EFFECTS OF SALINITY ON EGGS AND YOLK-SAC LARVAE OF ATLANTIC COD, ATLANTIC HALIBUT, HADDOCK AND WINTER FLOUNDER

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EFFECTS OF SALINITY ON EGGS AND YOLK-SAC LARVAE OF ATLANTIC COD, ATLANTIC HALIBUT, HADDOCK AND WINTER FLOUNDER

by

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ABSTRACT

Recent interest in the culture of cold water marine fish has prompted many questions with regards to water quality during early culture. Although temperature is probably the most important parameter of water quality, another key factor in determining site locations as well as optimizing success is salinity. Since salinity may vary dramatically from one location (e.g. estuarine) to another (eg. open ocean), determination of the optimal salinity for a species is important in selecting site locations for prospective marine hatcheries. The current study investigated salinity effects in four prospective aquaculture species: winter flounder, haddock, cod, and halibut.

Studies were undertaken to determine the optimal salinities for egg survival and their effects on the viability, size, and behaviour of newly-hatched larvae. Eggs from each species were incubated at salinities of 15, 20, 25, 30, and 35 ppt and a variety of parameters including hatch rate, % viable larvae, larval size, yolk volume and hatching problems were measured. In addition, overall larval condition was assessed using a temperature stress test and cell cleavage patterns among haddock and halibut eggs were investigated as indicators of larval viability.

Halibut eggs failed to develop past blastopore closure when incubated in salinities of 25 ppt or less, resulting in 0% hatch rates. Eggs incubated at 30 and 35 ppt demonstrated significantly higher hatch rates and there were no noticeable differences in early larval success at these two salinities.

Among haddock embryos, hatch and viability rates were found to be high at all salinities tested, although there was a slight decrease at 15 ppt. Haddock larval length and yolk size was found to be significantly greater if eggs were incubated at lower salinities. Larvae expressed greater tolerance to temperature shock if eggs had been incubated at lower salinities.

Cod eggs hatched successfully at all salinities although larval viability was better above 25 ppt, being maximal at 35 ppt. Larval size was generally unaffected by salinity although yolk area was significantly larger at lower salinities. Temperature stress tests revealed greater larval tolerance if eggs had been incubated at higher salinities.

Winter flounder embryos displayed significantly higher hatch and viability rates if incubated at 15-20 ppt compared to 30 and 35 ppt. Larval length tended to be maximal at the mid salinities (25 ppt) whiie as seen in the other species, yolk area was largest at 15 and 20 ppt. Larvae subjected to temperature stress survived significantly longer if eggs had been incubated at 15 ppt.

Early egg cleavage patterns (symmetrical vs. asymmetrical) were not found to be reliable indicators of larval viability in either halibut or haddock eggs.

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1.0 INTRODUCTION

The recent decline in harvest fisheries, as well as sustained demand for high quality protein has led to increased interest in the culture of marine fish species. Despite the heightened enthusiasm and effort, the development of marine fish culture has been slow and many problems have been encountered, especially during "critical stages" such as the switch from endogenous to exogenous food (Hjort, 1914; Laurence, 1974; Solberg and Tilseth, 1984; Quantz, 1985). Many of the problems associated with this critical stage have been attributed to the nutritional quality and quantity of initial food organisms (Ivlev, 1961; Laurence, 1974; Watanabe et al., 1983; Naas et al., 1992; Segner et al., 1993). Although nutritional quality of the food is of extreme importance so is the actual quality of first feeding larvae, which depends on initial egg quality (Holliday, 1969; Kjorsvik et al., 1984; Blaxter, 1988; Kjorsvik et al., 1990; Bromage et al., 1991; Solemdal et al., 1991; Kjorsvik, 1994; Vallin and Nissling, 1994).

One of the most important aspects of any successful culture system is a reliable supply of healthy eggs. Since the egg is the starting point of the entire culturing process, it is imperative that the eggs utilized are of the highest quality. Factors affecting egg quality are expansive and include: nutrition and genetic make-up of the broodstock (Hempel and Blaxter, 1967; Hislop et al., 1978; Thorpe et al., 1984; Watanabe, 1985; Kjorsvik et al., 1990), temporal stage of the spawning cycle (Craik and Harvey, 1984; Kjorsvik et al., 1990; Bromage et al., 1991; Bromage et al., 1994), water quality and husbandry practices (Billard et al., 1981; Whipple et al., 1981), as well as environmental parameters such as temperature, light, and dissolved oxygen levels (Rosenthal and

Alderdice, 1976; Kuhlmann et al., 1980; Buckley et al., 1990; Helvik and Walther, 1993; Liu et al., 1994)

Another important environmental factor affecting egg quality, which will be the focus of my thesis is salinity. Since, the marine environment offers varying salinity conditions, this factor likely has a significant effect on the survival of many marine embryos and larvae. For example, a coastal species, such as winter flounder, which spawns near shore (Scott and Scott, 1988) may expose its eggs to a range of salinities, especially under estuarine conditions. An understanding of optimal salinity conditions for eggs of a given species would provide information on a suitable egg incubation regime as well as prospective suitable locations for marine finfish hatcheries.

Salinity exerts its effects on eggs and larvae in a number of ways including: total osmotic concentration, buoyancy, oxygen availability, and disease (Holliday, 1969). The effects of salinity on embryos begin at the broodstock stage.

1.1 Broodstock, Gametes and Fertilization

Adult marine teleosts have an ability to maintain osmotic concentrations different from that of their external medium (Riis-Vestergaard, 1984). For example, in euryhaline teleosts such as plaice and flounder, osmolarity of blood and tissue fluids is ~ 350-400 mOsm compared to ~ 1000 mOsm of ambient seawater. The major component of osmolarity in both tissue fluid (150-200 mMol/l) and seawater (~ 500 mMol/l) is sodium chloride (Holmes and Donaldson, 1969). This concentration difference results in an uptake of chloride ions as well as a loss of water by the fish. The fish counteracts this

process by actively drinking seawater (Smith, 1930). Upon entry into the gut, most of the NaCl in seawater is transported to nearby tissues via active transport. The result is a lowering of gut fluid osmolarity, creating a passive flow of water which replaces the water lost through the external surfaces. This entire process creates salt loading in the fish in addition to that entering through external surfaces. To combat this excess salt, fish have chloride cells, located primarily in the gills, which excrete the ions (Keys, 1931; Keys and Wilmer, 1932).

Prior to spawning, gametes are usually isosmotic or slightly hyposmotic to the females's body fluids (Dakin, 1911; Hayes, 1949; Holliday, 1965; Davenport et al., 1981). Therefore, marine eggs, like the broodstock, are subject to the problems of salt uptake and water loss once they are released into the marine environment. In addition, it has been found that salinity of the external medium influences some aspects of the oocytes subsequent to release from the ovarian cavity. Solemdal (1967) discovered if females of Pleuronectes flesus were transferred from water of 34.5 ppt to 5 ppt three weeks before spawning, resulting eggs were larger and had a lower osmotic pressure. The larger size of the egg was attributed to an increased water content due to the lower osmotic pressure of the parent blood. This finding suggests that varying egg specific gravities may result from parental responses to salinity and may impact egg survival, especially in brackish water areas. Females from low salinities have a tendency to spawn eggs that are neutrally buoyant at lower salinities (Blaxter, 1988).

The final ovarian maturation in marine species such as Atlantic cod, whiting, haddock and plaice involves a massive water uptake and accompanying reduction in

protein phosphate (Craik and Harvey, 1984). This influx of water, which may be as much as 92% of total egg weight, creates a hypotonic egg fluid causing the eggs to be buoyant when released into seawater (Blaxter, 1988).

Fertilization among teleost eggs is a process initiated by the entrance of a single sperm into the egg's micropyle (Yamamoto, 1939). Since the micropyle is only 1.5 um in diameter (Ginzburg, 1968) sperm must enter single file, thus the first sperm penetrating the canal and contacting the plasma membrane essentially fertilizes the egg. Once one sperm reaches the plasma membrane, further sperm entry is prevented by a discharge of material from the cortical alveoli (Vacquier, 1975). The substance is forced into the micropylar canal, forming a plug that prevents further sperm entry (Nakano, 1969).

At the time of sperm contact with the plasma membrane, evidence from studies on sea urchin eggs suggests the occurrence of three consecutive events: 1) an initial depolarization; 2) a Na^{+}-Ca^{2+} action potential and 3) an activation potential that lasts until the egg repolarizes (Whitaker and Steinhardt, 1985). The first depolarization seems to be sperm dependant and it appears to initiate an action potential that depends on external Na^{+} and Ca^{2+}, suggesting that a calcium-carrying, potential-gated membrane channel is fundamentally responsible (Alderdice, 1988). The presence of calcium is important in the activation process and although calcium is released from the inner surface of the plasma membrane, the calcium level of the external fluid may influence this process (Mangor-Jensen and Jelmert, 1986; Alderdice, 1988). Immediately following fertilization there is a separation of the cortex from the chorion leading to the formation of the perivitelline space (PVS) (Blaxter, 1969). The manner of formation of the PVS

varies between species. For example, in cod the space is created by a reduction of the ovoplasm, while in the long rough dab, it is formed by a distension of the chorion (Laale, 1980). The chorion is freely permeable to water and minute molecules but larger colloidal-type molecules tend to be retained in the perivitelline fluid. It is the extremely low permeability of the vitelline membrane, and not the egg chorion, which plays a major role in the osmoregulation and water balance of teleost eggs (Alderdice, 1988).

During fertilization, gametes are often exposed to sudden changes in salinity, depending on the salinity of the external medium in relation to the osmolarity of the ovarian fluid. Depending on the degree of salinity change, eggs might be expected to suffer osmotic shock resulting in death or at least significant reductions in fertilization rates. However, in many species, gametes demonstrate tremendous tolerance to salinity change. In studies of gametes of herring (Clupea harengus) and plaice (Pleuronectes platessa), Holliday and Blaxter (1960) and Holliday (1965), found extreme tolerance to high salinities. Fertilization rates remained high even at salinities up to 55 ppt, being 100% for herring and 70 % for plaice. Yanagimachi (1958) found that sperm of Clupea pallasi, a species which usually spawns inshore, remains fertile after a 24 hour exposure to 50 ppt seawater. Rockwell (1956), in a study of Oncorhyncus, found normal fertilization to occur at salinities of 18 ppt or less. Salinities above 24 ppt inhibited fertilization, although a few eggs were fertilized in salinities as high as 30 ppt. These findings indicate an extreme tolerance to salinity and similar findings have been reported by Rutter (1902).

Westin and Nissling (1991), in a study of Baltic cod, found sperm activity in salinities as low as 12.5 ppt (maximum sperm activity at 15.5 - 26 ppt) and 100% fertilization rates in salinities as low as 11 ppt. In an earlier study on the same species, Westernhagen et al.(1988), found that eggs could be fertilized at 15 ppt but died at 10 ppt. Research by Mangor-Jensen and Jelmert (1986) on fertilization rates in Atlantic halibut suggested normal fertilization down to 27 ppt (17 ppt if calcium was added). This finding suggests that halibut, with respect to fertilization, are much more stenohaline than cod and that calcium levels appear to play an important role in the fertilization process.

The above results, with the probable exception of halibut, imply that marine teleost gametes are able to tolerate a wide range of salinities at fertilization. This adaptation probably results in increased embryo survival, especially for species dwelling in coastal areas that may be subject to salinity change.

1.2 Teleost Egg Hatching

The time to hatching is largely controlled by environmental variables such as temperature and dissolved oxygen levels. Alderdice and Forrester (1971) found that Pacific cod eggs kept at 2.7^{0}C and 9.3^{0}C hatched in 24 and 10.4 days, respectively. In addition eggs kept at 2.7^{0}C demonstrated differing hatch times depending on dissolved oxygen concentration. An increase in oxygen concentration from 3.6 ppm to 8.6 ppm increased hatching time by approximately 2 days.

Actual hatching is the result of chorion softening due to the secretion of enzymatic and/or chemical substances from the ectodermal glands on the anterior surface or from the endodermal glands in the pharynx (Blaxter, 1969). In addition, larval activity, usually increased by temperature and light increments or a reduction in oxygen levels, appears to assist in rupturing of the chorion (Yamagami, 1988). Hatching enzyme synthesis, in general, appears to be initiated just after lens formation but usually prior to eye pigmentation (Yamagami, 1988). The hatching enzyme has both proteolytic and choriolytic (egg envelope dissolving) properties and it appears that nutrient material in the disintegrating chorion may be utilized by the embryo via the perivitelline fluid (PVF) (Ishida, 1944; Smith, 1957; Kaighn, 1964).

The period of egg development is one of vast and rapid change, and is influenced by environmental parameters such as salinity. Researchers have investigated the effects of salinity on embryo function and development in a number of teleost species.

Several studies have examined the effect of salinity on the duration of development. Studies on Clupea harengus (Holliday and Blaxter, 1960), Limanda ferruginea (Laurence and Howell, 1981) and Gadus macrocephalus (Forrester and Alderdice, 1966) demonstrated faster development at higher salinities. A study by Liu et al. (1994) on Pacific halibut found no differences in development rates over a salinity range of 30-39 ppt, while Rubin (1994), in a study of sea trout (Salmo trutta) found increased development times at higher salinities (salinity range - 0-7 ppt). Heuts (1947) found development of Gasterosteus aculeatus eggs in different salinities was partially dependant on broodstock genetics, in some cases development was faster in higher

salinities and in other cases it was slower. It was also discovered that salinity effects could be greatly modified by temperature. A similar finding was produced by Alderdice and Forrester (1968) in Parophrys vetulus, they found most rapid development occurred at a salinity of about 25 ppt combined with temperatures between 6 and 12^0C.

The chorion of teleost eggs is highly permeable to water, so much that the salinity of the perivetelline fluid (PVF) often assumes the salinity of the surrounding environment in 4-6 hours (Lasker and Theilacker, 1962; Holliday, 1965; Weisbart, 1968). Despite the high permeability of the chorion, many researchers have found that egg yolk osmolarity is often uninfluenced by external salinity and it appears that the chorion provides at least some protection. Weisbart (1968) found a decreased survival time in salmon embryos that were dechorionated.

Eggs are often found to be larger at lower salinities, which has been attributed to an increased water content (Holliday and Blaxter, 1960; Solemdal, 1967). Quite often the cells of the blastodermal cap swell as much as 20% in response to lowered salinities and may shrink as much as 8% in higher salinities (Holliday, 1965; Holliday and Jones, 1967). Holliday (1965), in a study of herring, plaice and cod eggs, found that cells of eggs in low salinities became bloated, tightly squeezed together, and irregular in shape, while high salinities produced shrunken, sharply defined boundaries and roundness. In extremely low salinities (< 5 ppt) blastula cap cells of plaice underwent abnormal cleavages which appeared to result from the compression of the enlarged cells. However, once plaice eggs completed gastrulation there was a greater tolerance of low salinities. McMynn and Hoar (1953) found Clupea pallasii eggs became much more tolerant to low

salinities once gastrulation was complete. Holliday (1965) suggests that osmoregulatory power is increased upon completion of gastrulation because the entire egg surface is now covered by ecto and endodermal layers. The cells of these layers appear to be the site of osmoregulation.

The yolk of eggs also appears to undergo changes that may be very prominent in some species such as herring Clupea harengus (Holliday and Jones, 1965). Within this species, yolk osmolarity is higher in eggs incubated at higher salinities. However, once the blastopore is closed, yolk osmolarities become quite similar regardless of incubation salinity. In contrast, the yolk osmolarity of plaice eggs appear to be relatively unaffected by incubation salinity. A salinity range of 15 ppt to 45 ppt caused an internal osmolarity change of only 3-4% (Riis-Vestergaard, 1987). Similarly, the eggs of Pacific herring appeared to maintain a near constant yolk osmolality despite external salinity (Alderdice et al., 1979). It appears that plaice and Pacific herring possess some type of regulation mechanism from the time of fertilization, while Clupea harengus does not attain regulation until the end of gastrulation. Although some species do not show much change in yolk osmolality, Riis-Vestergaard (1987), suggests there is probably ongoing balancing of water loss and excretion of solutes to maintain this constancy. Evidence for this type of system has been shown in halibut embryos, where water volume continues to decline throughout development despite a near constant yolk osmolality (Riis-Vestergaard, 1982). There has even been some indication that cod embryos, despite some water loss during the first week of development, have an ability to increase water volume during later development (Mangor-Jensen, 1987). This volume increase, despite existence in a

hyper-osmotic environment, suggests some type of active fluid uptake which exceeds water loss to osmosis.

Survival to hatch (hatch rate) is often used as a tolerance index for eggs being incubated in a range of salinities. Hatch rate does not appear to have any universal trend and seems to be species-specific. Studies on herring eggs (Clupea harengus) demonstrate high hatching rates (>75%) in the salinity range of 20-35 ppt (Holliday and Blaxter, 1960; Holliday, 1965). A number of investigations have been completed with gadoid eggs of a variety of species and populations and salinity tolerance has been found to vary depending on species or location. Nissling and Westin (1991), in a study of Baltic cod eggs, discovered normal hatch rates of ~40-50% in salinities as low as 10 ppt. Most mortality occurred prior to completion of gastrulation. An examination of Atlantic cod (Gadus morhua) embryos, by Laurence and Rogers (1976), demonstrated increased hatching at higher salinities. Hatch rate at 36 ppt was 50% compared to 30% at 26 ppt, however the salinity range used was limited, with 26 ppt being the lowest. In contrast to the previous study, the highest embryo mortality occurred just prior to hatch. Studies of hatch rates in Pacific cod (Gadus macrocephalus) indicate that within a salinity range of ~15-31 ppt, the lowest salinities provide the greatest survival (Forrester and Alderdice, 1966; Alderdice and Forrester, 1971). This finding is somewhat surprising, considering salinities at Pacific cod spawning areas are ~29-30 ppt. The researchers postulate that the higher survival at 15-20 ppt is probably associated with the minimal osmotic stress at these isotonic salinities.

A variety of flatfish eggs have been investigated with respect to salinity tolerance and again results have been variable. Laurence and Howell (1981) incubated yellowtail flounder (Limanda ferruginea) embryos in salinities of 28-38 ppt and found survival to be greatest at the higher salinities, with the bulk of mortality occurring at the time of gastrulation. Holliday and Jones (1967) incubated plaice (Pleuronectes platessa) eggs in salinities of 5, 17.5, 35, and 50 ppt. High mortality was found in all egg stages at 5 ppt, while at the other salinities the majority of mortality occurred during gastrulation, just prior to blastopore closure. In a study of Atlantic halibut (Hippoglossus hippoglossus), Lonning et al. (1982), found no survival differences in eggs incubated at 33 ppt vs. 39 ppt. The scope of this experiment was somewhat limited since 33 ppt was the lowest salinity tested. A comparable study on eggs of the Pacific halibut (Hippoglossus stenolepis) examined hatching rate in a salinity range of 21-39 ppt. No significant differences were found in survival rates above 30 ppt, with hatch rates ranging from 25-46%. All salinities below 30 ppt provided 0% hatch. Although there were no apparent differences in mortality rate during the earliest egg stages, no eggs incubated below 30 ppt survived beyond the completion of gastrulation. Kuhlman et al. (1980) discovered highest viable hatch in turbot (Scopthalamus maximus) eggs to occur at a salinity of 20 ppt (>50%) while highest mortality took place at 30 ppt (~90%).

Although mortality in response to salinity in the above studies appears species dependant, there seems to be a trend with respect to the time of mortality. Both pre-blastopore closure and pre-hatch stages appear to be susceptible times for developing embryos. As mentioned previously, blastopore closure is often accompanied by increased

osmoregulatory capability which may result in a less vulnerable embryo once the process is complete. Mortality at or just prior to the time of hatching is a phenomenon often associated with lower salinities (Battle, 1930; Alderdice and Forrester, 1968). Studies on Atlantic and Pacific herring, by Ford (1929) and McMynn and Hoar (1953), found increased mortality at the time of hatching, with many larvae dying partially emerged from the egg if incubation occurred at low salinities. A similar result was found when Baltic cod eggs were incubated at salinities of 5 and 7 ppt (Nissling and Westin, 1991). Battle (1930) suggested that partially emerged larva was a result of poor tail musculature development at low salinities, disabling the larvae to wiggle free. Holliday (1965) postulated that partial emergence may actually be due to the low specific gravity of low salinity water, making it difficult for the larvae to escape or that the chorion may be "tougher" making rupturing more difficult.

1.3 Yolk-sac Larvae

The newly hatched, marine larvae is usually transparent, has some pigmentation, and may have pigmented or unpigmented eyes depending on species. Quite often the mouth and jaws are not formed, the yolk sac is large, often causing hydro-dynamic problems and a large primordial finfold is present. The notochord and myotomes are usually transparent with insignificant cartilage or skeletal formation. Although the heart is functioning before hatch, the blood is colourless and in most species circulatory and respiratory systems are poorly developed. The kidney is usually pronephric with few glomeruli and there are no gills present, thus most respiratory processes take place

through the skin, which is a thin, two-layered epithelium. As a result, external water salinity is likely to have an impact on osmotic and ionic movement (Jones et al.,1966; Threadgold and Lasker, 1967; Blaxter, 1969,1988).

The degree to which newly-hatched larvae tolerate salinity changes will depend on the ability of body fluids to function under salinity extremes and the ability of the larvae to restore osmotic pressures to normal concentrations through regulation (Holliday, 1969). In addition, it appears that a larva's tolerance to salinity is also affected by the salinity at which the eggs were incubated. Duenas (1981) performed salinity tolerance tests on Pacific herring larvae incubated at 13, 21, and 29 ppt and found respective upper tolerances to be 41.7, 43.6, and 48.2 ppt. Hwang and Hirano (1985) found the extent of chloride cell development can be related to the magnitude of salinity change involved in a test. Thus increased larval survival associated with higher incubation salinities may actually result from increased regulative tissue differentiation, possibly a greater density of chloride cells (Alderdice, 1988).

Yolk-sac larvae of many marine species appear to have a wide salinity tolerance. Holliday and Jones (1967) found plaice (Pleuronectes platessa) larvae could tolerate salinities from 15 ppt to 60 ppt for 168 hours. Yin and Blaxter (1987) found low salinity tolerance (50% of larvae active after 24 hours) in herring (Clupea harengus), cod (Gadus morhua) and flounder (Platicthys flesus) to be 1-1.5 ppt, 2-3 ppt and 0-1 ppt, respectively. Holliday and Blaxter (1960) and Holliday (1965), in a study of herring, plaice, and cod (Gadus callarias) found lower tolerance limits of 1-2 ppt, 5 ppt and 10 ppt, respectively, while all species could tolerate salinities as high as 60-65 ppt. Tolerance was based on

50% active larvae after 24 hours exposure. Holliday (1965) demonstrated that gradual changes in salinity tolerance of herring and plaice occurred with age, such that by metamorphosis, tolerance levels were similar to adults. This change in tolerance might actually reflect a shift in regulatory capability, possibly a switch to adult-type regulation involving the kidney, gills, gut, and water uptake through drinking.

The most pronounced effects of salinity on larval physiology involves its influence on osmotic and ionic concentrations of body fluids (Holliday, 1965). As mentioned previously, the larva's tissue tolerance and regulation capacities will probably determine survival. The importance of these abilities was shown by Weisbart (1968) in an examination of Pacific salmon. He found survival of Oncorhyncus tschawytscha alevins in seawater to be longer than that of O. kistutch and O. nerka due to a higher tissue tolerance but O. gorhuscha and O. keta demonstrated longer survival because of a better regulatory capacity for serum sodium and chloride concentrations, and osmotic pressure.

Another demonstration of the regulatory capacity of yolk-sac larvae is provided by Holliday and Blaxter (1960), in an investigation of herring (Clupea harengus). Larvae held in ambient seawater (~34 ppt) had body fluids of ~12 ppt NaCl but sudden transfer to 50 ppt water caused the osmotic concentration of body fluids to increase to ~22.5 ppt. Body fluids remained at this level for 3-6 hours but by 24 hours some regulatory mechanism returned the concentration to ~ 15 ppt. A similar study on plaice, by Holliday (1965), revealed that abrupt transfer of larvae into 50 ppt water caused body fluid concentrations to increase from ~12 ppt to ~25 ppt. Again regulation restored the value to

~15 ppt within 24 hours. Transfer of the larvae to a salinity of 5 ppt, caused a drop of body fluid concentration to ~9 ppt, this concentration rebounded to ~11 ppt within 24 hours. It was also found that the body weight of larvae changed in response to body fluid osmotic concentration, such that larvae could lose up to 25% of their weight in high salinities and gain up to 30% in low salinities. These changes were found to be non-permanent and could be reversed if salinity was altered, suggesting that water movement in and out of the larvae was responsible.

The above studies suggest larvae have some regulatory capacity and prompt a further question - "What is the metabolic cost of regulation ?" Studies by Lasker and Theilacker (1962) and Holliday et al. (1964) investigated oxygen uptake in Sardinops carerulea and Clupea harengus larvae in varying degrees of salinity from 5 to 60 ppt. It was found that fully adapted larvae show no differences in oxygen uptake across the salinities tested but some differences did occur immediately following transfer, when body fluids were undergoing osmotic changes. Once regulation was complete, levels of oxygen uptake returned to normal.

Some investigations have also been made with respect to the effect of salinity on heartrate in teleost larvae. Helle and Holliday (1965), unpublished data cited in Holliday (1969), found heartbeat in alevins of Salmo salar, which normally live in freshwater, to be less rapid in brackish seawater, for example, heartbeat per minute was 98 at 0 ppt compared to 31 at 24 ppt. Kryzhanousky (1956), as cited in Holliday (1969), found Clupea harengus membras to have a slightly reduced heart rate in lower salinities, 86 beats/min. at 4-5 ppt compared to 90 beats/min. at 25 ppt. Holliday (1969) suggests that

these effects may be the result of two factors: 1) response of the heart muscle to sub-optimal conditions and 2) changes in blood viscosity resulting from osmotic effects. In addition, energy expenditure among newly hatched larvae is likely to be influenced by salinity, through its effects on behaviour. Holliday (1965) revealed that herring and plaice larvae kept in seawater (34 ppt) swam continuously, while in salinities less than 12 ppt the larvae often lie on the bottom for a considerable amount of time. At salinities above 45 ppt, larvae spent substantial time trying to swim downward. These different behavioural patterns seem undoubtedly related to larval buoyancy, and suggest that in some instances, low salinities probably result in lower metabolic demands.

A number of investigations have been completed about the effects of salinity on structure and size of teleost larvae. Some studies have implicated salinity of egg incubation as a precursor for a variety of abnormalities. Battle (1930) found tail and cardiac region deformities in Enchelyopus cimbrius larvae hatching in salinities up to 70 ppt. Alderdice and Forrester (1968) found increased numbers of weak Parophrys vetulus larvae in salinities below 20 ppt. In addition, there were significantly more larvae expressing spinal curvatures, if eggs were reared outside the optimal salinity range of 20-30 ppt. Kryzhanousky (1956), in a study of Baltic herring (Clupea harengus membras), found abnormalities of the cardiac region, otic region, yolk sac, alimentary canal and associated structures such as the liver, if eggs had been incubated in 25 ppt instead of 4-5 ppt where they are normally found. Holliday and Blaxter (1960) discovered the yolk sacs of (Clupea harengus) hatched in low salinities were pale yellow and swollen compared to the bright yellow, firm yolk sacs found in salinities above 35 ppt.

Experimentation has also been conducted regarding the effects of salinity on larval cell size. Lasker and Threadgold (1968) found initial swelling in the epidermal cells of Sardinops caerulea larvae, which were transferred from 35 ppt water to either 5 ppt or 50 ppt. Although most of the cells were near normal within 6 hours, a few continued to swell even after all the epidermal cells had returned to normal. They referred to these cells as "chloride cells", which did not return to normal until between 6-24 hours after transfer. The cells were packed with microtubules which increased in diameter and the cell cytoplasms appear jammed. These findings suggest that the epidermal cells of larvae are important to the regulatory process, at least in the early stages.

A further consequence of differing salinities on larval life is the effect it may have on larval size, which could undoubtedly influence larval survival. Holliday (1965) and Holliday and Blaxter (1960) discovered Pleuronectes platessa and Clupea harengus larvae were 23% longer and 33% heavier if hatched in salinities of 5-25 ppt compared to 35-55 ppt. In a study of Pacific cod (Gadus macrocephalus), Forrester and Alderdice (1966), found the largest larvae to occur at a salinity of 19 ppt over the range of 19-31 ppt. By contrast, Laurence and Rogers (1976), in a study of Atlantic cod (Gadus morhua) and haddock (Melanogrammus aeglefinus), exposed larvae to salinities of 26-36 ppt and found cod larvae had longer total lengths with increasing salinity while no relationship was found for haddock larvae. Laurence and Howell (1981) found newly hatched yellowtail flounder (Pleuronectes ferruginea) larvae to have a maximum length at the mid-range salinities (33-34 ppt), when eggs were incubated in salinities ranging from 28-

38 ppt. Sweet and Kinne (1964) discovered Cyprinidon macularius larvae reared at salinities of 0, 35, and 75 ppt demonstrated increased body lengths, depths and widths with decreasing salinity. Although studies vary, most report larger larvae at hatch if eggs are incubated at lower salinities. However, discretion must be exercised when interpreting such results, since optimal salinity is probably affected by the salinity at which broodstock and eggs normally occur.

It is clear that salinity can have substantial impacts on the structure and function of newly-hatched larvae. Although some of these effects can be reversed others may result in abnormalities or deformities that are often life threatening.

The current study examines some effects of salinity on eggs and larvae of Atlantic cod (Gadus morhua), Atlantic halibut (Hippoglossus hippoglossus), haddock (Melanogrammus aeglefinus) and winter flounder (Pseudopleuronectes americanus). Although all of these species reside in the marine environment, there are some noteworthy differences in their specific habitat, distribution, and life history.

Atlantic halibut occur on both sides of the North Atlantic, extending from the west coast of Greenland to Virginia in the west and from the Bay of Biscay to the Barents Sea in the east. Although young halibut have been found in shallow waters (37-55 m), most mature individuals live in deeper waters of 165-229 m. Even though halibut are not highly migratory (Scott, 1982) they are strong swimmers capable of moving great distances. The halibut is the largest of the flatfishes, with average sizes of 4.5 kg, although individuals larger than 200 kg have been caught (Bigelow and Schroeder, 1953). Females grow faster than males and tend to attain a much larger maximum size, length at

sexual maturity is dependant on stock, one example being ~66 cm for males and ~70 cm for females (Beacham, 1982).

Halibut tend to spawn from late winter to early spring, spawning in Canadian waters usually occurs from February to April (McCracken, 1958; Kohler, 1967). Actual spawning grounds are not clearly defined and reported spawning may occur anywhere from a depth of 183 m to depths greater than 1000 m. Halibut are batch spawners, releasing up to 10-15 batches per female per year, with a 90 kg female producing approximately 2 million eggs. Eggs are fairly large, compared to other marine species, with a mean diameter of ~3.0 mm and appear to develop bathypelagically. Haug et al.(1984) found eggs are usually most abundant where temperatures and salinities range between 4.5-7.0^{0}C and 33.8-35.0 ppt.

Atlantic cod are found on both sides of the North Atlantic, extending from Iceland to the Baltic Sea and Bay of Biscay in the east, and from Greenland and Southern Baffin Island to Cape Hatteras in the west. There are at least 12-14 recognized cod stocks, of which the Southern Labrador-Eastern Newfoundland is probably best known (Jean, 1964). Cod inhabit cool waters (-0.5-10^{0}C) ranging from inshore to the edge of the continental shelf and although adapted for bottom feeding, may spend considerable time off the bottom (range-0-457m). Cod tend to migrate in schools, often moving onshore in summer and offshore in winter (Jean, 1964).

Although growth varies from stock to stock, 5-6 year olds average ~50-60 cm in length and ~1.1-2.3 kg in weight. The average weight of commercially caught cod is

under 4.5 kg. However, individuals are capable of attaining sizes as large as 95.9 kg (Anon, 1984).

In Canadian waters, cod tend to spawn as early as February in the north and as late as December in the south. The depth at which spawning occurs depends on stock and usually falls within 100-182 m. Fecundity depends, to a large extent, on female size, for example, a 51 cm female may deposit 200,000 eggs while a 140 cm female may release 12 million eggs (Powles, 1958; May, 1967). Eggs are spherical, transparent, pelagic and have a diameter of ~1.2-1.6 mm, and like halibut, cod are batch spawners. Larvae are usually 3.3-5.7 mm at hatch and remain pelagic until they are ~25-50 mm long (Fahay, 1983).

Haddock, like cod, occur on both sides of the North Atlantic stretching from Iceland to the English Channel in the east and from the Strait of Belle Isle to Cape Hatteras in the west. Haddock inhabit cool, temperate waters (1-13^{0}C) from inshore areas to the edge of the continental shelf, over a depth range of 27-366 m. In summer, haddock move into the shallow, warmer water of the banks, moving to deeper waters in the winter (Scott, 1982).

Growth rates tend to differ depending on location, with northern populations growing slower. Four year old haddock generally average 51 cm in length, with the largest haddock on record being 112 cm long and weighing 16.8 kg.

Haddock generally reach sexual maturity in 3-5 years with males usually being slightly younger than females. Spawning occurs from January to July in Canadian waters and February to May off New England. Fecundity, as in cod, is related to female size,

Hodder (1965), found egg production to be 228,000 eggs - 40 cm female, 704,000 eggs - 50 cm female, and 1,773,000 eggs - 60 cm female. Eggs are spherical, transparent, pelagic, and 1.3-1.6 mm in diameter. Hatching usually occurs when larvae are 3.0-4.0 mm in length, they remain at the surface and metamorphose (~25 mm), before seeking the bottom (~50 mm)(Fahay, 1983).

Winter flounder is a member of the right-eyed flounder family and inhabits waters from Battle Harbour, Labrador in the north to Georgia, U.S.A. in the south. The winter flounder is a shallow water, bottom species which usually resides in depths of 1.8-36.6 m but have been found as deep as 143 m (McCracken, 1954). Winter flounder generally make regular onshore (summer) - offshore (winter) migrations withstanding a wide range of temperatures, due particularly to their accumulation of antifreeze proteins in the blood (Dunan and De Vries, 1974; Fletcher, 1977).

Growth rates vary depending on region with southern populations being generally larger than northern populations. McCracken (1954) found 5 year old winter flounder to have mean lengths of 25.4-37.7 cm, adults rarely exceed 45 cm in length or 1.4 kg in weight.

Winter flounder usually reach sexual maturity in 3-4 years at average lengths of 20 and 25 cm for males and females, respectively. Over its entire range, spawning usually occurs between December and June. Average fecundity is ~500,000 eggs per female, although Topp (1968) found egg numbers in a sample of 30 fish from Narragansett Bay to range from 435,000 eggs to 3,329,000 eggs. Fertilized eggs are adhesive and demersal, often settling to the bottom singly or in clumps. Average egg diameter is 0.8 mm and

hatched larvae are ~3 mm in length. Larvae undergo a period of larval drift in surface waters and metamorphose into a flatfish form in ~2.5-3.5 months (Frank and Legget, 1983).

The intent of the current investigation was to determine the optimum salinity (or range of salinities) for egg incubation in four marine species; Atlantic cod, Atlantic halibut, haddock and winter flounder. It was believed data obtained would assist in both the development of a suitable salinity regime for egg incubation of these potentially valuable aquaculture species and the selection of prospective areas for marine finfish hatcheries.

2.0 MATERIALS AND METHODS

2.1 Atlantic Halibut

Halibut were caught in 1989 using otter trawl in Sable Island Gulley, off the coast of Nova Scotia. The mean weight of the fish was ~ 500 g. Halibut were used in various weir and cage growth studies at Campobello Island, N.B. from 1990-1994. In 1994, 33 halibut were returned to the St. Andrews Biological Station, St. Andrews, N.B. and placed in three 35,579 l circular, fiberglass tanks with a water inflow of 100 l/min. Mean weight at the time of transfer was ~12 kg and ~18 kg for males and females, respectively. Broodstock were fed a diet of mackerel, herring, and squid and maintained at ambient temperature and salinity.

Spawning took place in February and March of 1995 and represented the first captive spawning of this particular broodstock. Females were visually monitored to determine spawning readiness; once a batch of eggs was released from a prospective female; the female was then checked at various times (e.g. 70, 90, 110, 120 hours from previous release) for presence of ripe eggs. Actual spawning involved: 1) lowering water level of the tank to ~ 90 cm; 2) placement of a neoprene-covered, PVC spawning table (173 cm x 82 cm x 80 cm) into the tank; 3) gentle lifting of halibut (individually) onto the table for stripping of eggs or milt.

Eggs from a single female were stripped into a pre-cooled, dry, 1l, plastic beaker while milt from three males was collected in separate 40 ml plastic vials. Eggs and milt were placed separately into a pre-cooled, insulated cooler and transferred to a dark (flashlight only) cold room (5^0C) for fertilization. Subsequent to egg collection water of

the five test salinities (15, 20, 25, 30 and 35 ppt) was produced. This salinity range was chosen for three reasons. First, it provided a spectrum from brackish to full strength seawater, thus covering any salinity these fish would be exposed to under natural conditions. Second, sites for possible marine hatcheries would most likely fall within this range, even if located near large river sheds. Third, providing extreme salinities such as 15 ppt and 35 ppt made it easier to test high and low salinity tolerance. Salinities below 30 ppt were achieved by mixing distilled water with 0.2 um, filtered, u.v. sterilized ambient seawater (~30 ppt). Salinities above 30 ppt were attained by mixing distilled water with artificial sea salt (Instant Ocean) to create a high salt brine (~50 ppt). This solution was allowed to settle prior to being mixed with distilled water to create a 35 ppt solution. Exact salinities were determined using a Fischer hydrometer in combination with a thermometer and salinity-temperature charts. Once water batches of each salinity were created, accuracy of hydrometer readings were cross checked with a Guildline autosal. Stock water solutions were stored in 20 l plastic jugs and held at 5^0C.

Twenty - five, 300 ml glass beakers containing 200 ml of 15, 20, 25, 30, or 35 ppt salinity, 0.2 um filtered, u.v. sterilized, autoclaved water (5 per salinity) were inoculated with 0.5 ml of milt. The seawater-milt mixture was stirred for ~ 5 seconds before ~100 eggs were added to each beaker and swirled. Eggs were left for 30 minutes to water harden, before excess milt was removed and replenished with clean water of the appropriate salinity. These beakers were covered loosely with aluminum foil and left at 5^0C for 20 hours, at which time fertilization rates were determined (16-32 cell stage).

At the time of fertilization an additional two 1L, glass beakers containing ~ 800 ml of ambient salinity water (~30 ppt) were inoculated with milt and eggs as aforementioned. In addition, these fertilized eggs were treated with 200 ppm glutaraldehyde for 10 min. as a disinfectant. Glutaraldehyde was then decanted and eggs were rinsed twice with 30 ppt water. Once rinsing was complete ~ 200 eggs were transferred to beakers of 15, 20, 25, 30 or 35 ppt salinity water and used in the developmental study.

2.2 Winter Flounder

Winter flounder were caught in 1994 and 1995 by otter trawl in Western Passage, Passamaquoddy Bay, N.B. and held in two 10,752 l circular, fiberglass tanks with water inflow of 45 l/min.

Spawning occurred in May and June of 1995 and involved: 1) removing fish (individually) from the tank with a dip net; 2) placement of the fish on a neoprene-covered spawning table; 3) removal of eggs or milt. Eggs from one ripe female were stripped into a pre-cooled 500 ml beaker and milt from three males was collected separately into 40 ml plastic vials. Eggs and milt were separately placed into a pre-cooled, insulated cooler and transferred to a dark cold room (5^0C) for subsequent fertilization. Due to the adhesiveness of winter flounder eggs the fertilization procedure was different from that of halibut.

The fertilization procedure followed was similar to that used by Harmin and Crim (1992). Aliquots of 200-300 eggs were pipetted into twenty-five plastic petri-dishes (five

per salinity of 15, 20, 25, 30, and 35 ppt). Approximately 30-50 ul of milt (one drop) was placed on each aliquot of eggs, eggs and milt were mixed and 1 ml of appropriate salinity water (0.2 um filtered, u.v. sterilized, and autoclaved) was placed in each dish to activate the sperm. The egg, milt and water mixture was swirled before addition of another 4-5 ml of clean, appropriate salinity water, at this point, the eggs began sticking to the bottom of the petri-dish. An additional two washes with clean water of corresponding salinity removed excess milt, dishes were then filled with water and left for 20 hours, at which time fertilization rate was determined.

For the developmental study, fertilization procedure was similar to that above except all eggs were fertilized in ambient seawater (~30 ppt), left to water harden for 30 minutes and then water was replaced with water of 15, 20, 25, 30 or 35 ppt salinity.

2.3 Haddock

Haddock broodstock were captured in 1992 using longline and otter trawl in St. Mary's Bay, Nova Scotia and on George's Bank. Fish were held in three 35,579 l, circular, fiberglass tanks with a water inflow of 100 l/min., under ambient salinity and temperature regimes. Fish were maintained on a diet of squid, mackerel, and herring and were previously spawned in 1993 and 1994. Spawning in 1995 began in early March and ended in early June.

Fertilization of haddock eggs occurred naturally in the broodstock tanks and the pelagic eggs were collected with floating collectors. The egg collectors were constructed of black Coroplast™ and formed a rectangular box. They had an opening scoop (50 cm x

100 cm) which allowed eggs and water to flow in. The bottom portion of the collector was fitted with 500 um screening, this allowed water to flow through while retaining the eggs. The collector was attached to the side of the tank via c-clamps and positioned such that the circular water flow in the tank directed eggs into the collector. Egg batches accumulating in the collector were sampled for fertilization rates and development stages prior to being utilized. Only egg batches with 80% or higher fertilization and at the four cell stage or lower were accepted. Eggs were retrieved from the collectors using a glass beaker to skim an egg-water mixture off the surface, care was taken to handle the eggs gently and avoid moving the eggs without a water medium. Collected eggs were placed in a pre-cooled, insulated cooler and immediately transferred to a dark cold room (5^0C). Eggs were disinfected with 200 ppm glutaraldehyde for 10 minutes, after which the glutaraldehyde was decanted and the eggs rinsed twice in 30 ppt salinity water. Next, eggs were transferred to 500 ml beakers containing ~300 ml of 0.2 um filtered, u.v. sterilized, autoclaved water of 15, 20, 25, 30 and 35 ppt salinity (one beaker per salinity).

2.4 Atlantic cod

Cod broodstock were caught on the George's Bank using otter trawl and held in two 10,752 l, circular, fiberglass tanks with a water inflow of 45 l/min. Cod spawn in broodstock tanks (like haddock) producing fertilized eggs which are accumulated in floating egg collectors. Procedures for egg collection, transfer, and incubation were identical to those for haddock.

2.5 Developmental Study

Fertilized eggs were placed in beakers or petri-dishes (winter flounder) containing water of the appropriate salinity (15, 20, 25, 30 or 35 ppt) and incubation methods were the same for all species.

For each experiment, eggs were incubated at five salinities (15, 20, 25, 30 and 35 ppt) in 24-welled, polystyrene tissue culture trays (PTC trays) which were kept in a cold room at a constant temperature of 5^0 C. Trays measured 12.5 cm x 8 cm x 2 cm and each well held a volume of 2 ml. Eight PTC trays were used for each salinity, providing an initial total of 192 eggs per salinity condition. Prior to the initiation of each experiment, wells of PTC trays were filled with 2 ml of appropriate salinity water via a 10 ml Repipete™ dispenser. Fertilized eggs were added to tray wells at a density of one egg per well using a 10 ml glass pipette. The pipette was inverted, a rubber bulb attached to the pointed end and eggs were drawn up (5-6 at once) from beakers and gently transferred to tray wells. This process was completed under dim-light conditions and once completed trays were left in complete darkness for the entire incubation process except for water changes and daily microscopic examination. Water in the cell wells was completely exchanged with clean water every fourth day. Water was removed using a 10 ml pipette and replenished with 2 ml of clean water using the Repipete dispenser mentioned previously.

Each day developing eggs were examined using a Zeiss 47-50-22 dissecting microscope. Daily records were made of mortalities, abnormalities, and development

stage. Development stage classification used in the current study is similar to that of Laurence and Rogers (1976) and is as follows:

Stage I from fertilization to the formation of a complete blastodermal cap.

Stage II from formation of a completed blastodermal cap through to the first appearance of the germinal ring and embryonic shield/axis.

Stage III from first appearance of the germinal ring and embryonic shield/axis to the closure of the blastopore, marking the end of gastrulation.

Stage IV from the closure of the blastopore to the start of hatching.

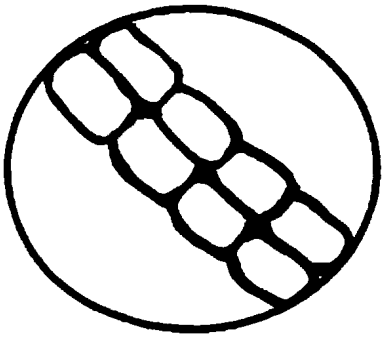
Stage V the hatching process (classification of eggs which only partially hatch).

Stage VI day on which hatching is complete for a particular larvae.

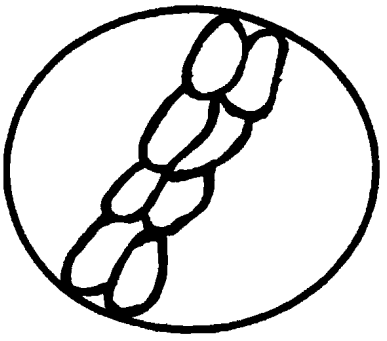
Stage VII one day-old yolk sac larvae.

In addition, the initial cleavage symmetry of halibut and haddock embryos was recorded to relate this parameter to subsequent larval viability. Since individual eggs were identifiable in the PTC trays, such an assessment was easily completed. Ninety-six eggs for each species were classified as symmetrical, slightly asymmetrical, or asymmetrical (Fig. 1).

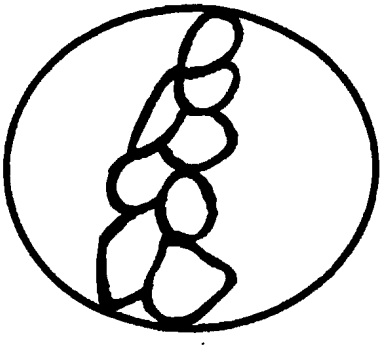
In all species, once embryos began hatching, records were kept of hatch period duration (up to 125 hrs, longer was considered failure to hatch); any hatching difficulties that occurred (e.g. partial hatch resulting in death); percentage hatch; percentage survival to 1 day post-hatch (DPH); percentage viable larvae (free of visible abnormalities); total larval length (from snout to posterior finfold); larval height (measured vertically at the



Symmetrical



Slightly Assymmetrical



Assymmetrical

Figure 1: Schematic diagrams used to classify cell symmetry of newly hatched eggs.

posterior end of yolk sac); yolk sac area; fertilization rates; developmental stage of egg mortality and egg cell cleavage symmetry.

Chorions of hatched eggs were immediately removed to avoid water quality degradation and possible obstruction to larvae. Hatched larvae were observed for spinal abnormalities and yolk abnormalities. At one day post-hatch (1DPH), larvae were removed from PTC trays and examined using an Olympus™ SZH-ILLD dissecting microscope in conjunction with an Ikegami™ ICD 40 video camera and a Panasonic™ H13150Y colour monitor. Measurements were made of larval total length, total height and yolk sac area (cursor was "clicked" around the entire circumference of the yolk sac). Also, at the time of measurement, overall larval activity was assessed. In some salinity conditions, larvae were very inactive showing no movement, all larvae were recorded as active or inactive.

2.6 Fertilization Study

Twenty hours post-fertilization, eggs fertilized at 15, 20, 25, 30 and 35 ppt, as previously mentioned, were observed for fertilization rates. A total of 300 eggs for each salinity condition were randomly selected from the beakers and assessed for fertilization. An egg was considered fertilized if development of the blastula cap (cell-division) was visible. Fertilization vs. salinity was assessed for halibut and winter flounder only, since both haddock and cod spawned naturally in broodstock tanks of ambient salinity (~30 ppt).

2.7 Stress Study

For each species studied, an additional two PTC trays were incubated at each salinity (15, 20, 25, 30 and 35 ppt) using the same method described earlier for the developmental study. At 1DPH, larvae were removed and placed directly into PTC trays containing 30^0C water (of appropriate salinity). Water temperature was maintained at 30^0C by placing the trays in a Precision Scientific™, model 6M incubator. Time was recorded from initial contact with the high temperature water until death occurrence. Microscopic observations were completed once every 15 minutes and criterion for death was cessation of heartbeat.

The parameters recorded in the above protocols were: 1) Fertilization rates (halibut and winter flounder); 2) Initial cleavage vs. larval viability (haddock and halibut); 3) Hatch time (mean time to complete hatching); 4) Hatch duration (length of time for hatching of first to last egg) ; 5) Hatching difficulties/abnormalities; 6) Length, height and yolk area of larvae 1DPH; 7) Percentage inactive larvae; 8) Development stage of embryo at mortality; 9) Cumulative egg and larval mortality; 10) An assessment of larval condition based on temperature stress; 11) Percentage viable larvae. Statistical analyses were completed using Statgraphics Plus™ for Windows™. The effects of salinity on the various parameters listed above were determined using one - way analysis of variance (Anova). Once Anovas were found significant, multiple range tests were used to pinpoint differences. In addition, regression analyses (simple regression) were completeted on data demonstrating strong linear trends. All data was tested for normality

and log or arcsin transformed as required. Ninety - five percent confidence level was used as a determinant of significance (alpha = 0.05).

3.0 RESULTS

3.1 Development Study

3.1.1 Percentage Hatch

Halibut eggs demonstrated significantly higher hatch rates at 30 and 35 ppt compared to 15, 20, and 25 ppt ($F = 23.21$; $df = 4,35$; $p<0.0001$; Fig 2). Regression analysis showed a significant positive salinity trend (Linear regression: $F = 47.68$; $df = 1,38$; $p<0.0001$; r squared = 47.26%; Fig 3).

Hatch rates among haddock eggs were different than those found for halibut. No significant differences between incubation salinity and mean hatch rates were found ($F = 1.98$; $df = 4,34$; $p > 0.05$; Fig. 2).

Cod eggs displayed no significant differences ($F = 0.92$; $df = 4, 35$; $p = 0.467$) in hatch rates across salinities with no particular salinity trends being established although 15 ppt produced the lowest result (Fig.2).

Winter flounder embryos generally expressed lower hatch rates with increasing salinity (Fig.2). Percentage hatch at 30 ppt and 35 ppt, was significantly less than at 15 ppt, 20 ppt and 25 ppt ($F = 4.67$; $df = 4,35$; $p<0.005$). Regression analysis also showed a significant negative trend of salinity on hatching performance ($R^2 = 25.04\%$; $p<.001$; Fig.3).

3.1.2 Mean Hatch Time

There were no significant differences in hatch time for halibut eggs reared at 30 or 35 ppt (Table 1). Hatch times were not available for salinities of 25 ppt and below since eggs failed to survive to the point of hatching at these salinities.

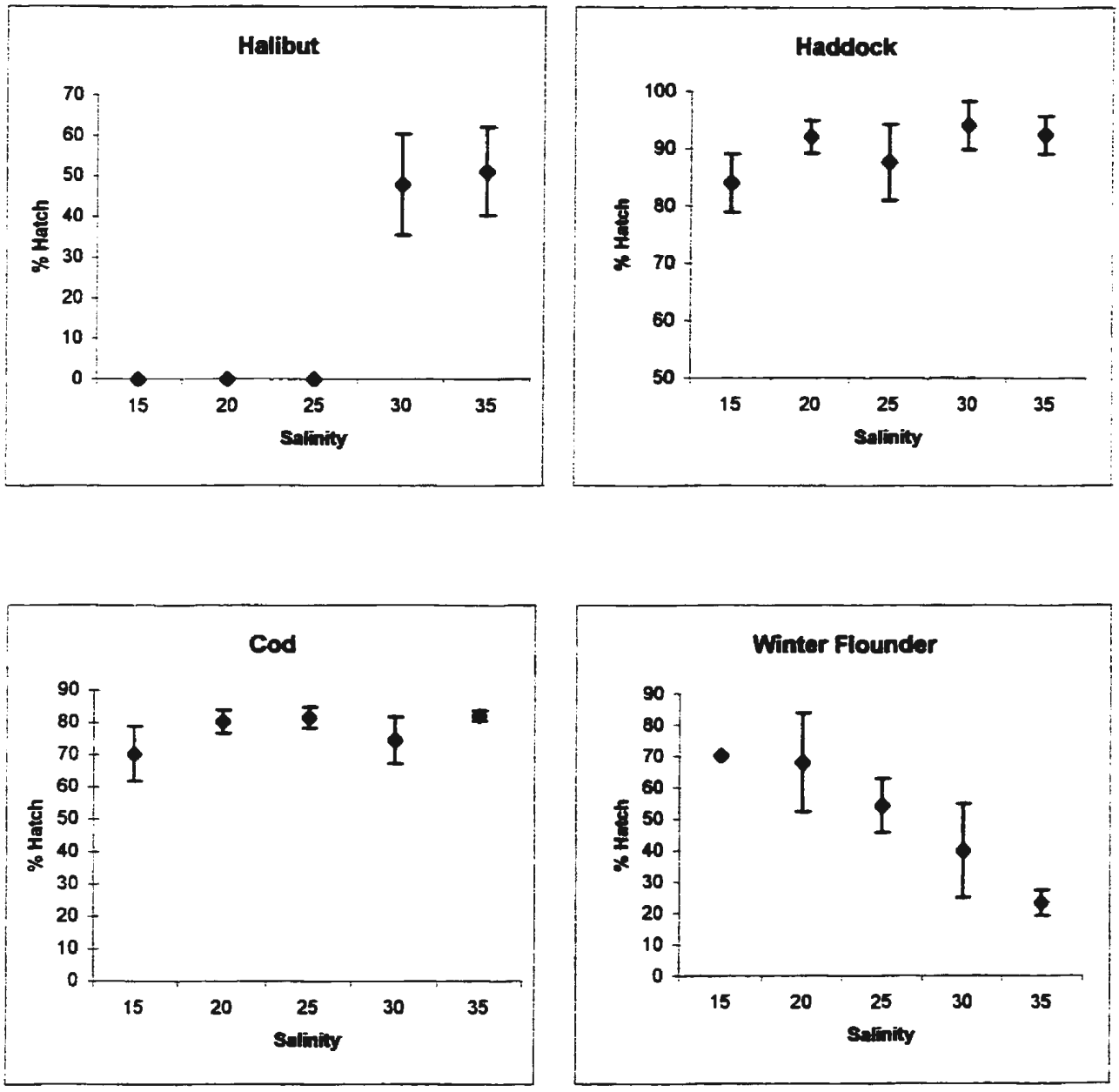
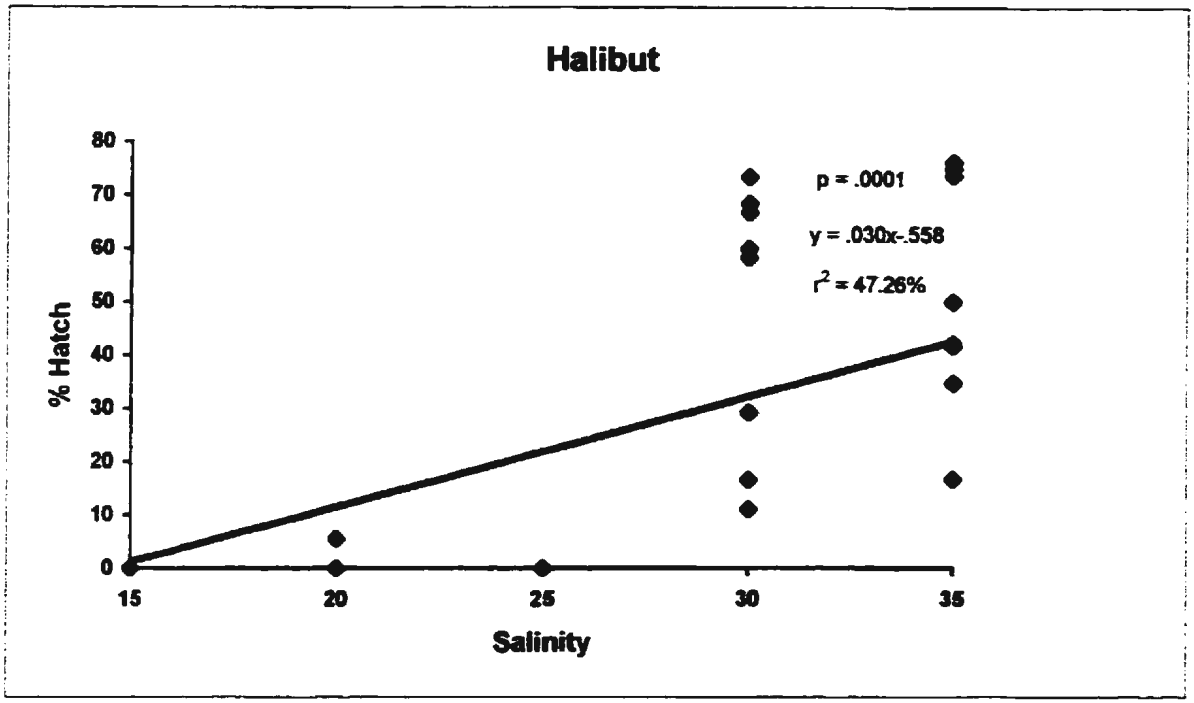


Figure 2: Hatching rates (%) of eggs incubated at the various salinity treatments. Data are shown as mean (+/-) one standard deviation (n=24).



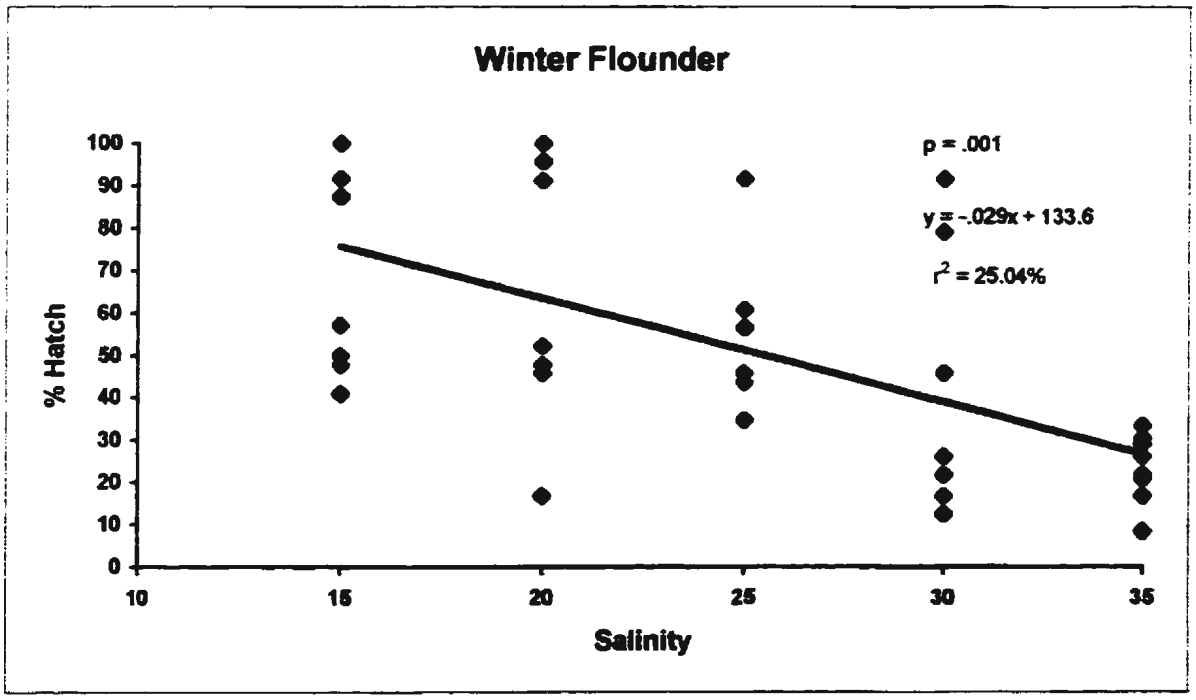


Figure 3: Scatterplot of salinity versus % hatch with regression line illustrated (n=24).

Table 1: Mean hatch time (hrs; from fertilization until complete hatch) for eggs incubated at the various salinity treatments. Standard deviation is shown in superscripts (n = 8).

Salinity	Cod	Haddock	Halibut	Winter Flounder
15	$489.59^{16.40}$	$412.82^{16.82}$		$344.22^{16.99}$
20	$464.24^{35.99}$	$416.83^{8.42}$		$351.65^{15.36}$
25	$463.85^{16.77}$	$395.24^{8.75}$		$365.60^{21.97}$
30	$454.32^{41.52}$	$415.22^{18.56}$	$382.28^{27.05}$	$357.57^{36.46}$
35	$485.58^{20.54}$	$399.25^{8.46}$	$381.24^{23.60}$	$350.03^{28.18}$

Table 2: Mean hatch duration (average number of hours for hatch to be complete from first until last egg) for eggs incubated at the various salinity treatments. Standard deviation is shown in superscripts (n = 8)

Salinity	Cod	Haddock	Halibut	Winter Flounder
15	$116.00^{80.91}$	$54.00^{16.97}$		$102.00^{52.50}$
20	$124.00^{68.59}$	$57.00^{21.99}$		$90.00^{24.84}$
25	$114.00^{58.67}$	$84.00^{46.25}$		$138.00^{93.17}$
30	$138.00^{114.54}$	$93.00^{67.20}$	$48.00^{36.28}$	$105.00^{96.80}$
35	$44.00^{23.60}$	$78.86^{61.52}$	$39.00^{17.86}$	$102.00^{68.79}$

Haddock eggs, unlike halibut, did show salinity trends with respect to hatch time. Mean hatch times at 25 ppt and 35 ppt were significantly less than those at 15, 20 and 30 ($F = 4.57$; $df = 4,34$; $p<0.005$; Table 1).

Hatch times among cod eggs failed to demonstrate any significant differences with incubation salinity ($F = 0.21$; $df = 1,28$; $p = 0.651$; Table 1).

Winter flounder embryos incubated at the various salinities did not show significant differences in hatch times ($F = 0.8$; $df = 4,35$; $p = 0.535$; Table 1).

3.1.3 Hatch Duration

Halibut and winter flounder eggs displayed no significant differences in duration of hatch with respect to incubation salinity (Table 2).

Haddock eggs displayed a generally longer hatch period with increasing salinity, however, this trend was not significant ($F = 0.5$; $df = 4,34$; $p = 0.734$; Table 2).

Following a similar trend as the rest of the species, cod eggs failed to express any significant differences in hatch duration ($F = 2.66$; $df = 4,25$; $p = .057$; Table 2). There was a large amount of variability within each condition and no salinity trends were evident.

3.1.4 Stage Specific Mortality

Based on the developmental stage classification system described earlier, mortality was recorded for each step from early cell cleavage through to 1 day post-hatch. Since there were no salinity effects on stage of mortality, results from all salinities were combined in an effort to determine the most susceptible stage for each species.

Halibut embryos demonstrated low mortality rates from stage 4 onwards (Fig. 4). Mortality was significantly higher in stages 1,2 and 3 ($F = 19.35$; $df = 6,245$; $p<0.0001$; Fig. 4). Mortality at stages 1 and 3 was significantly higher than at any other stage. There were no significant differences from stage 4 onwards.

Haddock eggs showed significantly higher mortality at stages 4,5 and 7 ($F = 7.25$; $df = 6,266$; $p<0.0001$; Fig. 4). There were no significant differences between mortality at these three stages and mortality was generally low, being less than 5% in any case.

Cod embryos demonstrated significantly higher mortality at stages 2, 3 and 4 ($F = 10.99$; $df = 6,203$; $p<0.0001$; Fig. 4). There were no significant differences among these three stages although mortality was slightly higher at stage 4.

Winter flounder embryos exhibited mortality specific to two developmental stages, stage 2 and 4. Mortality at these stages was significantly higher than all other stages ($F = 20.57$; $df = 6,273$; $p<0.0001$; Fig. 4).

3.1.5 Percentage Viable Larvae

Since halibut eggs failed to develop and hatch at salinities of 25 ppt and lower, larval viability was significantly higher at salinities of 30 and 35 ppt ($F = 24.71$; $df = 4,35$; $p<0.0001$; Fig.5). There were no differences in respective means viability rates at 30 and 35 ppt.

Viability rates among newly-hatched haddock larvae were not significantly different ($F = 2.26$; $df = 4,34$; $p = 0.083$) although lowest mean viability occurred at 15

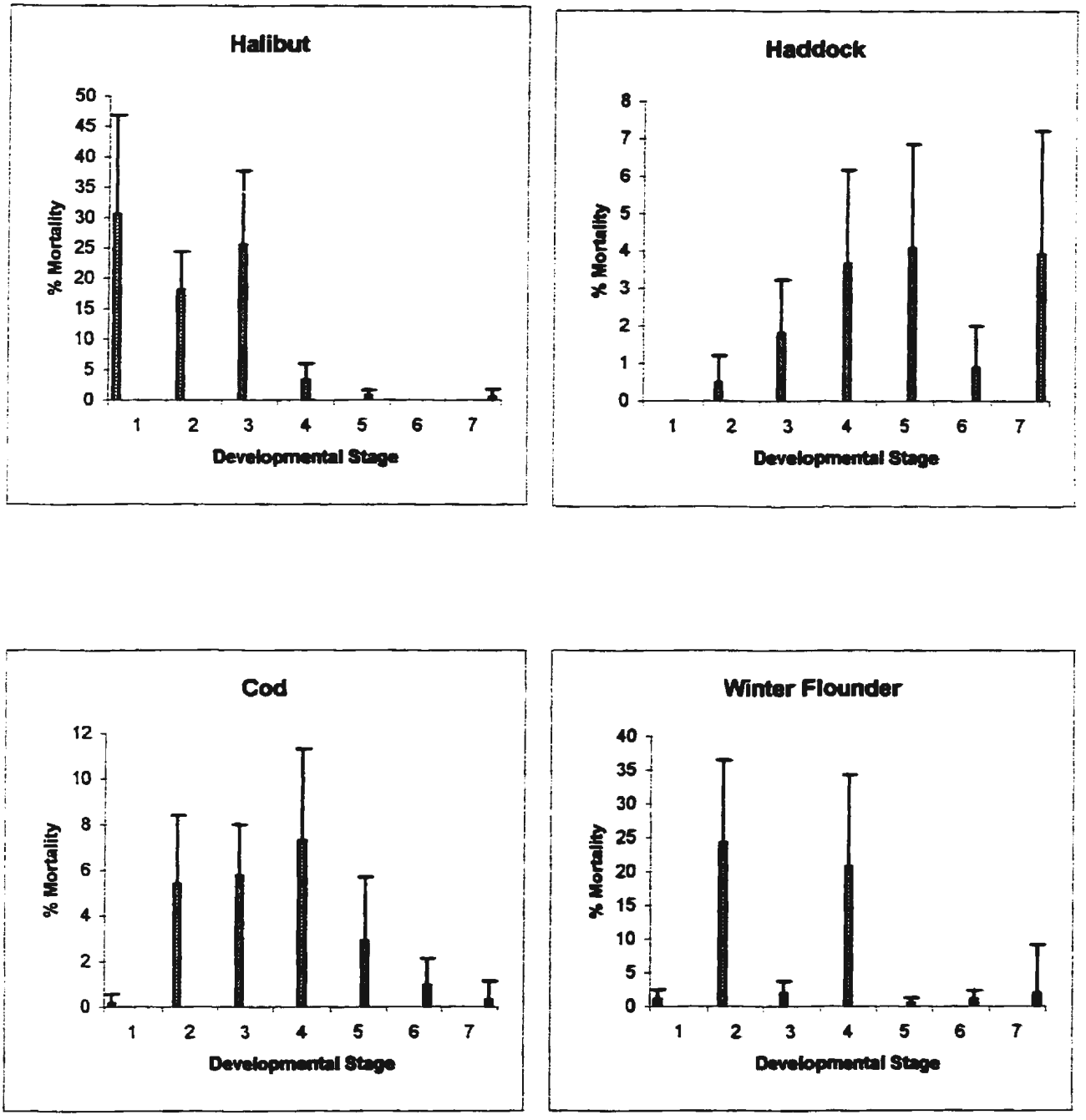


Figure 4: Mean percentage mortality at specific developmental stages of eggs and early larvae. Bars represent standard errors (n=24).

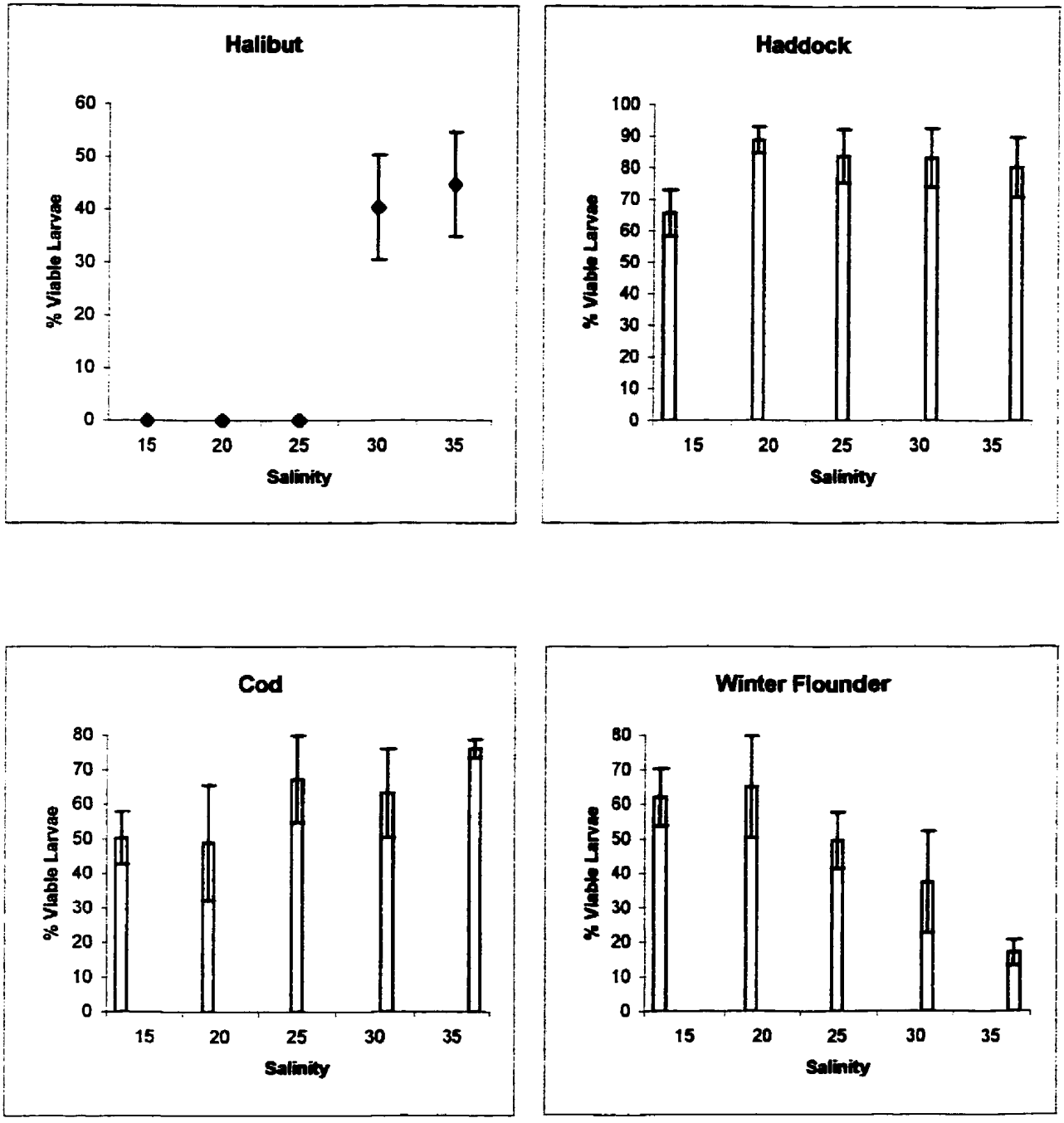


Figure 5: Mean percentage viable larvae resulting from subsequent salinity of egg incubation. Bars indicate standard deviations (n=24).

ppt (Fig. 5). Viability within the other four conditions was greater than 80% and did not appear to display a distinct salinity trend.

Cod larvae showed no significant differences in viability rates ($F = 1.47$; $df = 4,25$; $p = 0.24$; Fig. 5).

Winter flounder larvae demonstrated a significant decreased viability at the higher salinities, especially 35 ppt ($F = 5.36$; $df = 4,35$; $p<0.005$; Fig. 5). Viability at 30 ppt and 35 ppt was significantly less than at 15, 20 and 25 ppt. In addition, regression analysis showed a significant inverse relationship between viability and salinity ($R^2 = 21.84\%$; $p<.005$; Fig. 6).

3.1.6 Total Length

No significant differences ($F = 0.15$; $df = 1,128$; $p = 0.701$) were found in lengths of halibut larvae hatched from eggs incubated at either 30 or 35 ppt (Fig. 7).

Newly-hatched haddock larvae expressed longer overall lengths when eggs were incubated at 15 and 20 ppt (Fig. 7). Larvae were significantly longer at 15 ppt and 20 ppt compared to 25, 30 ppt and 35 ppt ($F = 33.87$; $df = 4, 711$; $p<0.0001$).

Cod larvae hatched from eggs incubated at the various salinities, did not show any significant differences in total length ($F = 0.6$; $df = 4, 322$; $p = 0.659$; Fig. 7).

Mean lengths of winter flounder larvae hatched at the various salinities showed no significant differences ($F = 2.42$; $df = 4,144$; $p>0.05$; Fig. 7). However, there appeared to be a weak salinity trend with shortest lengths occurring at 30 and 35 ppt.

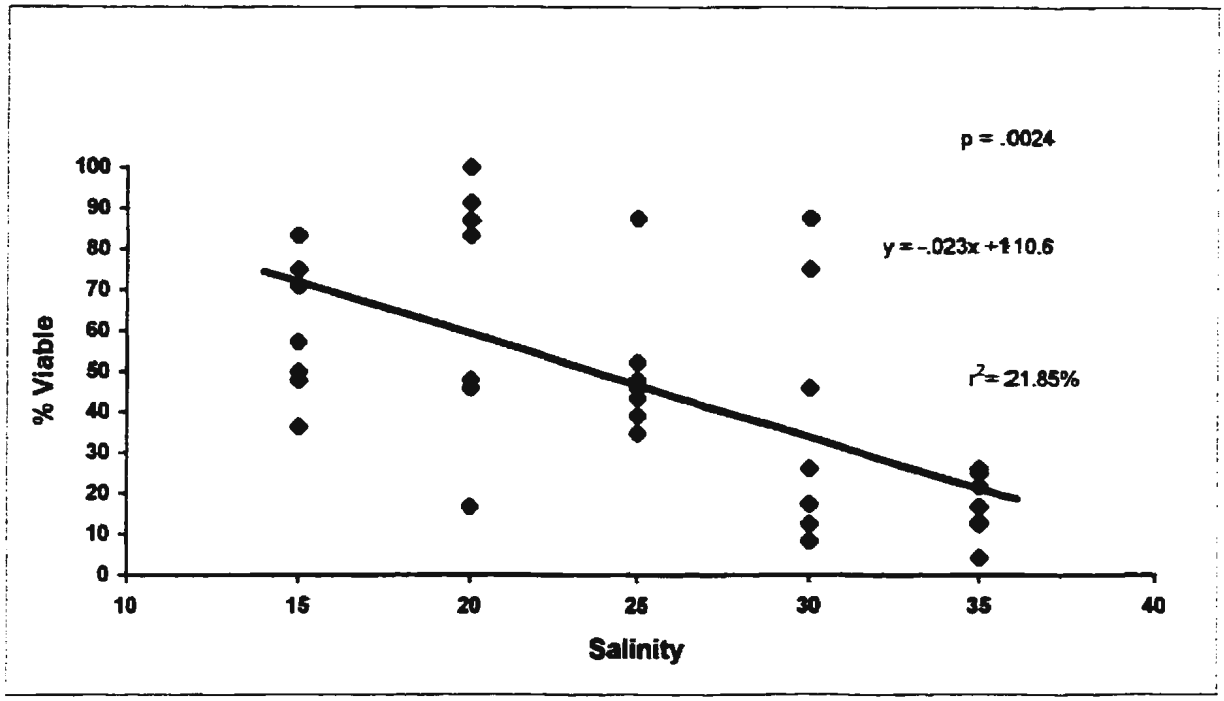


Figure 6: Scatterplot of viable larvae versus egg incubation salinity for winter flounder. Linear regression is also illustrated (n= 24).

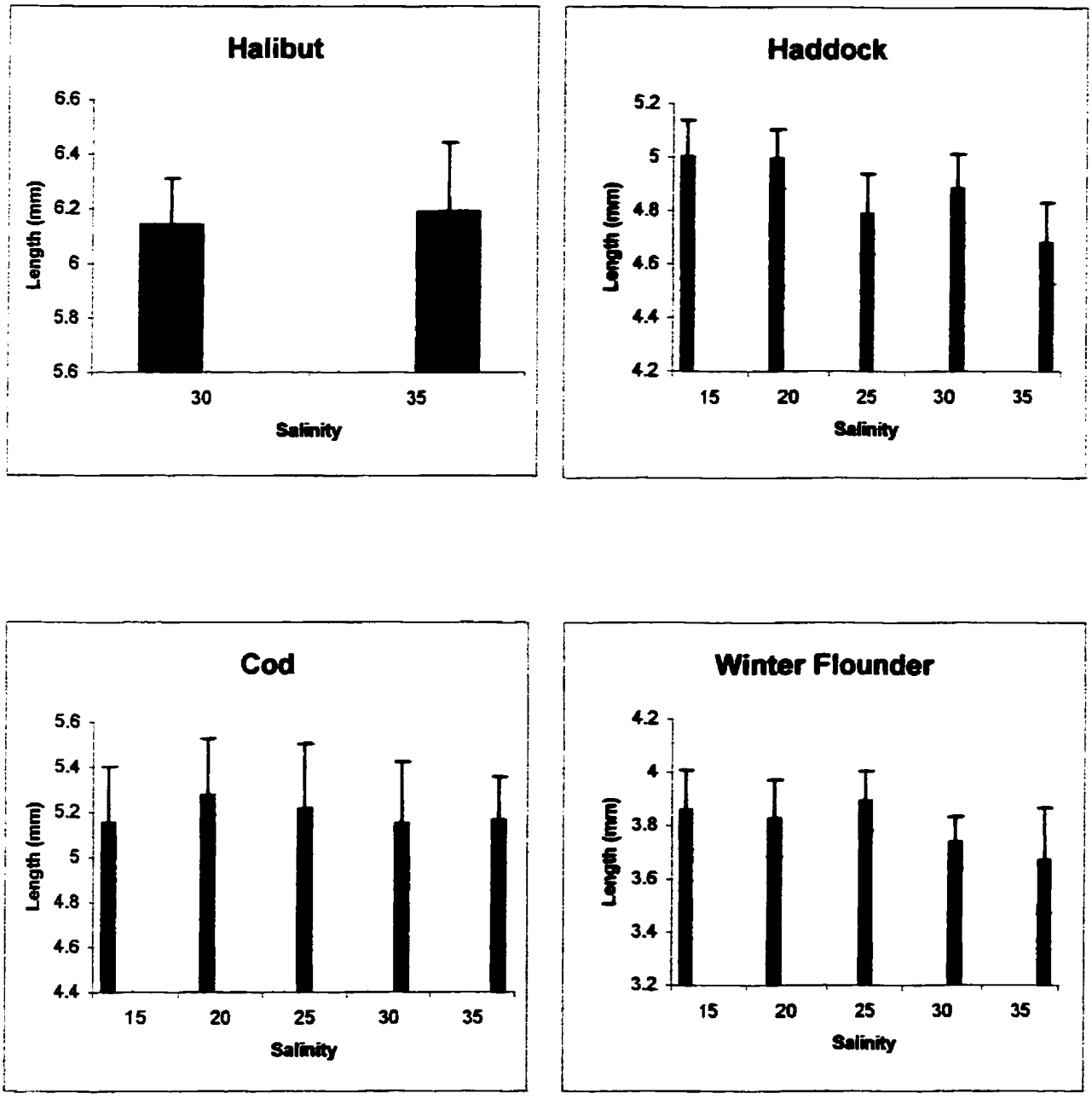


Figure 7: Length (mm) of larvae (one day post hatch) resulting from egg incubation at the various salinities. Data are shown as mean (+/-) one standard deviation (n= 327,cod; 716, haddock; 149, winter flounder; 130, halibut).

3.1.7 Yolk Size

Yolk areas of halibut larvae derived from eggs held at the various salinities were not significantly different ($F = 2.77$; $df = 1, 222$; $p = 0.094$; Fig. 8).

Haddock larvae displayed larger yolk sacs if eggs had been incubated at lower salinities. Eggs incubated at 15 ppt produced larvae with yolk sacs significantly larger than all other conditions ($F = 86.55$; $df = 4, 627$; $p<0.0001$; Fig. 8). Mean yolk size tended to get smaller with increasing salinity with the exception of 35 ppt where the yolk size was similar to that at 20 ppt (Fig. 8). Regression analysis demonstrated a significant inverse trend between yolk size and salinity ($R^2 = 19.05\%$; $p<0.0001$; Fig. 9). For cod, larval yolk area was significantly larger if eggs had been incubated at 15 ppt compared to all other conditions. In addition, yolks of larvae from eggs held at 20 ppt and 25 ppt were significantly larger than those at 30 and 35 ppt ($F = 87.41$; $df = 4, 326$; $p<0.0001$; Fig. 8). The inverse trend of larger yolk size with decreasing salinity was significant ($R^2 = 48.38\%$; $p<0.0001$; Fig. 9).

Among winter flounder embryos, larvae hatched from 15 ppt had significantly larger yolks than all other conditions while those at 20 ppt were larger than at 35 ppt ($F = 5.79$; $df = 4, 347$; $p <0.0005$; Fig. 8).

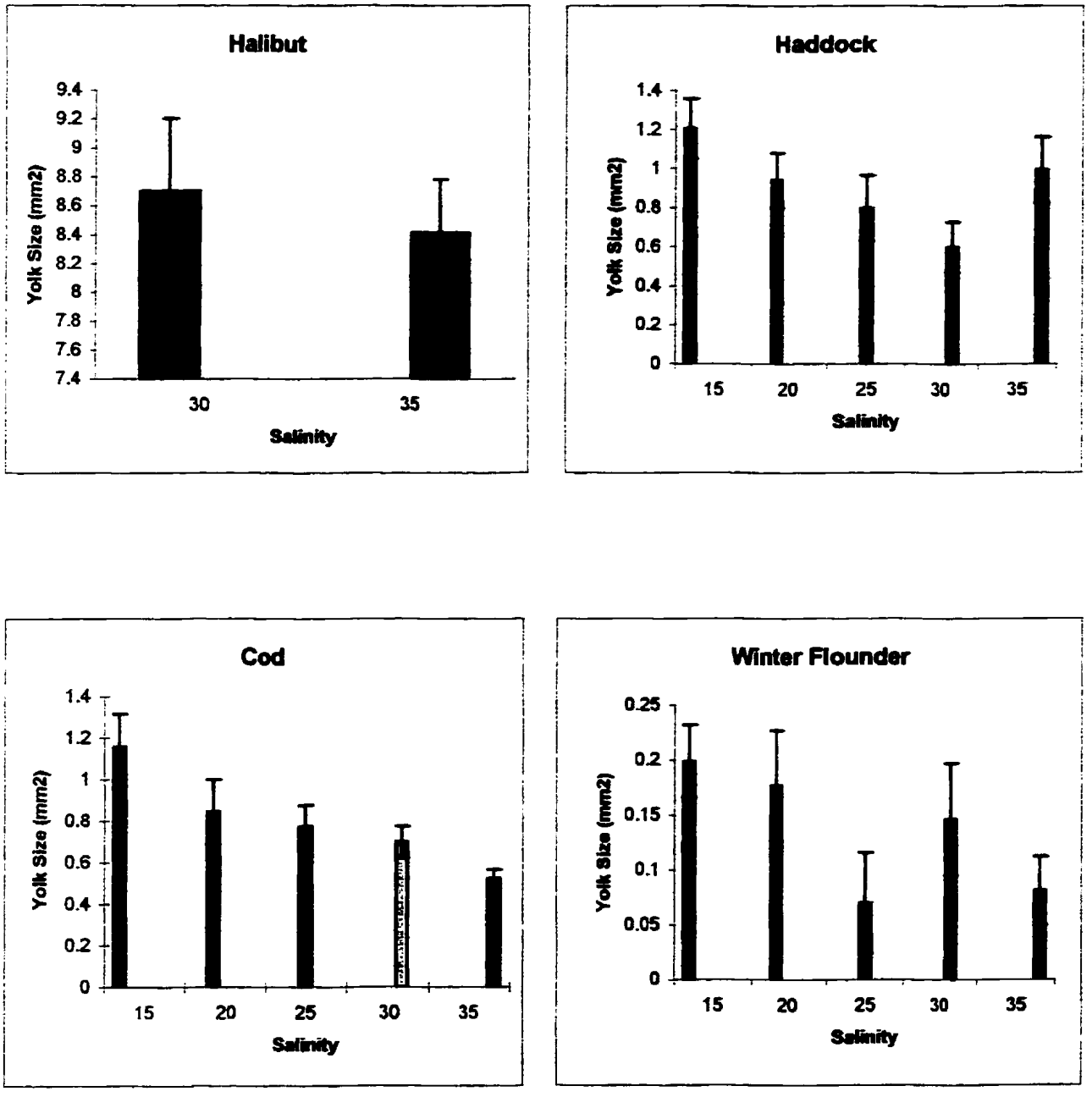
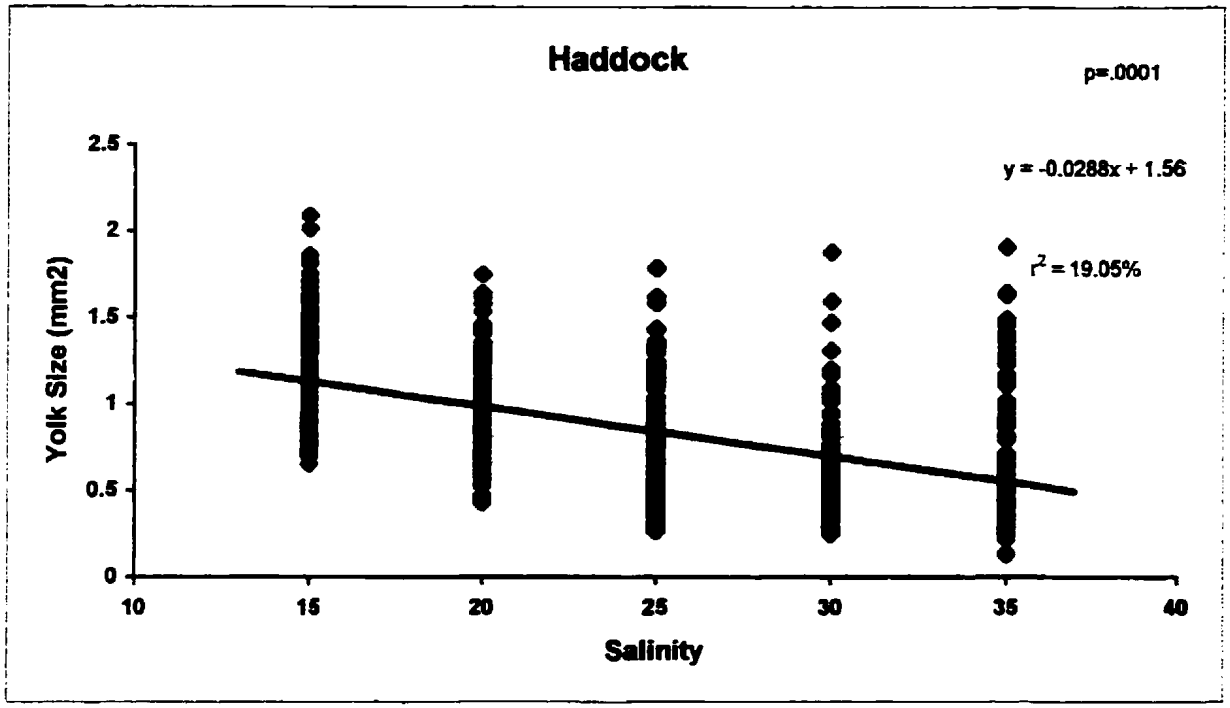


Figure 8: Mean yolk-sac size of larvae (1DPH) from eggs incubated at the various test salinities. Bars indicate standard deviations (n=714, haddock; 331, cod; 472, winter flounder; 124, halibut).



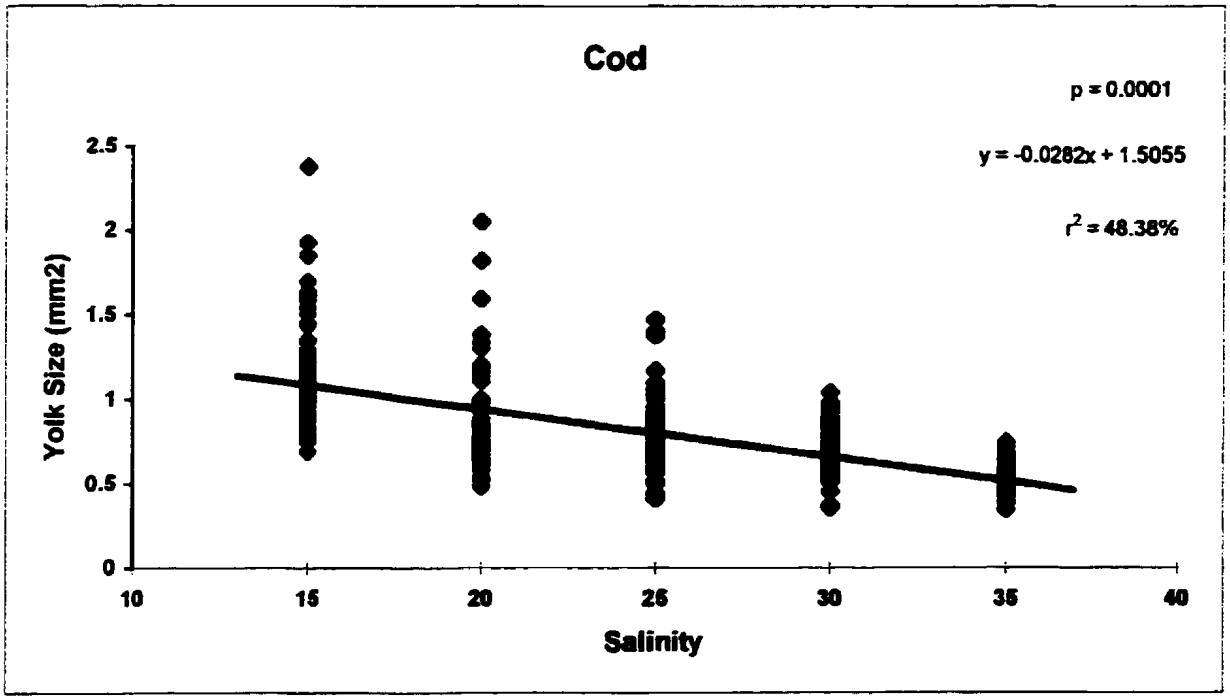


Figure 9: Scatterplot of larval yolk size vs. egg incubation salinity. Linear regression is illustrated (n= 714, haddock; 331,cod).

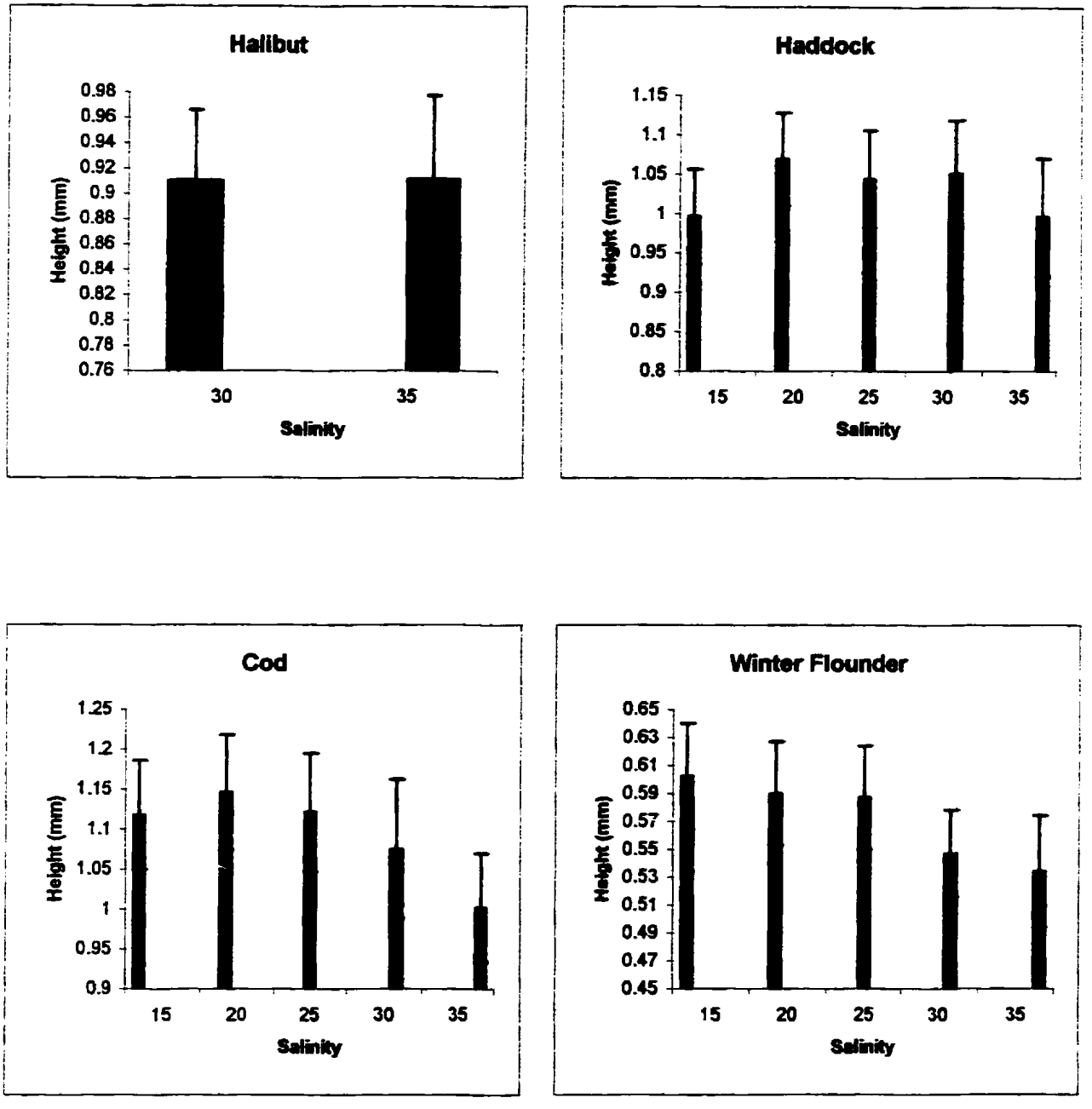


Figure 10: Height (mm) of larvae resulting from eggs incubated at the respective salinities. Data are shown as mean (+/-) one standard deviation (n= 697, haddock; 295, cod; 385, winter flounder; 102, halibut).

3.1.8 Larval Height

Salinity of egg incubation did not have any significant effect on larval height among halibut larvae ($F = 0.01$; $df = 1,100$; $p = 0.915$; Fig. 10). Haddock larval height was significantly higher at 20, 25 and 30 ppt compared to 15 and 35 ppt ($F = 5.33$; $df = 4,692$, $p<0.0005$; Fig. 10).

Larval height among cod larvae was significantly lower in eggs reared at 35 ppt compared to all other salinities ($F = 8.30$; $df = 4, 290$; $p<0.0001$; Fig.10). Means suggested an inverse trend of decreasing height with increasing salinity.

Winter flounder embryos incubated at salinities of 15, 20 and 25 ppt had significantly increased larval heights compared to 30 ppt and 35 ppt ($F = 5.68$; $df = 4, 380$; $p<0.0005$; Fig. 10).

3.1.9 Percentage Inactive Larvae

No halibut larvae were recorded as being inactive in any of the treatments tested.

Hatched haddock larvae from salinities of 15 and 20 ppt were significantly less active compared to larvae from higher salinities ($F = 96.37$; $df = 4, 34$; $p<0.0001$; Fig. 11). Regression analysis demonstrated a significant inverse trend of increased inactivity especially at the lowest salinities of 15 and 20 ppt (R^2 squared = 59.43%; $p<0.0005$; Fig. 12).

Differences in larval activity versus egg incubation salinity were not significant among cod treatments. Inactivity was 0% in all conditions except 20 ppt were it was 1.11%.

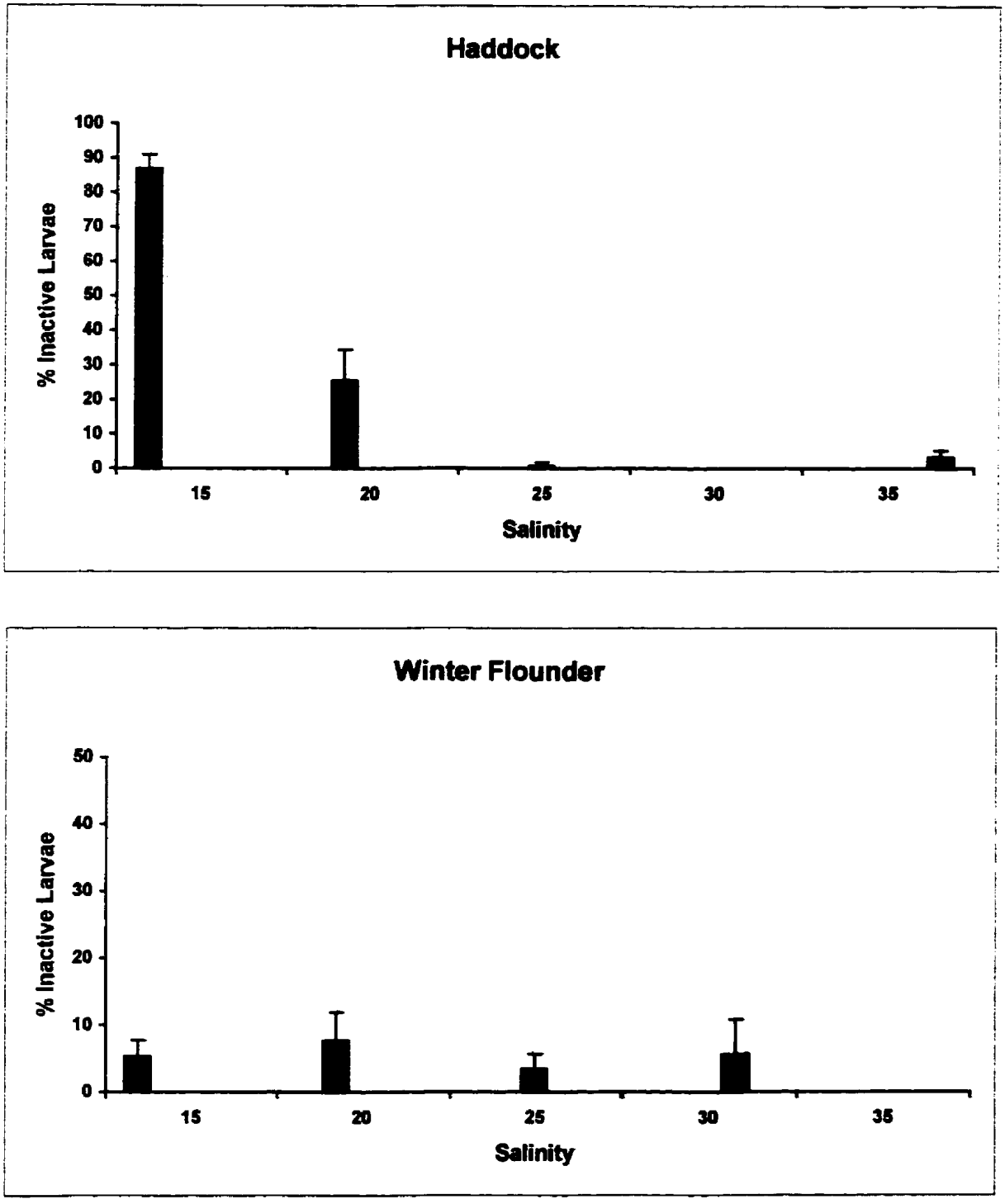


Figure 11: Percentage of inactive larvae resulting from the various egg incubation salinities. Bars indicate standard deviations (n=24).

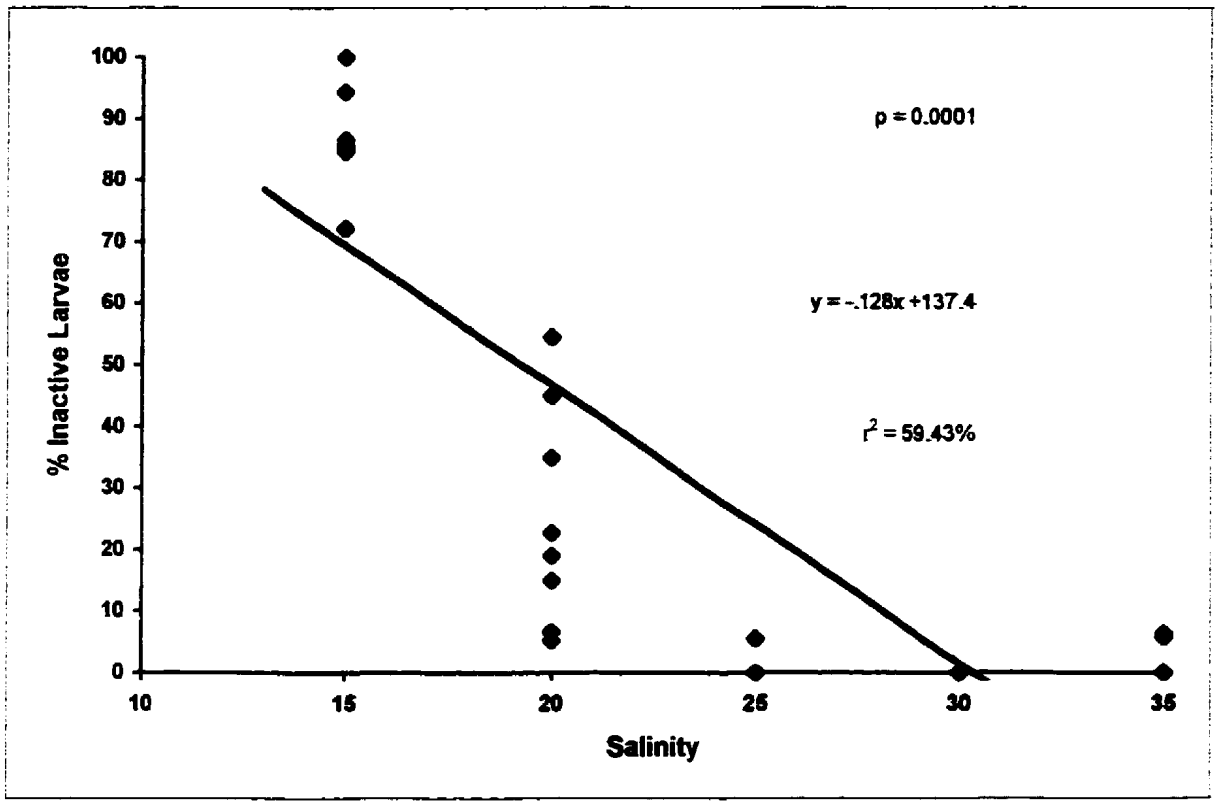


Figure 12: Regression results illustrating the negative trend with respect to % larval inactivity and salinity of egg incubation for Haddock (n=24).

Winter flounder larvae failed to demonstrate any significant difference ($F = 1.46$; $df = 4,35$; $p = 0.235$) in activity regardless of incubation salinity, although inactivity was lowest at 35 ppt (Fig. 11).

3.1.10 Percentage Partial Emergence

Partial hatching was not a significant problem among halibut larvae at either 30 ppt or 35 ppt ($F = 3.34$; $df = 1,14$; $p = 0.089$; Fig.13).

There were no significant differences in % partial hatching of haddock larvae across all salinities ($F = 2.3$; $df = 4,34$; $p = 0.079$; Fig.13).

Cod hatching at 15 ppt showed significantly higher partial emergence compared to near zero percentages at other salinities ($F = 4.07$; $df = 4,25$; $p<0.05$; Fig. 13).

Incidences of partial hatching were low among all winter flounder eggs examined and no significant differences were found ($F = 1.14$; $df = 4,35$; $p>.05$; Fig. 13).

3.1.11 Cell Symmetry as an Indicator of Larval Viability

Early cell cleavage patterns were assessed for halibut and haddock embryos only. No significant relationship was found between early cleavage patterns and resulting larval viability in either halibut ($F = 1.17$; $df = 2,9$; $p = 0.355$) or haddock ($F = 3.84$; $df = 2,12$; $p = 0.052$) embryos (Fig.14).

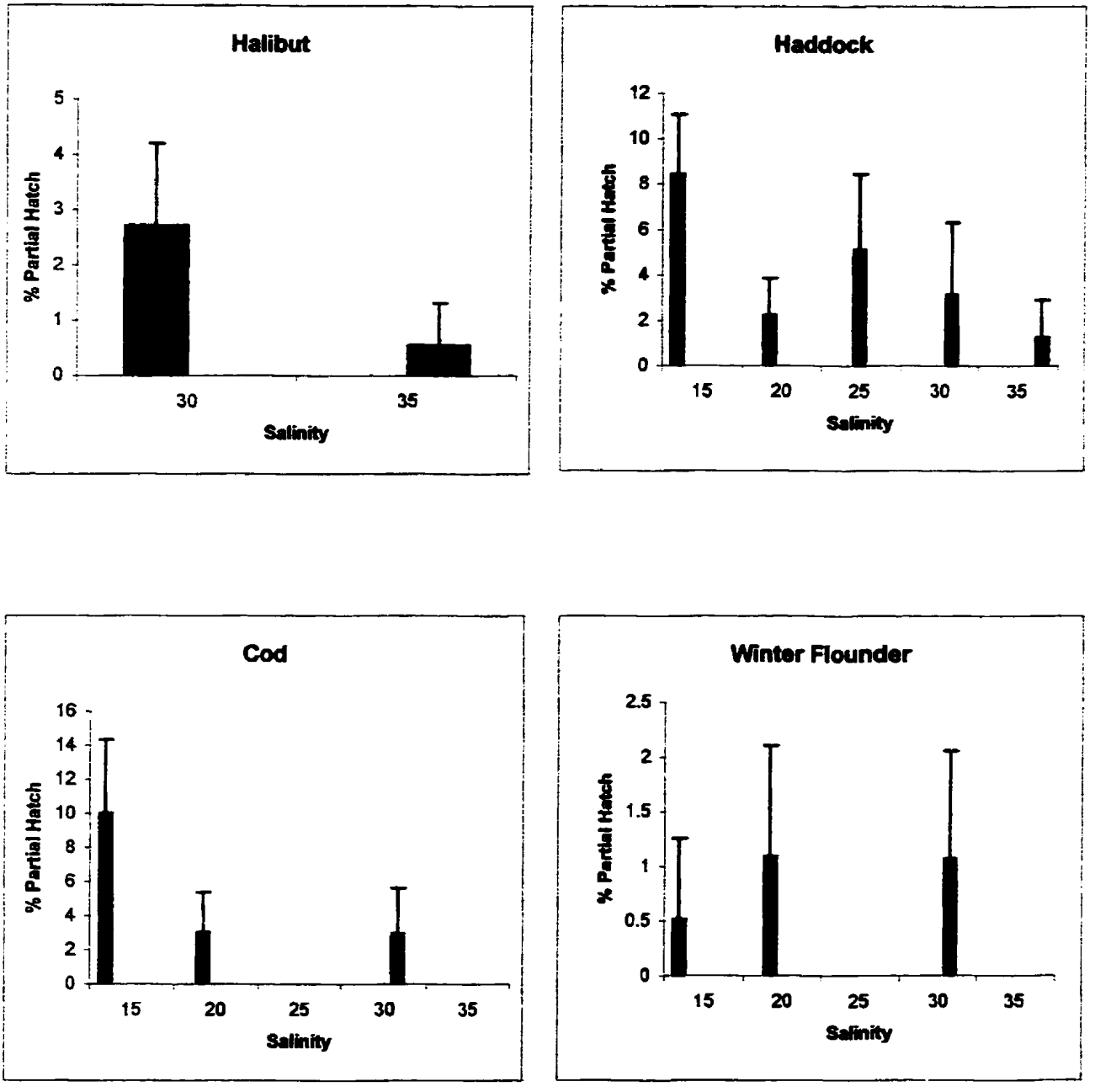


Figure 13: Percentage of larvae demonstrating partial hatch as a result of incubation salinity. Bars demonstrate standard deviations (n=24).

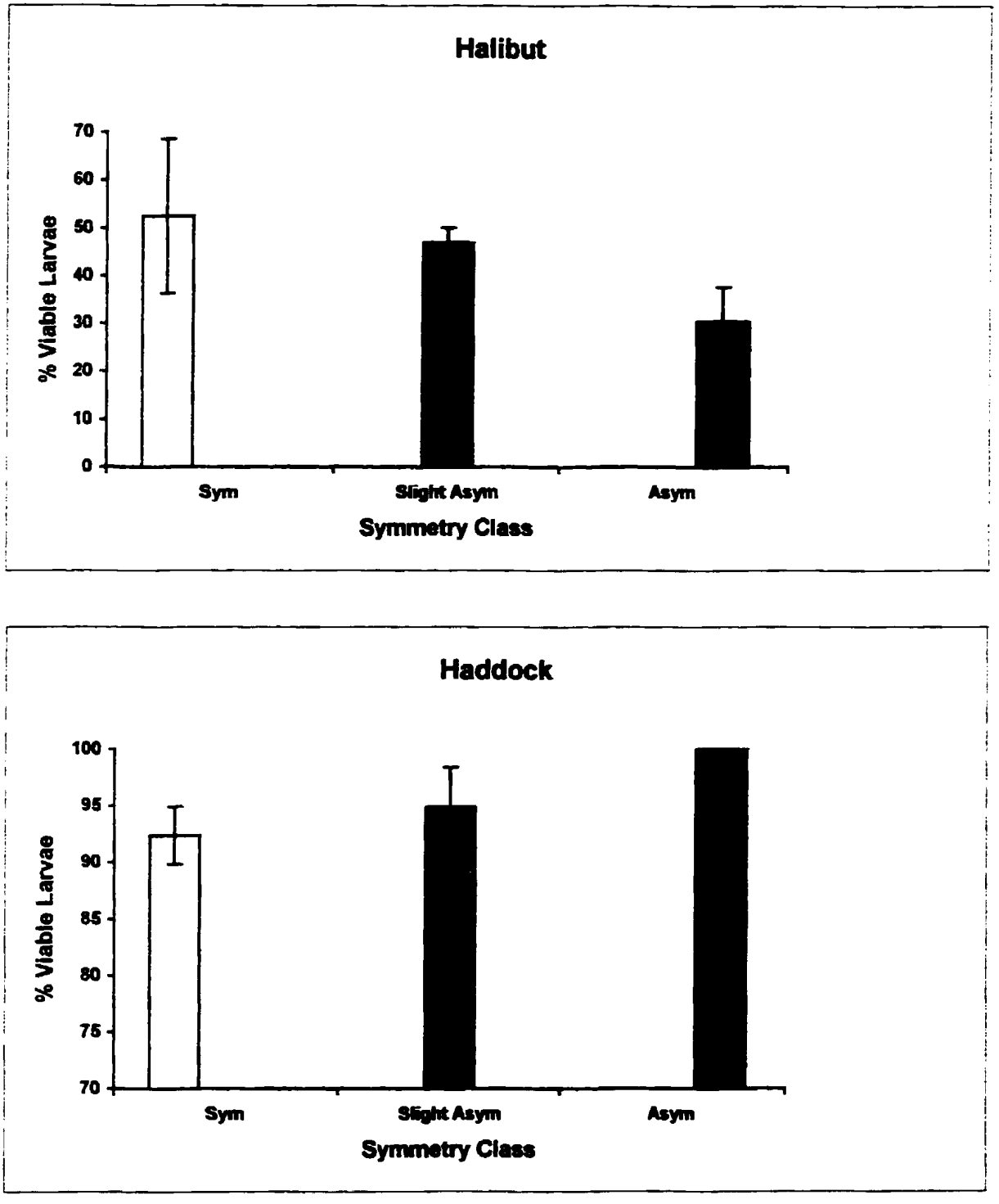


Figure 14: The relationship between viability of resulting larvae and initial symmetry of the egg. Data shown are mean (+/-) one standard deviation (n=24).

3.2 Fertilization Study

Fertilization rate versus salinity of egg incubation was assessed for halibut and winter flounder embryos (Fig.15). Winter flounder eggs expressed no significant differences ($F = 0.26$; $df = 4,5$; $p = 0.895$) in fertilization rates across salinities (Fig.15). Halibut fertilization rates were significantly lower at 15 ppt compared to all other salinity conditions ($F = 5.37$; $df = 4,5$; $p<0.05$: Fig.15). Although there were no significant differences in fertilization rates from 20 ppt-35 ppt, percentages were higher at 30 and 35 ppt.

3.3 Temperature Stress

Halibut larvae, incubated as eggs at 30 or 35 ppt, did not show any significant differences in stress tolerance ($F = 0.23$; $df = 1,94$; $p = 0.639$; Fig. 16).

Haddock larvae demonstrated a trend of increased stress tolerance with decreasing egg incubation salinity. Eggs incubated at 15, 20 and 25 ppt produced larvae with significantly longer death times compared to 30 ppt and 35 ppt ($F = 34.78$; $df = 4,235$; $p<0.0001$; Fig. 16). Death time at 35 ppt was also significantly less than at 30 ppt. Regression analysis also demonstrated the inverse trend ($R^2 = 27.61\%$, $p<0.0001$; Fig. 17).

Cod larvae exhibited an opposite trend compared to that of haddock larvae. Cod larvae survived significantly longer when incubated at increasingly high salinities, except for 30 ppt, which produced shorter death times than 25 ppt ($F = 283.78$; $df = 4, 235$; $p<0.0001$; Fig. 16). Regression analysis showed this trend to be significant ($R^2 = 70.46\%$;

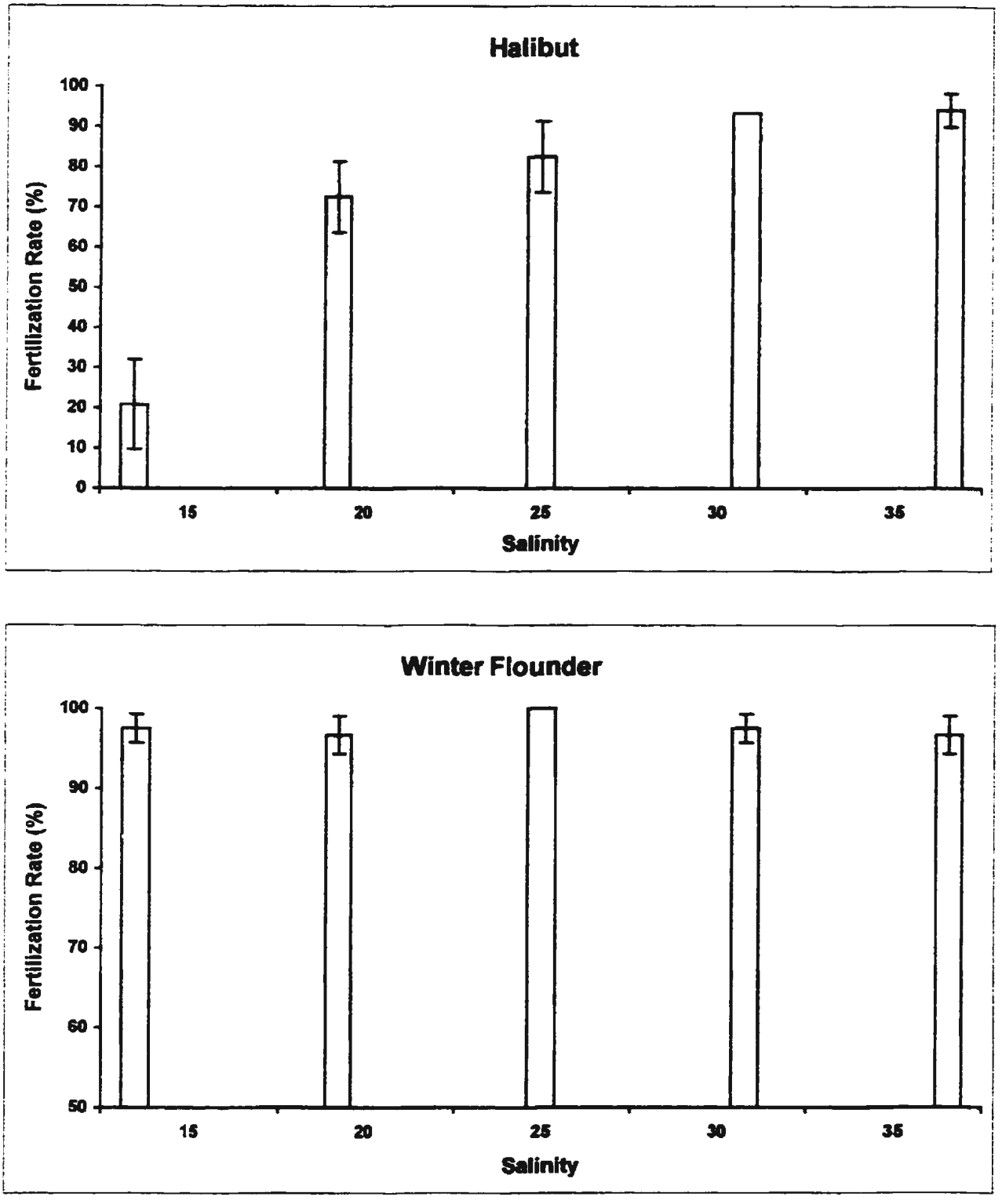


Figure 15: Fertilization rates (%) for halibut and winter flounder eggs as a function of incubation salinity. Data are shown as mean (+/-) one standard deviation (n=60)

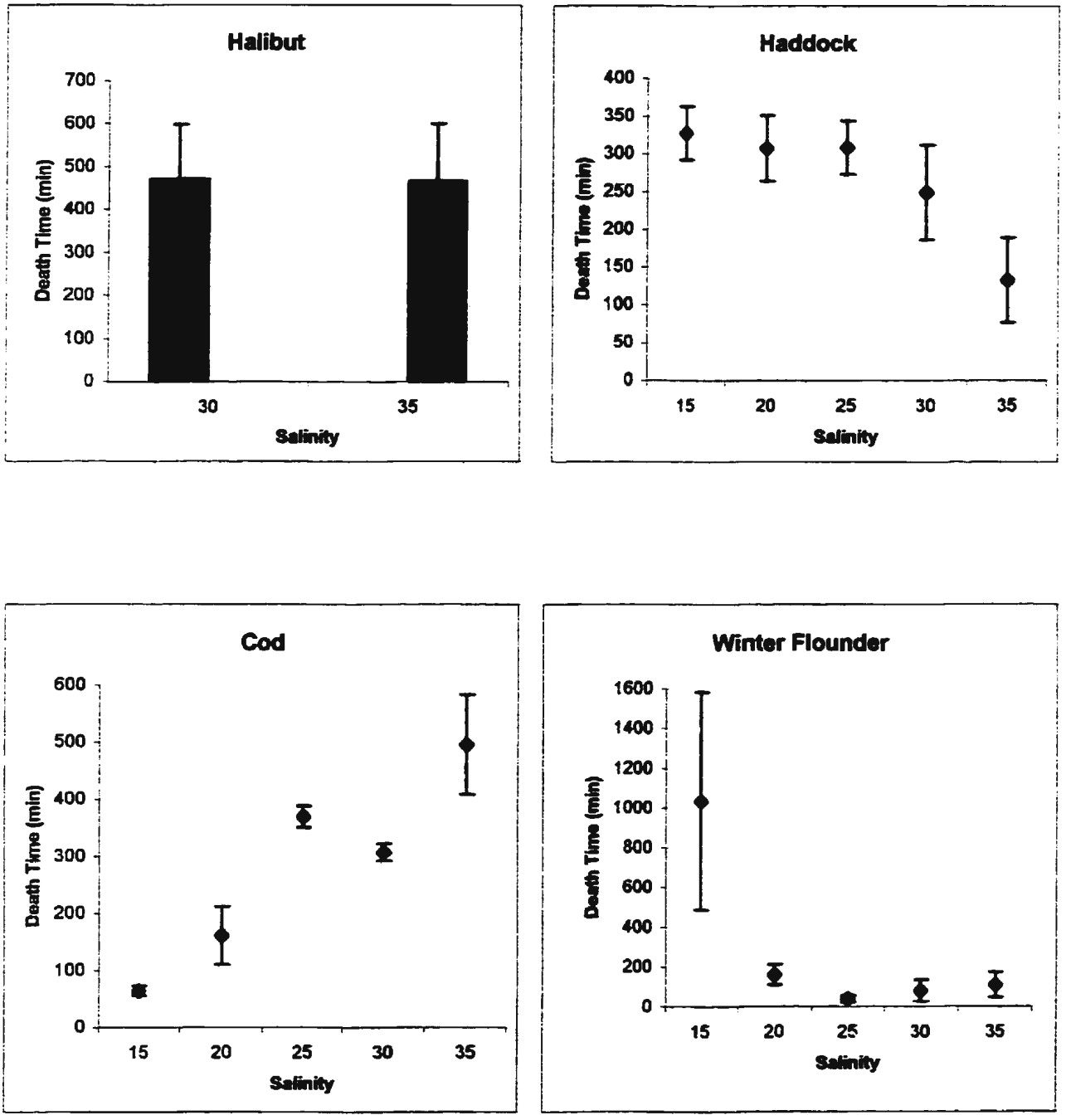
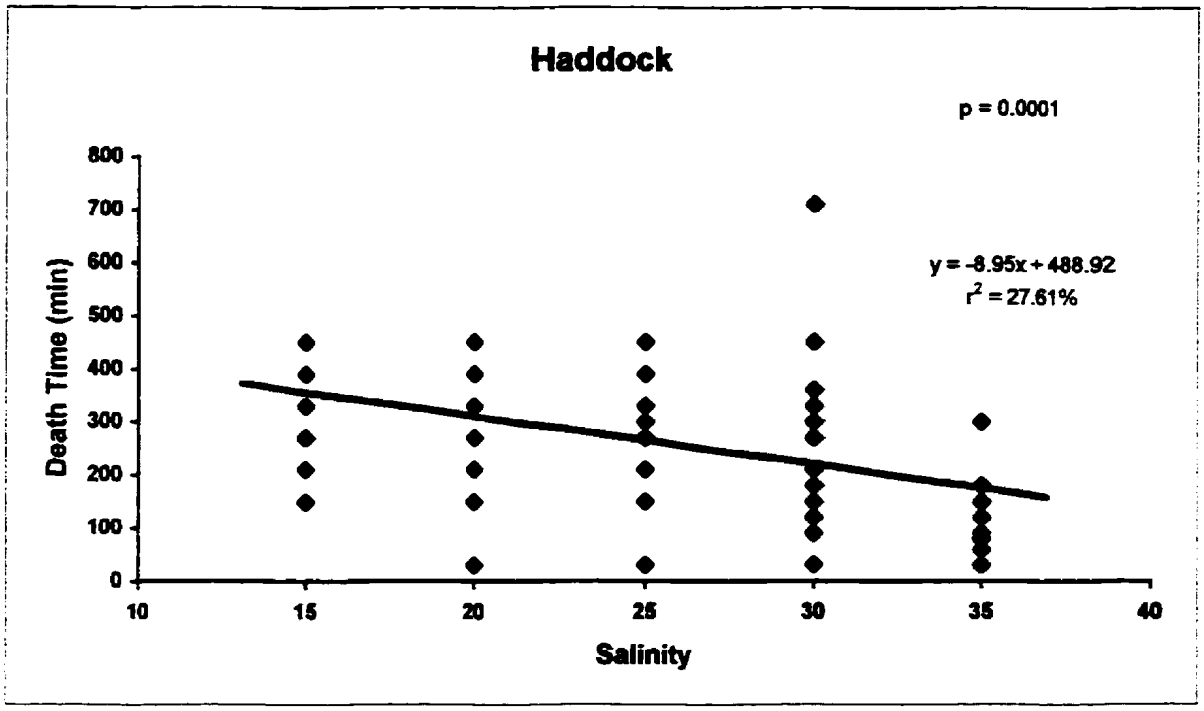


Figure 16: Mean death time (min) of larvae exposed to a temperature stress of 30^0 C. Bars indicate one standard deviation (n=48).



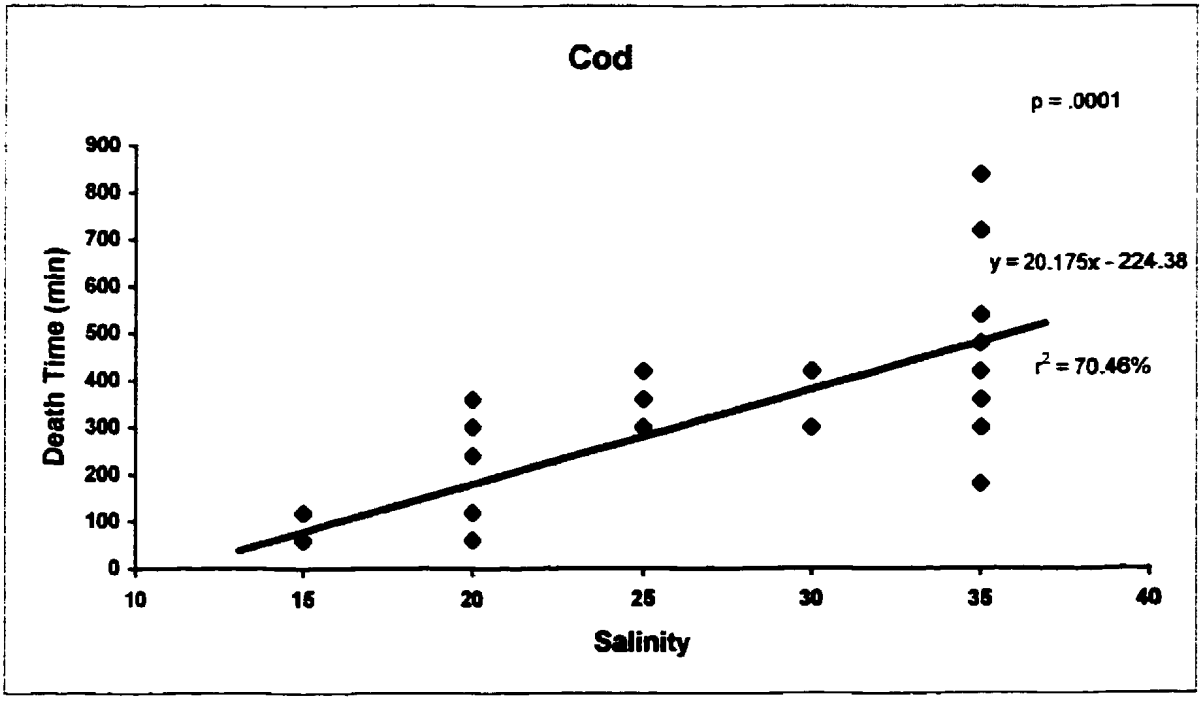


Figure 17: Scatterplot of relationship between egg incubation salinity and death time of larvae under high temperature stress. Line illustrates linear regression (n= 48).

$p<0.0001$; Fig. 17).

Winter flounder larvae demonstrated a significant increase in survival time when eggs had been incubated at 15 ppt ($F = 24.25$; $df = 4,235$; $p<0.0001$; Fig.16).

4.0 DISCUSSION

4.1 Fertilization

As mentioned, the effect of salinity on fertilization was not assessed in cod or haddock. Halibut and winter flounder demonstrated somewhat different patterns of fertilization with respect to salinity. As with previous findings, halibut fertilization was highest at 30 and 35 ppt, becoming progressively lower with decreasing salinities. Mangor-Jensen and Jelmert (1986), in a study of Atlantic halibut, found fertilization rates of 5,20, 84, and 92% for salinities of 17, 23, 27, and 34 ppt, respectively. They also found that fertilization rates at low salinities could be significantly increased if $CaCl_2$ was added at a final concentration of 10 mM. The resulting fertilization rates were 89, 94, and 90% for 17, 23 and 27 ppt. Except for a low fertilization rate of 20.84% at 15 ppt, the current study produced relatively high fertilization rates (>70%). One possible explanation for the differences between the two studies may be a difference in natural calcium content of the water, with waters in the current study possibly containing more. Calcium is important in egg activation and hardening processes, and although internal calcium release is important, external calcium levels also play a major role. Nevertheless, both studies demonstrate decreased fertilization at lower salinities (e.g. 15 ppt), suggesting these are suboptimal for halibut gamete release.

In contrast to halibut, fertilization rates among winter flounder embryos remained high (>96%) in all salinities examined. Similar results have been found in other marine finfish embryos. Westin and Nissling (1991) reported that Baltic cod (Gadus morhua) eggs could be successfully fertilized (rates = 99-100%) in salinities of 12-30 ppt. At

salinities of 11.5-12 ppt (as in the high salinities) they found sperm were unable to swim and vibrated or ceased all movements. A wide salinity tolerance, with respect to fertilization, was also noted for Atlantic herring (Clupea harengus) by Holliday and Blaxter (1966). They found successful fertilization (~100%) from 22.7-52.5 ppt.

If eggs from species such as herring, cod and flounder can be fertilized successfully at lower salinities then "why the difference with respect to halibut ?" Some researchers have suggested that the salinity in which broodstock reside has an impact on the tolerance of newly released gametes. Solemdal (1967) studied fertilization rates of two populations of flounder (Pleuronectes flesus), one from an area with 34.5 ppt salinity and one from an area with 6.5 ppt salinity. Flounder from the high salinity location were found to fertilize and develop successfully in salinities down to 11 ppt compared to a much lower level of 6.5 ppt in the low salinity. Success at such a low salinity was attributed to the fact that flounders living at low salinities will have ovarian fluid of very low concentration, thus allowing eggs to be fertilizable and often buoyant in quite low salinities. This allows speculation that poor performance of halibut embryos in low salinities is at least partially due to the high natural salinity of broodstock. Since halibut broodstock used in the current study reside in water of 30 ppt, one would expect egg success possibly down to 25 ppt, but this was not the case and questions the influence of broodstock salinity on subsequent egg performance. However, one cannot forget the natural ecology/ physiology of a particular species either. In nature, halibut reside in deep waters which rarely undergo fluctuations in salinity thus providing a stable salinity regime of 33 - 34 ppt. Thus halibut do not require the ability to withstand salinity

fluctuations since they are never exposed to such an event in nature. It may be foreseeable that cultured stocks of halibut will eventually adapt to the salinity conditions in which they are reared. In contrast, winter flounder is a inshore species often exposed to variations in salinity (e.g . spring run off or estuary conditions) thus it is not surprising that fertilization of its gametes is successful over a wide salinity range.

Fertilization rate is an early indicator of gamete salinity tolerance. Generally, salinities producing low fertilization are not usually conducive to subsequent egg development. However, as evidenced by the current study, salinities which produce successful fertilization are not necessarily adequate for egg incubation.

4.2 Hatching

Halibut embryos failed to hatch at salinities of 25 ppt and lower. Again this result is not surprising since halibut inhabit a stenohaline environment in nature. Hatch rates at 30 and 35 ppt were not significantly different and were 48% and 51%, respectively. The low overall hatch rate may be attributed to the broodstock's first captive spawning, as other studies have demonstrated this phenomenon (Kjorsvik, 1994). A somewhat similar study on Atlantic halibut, by Lonning et al.(1982), compared egg development rates at two salinities, 33 and 39 ppt. They found no differences in hatch rate or mortality of eggs during the early stages. However, during later development, eggs kept at 39 ppt had lower mortality than those at 33 ppt. The authors suggest since eggs were not incubated individually the higher mortality at 33 ppt may be attributable to accumulation of dead material in the bottom of incubators, resulting in bacterial contamination. This would not

be such a problem at 39 ppt since eggs are buoyant at this salinity. This confounds the experiment making it difficult to attribute the mortality to salinity effects or to bacterial contamination. A study on the closely related Pacific halibut (Hippoglossus stenolepis), by Liu et al.(1994), incubated eggs at 21, 24, 28, 30, 33, 36, and 39 ppt. Similar to the present study, they found no differences in hatching or development rate at 30 ppt and above while all salinities below 30 ppt resulted in 0% hatch. The magnitude of hatch was also similar to the current study, being ~ 40% at salinities above 30 ppt. The Pacific halibut eggs were found to have a lower neutral buoyancy of 30 ppt compared to ~35 ppt for Atlantic halibut eggs in the present investigation. This may be attributed to the salinity in which the natural broodstock live, which is generally lower (27-28 ppt) for Pacific halibut.

Failure of eggs to hatch at 25 ppt and below did not appear to be a function of hatching difficulties per se, since these eggs expressed close to 100% mortality by blastopore closure. Partial hatching was low (<4%) at 30 and 35 ppt in all eggs examined. It appears that the specific gravities of these salinities pose no problems with respect to chorion break-down or larval emergence.

Haddock eggs displayed high hatch rates over all salinities tested. Although hatching was lower at 15 ppt, it was not significantly lower and was still above 84%. These results suggest that haddock eggs can be successfully hatched at all salinities from 15-35 ppt. However, haddock eggs did appear to experience some hatching difficulty at 15 ppt compared to higher salinities. Partially-emerged larvae amounted to 8% which is higher than most other conditions, next highest being 5% at 25 ppt. Incubation at 15 ppt

may be sub-optimal and should possibly be avoided. Similar data on the effects of salinity on haddock embryos is sparse with the exception of Laurence and Rogers (1976). They exposed eggs to salinities ranging from 26-36 ppt and found increased hatch rates with increasing salinity. However, overall mean egg mortality in their study was high (~66.1%) suggesting a possible problem with oxygen levels or bacterial contamination. Since eggs were incubated in 100 ml containers (25 eggs/container) with no water exchange for the entire experiment, bacterial contamination was likely high, especially at salinities lower than 30 ppt where eggs settled to the bottom. Additional evidence for such a problem comes from the large number of abnormal larvae (e.g. spinal curvatures) found in the study (mean = 21.5%). The mean percentage of partially-emerged larvae, across all salinities, was low at 2.7%. They did not provide partial-emergence percentages for each salinity thus it was difficult to determine salinity effects on this phenomenon.

Cod eggs, like those of haddock, appeared to be euryhaline with respect to hatching. There were no significant differences in hatch rate although it was noticeably lower at 15 ppt (mean = 81%). Overall hatch rates were fairly high at 81-97%. Several studies have been completed on the effects of salinity on cod embryos, although much of the research has been on Baltic cod (Gadus morhua) and Pacific cod (Gadus macrocephalus). Westin and Nissling (1991) incubated Baltic cod eggs at salinities ranging from 5-30 ppt. They discovered the majority (mean = 71-92%) of eggs developed normally in salinities of 11-30 ppt. Salinities lower than 11 ppt resulted in 100% mortality. Since Baltic cod often inhabit waters of decreased salinities (~15 ppt) it is not

surprising that eggs would develop at such low salinities, and overall, results are agreeable with those of the present study. A similar study on Pacific cod, by Forrester and Alderdice (1966), found highest hatch rates at 19 ppt (mean = 25.07%, 6^0C) compared to 22, 25, 28, and 31 ppt with respective means of 11.62%(4^0C), 10.68%(6^0C), 1.28%(4^0C), and 0.94%(6^0C). Hatch rates in the study were low and the researchers suggested salinity effects may have been biased by hypoxial conditions in the 35 l tanks used. Nonetheless, the study did show cod eggs could perform well at salinities far below those of their natural environment.

In a follow-up study, Alderdice and Forrester (1971), found total hatch to be highest in salinities of 12.7-20 ppt, over the range of 12.7-27.9 ppt. Again, results suggest that the highest hatch rates occur at salinities much lower than that of ambient seawater. The two studies together, suggest that salinities of ~15-20 ppt may be optimal for incubation of Pacific cod eggs. In contrast, a study of Atlantic cod eggs, by Laurence and Rogers (1976), found hatching to be greatest at the high end of incubation salinities ranging from 26-36 ppt. However, the results of the latter study are questionable since eggs below 30 ppt were likely exposed to high bacterial conditions.

Although hatch rates in the current study of cod eggs were fairly uniform, there appears to be a problem of partial-emergence at 15 ppt (10.1%). As previously mentioned, this problem may be associated with the low specific gravity making it more difficult for the larvae to free themselves or there may be some difference in the "toughness" of the chorion (Holliday, 1965). McMynn and Hoar (1953) discovered a preponderance of partial-emergence in both Atlantic and Pacific herring eggs incubated

at low salinities. As well, Holliday (1965,1969) discovered similar occurrences in cod (Gadus callarius) and plaice (Pleuronectes Platessa).

Hatch rates among winter flounder embryos showed a significant trend towards lower hatch at higher salinities. A comparable study examining salinity effects on winter flounder eggs was conducted by Rogers (1976). She incubated eggs at various salinities ranging from 0.5-45 ppt and found total hatch to be within acceptable ranges (>54%) from 5-30 ppt. Highest total hatch occurred at 10-20 ppt, dropping off slightly at 25 ppt. These results agree with the present study where highest hatch occurred at 15-20 ppt. Since winter flounder eggs are demersal, even at salinities of 35 ppt, results from the Rogers' study are probably not confounded by increased bacterial loading of eggs held at lower salinities. Both studies suggest winter flounder embryos are euryhaline and both suggest 35 ppt is approaching the upper limit with respect to optimal egg incubation.

The current study did not detect any prevalence of partial emergence at the lowest salinity of 15 ppt. However, Rogers (1976) found that at salinities below 15 ppt many winter flounder larvae were observed in a partially-emerged state. These results suggest feasible hatching of eggs down to at least 15 ppt with lower salinities approaching the lower limit. Since winter flounder, in the wild, often occur from brackish (inshore/estuary) areas to full seawater (offshore) areas (Dovel, 1971) it is not surprising that these gametes are able to withstand such a range of salinities under captive conditions.

4.3 Hatch Time

Hatching time of marine eggs held under controlled conditions is probably not as critical as in the natural environment. In nature, eggs and newly-hatched larvae are subject to a variety of conditions which could be life threatening if the timing is not right. A shortened egg incubation period could be positive for a couple of reasons: 1) Newly-hatched larvae are more capable of avoiding predators than an egg. 2) Extended egg incubation may mean less yolk availability when the larvae hatch. With this scenario, the larvae has less time to find exogenous food. Under culture conditions, the threat of predators, food unavailability, and temperature fluctuations are minimized and pose no threat to survival. However, since marine eggs are fragile, not withstanding high water flow rates, the threat of bacterial contamination is ever present. Any condition which extends egg incubation significantly could possibly increase egg mortality due to increased chances of bacterial overloading of the egg. The effect of salinity on hatch time does not appear to follow any patented trend but appears to be more species specific.

Since halibut embryos hatched only at 30 and 35 ppt, the effect of low salinity on hatch time is not a factor. There were no differences between hatch times at 30 and 35 ppt, means for each salinity were within 1 hour. Lonning et al., (1982) also found no differences in hatch times of Atlantic halibut eggs incubated at 33 and 39 ppt. However, as previously mentioned, eggs kept at 33 ppt in the Lonning study were subject to hypoxial conditions in the bottom of incubators. This may have distorted hatching time. Kinne and Kinne (1962) found that hatch time under high salinities may be altered by oxygen availability. Liu et al., (1994), in a study of the closely-related Pacific halibut,

found no differences in hatch times at salinities of 30 ppt or greater. Results of the current investigation are in agreement with similar studies and suggests time to hatching for halibut embryos is not different for 30 and 35 ppt.

Haddock embryos displayed no real salinity trends in hatch times over the 15-35 ppt range, although times were slightly shorter (~ 15 hrs.) at 25 and 35 ppt. The reason for this slight difference is unknown. Mean hatch time over all salinities was ~16.5-17 days. Similarly, Laurence and Rogers (1976) found no relationship between salinity and hatching time over the range 26-38 ppt. From these findings one can assume that hatch time will not be significantly lessened or enhanced at salinities of 15-35 ppt.

Cod eggs demonstrated a slightly extended hatching period (1-1.5 days) at the salinity extremes (15 and 35 ppt) although this was not statistically significant. As mentioned previously, extended egg incubation intervals can sometimes have negative effects especially where chances of bacterial contamination are high. However, it is not likely that hatching periods extended by a duration as short as 1 day are going to have an impact. A previous study, by Laurence and Rogers (1976), found that cod eggs held at 2 and 4^{0}C showed progressively longer hatch times at lower salinities, in the range 26-36 ppt. These differences were as high as 5 days between 26-28 ppt and 32-36 ppt at 4^{0}C. However, because of the static water conditions (no exchange), as previously mentioned, hatch times were likely more influenced by oxygen availability than salinity. A similar study completed on Pacific cod by Forrester and Alderdice (1966), produced results similar to those of Laurence and Rogers. At 6^{0}C, hatch time at 19 ppt (mean = 15.7 days) was significantly longer than at 31 ppt (mean = 13.8 days). Again, the researchers in this

study suggest that oxygen availability, which tends to be less at higher salinities, may have played a role. Nevertheless, all studies point to a slightly extended hatch time at lower salinities and the current study also suggests a slight extension at 35 ppt. These hatching delays of 1-2 days are not likely to have dramatic effects for resulting larvae, especially under controlled culture conditions.

No significant differences were observed in hatch times for winter flounder eggs incubated at the various test salinities. Mean hatch time for all conditions was ~14.5-15 days. Rogers (1976), studying winter flounder eggs, found a slight decrease in hatch time over the range 5-40 ppt, although hatch rates were quite stable from 15-35 ppt (maximal decrease = 2 days). Slight differences in results may be attributed to differing experimental methods, as previously discussed. A study completed on a similar flatfish, the yellowtail (Pleuronectes ferruginea), by Laurence and Howell (1981), also found extended hatch times with decreasing salinities, over the range 28-38 ppt. However, over the entire salinity range, hatch times were only extended by ~14 hours, which is unlikely to have any significance in fish culture operations.

Over the scope of salinities tested (15-35 ppt), hatch times were not severely different in any of the species. The only probable exception was the extension in hatch times at the salinity extremes for cod eggs. However, increased hatch times here were only ~1-1.5 days longer and not likely influential. Although some studies demonstrate longer hatch periods at lower salinities, extended duration is often not longer than 1.5-2 days. Such a difference is probably attributable to different oxygen levels between experimental conditions. Similar studies on other species such as that by Holliday and

Blaxter (1960) on Atlantic herring, have found minimal differences except at extremely low salinities such as 5.9 and 11.5 ppt. Even then, hatch times were only extended by ~2 days.

The actual duration of hatching (period from first hatched egg until hatching was complete) was not greatly altered by salinity in any of the eggs examined. A previous study by Laurence and Rogers (1976), on cod and haddock, found no significant differences in duration of hatching period related to salinities of 26-36 ppt. This is in agreement with the current study.

4.4 Stage-Specific Mortality

Through close observations of the precise time of embryo mortality, researchers, studying a variety of species, have been able to identify certain susceptible developmental stages. Mortality at these particular stages is generally higher than at other stages, even under normal environmental conditions. When environmental conditions are abnormal (e.g. extremely high or low salinities) mortality at these stages might be very pronounced. The majority of research on this topic elucidates two particular stages of increased embryo mortality: 1) Gastrulation (McMynn and Hoar, 1953; Holliday and Jones, 1967; Holmefjord et al.1993) and 2) Hatching (or just prior; Battle, 1930; McMynn and Hoar, 1953; Rogers, 1976; Laurence and Rogers, 1976).

Prior to the completion of gastrulation (closure of the blastopore) the only regulatory capacity available to embryos is the low permeability achieved through the presence of a compact plasma membrane (Alderdice, 1988). This limits water and ion

fluxes and will, if only for a limited period, shield and protect embryos against stresses such as being immersed in a fluid of high or low salinity. The second degree regulatory system appears to develop in temporal contiguity with the end of gastrulation (closure of the blastopore) and involves the development of "chloride cells" (Alderdice, 1988). The presence of "chloride cells" was first proposed by Keys and Willmer (1932) but until recently have been controversal. Marshall and Nishioka (1980) found chloride current in the skin of Gillichthys mirabilis varied directly with the density of chloride cells in the epithelium. Further, Foskett and Scheffey (1982) discovered chloride cells as the site of salt transport in the tilapia (Oreochromis mossambicus). Numerous studies have demonstrated an increased tolerance in marine embryos, through decreased mortality, once gastrulation is complete (Holliday and Jones, 1967; Blaxter et al. 1983; Pittman et al. 1990; Nissling and Westin, 1991).

As previously mentioned, a period of increased mortality is often seen just prior to or at the time of hatching. Battle (1930), in a study of Enchelyopus cimbrius reared at low salinities, found increased mortality or preponderance of partially-emerged larvae during hatch. He attributed the problem to poorly developed tail musculature, with the larvae being unable to free itself from the egg membrane. Holliday (1969), however, suggests that the problem may actually result from the low specific gravities of low salinity water, making it difficult for the larvae to free itself, probably due to a lack of buoyancy. A plausible explanation for the increased mortality often experienced just prior to hatch (stage 4) is provided by Riis-Vestergaard (1984) and Mangor-Jensen (1987). They both studied cod and found from day 8-10 post-fertilization onwards, the

permeability of the perivitelline membrane and volume of the embryo both increase suggesting an uptake of water. This increase in water uptake coincides with the period of increased mortality at or near hatch, especially if the water is of low salinity.

Halibut eggs, in the current study, demonstrated the majority of mortality during the first three embryonic stages and death was negligible thereafter. This agrees well with previous studies on Atlantic halibut by Blaxter et al.(1983) and Pittman et al. (1990), whom both found egg mortality to fall dramatically once the blastopore had been closed, corresponding to the end of stage 3 in the present work. By closely examining the effect of salinity on egg mortality time (daily observations) it was seen that eggs died more quickly when exposed to lower salinities, which may reflect some minimal salinity tolerance during the earliest egg stages. At 15 ppt, eggs showed complete mortality by stage 2 (beginning of gastrulation). Eggs held at 20 and 25 ppt showed early mortality as well, although progressively more eggs reached stage 3, however, all eggs in both salinities were dead by the end of stage 3 (most died during blastopore closure). Incubation of eggs at 30 and 35 ppt produced much less mortality in the early stages, with highest mortality occurring at stage 3. Once stage 3 was complete, mortality was much lower and near zero from stage 5 onwards. Similar studies of salinity effects on embryo death in Atlantic halibut have not been done although some work has been done with Pacific halibut. Liu et al.(1994) found Pacific halibut embryos incubated at low salinities (21 and 24 ppt) produced high mortality during the late blastula stage (stage 2). None of these embryos survived past the 1/2 epiboly stage. Eggs incubated at mid-salinities (27 and 30 ppt) began expressing mortality during the germ ring stage (late stage 2) with no

eggs surviving beyond blastopore closure. These results are remarkably similar to those found for Atlantic halibut in this study, with the exception of 30 ppt.

Halibut eggs reared at salinities of 25 ppt and lower in the current study, swelled and blastomeres were almost bursting as early as day 2. This swelling suggests that water permeability is no longer kept to a minimal and the eggs are losing all osmotic control, possibly due to the large osmotic gradient present.

Specific mortality of haddock eggs appears to be highest at stages 4, 5, and 7, although mortality levels were low at all stages. A previous study completed on embryo mortality in haddock, by Laurence and Rogers (1976), found increased mortality (31%) at stage 2 (beginning of gastrulation). The reason for such a discrepancy is open to discussion but may be due to differences in egg quality. Mean overall haddock mortality in the Laurence and Rogers (1976) study was high at 66.1%, in addition 21.5% of hatched larvae were abnormal. Thus, it may be possible that the eggs were of a lesser quality and died more quickly (e.g. stage 2) compared to the current study. Also, incubation methods may have led to increased bacterial loading, possibly causing earlier death.

A closer examination of mortality versus salinity shows that the pattern of mortality in haddock is approximately the same across all salinities. The only notable exception is the slightly higher mortality at 15 ppt in stages 5 and 7. Since stage 5 covers the hatching process, it is not surprising that mortality was slightly higher for 15 ppt. As mentioned earlier, previous studies have found partial-emergence of larvae and general hatching difficulty at low salinities. The increased mortality of the newly-hatched larvae

(7) suggests that although the eggs developed and hatched, resulting larvae may sometimes not be hardy enough to survive. Observations of inactivity in one day-old haddock larvae at 15 ppt (see Figure 12) are in good agreement with the present finding and may both suggest that haddock egg incubation at 15 ppt is suboptimal.

Cod eggs appear to be most susceptible at stage 4 although mortality was almost equally as high at stages 2 and 3 and slightly less at stage 5. An earlier study on cod embryos, by Laurence and Rogers (1976), demonstrated somewhat similar results with mean mortalities of 13.4, 12.4, 3.9, 23.4, and 4.4% for stages 1, 2, 3, 4 and 5, respectively. A similar study involving Baltic cod, by Nissling and Westin (1991), found high mortality for the first three days, at which time completion of gastrulation resulted in low subsequent mortality, except at 5 and 7 ppt. At these two low salinities there was an increased mortality at the time of hatch with many larvae dying in a partially-emerged state.

The cod eggs in the current study did not display a distinct stage of high mortality but rather showed a fairly even spread over stages 2,3,4 and 5. These results are in close parallel with those for North sea cod (Gadus morhua) described by Iversen and Danielssen (1984). They found that mortality was fairly constant from stage 2 onwards, rising slightly at the time of hatch.

Winter flounder embryos displayed increased mortality at two distinct stages (2 and 4) with respective mortality of 25.5 and 22.6 %. Mortality at all other stages was low (less than 2%).These two stages of susceptibility correspond well with findings of increased mortality during gastrulation (stage 2) and just prior to hatching (stage 4).

Rogers (1976) found, for winter flounder eggs in salinities of 15-30 ppt, most deaths occurred just prior to hatch (stage 4) except at temperatures above 10^0C where some mortalities usually occurred during gastrulation. The higher mortality at stage 4 corresponds well with our findings. In addition, the fact that Rogers (1976) found increased mortality at stage 2 in embryos kept above 10^0C suggests that if embryos are kept in suboptimal conditions (possibly stressed/abnormal), they die at an earlier stage. Investigations of yellowtail, a somewhat similar flatfish, by Laurence and Howell (1981), also found most embryo mortality to occur during stage 2.

It is interesting to note that over the salinity range of 15-35 ppt, the occurrence of partially-emerged winter flounder embryos was virtually nil. It appears that of the four species currently investigated, winter flounder produce eggs best suited to incubation in low salinities.

4.5 Larval Viability (1DPH)

Although percent hatch and percent survival (1DPH) provide some indication of salinity tolerance among eggs and larvae, the percentage viable larvae probably provides more critical information. Within the current experiment, viable larvae were those considered to be of normal physical appearance, in good condition, and having a detectable heartbeat. Actual movement (behaviour) of the larvae was considered separately (activity). The most notable abnormality of larvae in the current study was the presence of a curved spine, other known abnormalities in marine larvae such as yolk sac

hydropsy, and incomplete caudal formation were not observed. Thus viable larvae in the present study are those free of spinal abnormalities.

As mentioned previously, the ability of larvae to function properly in suboptimal salinities will depend on a combination of tissue tolerance and regulatory capacity (Holliday, 1969). Marine fish larvae are known to survive a wide range of salinities. For example, yolk sac larvae of Clupea harengus, Clupea pallasi, Pleuronectes platessa and Gadus callarias have been reported to tolerate upper salinities of 60-65 ppt, low salinity tolerance was found to be 1-4 ppt, 5 ppt and 10 ppt for Clupea harengus and Clupea pallasi, Pleuronectes platessa, and Gadus callarias, respectively (Kurata, 1959; Holliday and Blaxter, 1960; Holliday, 1965). Any failure of tolerance in newly-hatched larvae may be manifested in mortality as well as a number of physiological and structural changes. Mortality is straightforward and would indicate an inability to either tolerate or regulate, as previously mentioned.

Most physiological effects would involve the osmoregulatory capacities of the larvae. Most larvae hatching in normal seawater have body fluids with a concentration equal to that of ~12-15 ppt (Holliday, 1971; Tytler and Blaxter, 1988). Thus, if fish are held at a salinity isosmotic with internal fluids (e.g. 15 ppt), "they should not have to expend as much energy on osmoregulation and have more reserve energy available then those held at higher or lower salinities". If suboptimal salinities produce conditions where osmoregulation is difficult than larvae may become stressed and more susceptible to death or possible cell structure abnormalities.

Structural effects related to salinity are often manifested in various larval abnormalities, as a result of sub-optimal or stressful egg incubation (Holliday, 1969). Battle (1930) discovered tail and cardiac deformities in larvae of Enchelyopus cimbrius hatching in salinities up to 70 ppt. Kryzhanovsky (1956) found Baltic herring eggs incubated in water of 25 ppt resulted in abnormalities of the cardiac region, otic region, yolk sac, alimentary canal and liver. Other structural effects include changes in overall size and weight of larvae and their yolk sacs (Holliday, 1969), this will be discussed in a later section.

Although overall viability of halibut larvae was not high it was similar at both 30 and 35 ppt, 40.4 and 44.8 %, respectively. Spinal deformities did not account for more than 7% of larvae at both these salinities. Viability rates of larvae at salinities below 30 ppt are not available since eggs failed to hatch at 25 ppt and below. Similar studies relating larval viability to egg incubation salinity have not been completed with Atlantic halibut. Studies on newly-hatched halibut larvae (Bergh et al.,1989; Pittman et al.,1990) have found two noticeable deformities, incomplete caudal development and gaping mouth syndrome (lockjaw). Within these experiments both of the deformities were related to temperature, gaping at warm temperatures (e.g. 9^0C) and incomplete caudal development at cold temperatures (e.g. 3^0C), no salinity manipulation was exercised. In a study of Pacific halibut (Hippoglossus stenolepis), Liu et al.(1994) found larval viability (normal larvae) to be highest at salinities of 30, 33, and 36 ppt with respective means of 43.3, 29.4, and 41.8%. All eggs below 30 ppt failed to hatch and viability rate at 39 ppt was reduced to 16.6%. The occurrence of abnormal larvae, in the form of spinal

curvatures, was low at 30, 33, and 36 ppt (2.9, 3.6 and 4.0%, respectively) rising slightly at 39 ppt (8.9%). They postulated that increased abnormalities at 39 ppt was probably a response to the environmental stress elicited at this salinity extreme.

Viability among haddock larvae was high and peaked at salinities of 20 ppt (88.74.1%). Viability was significantly lower at 15 ppt (65.6%). This lowered viability at 15 ppt suggests that this salinity is reaching the lower tolerance limit for haddock, and provides enough stress to cause increased mortality. Also, the fact that viability was slightly reduced at 35 ppt cautions that this salinity may also be approaching an upper salinity limit for optimal rearing.

A previous study on the effects of salinity on yolk-sac haddock was completed by Laurence and Rogers (1976). They found larval viability to increase linearly with increasing salinity. Viability at salinities of 26, 28, 30, 32, 34 and 36 ppt was 13.4, 20.2, 27.5, 29.3, 33.4, and 42%, respectively. Although the range of salinities tested was not as broad as in the current study, they found a dramatic salinity effect. However, as mentioned previously their experimental set-up probably resulted in high bacterial loading especially at low salinities. Also, their generally low viability rates in all conditions, when compared to the present study, suggests there was probably oxygen deficiencies in all conditions. This is further supported by the high degree of larval abnormalities (shortened bodies, spinal curvatures, enlarged yolk sacs) observed in all salinity conditions. Mean abnormality rate was 21.5% with no real salinity trend, although highest at 26 ppt (mean = 33%). The differences in procedures and incubation techniques between the current study and that of Laurence and Rogers (1976) makes it

difficult to compare. Based on present findings, adequate haddock larval viability can be achieved by incubating eggs in salinities of 20, 25, 30 and 35 ppt.

In the current study, cod larval viability was not significantly affected by egg incubation salinity. An earlier study on cod larvae, by Laurence and Rogers (1976), did find slightly increasing viability with increasing salinity. Mean viability was 20.34, 24.55, 30.66, 28.35 and 30.40% for salinities of 26, 28, 30, 32, 34 and 36 ppt, respectively. However, as previously mentioned, the Laurence and Rogers (1976) study is probably biased due to high bacterial loading, especially at salinities below 30 ppt. Yin and Blaxter (1987) found North sea cod larvae (Gadus morhua) were able to tolerate salinities of 2-3 ppt between hatching and the end of the yolk-sac stage. From the results of the current study it appears that optimal cod larval viability can be achieved if eggs are incubated in salinities of 25, 30, or 35 ppt.

Viability of winter flounder larvae was found to be greater in low salinities (15, 20 and 25 ppt). Viability at 35 ppt (17%) was much lower than at all other salinities and appears to be the upper tolerance threshold with respect to salinity. Occurrence of spinal abnormalities was low, not exceeding 7%. Rogers (1976), also studying winter flounder, found highest larval viability at salinities of 15 ppt (mean = 75.7%, 5^0C), 20 ppt (mean = 79.3%, 5^0C) and 25 ppt (mean = 74.0%, 5^0C), over the range 0.5-45 ppt. Abnormality rates were also low from 15-35 ppt, not exceeding 10%; these findings are very similar to that of the current study. Laurence and Howell (1981), in an investigation of yellowtail flounder, found increasing larval viability if eggs had been incubated at higher salinities,

over the range 28-38 ppt. In addition, they found fairly low rates of abnormalities (<10%) at all salinities.

The low occurrence of abnormalities combined with acceptable viability rates over the salinity range 15-30 ppt, suggests that these salinities are conducive to winter flounder egg incubation. In addition it appears best viability can be achieved at 15 and 20 ppt.

4.6 Larval Size

Halibut larvae expressed no significant differences between 30 and 35 ppt in any of the size parameters examined. This result is not surprising given the narrow range of salinity (5 ppt). Although most studies on marine larvae have found increased length with lower salinities, some have found the opposite. Laurence and Rogers (1976) found cod larvae incubated as eggs in salinities of 26, 28, 30, 32, 34, and 36 ppt, attained significantly longer lengths at higher salinities. Alderdice and Forrester (1971) had similar results for petrale sole (Eopsetta jordani) over salinities of 20-35 ppt. In a study of the closely related Pacific halibut, Liu et al.(1994) found no differences in mean larval length when eggs were incubated at 30, 33, 36, and 39 ppt.

In view of the current results and those of Liu et al.(1994), it appears that the size of newly-hatched halibut larvae is not meaningfully different for salinities of 30 and 35 ppt.

Both total length and yolk area of haddock larvae displayed a significant trend of increased size at lower salinities. The relationship of larval height with salinity was less

clear and appeared to be maximal in the mid-salinity range. Several researchers found similar trends. Young and Duenas (1993), rabbitfish (Siganus guttatus); Holliday and Blaxter (1960), Atlantic herring (Clupea harengus) and Forrester and Alderdice (1966), Pacific cod (Gadus macrocephalus) have all found increased larval lengths at low egg incubation salinities. Increased yolk sizes in low salinities have been found by May (1974), Bairdiella icistia; Young and Duenas (1993), rabbitfish, and Holliday (1965), Atlantic herring. A previous study of larval length versus salinity in haddock larvae was done by Laurence and Rogers (1976) who found no differences in larval length at hatch for eggs incubated at 26-36 ppt. However, the lowest salinity in their study was 26 ppt compared to 15 ppt in the current investigation, which could account for the difference in results.

Results of the present study suggest optimal haddock length and yolk size at lower salinities.

Unlike haddock, cod larvae did not demonstrate significantly different larval lengths at one day post-hatch, regardless of incubation salinity. In contrast to the present study, Laurence and Rogers (1976) found increased cod larval lengths with increasing salinity. However, their salinities ranged from only 26-36 ppt, and possible findings of larger larvae at lower salinities would have negated the small increase in length (~0.1 mm) from 26-36 ppt.

Like haddock larvae, cod larvae hatched at lower salinities displayed significantly larger yolk sizes. Other studies on marine larvae have found similar results and will be discussed later. As for larval height, cod larvae showed increased height at lower

salinities and this is probably the result of increased subdermal spaces (water content) as acknowledged by Shelbourne (1956). He found that pelagic marine larvae have increased finfold heights in an effort to increase buoyancy (increased water content) and it appears salinity may enhance this process. Nissling and Vallin (1994) found increased heights of Baltic cod larvae, if eggs had been incubated in lower salinities.

It appears that yolk size and possibly height of cod larvae can be maximized by incubation in low salinities. Depending on the advantage or quality of the increased yolk, larval survival may actually be enhanced. Possible advantages of increased yolk and height are discussed later.

Winter flounder larvae, like haddock, demonstrated increased length and yolk size with decreasing incubation salinity and also showed this same trend with respect to height.

Several studies on a number of species have found larval size differences in relation to egg incubation salinity. For example, Holliday and Blaxter (1960) and Holliday (1965) found that herring, (Clupea harengus) and plaice (Pleuronectes platessa) larvae, incubated in salinities of 5-25 ppt, were up to 33% heavier and 23% longer than larvae from salinities of 35-55 ppt. In addition, yolk sacs of herring hatching in low salinities were swollen and pale compared to those incubated at 35 ppt. Young and Duenas (1993) found the size of rabbitfish larvae, hatching in salinities of 8-40 ppt, was greater at lower salinities. Similar findings have been reported in Pacific cod (Forrester and Alderdice, 1966), Baltic herring (Dushkina, 1973) and Baltic cod (Nissling and Vallin, 1994). Yolk volumes of rabbitfish (Young and Duenas, 1993), Atlantic herring

(Holliday and Blaxter, 1960), Clupea pallasi (Alderdice and Velsen, 1971) and Bairdiella icistia (May, 1974) all demonstrated increases in eggs incubated at lowered salinities. On the other hand, increased yolk volume at high salinities was found in killifish (Fundulus parvipinnis; Rao, 1974) and petrale sole (Eopsetta jordani; Alderdice and Forrester, 1971).

The larger lengths, heights and yolk sacs often seen in larvae hatching at lower salinities is, at least partially, attributed to an increased water content (Holliday, 1965; May, 1974; Lee et al.1981; Nissling and Vallin, 1994). At lower salinities, eggs are incubated in isosmotic conditions creating an inflow of water as opposed to water loss (hyperosmotic) at higher salinities. Smaller size (length, yolk, sometimes height) sometimes observed in larvae hatching at higher salinities may result from increased energy costs of maintenance metabolism (e.g. osmoregulation) and activity (e.g. increased movement) at the expense of growth. For example, Holliday (1965) found herring and plaice larvae held at 34 ppt swam actively, continously, while at salinities less than 12 ppt, larvae stayed near the bottom and were inactive for long periods. Young and Duenas (1993) found rabbitfish larvae held at 8-16 ppt were inactive and remained at the bottom of containers for 24 hrs following hatch. In the current investigation, decreased activity, as previously discussed, at lower salinities (15 and 20 ppt) for cod and haddock larvae provides support for decreased energy costs. In addition, during the temperature stress test (discussed later) larvae held at the lower salinities were found to be inactive and often laid on their sides. Haddock and winter flounder larvae held at

these low salinities were found to survive significantly longer compared to those hatched from eggs at higher salinities.

Newly-hatched marine larvae have body fluids with a concentration of ~12-15 ppt (Holliday, 1971; Tytler and Blaxter, 1988). Thus, a larvae held in salinities of ~ 15-20 ppt do not have to use as much energy for osmoregulation since their body fluids are nearly isosmotic with the external medium. As a result there is more reserve energy available for growth. In herring (Clupea harengus), plaice (Pleuronectes platessa) and cod (Gadus morhua) larvae, drinking rates at 32 ppt were about twice those at 16 ppt (Tytler and Blaxter, 1988). This increased energy demand could result in further yolk utilization, making the time frame for switching to exogenous food shorter and correspondingly decreasing chances for survival.

The implications of increased larval size (length, height, yolk size) often depends on the magnitude of change. If increase in size is minimal, as that found by Laurence and Howell (1981), for yellowtail, then advantages with respect to survival are likely not significant. Larger differences may have substantial impacts on foraging processes and predator avoidance, and are likely critical in determining survival (Bailey and Houde, 1989).

Larger larvae (longer and/or higher) will probably have the advantage of increased swimming ability thus being better able to capture prey organisms and avoid starvation during the critical switch from endogenous to exogenous food (Blaxter and Hempel, 1963). Also, smaller larvae at hatch require a longer growth period to achieve

the initial size of larvae reared under more favourable conditions (e.g. optimal salinity); this could be detrimental under predator conditions (Rosenthal and Alderdice, 1976).

Although all species, except halibut, in the current study demonstrated significantly greater yolk supplies at lower salinities, actual benefits or quality of this increased yolk is unknown. Some researchers have suggested that larger yolk size is due mainly to increased water content and may not have any additional nutritional benefits for the larvae (Holliday, 1965). Others have observed that increased yolk at lower salinities may be due to decreased activity and thus less energy consumption. Tsukamoto and Kajihara (1984) found ayu larvae (Plecoglossus altivelis) expressed increased movement at higher salinities resulting in a 36% decrease in yolk volume. In an attempt to determine yolk volume and quality at lower salinities, May (1974) exposed sciaenid larvae (Bairdiella icistia) to salinities of 20, 30 and 40 ppt. He found that some of the increased size of yolk-sacs was due to water, however, dry weights and yolk utilization efficiency was higher for larvae at the low salinities (highest at 20 ppt - near isosmotic). In addition he found that unfed larvae survived longest in low salinities. Ryland and Nichols (1967) found plaice larvae could differ in length by as much as 10% at the time of first feeding, depending on yolk utilization efficiency.

In the current study, yolk-sacs at lower salinities were larger. In order to determine the advantages of the larger size, long term studies are required to track individuals through larval life, which would provide information on survival rates.

4.7 Temperature Stress

The temperature stress test was used as an index of larval condition. It was felt that any significant increases in survival time may be indicative of better quality larvae. Similar type tests using temperature and or salinity changes have been used to evaluate shrimp (Penaeus monodon) quality (Briggs, 1992).

Since marine fish larvae are poikilothermic, their body temperature is very similar to that of the water they inhabit. Increased temperatures result in enhanced biochemical reactions, producing faster metabolic rates and increased energy usage (Laurence, 1974). I felt that exposure of larvae (DPH), hatched from eggs incubated at 5^0C, to water of 30^0C would produce mortality and disclose any survival advantages related to salinity. Iversen and Danielssen (1984), in a study of cod larvae (Gadus morhua), found resorption of yolk sacs in less than one day if larvae were transferred from 6^0C to 18^0C at one day post-hatch. Sixty percent of the larvae were dead after one day. I felt if incubation salinity could somehow mediate yolk size, overall size of the larvae, or energy utilization efficiency, then maybe some larvae would be able to tolerate stressful conditions (e.g. temperature increase).

Halibut larvae, whether incubated as eggs in 30 or 35 ppt salinity, expressed no differences in tolerance to 30^0C water. This agrees well with other findings in the current study, which indicate very little difference in performance of halibut eggs and larvae when reared at 30 or 35 ppt. Since larvae in both salinity conditions were able to survive 465 plus minutes, it appears they were in good condition.

Haddock larvae displayed increased stress tolerance if eggs had been incubated at 15, 20 and 25 ppt compared to 30 and 35 ppt. This increased tolerance at the lower salinities may be attributed to a couple of factors. First, it was noticed that larvae at 15 and 20 ppt (less pronounced at 25 ppt) showed very little movement and laid on the bottom or otherwise remained inactive the entire time. Hence, these inactive larvae probably expended less energy than those at 30 and 35 ppt and were able to live significantly longer. Second, the fact that larvae at the lower salinities had larger yolk sacs may mean they had additional energy supply and could survive longer than larvae with smaller energy reserves. It appears that under stressful conditions, larvae from eggs incubated at lower salinities may have some survival advantage compared to larvae reared at higher salinities.

Cod larvae, unlike haddock, demonstrated increased stress tolerance at higher salinities, with 35 ppt producing the best results. The reason(s) for the difference between cod and haddock is not clear but possibilities are as follows: cod larvae did not express high levels of inactivity at the lower salinities, thus movement was probably about the same in all salinities. Also, cod larval viability in egg batch was significantly higher at 35 ppt compared to all other salinities. These findings imply that larvae held at 35 ppt are more hardy and have a survival advantage over those in lower salinities. However, cod larvae held at lower salinities were found to have increased yolk-sac size and possibly increased energy stores. Nevertheless, it is possible that cod larvae are more intolerant of low salinities compared to haddock, and that increased yolk stores do not compensate for the associated osmoregulatory problems. It appears cod larval quality may be somewhat

enhanced by incubation at salinities of 25 ppt and likely further optimized at 35 ppt. The degree to which this enhanced stress tolerance will affect overall survival is difficult to determine, however, anything that increases larval hardiness is beneficial to the process.

Among winter flounder larvae, tolerance to increased temperature was significantly higher for larvae hatched from eggs incubated at 15 ppt. There were no differences in survival times for all other salinities and survival at 15 ppt was highest. The reason for the increased stress tolerance at 15 ppt is unclear. Observations of various trials showed that unlike haddock larvae, winter flounder larvae were not more inactive when held at 15 ppt compared to other salinities. Accordingly, it would not appear that they were more active and using up more energy. Also, yolk sac reserves, although larger at 15 ppt, were not much larger and probably did not account for the increased tolerance. One possible explanation may be that at 15 ppt, body fluids are nearly isosmotic with the external medium, making osmoregulatory processes more efficient, thus lessening the demand on stored energy.

There appears to be no unequivocal relationship between larval yolk size/total length and temperature stress tolerance. Having a somewhat larger yolk sac and larger overall size does not necessarily mean a larva is more hardy. Another important component is how well the larva functions physiologically and osmotically at a particular salinity. Within my study, salinities which provided highest larval viability also seemed to provide fairly high stress tolerance.

One might argue that the trend towards decreased survival times at higher salinities (haddock, winter flounder) is due to decreased oxygen availability at these

salinities. However, I am quite confident that oxygen concentrations were above critical levels, especially given the short time span of the stress tests. In addition, the increased tolerance time of cod larvae at the higher salinities is suggestive of adequate oxygen levels.

4.8 Cell Symmetry

Determination of egg quality among marine finfish is not as straightforward or reliable as in species such as Atlantic salmon. With salmon eggs, researchers and commercial aquaculturists alike can attain considerable knowledge of quality through fertilization rates. It has been found that surviving fry from egg batches of lower fertilization perform poorly at all later stages of development (Springate et al.1984). Fertilization rates have been used as a quality indicator among marine eggs but they don't always correlate with good survival and development in later embryonic stages (Kjorsvik et al.1984; Hay, 1986; McEvoy, 1984).

A number of parameters including buoyancy (McEvoy, 1984; Carrillo et al.1989; Kjorsvik et al.1990), size (Solemdal et al.1991), hatch rate, transparency, and shape (Kjorsvik et al.1990) of marine eggs have been used, with variable consistency, to evaluate egg quality. Another more recent parameter that has been studied and examined in the current experiment, is cell cleavage symmetry to determine egg quality and subsequent larval viability.

In an effort to find a parameter which would provide early, reliable, and accurate information on egg quality, some researchers postulated that study of early blastula

cleavage (4-128 cell) could be a key. Since pelagic marine fish eggs are generally transparent, cell cleavage pattern is easy to observe, and it has been suggested that early malformations may influence further embryo development (Kjorsvik et al. 1990).

The current study investigated early cell cleavage (8-32 cell) in eggs of halibut and haddock. Since eggs were incubated individually, larval viability could be easily traced back to initial cleavage patterns. No significant relationship was found between early cell symmetry and subsequent larval viability in either halibut or haddock eggs. Within haddock eggs sampled, larval viability was actually slightly higher if initial cleavage was asymmetrical rather than symmetrical. Halibut eggs, on the other hand, showed slightly increased larval viability if eggs were symmetrical. Based on these results it appears cell symmetry alone is not an accurate predictor of subsequent egg and larval viability.

A few studies have been completed on the use of cell symmetry as an indicator of egg quality, and results have been mixed. In a study of cod, plaice, and flounder eggs, Westerhagen et al., (1988) found early asymmetrical cleavage to coincide with low hatching rates. The correlation was found to be significantly high for both cod (r_{38}:0.4656, $p<.01$) and plaice (r_{38}:0.7258, $p<.01$). Similarly, Devauchelle et al.(1988) discovered a significant relationship between deformities at the blastula stage and low hatching rates in turbot (Scopthalamus maximus) eggs. Vallin and Nissling (1994) found two populations of cod (Baltic and Skagerrak) to have significantly higher hatch from symmetrical rather than asymmetrical eggs. However, there was still a relatively high viable hatch from asymmetrical eggs in both cod groups (means = 89% and 85% for

Skagerrak and Baltic, respectively). In addition, they found wide variations in viability depending on the female, with some showing higher viable hatches for asymmetrical eggs. They suggested that the poor reliability of cell symmetry as a quality indicator may have resulted from classification too early (4 cell stage). They postulated that classification would be better at a later stage (e.g. 8 cell) since cleavage patterns may not be fixed at earlier stages.

Results of the current study in conjunction with others appear to question the relevance of early cell symmetry. Some discrepancies between studies may be the result of different symmetrical criteria (see Figure 1), species or population specificity, viability criteria, and experimental design. I am confident in the results of the current study since each egg was individually incubated, thus negating any possibility of misrepresentation. It is possible that early cell symmetry could be beneficial if used in conjunction with other indices such as shape, size, transparency, buoyancy, and fertilization rate.

The current study followed hatched larvae to one day post-hatch, future studies should track larvae further to see if they are in fact viable. In addition, new research should also aim at answering the question : "why do abnormal/asymmetrical cleavages occur ?"

4.9 Conclusions

Based on the findings of the current study, suitable salinity ranges for egg incubation and early larval rearing in the four species studied are as follows:

Atlantic halibut - both 30 and 35 ppt appear suitable and no distinct advantages were found for one or the other. Egg incubation at 25 ppt or less is not feasible.

Haddock - eggs performed well over the entire range of 15-35 ppt. Although survival was depressed at 15 ppt, the larger length, yolk size, and possible increased hardiness (stress test) at lower salinities (15,20,25 ppt) make these attractive possibilities for early culture.

Atlantic cod - successful hatching occurred at all salinities although viability appears best above 25 ppt. This is further supported by the increased temperature stress tolerance of eggs exposed to high salinities (35 ppt).

Winter flounder - optimal salinities appear to be 15-25 ppt, where viability rates, larval size and overall condition appear maximal. It appears that 35 ppt is approaching the upper limit and should be avoided during early culture.

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