.A. Brown, L. Boston, J.S. Strickland and D.C. Somerton. 1994. Fatty acid composition of Atlantic halibut eggs in relation to fertilization. Bull. Aquacult. Assoc. Can. 94-2:36-38.

Fatty acid analyses were performed on Atlantic halibut (*Hippoglossus hippoglossus*) eggs before and after fertilization. The levels of 16:1n-7, which accounted for 12.4±1.2% of total fatty acids, and three minor fatty acids (20:1n-7, 20:4n-3, and 22:4n-3 were correlated with survival. Energy derived from saturated fatty acids may be more important at the time of fertilization and at the initiation of embryogenesis while unsaturated fatty acids may become more important after fertilization.

Paul, A.J., J.M. Paul and R.L. Smith. 1994. Energy and ration requirements of juvenile Pacific halibut (*Hippoglossus stenolepis*) based on energy consumption and growth rates. J. Fish Biol. 44:1023-1031.

Growth of captive juvenile Pacific halibut, (*Hippoglossus stenolepis*) was found to be linearly related to energy consumption (J/g/day) at 4°C. Weight gain was independent of size for fish between 9 and 7,000 g when growth was expressed as a function of consumption in J/g/day. Maintenance ration determined in feeding-growth experiments averaged 27.4 J/g/day at 4.0°C. Single meals following two day fasts averaged 4.1% of body for halibut under 100 g, 1.72%, for 1 to 2 kg fish, and 1.1% for 6 to 8 kg fish.

Person Le Ruyet, J., P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Feeding of marine fish larvae: Microdiets or live preys? p. 168, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

Any diet that can reduce dependence on live prey production is of great interest to marine fish culturists. This paper demonstrated that early weaning of marine fish larvae, including Japanese flounder is technically feasible using efficient microdiets.

Rabben, H. 1991. Progress in methodology development for rearing of halibut fry. Aquacult. Eur. 16:6-12.

This report summarizes the results of experimental research conducted in Norway to develop rearing methods for the mass production of Atlantic halibut (*Hippoglossus hippoglossus*) fry. Broodstock and spawning, egg incubation, yolk sac larvae incubation, and handling, first feeding and weaning to artificial diets are discussed.

Rainuzzo, J.R., K.I. Reitan and L. Joergensen. 1992. Comparative study on the fatty acid and lipid composition of four marine fish larvae. Comp. Biochem. Physiol. 103B:21-26.

Fatty acid and lipid class composition were determined in larvae of four marine species, including thee flatfishes: Atlantic halibut (*Hippoglossus hippoglossus* L.), plaice (*Pleuronectes platessa*), and turbot (*Scophthalmus maximus*) at hatching and prior to first feeding. Total fatty acid content decreased in each species with up to 50% reduction in one of the halibut groups. Docosahexanaoic acid (22:6n-3) was highly utilized. Low lipid utilization was found in turbot in relation to the other species, which may relate to the fact that turbot larval development is optimized at a higher temperature than the others. Relative amounts of neutral lipids and phospholipids were similar in plaice and halibut, and were approximately constant during the yolksac stage. Neutral lipids were dominant for turbot at hatching, accounting for 53 to 55% of the total lipids, while phospholipids predominated prior to first feeding (56 to 59%). Phosphatidylcholine was catabolized in halibut and plaice but not in turbot, while phosphatidylethanolamine tended to be synthesized in each species.

Rainuzzo, J.R., K.I. Reitan, L. Joergensen, P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Fatty acid and lipid utilization in the yolk-sac stage of marine fish larvae. pp. 25-29, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

The yolksac period can be as long as 55 days at 5°C in Atlantic halibut (*Hippoglossus hippoglossus*) or as short as 5 days at 10°C in plaice (*Pleuronectes platessa*). This study discusses lipid utilization in the yolksac stage.

Reitan, K.I., S. Bolla and Y. Olsen. 1994. A study of the mechanism of algal uptake in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*). J. Fish Biol. 44:303-310.

The drinking rate of water, and the ingestion and assimilation rates of algae in yolksac larvae of Atlantic halibut (*Hippoglossus hippoglossus*) were examined. The drinking rate throughout the yolksac stage was in the range of 7-160 nl per larva/hour, with a slight increase towards the end of the period. The algae (*Tetraselmis* sp.) ingestion rate was very low before day 30 and showed a peak between day 43 and 48 at 5°C. The assimilation efficiency for the algae was in the range of 1 to 5%. The results suggested that the larvae act as filter feeders beginning on day 30 after hatch until they begin feeding on larger prey.

Reitan, K.I., J. Rodriguez Rainuzzo, L. Joergensen, R. Billard and N. de Pauw. 1989. Yolk utilization in larvae of halibut (*Hippoglossus hippoglossus*). p. 328, *In*: Aquaculture Europe '89. Spec. Publ. 10, European Aquacult. Soc., Gent, Belgium.

Decreases of carbon and nitrogen content were observed in Atlantic halibut during the yolksac stage under both light and dark incubation. Fatty acid analyses of individual larvae showed a very low variance among samples. There was gradual consumption of some fatty acids during the yolksac stage.

Rœnnestad, I. 1993. No efflux of free amino acids from yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.). J. Exp. Mar. Biol. Ecol. 167:39-45.

The loss of free amino acids from sac larvae of Atlantic halibut incubated for 116 or 94 hours in filtered seawater containing a broad spectrum antibiotic (Nitrofurazolidon, 0.5 ppm) was investigated. During incubation the larval pool of free amino acids declined but there was no concomitant increase in the free amino acid concentration of the incubation water. The results suggest that the decline in the larval free amino acid pools is not due to an efflux, but rather derives from metabolic transformations within the embryo.

Rœnnestad, I. and H.J. Fyhn. 1993. Importance of free amino acids in embryonic energy production of three marine flatfishes as revealed by measurements of oxygen consumption and ammonia production. pp. 285-289, *In*: B.T. Walther and H.J. Fyhn (Eds.). Physiology and Biochemical Aspects of Fish Development. University of Bergen, Bergen, Norway.

This study found that in turbot and Atlantic halibut, free amino acids are utilized as an energy substrate during the egg stage and early yolksac stage, while in lemon sole, the few available data suggest that free amino acids are important for energy production during the egg stage as well as during the yolksac stage. Other energy substrates, probably lipids, are used concurrently.

Rœnnestad, I. and K.E. Naas. 1993. Oxygen consumption and ammonia excretion in larval Atlantic halibut (*Hippoglossus hippoglossus* L.) at first feeding: A first

step towards an energetic model. pp. 279-284, *In*: B.T. Walther and H.J. Fyhn (Eds.). Phsyiology and Biochemical Aspects of Fish Development. University of Bergen, Bergen, Norway.

The metabolic rate of first feeding Atlantic halibut larvae was found to scale isometrically with body dry weight in fish from 0.7 to 7 mg). The weight specific metabolic rate was 1.5± 0.2 µl O_2/mg/hr. An allometric relationship was found between ammonia excretion and dry weight. The nitrogen quotient varied between 0.09 and 0.15 (mean 0.12±0.02), indicating that approximately 60% of the metabolic energy was derived from catabolism of amino acids.

Rœnnestad, I., E.P. Groot and H.J. Fyhn. 1993. Compartmental distribution of free amino acids and protein in developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*). Mar. Biol. 116:349-354.

At hatching the free amino acids and protein in Atlantic halibut larvae are mainly located in the yolk compartment. During the first 12 days of the yolksac stage more than 70% of the free amino acid pool disappeared from the yolk without any significant changes in the yolk protein pool. The data suggest that in the early yolksac stage free amino acids enter the embryo from the yolk and are utilized both for energy and protein synthesis. Once the free amino acid pool cannot fulfil the nutritional requirements of the larvae, additional amino acids are recruited from yolk protein. It was estimated that of the total amino acids present at hatching (including free amino acids and those bound in protein), 60% will be used as precursors for body protein synthesis and the remaining 40% a source of energy.

Rœnnestad, I., R.N. Finn, I. Lein and Ø. Lie. 1995. Compartmental changes in the contents of total lipid, lipid classes and their associated fatty acids in developing yolk-sac larvae of Atlantic halibut, *Hippoglossus hippoglossus* (L.). Aquacult. Nutr. 1:119-130.

At hatching larval Atlantic halibut contained 17 µg/individual of lipid (11% of larval body dry weight), while the yolk contained 190 µg. Phosphatidylcholine accounted for 57% of total yolk lipids while phosphatidylethanolamine, triacylglycerol, cholesterol, and sterol ester accounted for 12, 12, and 9, and 6% respectively. The main fatty acids in the phosphatidylcholine fraction were 22:6n-3, 16:0, and 20:5n-3. Between hatch and 200-degree days posthatch,. a net decline in total lipids of 29% was seen. An observed net synthesis of phosphatidylethanolamine in developing larvae and the preferential retention of 22:6n-3 into it may point to a high biological value of this compound.

Saitoh, K., M. Tanaka, R. Ueshima, T. Kamaishi, T. Kobayashi and K-I Numachi. 1995. Preliminary data on restriction mapping and detection of length variation in Japanese flounder mitochondrial DNA. Aquaculture, 136:109-116.

Individual Japanese flounders were found to possess discrete types of mitochondrial DNA. This implies that mitochondrial DNA typing could be useful for monitoring the effectiveness or genetic impact of ongoing enhancement stocking activities.

Samuelsen, O.B. and B.T. Lunestad. 1996. Bath treatment, an alternative method for the administration of the quinolones flumequine and oxolinic acid to halibut *Hippoglossus hippoglossus*, and in vitro antibacterial activity of the drugs against some *Vibrio* sp. Dis. Aquat. Org. 27:13-18.

Administration of flumequine and oxolinic acid to halibut *Hippoglossus hippoglossus* by bath resulted in significant tissue levels of both the antibacterials. Bath treatment using 150 mg/l of flumequine and 200 mg/l of oxolinic acid for 72 h resulted in flumequine concentrations of 14.2 µg/g in muscle and 85.4 µg/g in abdominal organ homogenate and oxolinic acid concentrations of 9.4 µg/g in muscle and 72.6µg/g in abdominal organ homogenate. Excretion of both antibacterials was rapid. The minimum inhibitory concentration against strains of *Vibrio* sp. ranged from 0.015 to 1.0 μg/ml for flumequine and 0.015 to 0.5 μg/ml for oxolinic acid.

Sano, T. and H. Fukuda. 1986. Principal microbial diseases of mariculture in Japan. Aquaculture, 67:59-69.

Vibriosis, pseudotuberculosis, streptococcicosis, nocardiosis and bacterial kidney disease (BKD) are described as the most important bacterial infections associated with Japanes mariculture. Viral infections with epizootic outbreaks have been observed in yellowtail fry and Japanese flounder. Drugs approved for use in Japan for treatment of diseases of marine fish are listed.

Sano, T., H. Fukuda and T. Komatsu. 1994. Thermal effect on lymphocystis of flounder (*Paralichthys olivaceus*). p. W-22.4, *In*: International Symposium on Aquatic Animal Health: Program and Abstracts. Univ. California School of Veterinary Medicine, Davis.

In the case of cultured fish, lymphocystis virus is non-lethal but has a socio-economic impact due to its appearance. In the work reported on here, lymphocystic lesions on flounder began appearing in autumn and disappeared in early summer. During that period, the incidence of lesions was as high as 24%. Development and regression of lymphocystis is closely related to rearing water temperature. Lesions become visible at water temperatures less than about 22°C in October and disappear at above about 20°C in June or July. Maintaining diseased animals at temperatures above 25°C caused remarkable regression in 3 to 5 weeks. Maintaing diseased fish at temperatures lower than 15° C led to further progression of lesions.

Sasaki, K., H. Kurokura and S. Kasahara. 1988. Changes in low temperature tolerance of the eggs of certain marine fish during embryonic development. Comp. Biochem. Physiol. 91A:183-187.

Changes in tolerance to low temperature during embryonic development were investigated in three fishes, including olive (Japanese) flounder, *Paralichthys olivaceus*. The tolerance decreased at cleavage stages, early gastrula stage, the stage of embryo appearance, and the stage of blastopore closure in each species. Mitotic division appeared to be obstructed when cleavage stages were exposed to low temperature. Cooling the early gastrula stage resulted in delay of epiboly of the periblast. No abnormalities were found in embryos exposed to low temperature at the stage of blastopore closure.

Schauer, P.S. and K.L. Simpson. 1985. Bioaccumulation and bioconversion of dietary labeled fatty acids in *Artemia* and winter flounder (*Pseudopleuronectes americanus*). Can. J. Fish. Aquat. Sci. 42:1430-1438.

One-day-old *Artemia* were fed for 14 days on nine different rice bran diets, along with various labelled fatty acids. The *Artemia* were then fed as to winter flounder (*Pseudopleuronectes americanus*). Bioaccumulation was observed for 18:2n-6, 18:3n-3, and 20:5n-3 and all *Artemia* groups contained some 22:6n-3, even when it was not present in the diet. The *Artemia* appeared to bioconvert small amounts of other fatty acids to 20:5n-3 and 22:6n-3. The bioconversion rate was substantially reduced when either 20:5n- 3 or 22:6n-3 was present in the diet. Winter flounder fed the *Artemia* bioaccumulated 20:5n-3 and 22:6n-3 and also bioconverted some of the 18:3n-3 to 20:5n-3 and 22:6n-3.

Schram, T.A. and T. Haug. 1988. Ectoparasites on the Atlantic halibut, *Hippoglossus Hippoglossus* (L.), from northern Norway - Potential pests in halibut aquaculture. Sarsia, 73:213-227.

A total of 174 adult and 72 immature wild Atlantic halibut were examined for skin parasites. Immature fish did not carry parasites. The prevalence of the caligid *Lepeophtheirus* *hippoglossi* infection at the two Norwegian localities, Soroysund and Malangen, was 78 and 66%. The monogenean *Udonella caligorum* was commensal mainly on *L. hippoglossi* from Soroysund. No less than 50 to 60 % of the halibut from Soroysund and Malangen were infected by the monogenean *Entobdella hippoglossi*. More females than males were parasitized.

Seikai, T. 1989. Albinism of hatchery-reared flounder (*Paralichthys olivaceus*) as a result of deformation of asymmetrical development of skin structure. p. 489, *In*: J.H.S. Blaxter, J.C. Gamble and H. vonWesternhagen (Eds.). The early life history of fish. Rapp. P-V. Reun. Ciem. 191.

Many metamorphosed flatfish show different chromatophore distribution and scale shape between the ocular and blind sides. Normally pigmented juvenile Japanese flounder, *Paralichthys olivaceus*, were produced by feeding wild zooplankton and nearly total albinism was produced by feeding the larvae Brazilian *Artemia* nauplii and rotifers. Albinism species may be induced by asymmetrical development of the skin structure.

Seikai, T. and J. Matsumoto. 1991. Mechanism of pseudoalbinism in flatfish: An association between pigment cell and skin differentiation. J. World Aquacult. Soc. 25:78-85.

Two groups of Japanese flounder larvae were reared under different conditions to provide either normally pigmented or pseudoalbinic metamorphosed juveniles. The results suggested that pseudoalbinism was evoked as a result of disruption of the mechanisms that controlled the establishment of asymmetric skin structures during metamorphosis.

Seikai, T., M. Shimozaki, M. and T. Watanabe. 1987. Jinko-saibyo hirame chigyo no hakka shutsugen o ketteisuru shigyo-ki hatsuiku dankai no suitei. [Estimation of larval stage determining the appearance of albinism in hatchery-reared juvenile flounder *Paralichthys olivaceus*.] Bull. Jpn. Soc. Sci. Fish. 53:1107-1114.

The larval stage in Japanese flounder, *Paralichthys olivaceus*, when the future body color (normal coloration or albinism) is determined, was estimated by a rearing experiment. High incidences of normal coloration development or albanism can be induced by feeding Brazilian *Artemia* and rotifers, or wild zooplankton and rotifers. Ten-day-old larvae were reared for 29-30 days to the juvenile stage on different feeding schedules with combinations of the two types of food organisms. The initial stage of metamorphosis was estimated to be the critical stage determining the ultimate juvenile coloration as influenced by the food received.

Seikai, T., T. Watanabe and M. Shimozaki. 1987. Sanchi no kotonaru 3-shu no arutemia kyuji ga hirame hakka kotai no shutsugen ni oyobosu eikyo. [Influence of three geographically different strains of *Artemia* nauplii on occurrence of albinism in hatchery-reared flounder *Paralichthys olivaceus*.] Bull. Jpn. Soc. Sci. Fish. 53:195-200.

Remarkable differences in the percentage occurrence of albinism were observed among three groups of hatchery-reared Japanese flounder, *Paralichthys olivaceus*, larvae fed different strains of *Artemia* nauplii (from Brazil, China and San Francisco Bay).

Seikai, T., J. Matsumoto, P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Mechanisms of albinism in flatfish with regard to pigment cells and skin differentiation. pp. 328-330, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium

A high percentage appearance of albinism is a serious problem in flatfish fingerling production. Malpigmented larvae demonstrate lower survival rates after release in the environment and marketable malpigmented fish bring lower market prices. This study was designed to analyze the mechanism of asymmetric pigmentation and albinism in the Japanese flounder, *Paralichthys olivaceus,* with regard to pigment cells and skin differentiation.

Shangguan, B. and L.W. Crim. 1995. The effect of stripping frequency on sperm quantity and quality in winter flounder (*Pleuronectes americanus* Walbaum). p. 142, *In*: F.W. Goetz and P. Thomas (Eds.). Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish. Fish Symposium 95, Austin, Tx.

Increased sampling frequency significantly raised the total sperm production collected in the first eight weeks from winter flounder, *Pleuronectes americanus,* and did not influence sperm motility and fertility. Delaying collection of milt reduced the quantity and quality of sperm.

Shields, R.J., J. Davenport, C. Young and P.L. Smith. 1992. Oocyte maturation and ovulation in the Atlantic halibut, *Hippoglossus hippoglossus* (L.), examined using ultrasonography. Aquacult. Fish. Manage. 24:181-186.

High resolution ultrasound scanning was evaluated as a non-invasive technique for monitoring oocyte maturation and ovulation in Atlantic halibut *Hippoglossus hippoglossus.* Female broodstock were examined prior to, and during spawning. Individual oocytes of spawning females were discernible during final maturation, due to the large increase in volume caused by water uptake. The resulting differences in depth of ultrasound penetration permitted easy distinction of pre-spawning from spawning females.

Silva, A.E. 1992. Egg production of Chilean flounder (*Paralichthys microps*, Gunther 1881) in captivity. p. 206, *In*: Growing Toward the 21st Century. Aquaculture '92, Orlando, Florida. World Aquaculture Society, Baton Rouge, Louisiana.

Natural spawning in tanks of five females and three male Chilean flounders was monitored over three years. Spawners were maintained under ambient conditions and fed with local fresh fish. Spawning did not occur until after two years of captivity. The total number of eggs collected varied seasonally and was from 7.01 to 11.67 million, of which 17.5 to 18.2% were fertile. The bulk of production of viable eggs occurred in spring at 14 to 15°C. The average egg production increased from 2.40 to 3.15 million/kg of female biomass. Egg diameter was negatively correlated with temperature.

Silva, A. 1994. Spawning of the Chilean flounder *Paralichthys microps* Gunther, 1881 in captivity. J. World Aquacult. Soc. 25:342-344.

The little eye flounder, *Paralichthys microps,* is one of the most important flounders in Chile and is of interest to aquaculturists. This papers reports on preliminary results associated with breeding *P. microps* in captivity under natural conditions of temperature and photoperiod.

Silva, A. and H. Flores. 1989. Consideraciones sobre el desarrollo y crecimiento larval del lenguado (*Paralichthys adspersus* Steindachner, 1987) cultivado en laboratorio. [Considerations on the development and larval growth of the flounder (*Paralichthys adspersus* Steindachner, 1987) cultured in laboratory]. Rev. Com. Perm. Pac. Sur. 1989:629-634.

Larval development, growth and survival of *Paralichthys adspersus* were investigated. The larvae measure 1.9 mm at hatching. Survival ranged from 90.7% to 98.8% during the first four to five days post-hatch, during which time the larvae reach 3.8 to 4.0 mm long. The hatched larvae were fed on rotifers (*Brachionus* sp.) supplemented with brine shrimp. Survival at 27 days of age ranged from 15.8 to 23.3%. Metamorphosis was complete by day 60, with the fish averaging between 13.4 and 14.6 mm. Survival ranged from 13.1 to 11.7%.

Sim, D.S., S.H. Jung, S.H. and S.D. Lee. 1995. Changes in blood parameters of the cultured flounder *Paralichthys olivaceus* naturally infected with *Staphylococcus*

***epidermidis*. Bull Natl. Fish. Res. Dev. Agency, 49:149-155.**

Changes in various hematological, hemochemical and electrolyte blood serum characteristics were examined in cultured olive (Japanese) flounders, *Paralichthys olivaceus*, naturally infected with *Staphylococcus epidermidis*. Fish with slight signs showed high values for red blood cell counts, hemoglobin and hematocrit in comparison with control fish. Flounders with serious signs showed low red cell counts and hemoglobin. Slight disease signs were associated with a low value of potassium, but serious signs were seen in conjunction with high values. The value of calcium was progressively lower than the control as disease signs increased.

Skiftesvik, A.B. and Ø Bergh. 1993. Changes in behavior of Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) yolk-sac larvae induced by bacterial infections. Can. J. Fish. Aquat. Sci. 50:2552-2557.

Eggs of Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) were exposed to *Flexibacter ovolyticus* and pathogenic *Vibrio* sp. prior to, and during hatching.. The halibut larvae showed reduced activity and increased mortality in response to the bacterial challenge. In addition, the infected halibut larvae showed increased specific density compared to the uninfected larvae. Similar responses were not found for turbot. However, turbot larvae infected with *Vibrio anguillarum* had lower activity than larvae infected with *F. ovolyticus*. The reduced activity of halibut larvae occurred 1 to 2 weeks prior to the increased mortality. Results suggest that the behavior of fish larvae is influenced by bacterial infection.

Skiftesvik, A.B. and Ø. Bergh. 1995. Impact of bacterial infection on activity, buoyancy and mortality of halibut and turbot yolk-sac larvae. ICES Mar. Sci. Symp. 201:204.

Eggs of Atlantic halibut (*Hippoglossus hippoglossus* L.) and turbot (*Scophthalmus maximus* L.) were exposed to *Flexibacter ovolyticus* and pathogenic *Vibrio* sp. prior to, and during hatching. Performance of the yolksac larvae were monitored from hatching to day 34 in halibut and to day 6 in turbot. Halibut larvae infected with both pathogens had

Skiftesvik, A.B., Ø Bergh and I. Opstad. 1994. Activity and swimming speed at time of first feeding of halibut (*Hippoglossus hippoglossus*) larvae. J. Fish Biol. 45: 349-351.

Atlantic halibut (*Hippoglossus hippoglossus*) larvae showed an increase in activity, together with a decrease in swimming speed during active periods, coinciding with the time of ~50% yolk absorption (day 26 post-hatch).

Skjermo, J., T. Defoort, M. Dehasque, T. Espevik, Y. Olsen, G. Skjaak-Brek, P. Sorgeloos and O. Vadstein. 1995. Immunostimulation of juvenile turbot (*Scophthalmus maximus* L.) using an alginate with high mannuronic acid content administered via the live food organism *Artemia*. Fish Shellfish Immunol. 5:531-534.

Poor control of the microbial conditions in the intensive rearing facilities may adversely affect the health of fish larvae, and is most likely one of the main factors restricting production of marine juveniles. This reserach demonstrated that increased protection against a pathogenic bacterium was provided to juvenile turbot (*Scophthalmus maximus*) juveniles fed *Artemia* enriched with a suspension of alginate microspheres containing an immunostimulant.

Soltani, M., B.L. Munday and C.M. Burke. 1996. The relative susceptibility of fish to infections by *Flexibacter columnaris* and *Flexibacter maritimus*. Aquaculture, 140:259-264.

The susceptibility of freshwater and marine fish to infection by *Flexibacter columnaris* and *Flexibacter maritimus* was assessed in this laboratory study. At 15 ppt salinity greenback flounders (*Rhombosolea tapirina*) were more resistant to *F. maritimus* than Atlantic salmon (*Salmo salar*).

Spedicato, M.T., G. Giorgetti, G. Lembo, G. Bertoja, G. Bovo and G. Ceschia, G. 1993. Introduzione di uova embriondate di *Paralichthys olivaceus* (Temminch e Schlegel) dal Giappone: Tecniche di quarantena e risultati preliminari dell'allevamento. (Introduction of fertilized eggs of *Paralichthys olivaceus* [*Temminch e Schlegel*] from Japan: Quarantine techniques and preliminary results of rearing.). Ital. Acquacolt. 28:25-37.

Requirements for warm water, a slow growth rate, and poor survival limit the development of turbot, *Scophthalmus maximus*, in Italy. Alternative species have been considered. This study evaluated the Japanese flounder, *Paralichthys olivaceus*, for suitability in Italy. Fertilized eggs of *P. olivaceus* were first imported from Japan in April 1992 and incubated under strict quarantine conditions. Survival rates at 16°C, 34 ppt salinity and pH 7.5 were satisfactory: 83% at ten days after hatching, 61% at 45 day, and 49% at 65 days. Larvae were first fed rotifers (*Brachionus*), followed by *Artemia* naupli. From 46 days after hatching only prepared feed was provided. Preliminary results indicate that the Japanese flounder may be profitably farmed in Italy.

Stickney, R.R. 1994. A review of the research efforts on Pacific halibut, *Hippoglossus stenolepis*, with emphasis on research and development needs. pp 4-6, *In*: T. Nosho and K. Freeman (Eds.). Marine fish culture and enhancement. Washington Sea Grant Program, Seattle, Washington.

Research conducted on Pacific halibut (*Hippoglossus stenolepis*) in Washington to date has been a collaborative effort of the University of Washington School of Fisheries (SOF), the International Pacific Halibut Commission (IPHC), the U.S. Fish and Wildlife Service (USFWS), the National Marine Fisheries Service (NMFS) and Stolt Sea Farms. We also have collaborated with scientists and conducted research at the Nanaimo Laboratory of the Department of Fisheries and Oceans (DFO) in Canada. Interest in the research community was inspired by a desire on the part of IPHC to study captively reared Pacific halibut larvae as a means of learning more about early life history and, in particular, growth rates and morphological changes at various ages. There also was interest in evaluating the potential of producing Pacific halibut postlarvae or juveniles for enhancement and for commercial culture. SOF investigators became involved with Pacific halibut culture in 1986.

Stickney, R.R. and H.W. Liu. 1991. Spawning and egg incubation of Pacific halibut. World Aquculture, 22(4):46-48.

The Pacific halibut (*Hippoglossus stenolepis*) is a highly regarded sport and commercial fish native to the North Pacific Ocean. It occurs on the continental shelf from southern California (latitude 34°N) northward into the Bering Sea (latitude 63°N). Halibut can reach weights of several hundred kilograms, with males maturing at about eight years and females at 12 years of age. The number of eggs that can be obtained from an adult halibut female will, of course, vary considerably with the size of the fish. Published reports indicate that a 25 kg fish can produce a half million eggs while a 120 kg fish may produce as many as four million eggs each year. Because of limitations in holding facilities the adults maintained usually do not exceed about 25 kg. At hatching, Pacific halibut larvae are about 6.25 mm long and have a dry weight of 210 μg. They have large yolk sacs that must support them for a few weeks while they develop from a relatively primitive stage at hatching to a stage where they can begin feeding. At hatching they lack pigmentation, their mouths have not formed, and they have very limited swimming ability. A few hours after hatching they can swim short distances, but they spend most of their time resting suspended in the water. By the end of 20 days, the larvae are over 9 mm long, a significant percentage of their yolk has been absorbed and the mouth is fully developed. Larval dry weight (excluding yolk sac) average 570 μg at 20 days of age. Conservation hatcheries charged with stocking juvenile halibut at sea to augment the commercial and recreational fisheries, as well as commercial aquaculture, could develop in the future. While our estimate may be optimistic, it should be possible to consider enhancement stocking within the current decade and also to have a good indication of the feasibility of commercial aquaculture in pond, tanks, or net-pens within the same time frame.

Stickney, R.R. and H.W. Liu. 1993. Culture of Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*Hippoglossus stenolepis*). Rev. Fish. Sci. 1:285-309.

Interest in the culture of Atlantic and Pacific (*Hippoglossus hypoglossus* and *H. stenolepis*) halibut developed in the 1980s. In general, information collected with respect to the culture requirements of one halibut species is applicable to the other. Maintenance of captive broodstock in circular culture tanks of sufficient size can be accomplished without difficulty. Broodfish can be induced to develop and spawn with or without the use of hormones. Various devices for incubating eggs have been developed and tested, with slow upwelling systems being among the most successful. Eggs are fragile and can be protected to some degree by incubating them at their salinity of neutral buoyancy. A major bottleneck to successful routine culture of the two species is in the area of larval development. The larvae are weak swimmers, extremely fragile, and slow to develop to the size of first-feeding. Larvae that will accept feed require several additional weeks before they metamorphose into juveniles. The environmental requirements of halibut larvae have been determined, in part. Of the two species, more progress toward commercial culture has been made with Atlantic than Pacific halibut.

Stickney, R.R. and H.W. Liu. 1995. Status and potential for Pacific halibut culture. Bull Aquacult. Assoc. Can. 95-4:5-9.

Pacific halibut, *Hippoglossus stenolepis*, culture research in the United States was initiated in the mid-1980s with the intentions of examining larval morphology and evaluating the potential of the species for enhancement or foodfish culture. Adult halibut have been acclimated to circular tanks and standard salmon net-pens and been trained to accept prepared feeds. Fish have been spawned routinely, eggs have hatched in significant numbers, and larvae reared to the time of first-feeding. Rearing through metamorphosis has remained an elusive goal. This paper summarizes accomplishments from the studies prior to 1994-1995 and presents a synopsis of information that was obtained during the 1995 spawning season. A few words on future plans are included.

Stickney, R.R. and D. Seawright. 1993. A bibliography on Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*Hippoglossus stenolepis*) culture, with abstracts. Tech. Rep.. No. 29, International Pacific Halibut Commission, Seattle, WA. 32 p.

This report provides citations and abstracts of studies conducted on Atlantic and Pacific halibut up to the year of publication.

Stoettrup, J. 1996. Stocking: Actual situation and prospects for the marine environment. Aquacult. Eur. 20:6-11.

Present day releases of marine fishes incorporate primarily releases of hatchery-reared juveniles of several species. Included among European species are flounder (*Pleuronectes flesus*), plaice (*P. platessa*) and turbot (*Psetta maxima*).

Strout, R.G., E.S. Sawyer and B.A. Coutermarsh. 1978. Pathogenic vibrios in con-

finement-reared and feral fishes of the Maine-New Hampshire coast. J. Fish. Res. Board Can. 35:403-408

Vibrio anguillarum was isolated from moribund or fresh dead captive and wild fishes from the Maine-New Hampshire coast. All *Vibrio* isolates were tested for pathogenicity in coho salmon smolts reared in freshwater. One group of *Vibrio* isolates from winter flounder (*Pseudopleuronectes americanus*) killed all salmon smolts the same day of injection.

Sudo, H., T. Goto, R. Ikemoto, M. Tomiyama and M. Azeta. 1993. Shijiki wan ni okeru hirame horyu shubyo no genmo katei. [Mortality of reared flounder (*Paralichthys olivaceus*) juveniles released in Shijiki Bay.]. Bull. Seikai Natl. Fish. Res. Inst. 70:29-37.

Experimental releases involved 37,000 (33.7 mm TL) and 24,600 (81.2 mm TL) flounder juveniles into Shijiki Bay in 1985 and 1986. Recaptures amounted to 577 and 450 in the two years, with most being caught within 10 days after release. In 1985, the rate of decrease in abundance within the bay of small fish (TL < 35 mm) was higher than that of large ones, albino decreased abundance was higher than that of normal fish, and feeding incidence was low. In 1986, there was no difference in the rate of abundance decrease between small and large fish, the rates were similar for both albino and normal fish, and feeding incidence was high. It is suggested that the most significant causes of differences in the rates of decrease in the populations were predation in 1985 and dispersal in 1986. The conclusion is based on the fact that it is easy for predators to catch and handle small and starving fish and to detect albinic fish.

Sugiyama, M. and Y. Yano. 1989. Hirame (*Paralichthys olivaceus*) chigyo no ryotaisoku taishoku hatsugen ni oyobosu chiourea no eikyo ni tsuite. [Effect of thiourea on the abnormal pigmentation in flounder (*Paralichthys olivaceus*) larvae.] Bull. Natl. Res. Inst. Aquacult. 16:109-112.

Treatment of Japanese flounder, *Paralichthys olivaceus*, larvae during metamorphosis with 30 ppm thiourea increased the incidence of albinism on the ocular side and the appearance of abnormal heavy pigmentation of the blind side. The observed result may be caused by the inhibiting effects of thiourea on tyrosinase activity and/or thyroid function.

Suprapto, H., T. Nakai and K. Muroga. 1995. Toxicity of extracellular product and intracellular components of *Edwardsiella tarda* in the Japanese eel and flounder. J. Aquat. Anim. Health, 7:292-297.

Virulent *E. tarda* strains belonging to serotype A produced heat-labile substance which was lethal to Japanese eel and Japanese flounder. The susceptibility of Japanese flounder to the toxin was approximately 15 times higher than that of the Japanese eel. The susceptibility to the live *E. tarda* was much higher in the flounder than in the eel. These results indicate that the toxin plays an important role in the virulence of *E. tarda*.

Suzuki, N. 1994. Yoshoku hirame mugansoku hifu no bisaikozo to sono taishokuijosei no soshikigaku-teki kento. (Ultrastructure of the skin on reverse side of hatchery-reared Japanese flounder, *Paralichtys olivaceus* with reference to the pigmentation.). Bull. Nansi Natl. Fish. Res. Inst. 27:113-128.

The ultrastructure of the skin on the reverse side of a hatchery-reared pseudoalbinic Japanese flounder, *Paralicthys olivaceus*, after metamorphosis was studied. Many melanophages migrating around a melanocyte or melanoblast were observed in unpigmented areas. They are involved in phagocytosis of melanin. The asymmetrical formation process is thought to be due the cell differentiation on the obverse side and pedomorphism on the reverse side through the adaptive characteristics in relation to a bottom life style.

Tagawa, M., S. Miwa, Y Inui, E. Grace de Jesus and T. Hirano. 1990. Changes in

thyroid hormone concentrations during early development and metamorphosis of the flounder, *Paralichthys olivaceus*. Zool. Sci. 7:93-96.

Changes in the whole body concentrations of thyroid hormones were examined during early development and metamorphosis of Japanese flounder (*Paralichthys olivaceus*). Thyroxine (T_4) and triiodothyronine (T_3) were detected in eggs just after fertilization. The concentration of T_3 declined gradually until hatching, decreased sharply within one day after hatching, and became non-detectable thereafter. In contrast, T_4 concentration did not show marked changes until 10 days after fertilization. Until the climax of metamorphosis, T_3 was undetectable and T_4 concentration was less than 1 ng/g.

Takahashi, Y-I. 1994. Hirame chigyo no mugangawa no taishoku ijo ni oyobosu shiiku mitsudo to shijiryo no eikyo. [Influence of stocking density and food at late phase of larval period on hypermelanosis on the blind body side in juvenile Japanese flounder]. Bull. Jpn. Soc. Sci. Fish. 60:593-598.

The influence of stocking density and food on hypermelanosis on the blind body side was investigated in late phase larval Japanese flounder. Four stocking densities (1,000, 5,000, 10,000, and 30,000 fish/m^3) and two types of food (a commercial feed and *Artemia* nauplii) were tested. Larvae 27 days of age (11.5 mm total length) to 42 days of age (about 20 mm TL). Pigmentation on the blind and ocular was monitored and scored. High stocking density and artificial feed reduced the occurrence of fish with normal coloration on the blind side. Stocking density appeared to have no effect on ocular side coloration, while feeding of *Artemia* nauplii resulted in a lower percentage of normal coloration on the ocular side.

Takaji I. and W. Hisatsugu. 1992. Preliminary trial of vaccination against edwardsiellosis of hirame (Japanese flounder), *Paralichthys olivaceus*. pp. 407-412, *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Proceedings of the First Symposium on Diseases in Asian Aquaculture. Fish Health Section, Asian Fisheries Society, Manila.

Japanese flounder (*Paralichthys olivaceus*) were vaccinated against edwardsiellosis. One group of fish was injectedwith *Edwardsiella tarda* and another was immersed for five minutes into the bacterin. Booster vaccinations were delivered one week later. Serum agglutination antibody titre was measured 1, 4, 7 and 8 weeks after booster. The titre was not detected in the unvaccinated control fish, but the injected fish varied in titre from 256 to 4,096. Titres in the injected fish were much higher than those of immersed fish, which ranged 4 to 128. Phagocytic rates of the neutrophils from the injected fish and the immersed fish were 13.1 and 12.5%, respectively, and were higher than that from the unvaccinated control fish (7.1%).

Takeuchi, T., J. Dedi, C. Ebisawa, T. Watanabe, T. Seikai K. Hosoya, and J-I Nakazone. 1995. The effect of ß-carotene and vitamin A enriched *Artemia* nauplii on the malformation and color abnormality of larval Japanese flounder. Fish. Sci. 61:141-148.

Two experiments were conducted to determine if the occurrence of color abnormality and malformation in larval Japanese flounder could be prevented when enriching *Artemia* nauplii with vitamin A and its precursor. Ten-day-old larval flounder were fed different the Utah *Artemia* strain in one experiment and the Tien-tsin strain in another. Vitamin A appeared to be effective in preventing the abnormal coloration caused by Tien-tsin *Artemia*. Hypervitaminosis results if excessive amounts of the vitamin are employed.

Takeuchi, A. T. Okano, M. Tanda and T. Kobayashi. 1991. Possible origin of extremely high contents of vitamin D sub(3) in some kinds of fish liver. Comp. Biochem. Physiol. 100A:483-487.

Comparative studies on the possible origin of extremely high contents of vitamin D sub(3) in some kinds of fish liver were performed. Neither photochemical formation of vitamin D sub(3) in fish skin by solar radiation of 7-dehydrocholesterol (7-DHC) nor nonphotochemical enzymatic formation of vitamin D sub(3) from 7-DHC in fish liver was demonstrated as the origin of vitamin D sub(3). On the other hand, when bastard halibuts and carps were farmed from fingerlings to adults with feedstuffs containing vitamin D sub(2) or D sub(3), significant amounts of the vitamins were accumulated in the fish liver. The contents of vitamins D sub(2) and D sub(3) in bastard halibut liver increased according to the duration of farming and dose responses of the vitamins in carp liver were observed. Significant amounts of vitamins D sub(2) and D sub(3) in phytoplankton and vitamin D sub(3) in zooplankton and small fish were detected. Therefore, we have concluded that the most probable origin of vitamin D sub(3) in fish liver is a result of food chains from plankton.

Tanaka, H. 1987. Hirame no seishokusen no sei bunka katei. [Gonadal sex differentiation in flounder, *Paralichthys olivaceus.*] Bull. Natl. Res. Inst. Aquacult. 11:7-19.

The process of gonadal sex differentiation in the Japanese flounder, *Paralichthys olivaceus* was studied histologically. In larvae of 5.6 mm total length the primordial germ cell was found between the mesonephric duct and the gut. Genital ridges containing a few germ cells and somatic cells were found beneath the dorsal peritoneal wall in 7.3 mm larva. In 13-18 mm larvae, the gonadal germ cells gradually multiplied. Oocytes in early meiosis were first observed from the ovary of 55 mm females. Oocytes in the early perinucleolus stage were found in females longer than 150 mm. Multiplication of the spermatogonia and active spermatogenesis were observed, respectively, in the testes of 100 mm and 200 mm total length males.

Tanaka, H. 1988. Hirame no seishokusen no sei bunka ni oyobosu esutorajioru-17ß no eikyo. [Effects of estradiol-17ß on gonadal sex differentiation in flounder, *Paralichthys olivaceus.*] Bull. Natl. Res. Inst. Aquacult. 13:17-23.

Estradiol-17ß was orally administrated to juvenile Japanese flounder, *Paralichthys olivaceus*, for a period from 56 to 90 days after hatching at concentrations of 0 to 100 µg/g diet treatment to examine its effects on gonadal sex differentiation. At age 116 days, higher percentages of females were observed in groups receiving a hormone dose of 0.1/ µg/g diet compared with controls. In groups treated with more than 1µ g/g diet, all the fish examined were female. Sterilized ovaries and hyperplasia of connective tissue around the gonads were found in fish treated with high doses of estradiol amd high rates of mortality occurred in groups treated with 100 µg/g diet.

Tanaka, M., J.B. Tanangonan, M. Tagawa and T. Hirano. 1989. Ontogenetic changes in thyroid hormone concentrations related to morphological and ecological events during the early life history of fish. p. 490, *In:* J.H.S. Blaxter, J.C. Gamble and H. von Westernhagen (Eds.). The early life history of fish. Rapp. P-V. Reun. Ciem. 191.

Ontogenetic development of the thyroid hormones during egg, larval, and early juvenile periods of teleosts was investigated in a number of species, including Japanese flounder (*Paralichthys olivaceus*), stone flounder (*Kareius bicoloratus*) and marbled sole (*Limanda yokohamae*). The presence of thyroid hormones throughout the early ontogenetic period and diagnostic changes in their concentrations showed that the hormones play a fundamental role in the metamorphosis of pleuronectiforms.

Tanaka, Y. and S. Kadowaki. 1995. Kinetics of nitrogen excretion by cultured flounder *Paralichthys olivaceus.* J. World Aquacult. Soc. 26:188-193.

The rate of nitrogen excretion by the cultured Japanese flounder as a function of body weight and water temperature was studied. Diurnal rates of nitrogen excretion were measured at mean water temperatures of about 16, 19, and 23°C. Nitrogen excretion rate was estimated from total nitrogen excreted as ammonia and urea. Feeding time governed the diurnal pattern in nitrogen excretion rate. The percentages of total nitrogen excreted as ammonia were 77.9% at 16°C, 84.5% at 19°C and 83.2% at 23°C. A formula for the relationship between fish body weight and nitrogen excretion rate was developed.

Tanaka, M., P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Functional development of the endocrine system during early ontogeny of marine teleost fishes. pp. 199-200, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc. Gent, Belgium.

This paper reviews information on the functional development of the endocrine system, particularly of studies recently conducted by the authors. Information is included on development of the endocrine organs in the following flatfishes: Japanese flounder, stone flounder, marbled sole, plaice, and bamboo sole, reared from fertilization beyond metamorphosis.

Tanasomwang, V. and K. Muroga. 1988. Intestinal microflora of larval and juvenile stages in Japanese flounder (*Paralichthys olivaceus*). Fish Pathol. 23:77-83.

The number of bacteria in the intestine of larval and juvenile Japanese flounder from 10 mm up to 19 mm total length decreased with increasing fish size. The decrease hypothesized to be due to the change in feeds from live diets (rotifers and brine shrimp) to prepared feeds. The composition of intestinal flora was characterized by two predominating groups of *Vibrio* and *Pseudomonas* followed by *Moraxella*, *Cytophaga*, and *Alcaligenes*. Similar generic composition was recovered in water and live diets, while bacteria in the prepared feeds were *Acinetobacter* and Gram-positive bacteria in addition to *Moraxella*. *Vibrio alginolyticus* was frequently isolated from fish examined in one station where no apparent fish mortality occurred.

Tanda, M. 1990. Makogarei oyobi hirame shubyo no sensa-noryoku to teishitsu-sentaku. [Studies on burying ability in sand and selection to the grain size for hatchery-reared marbled sole and Japanese flounder.] Bull. Jpn. Soc. Sci. Fish. 56:1543-1548.

The burying ability and grain size selection of hatchery-reared marbled sole (*Limanda yokohamae*) and Japanese flounder (*Paralichthys olivaceus*) juveniles were studied in sands varying in grain sizes. Burying ability increased with growth.

Teshima, S., A. Kanazawa and S. Koshio. 1991. Potentiel de bioconversion des acides gras de la serie n-3 chez les poissons et les crustaces. Oceanis, 18:67-75.

The ability for bioconversion of 18:3n-3 to other fatty acids, especially highly unsaturated fatty acids (HUFA) was examined in various fishes and shrimp, including Japanese flounder *Paralichthys olivaceus* to determine essential fatty acid requirements. The test animals were fed on diets containing ^{14}C-labeled 18:3n-3, and lipids were extracted from the whole bodies 12 hours after feeding. Radio-gas chromatographic techniques were used to determine the percentage distribution of radioactive fatty acids in the whole bodies (% of total radioactive body lipids).

Teshima, S.I., A. Kanazawa, S. Koshio and S. Itoh. 1993. L-ascorbyl-2-phosphate-Mg as vitamin C source for the Japanese flounder (*Paralichthys olivaceus*). pp. 157-166, *In*: S.J. Kanshik and P. Luquet, P (Eds.). Fish Nutrition in Practice. Institut National de la Recherche Agronomique, Paris.

A relatively stable form of vitamin, L-ascorbyl-2-phosphate-Mg was evaluated as a vitamin C source for Japanese flounder, *Paralichthys olivaceus*. Two feeding experiments were conducted in a flow-through system at 19 to 22°C. In the first, fish weighing ~3 g were fed two casein-based diets containing the vitamin at 788 mg/100 g of diet or no supplemental ascorbic acid for 90 days. Fish receiving the diet without supplementation showed retarded growth and high mortality within 45 days, whereas fish receiving the reached ~50 g after 90 days. In a second experiment, fish weighing ~43 g were fed 5 diets containing graded levels of the vitamin source (0, 1, 3, 6, and 10 mg/100 g diet) for 24 weeks. The basal diet was a casein-white fish meal (1:1) diet. There were no significant differences in body lengths or survival rates after 24 weeks among the experimental groups, but the fish receiving diet without supplemental ascorbic acid led to poor growth. Although no significant difference was found among the diets containing 1 to 10 mg /100 g ascorbic acid, higher weight gains (%) and feed conversion efficiencies were obtained in fish on the diets containing 6 to 10 mg/100 g than those fed lower vitamin levels. The results indicated that 6 to 10 mg of APM/100 g diet was sufficient to support good growth and survival of the Japanese flounder.

Tuene, S. and R. Nortvedt. 1995. Feed intake, growth and feed conversion efficiency of Atlantic halibut, *Hippoglossus hippoglossus* (L.). Aquacult. Nutr. 1:27-35.

The food intake of individual Atlantic halibut, *Hippoglossus hippoglossus* (mean weight = 422 g) was monitored by direct observation for 21 days. Feed conversion effiency and maintenance ration requirement were estimated. Maintenance ration was 0.126% of body weight/day. The gross feed conversion efficiency increased asymptotically with increasing feed intakes and growth rates, and was found to approach 1.9 at high growth rates. One feeding per day seemed to be sufficient for maximum food intake and growth rate.

Uchino, K. and M. Nakanishi. 1983. Wakasa-wan seibu kaiiki (Tango-kai) ni okeru shubyo seisan hirame no hyoshikihory. [Results of tagging experiments of hatchery-reared flounder, *Paralichthys olivaceus*, in the western part of Wakasa Bay (Tango-kai).] Bull. Kyoto Inst. Ocean. Fish. Sci. 7:17-27.

Tagging experiments were conducted on hatchery-reared Japanese flounder, *Paralichthys olivaceus*, in Wakasa Bay, Japan. Nearly 4,000 age 0 and 300 age 1 fish were tagged and released, and 470 fish were recaptured. Growth of hatchery-reared flounder was nearly the same as that of wild flounders in the same waters. Flounder recaptured after nine months had recovered from color anomalies on the ocular side but not on the nonocular side.

Ugelstad, I., I. Lein and K. Hjelmeland. 1995. Synthesis of the digestive enzyme trypsin in halibut (*Hippoglossus hippoglossus* L.) larvae and juveniles. ICES Mar. Sci. Symp. 201:205.

Radioimmunoassay of trypsin in Atlantic halibut larvae strongly indicates that significant synthesis of trypsin starts at 160 degree days after hatching.

van Leeuwen, P.I. and D. Vethaak. 1988. Growth of European flounder (*Platichthys flesus*) and common dab (*Limanda Limanda*) in Dutch coastal waters with reference to healthy and diseased fish. Collected Papers, ICES Council Meeting, 1988, Copenhagen. 12 pp.

Growth was studied by back calculation of otoliths in conjunction with the European flounder (*Platichthys flesus*) in marine, brackish and freshwater areas and dab (*Limanda limanda*) in coastal and offshore waters of the Netherlands. A total of 2,015 otoliths from flounder and 931 from dab collected during August 1987 were examined. Sexual dimorphism in growth was observed in flounder but not in dab. Length at age appeared to differ between in both species with sampling location. Flounder populations in fresh and brackish waters generally showed higher growth rates than those in estuarine and coastal areas. Highest dab growth rates were found offshore. The data did not appear to indicate reduced growth in flounder affected with Lymphocystis disease or skin ulcers. The occurrence of *Glugea stephani* in dab did not appear to affect growth.

Wall, A.E., D.L. Williams, E.J. Branson, W.M. Brancker and M. Williamson. 1994. Ocu-

lar abnormalities in farmed halibut, *Hippoglossus hippoglossus*. p. P-90, *In*: International Symposium on Aquatic Animal Health. University of California School of Veterinary Medicine, Davis, California.

Ocular abnormalities have been recorded in farmed halibut from both cultured and wild caught fish. Large fluid-filled and gas-filled cysts have been consistently seen in both the choroid and the superficial periocular areas. Gas bubbles in the anterior chamber have been recorded. Other ocular changes including cataracts, lens dislocations and total disruption of the globe.

Waring, C.P., R.M. Stagg and M.G. Poxton. 1992. The effects of handling on flounder (*Platichthys flesus* L.) and Atlantic salmon (*Salmo salar* L.). J. Fish Biol. 41:131-144.

The effect of nine minutes of net confinement on the flounder *Platichthys flesus* and the Atlantic salmon was investigated to compare how different species respond to the same stress stimulus. Significant elevations in plasma cortisol, glucose, lactate, osmolality, and monovalent ion levels occurred in both species, though the responses to net confinement in salmon were generally of a greater magnitude. Plasma protein levels were significantly elevated in flounders, but not in salmon.

Watanabe, T. 1993. Importance of docosahexaenoic acid in marine larval fish. J. World Aquacult. Soc. 24:152-161.

This study revealed that in marine larval fish, docosahexaenoix acid is superior to eicosapentaenoic acid as an essential fatty acid in marine fish larvae, including Japanese flounder. A functional difference between the two fatty acids was hypothesized.

Watanabe, T., M. Ohta, C. Kitajima and S. Fujita. 1982. Improvement of dietary value of brine shrimp *Artemia* salina for fish larvae by feeding them on $\omega 3$ highly unsaturated fatty acids. Bull. Jpn. Soc. Sci. Fish. 48:1775-1782.

Experiments aimed at improveing the nutritional value of *Artemia* nauplii for marine fish by feeding the nauplii n-3 highly unsaturated fatty acids (HUFA). The nutritional value of the nauplii for juveniles of flounders and other species was compared with fish fed on various kinds of emulsified lipids or on baker's yeast. Newly-hatched nauplii easily incorporated the lipids when the HUFA were emulsified in a slurry containing water, a small amount of egg yolk and baker's yeast. Feeding on the newly-hatched nauplii of the freshwater type or on nauplii fed either baker's yeast or corn oil resulted in poor growth and survival.

Watanabe, T., P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier. 1991. Importance of docosahexaenoic acid in marine larval fish. p. 19, *In*: LARVI '91. Spec. Publ. 15, European Aquacult. Soc., Gent, Belgium.

Recent studies on essential fatty acids of marine fish have shown that docosahexaenoic acid is superior to eicosapentaenoic acid as an essential fatty acid for larval fish, including flounders. Research suggests that the physiological functions of eicosapentaenoic acid differ from those of docosahexaenoic acid.

White, D.B. and R.R. Stickney. 1973. A bibliography of flatfish (Pleuronectiformes) research with partial annotation. Georgia Marine Science Center Technical Report Series 73-6. Skidaway Institute of Oceanography, Savannah, Georgia. 77 p.

Contains 433 citations of papers published prior to 1973 on the culture of pleuronectid flatfishes. Some citations include a brief summary of results.

Whyte, J.N.C., W.C. Clarke, N.G. Ginther and J.O.T. Jensen. 1993. Biochemical changes during embryogenesis of the Pacific halibut, *Hippoglossus stenolepis*. Aquacult. Fish. Man. 24:193-201.

Eggs of Pacific halibut held in a conical incubator at 6°C hatched 422 hours after

fertilization. Levels of moisture, monosaccharides and total carbohydrates in the embryos demonstrated significant linear increases during development. Significant linear declines in the levels of total lipid and ash occurred, while polysaccharide and protein contents were virtually unchanged. There was a slight increase in relative proportions of the essential eicosapentaenoic acid and docosahexaenoic that was offset by a decline in palmitic acid and indicated that only saturated fats were being used as a source of energy.

Yamamoto, T. and T. Akiyama. 1995. Effect of carboxymethylcellulose, α-starch, and wheat gluten incorporated in diets as binders on growth, feed efficiency, and digestive enzyme activity of fingerling Japanese flounder. Fish. Sci. 61:309-313.

A feeding experiment was conducted to examine the effect of several feed ingredient binders on the growth, feed efficiency, and digestive enzyme activities of fingerling Japanese flounder. Carboxymethylcellulose, gelatinized potato starch (a-starch), and wheat gluten were separately incorporated in flounder diets as binders at the 5% level and the diets were fed to flounder with an initial mean weight of 15 g for 4 weeks at 20°C. Weight gain, feed conversion efficiency, and protein retention were best in fish fed a-starch or wheat gluten. Apparent protein digestibility in the diet containing CMC was significantly lower than the diet containing wheat gluten. Proteolitic enzyme activities such as pepsin-like and trypsin-like enzymes were lower in fish fed the CMC diet than those in fish fed the other two diets. These results suggest that a-starch and wheat gluten are suitable binders for fingerling flounder diets.

Yamamoto, T., T. Unuma and T. Akiyama. 1995. Utilization of malt protein flour in fingerling Japanese flounder diets. Bull. Natl. Res. Inst. Aquacult. 24:33-42.

This study was conducted to determine the maximum substitution level of white fish meal protein by malt protein flour and effect of combined use of malt protein flour and soybean meal in fingerling Japanese flounder diets. Graded levels of white fish meal protein were isonitrogenously replaced by malt protein flour or the combination of malt protein flouur and soybean meal. Fish of 15 g initial weight were fed for 4 weeks at 20°C. Weight gains and feed conversion efficiencies of fish fed diets containing 10 and 20% malt protein flour were not significantly different from those of the control group. The performance of fish fed diets containing 30 and 40% malt protein flour was poorer than that of fish fed a combination of malt protein flour and soybean meal.

Yamamoto, T., K. Fukusho, M. Okauchi, H. Tanaka, W.D. Nagata, T. Seikai, T. and T. Watanabe. 1992. Hirame no hakuka-kotai-shutsugen ni oyobosu hentaiki no shiryo no eikyo. [Effect of various foods during metamorphosis on albinism in juvenile of flounder]. Bull. Jpn. Soc. Sci. Fish. 58:499-508.

The dietary-related albinism in juvenile Japanese flounder, *Paralichthys olivaceus*, was investigated. Newly hatched larvae were reared on rotifers, *Brachionus plicatilis*, alone until they reached 6.8 mm in length. Thereafter, the larvae were divided into eight groups and reared on rotifers, vitamin-enriched rotifers, *Artemia* nauplii, vitamin-enriched *Artemia* nauplii, fertilized red sea bream (*Pagrus major*) eggs and a micro-formulated diet, singly or combined, until the juveniles reached nearly 10 mm. They were then reared on rotifers and *Artemia* nauplii until they had completely metamorphosed. Albinism was highest in the juveniles fed rotifers and/or *Artemia* (88 to 98%).

Yamano, K., S. Miwa, T. Obinata and Y. Inui. 1991. Thyroid hormone regulates developmental changes in muscle during flounder metamorphosis. Gen. Comp. Endocrinol. 81:464-472.

Morphological and biochemical changes in muscle tissue of metamorphosing Japanese flounder (*Paralichthys olivaceus*) were studied in relation to the regulatory role of thyroid hormone. Premetamorphic larvae were reared in unaltered seawater or in seawater contain-

ing either thyroxine (T_4) or an antithyroid drug (thiourea). The muscle tissue was characterized by abundant vacuoles and basophilic sarcoplasm. Results suggest that developmental changes in muscular tissue of metamorphosing flounder are regulated by thyroid hormone.

Yamashita, Y., S. Nagahora, H. Yamada and D. Kitagawa. 1994. Effects of release size on survival and growth of Japanese flounder *Paralichthys olivaceus* in coastal waters off Iwate Prefecture, northeastern Japan. Mar. Ecol. Prog. Ser. 105:269-276.

The totals of otolith-marked, hatchery-raised Japanese flounder from 4 to 15 cm total length released into water of 4 to 8 m at Ohno Bay, northeastern Japan were 35,900 in 1991 and 73,400 in 1990. Alizarin complexone fluorescent marker was used to tag the otoliths 1 to 3 days prior to release. Flounder fry >9 cm at release recruited to the commercial fishery. The primary cause of mortality was thought to be predation by large flounder and greenling.

Yang, B-S, H-M Lee and B-G Jeong. 1992. Water quality control in the semiclosed culture system growing a flounder, *Paralichthys olivaceus*. Bull. Korean Fish. Soc. 25:197-204.

A comparative evaluation of water quality in a semiclosed culture system for Japanese flounder, *Paralichthys olivaceus*, was made to improve the system management. Partial flow-through and recycle modes were contrasted. Peak concentrations of COD, PO_4 and suspended solids in the fish tanks appeared just after feeding, and then decreased to normal levels within 2 hrs. However, the NH^{4+} concentrations increased slightly after 2 hrs of feeding in the recycle mode. The water exchange rate was directly related to the water quality in the semiclosed fish culture system.

Yoshimizu, M. and T. Kimura. 1990. Viral infections of cultured fishes in Japan. pp. 959-962, *In*: R. Hirano and I. Hanyu (Eds.). Proceedings of the Second Asian Fisheries Forum, Tokyo, Japan, 17-22 April 1989.

Six viral diseases are major problems and cause economic losses among cultured fishes in Japan. Included are rhabdovirus infection and epidermal necrosis of Japanese flounder.

Van der Meeren, T. 1991. Production of marine fish fry in Norway. 1991. World Aquacult. 22(2):37-43.

Extensive and semi-intensive production methods for marine fish larvae are not yet fully developed at a commercial scale. At the moment, they are superior for halibut and cod, but for turbot the choice of production system is a matter of financial support and suitability of the locality for extensive zooplankton production. However, semi-intensive farms might be the first to make a profit from turbot fry production. Today, live food for marine fish fry production can be obtained either by intensive culture of rotifers (*Brachionus plicatilis*) on algae and bakers yeast, by decapsulation and hatching of brine shrimp (*Artemia salina*), or by collection of wild zooplankton both from extensive production lagoons and directly from the sea. In central Europe, intensive production of rotifers and *Artemia* has become the main focus of research. Trials in the 1970s quickly revealed that although rotifers and *Artemia* can be easily produced in great numbers, their nutritional insuf- the extensive or the semi-intensive method, these food shortages have probably caused cannibalism (cod) or malnutrition (turbot) among the developing fish larvae. The effects of food shortage can be reduced by supplements of Artemia. Semi-intensive fry production is a "green water" system based on a diverse composition of natural algal species, enabling added Artemia to be nutritionally enriched and added zooplankton to retain their nutritive value until they are eaten by the fish larvae.

Zhuravleva, N.G. 1995. Mariculture and its potential along the Murmansk coast. ICES Mar. Sci. Symp. 201:187-188.

The Murmansk Institute of Marine Biology has been conducting experiments on the rearing of marine fish since 1978. The marine fish rearing programme has two major goals: (a) to develop mass-rearing technologies for commercially important species for restocking trials and (b) to improve culture techniques for commercial growout. Moiseev and Karpevich (1977) have discussed the prospects for mariculture development in this region, and species such as cod, herring, plaice, and halibut have been identified as potential candidates (Dushkina *et al.*, 1977), along with other species (Matishov, 1990). From 1978 to 1992, the Murmansk Marine Institute conducted experiments on potential candidates for mariculture, and has concentrated on the rearing of plaice (*Pleuronectes platessa*), cod (*Gadus morhua*), capelin (*Mallotus villosus*), wolffish (*Anarhichus lupus*) and goby (*Myoxocephalus scorpius*), with emphasis on the first two species. All species were kept up to 2 years under artificial conditions.

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