

**Theoretical and experimental studies of
Atlantic cod (*Gadus morhua* L.) egg buoyancy
within a metapopulation framework**

Kyung-Mi Jung



Dissertation for the degree of philosophiae doctor (PhD)
at the University of Bergen, Norway

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List of papers

Paper I

Jung, K.-M., Folkvord, A., Kjesbu, O.S., Sundby, S. Physiological determinants of egg specific gravity of Atlantic cod (*Gadus morhua* L.). Manuscript.

Paper II

Jung, K.-M., Svardal, A.M., Eide, T., Thorsen, A., Kjesbu, O.S. 2012. Seasonal trends in adenylate nucleotide content in eggs of recruit and repeat spawning Atlantic cod (*Gadus morhua* L.) and implications for egg quality and buoyancy. *Journal of Sea Research*, 73: 63-73.

Paper III

Jung, K.-M., Folkvord, A., Kjesbu, O.S., Agnalt, A.L., Thorsen, A., Sundby, S. 2012. Egg buoyancy variability in local populations of Atlantic cod (*Gadus morhua* L.). *Marine Biology*, 159: 1969-1980.

Paper IV

Myksvoll, M.S., **Jung, K.-M.**, Albretsen, J., Sundby, S. Modeling dispersal of eggs and quantifying connectivity among Norwegian coastal cod subpopulations. Manuscript.

Abstract

Marine fishes are subdivided into genetically separated units at various geographical scales. The mechanisms contributing to distinct populations may include year-round site fidelity or natal homing of juvenile and adult stages. Restricted dispersal of pelagic eggs and larvae, associated with ocean environmental conditions, can also enhance distinction between populations. In Norwegian waters, Norwegian coastal cod (NCC) and Northeast Arctic cod (NEAC) are managed as separate units, but increasing evidence demonstrates that the NCC may be structured into local populations on a surprisingly small scale, and that each single fjord population should be preserved for genetic diversity and population persistence. Until now, few studies have been done on ecological and physiological differences among local populations.

This thesis was motivated by gaining increased understanding of how retention mechanisms of early life stages along the coast and fjord areas of Norway might contribute to the diversity in coastal cod population structure. A key factor here is believed to be egg buoyancy, i.e. the ability of the eggs to float naturally in seawater. Therefore, this thesis addresses how basic physical, chemical and biological properties influence egg buoyancy to ultimately understand population structuring of Atlantic cod. Specifically it is studied whether or not the phenotypes of egg specific gravity (ρ_{egg}), which is a key biological factor determining egg vertical distribution, are different among local populations. Regarding basic knowledge of egg buoyancy, it is examined what are physiological and physical determinants of ρ_{egg} , i.e., in light of the important roles of egg osmoregulatory capacity influencing ooplasm specific gravity and the chorion (eggshell) thickness and specific gravity, respectively. The data of ρ_{egg} are applied to model egg dispersal during the spawning season in Northern Norway. The modelling results are discussed in the context of metapopulation structure (defined here as a set of semi-independent local populations within a larger area, where typically migration from one local population to another is possible).

The eggs used in this thesis were naturally spawned from broodfish and incubated under optimal condition in flow-through aquaria until hatching. In general terms, fertilized eggs showed a slight rise in ρ_{egg} until gastrulation (~4 days old) and then a gradual decline in ρ_{egg} was apparent until full development of main organs (~10 days old), but ρ_{egg} suddenly

increased just before hatching (~13 days old). This temporal trend during development seems to be typical for pelagic marine fish eggs. The long-lasting decline of ρ_{egg} was attributable to the changes in yolk volume. The initial value of ρ_{egg} before spawning was largely determined by the volume fraction of chorion in an egg. When the eggs reduced the specific gravity at later stages, adenylate nucleotides (ATP and ADP) significantly increased. The indicated higher content of energy source might reflect an improved ability for energy-demanding osmoregulation. There was no seasonal trend in ρ_{egg} within females, but considerable variation in ρ_{egg} was observed among females. Nevertheless, no significant difference was noted in ρ_{egg} among local populations. The mean ρ_{egg} of the NEAC was similar to that of the NCC, indicating that eggs from the two stocks should have similar vertical distribution under the same environmental conditions. The use of egg dispersal models led to the conclusion that the degree of egg retention is high in fjords, intermediate in coastal areas, and zero in offshore areas. Demographic exchange by egg dispersal could be low not only between fjords and coastal areas but also among neighbouring fjords. With the evidence of resident behaviour in NCC populations, each fjord population may evolve as a discrete population in a metapopulation context.

Introduction

Early life stages, in many cases, are separated spatially from later stages, and they drift passively with ocean currents. The offspring grow, develop, and mature in appropriate nursery grounds to join their parental spawning populations, as described in the Migration Triangle Hypothesis (Harden Jones 1968). Sinclair (1988) proposes the Member-Vagrant Hypothesis that local retention of eggs and larvae by physical ocean structural features helps maintain marine populations. He refers to the individuals retained within appropriate habitat as “members”, and those lost to the local spawning population as “vagrants”. As seen in the two hypotheses, connections from one life stage to another is important to close life cycles, and ocean hydrodynamics are closely related to marine population dynamics.

Knowledge of the vertical distribution of fish eggs and larvae is crucial for understanding transport patterns and potential survival. The vertical distribution is determined by interaction between biological (buoyancy and size) and physical (water density, viscosity, turbulence) factors (Sundby 1991). Sprat (*Sprattus sprattus*) eggs in the Baltic Sea show a seasonal decline in egg specific gravity during spawning season (May–June). Due to this variation in egg buoyancy, eggs are distributed mainly in the deep layers early in the season, occurring in and above the halocline during peak spawning and above the halocline towards the end of the spawning season (Nissling *et al.* 2003). It is also expected that if wind-driven currents transport eggs eastward into low salinity regions, the eggs would sink and die due to low oxygen conditions at depth (Petereit *et al.* 2009). In the Northern Benguela upwelling ecosystem, hake (*Merluccius capensis*) have developed a robust spawning strategy based on spawning depth and egg buoyancy, which bring the eggs and larvae inshore to beneficial survival areas of high prey concentration and prevent offshore larval loss (Sundby *et al.* 2001). Sardine (*Sardinops sagax*) eggs and larvae are well adapted to circulation systems in the Northern Benguela in a way that promotes retention of the larvae in inshore nursery areas (Stenevik *et al.* 2003). In the study of episodic wind effects on planktonic stages, Asplin *et al.* (1999) argue that fish spawning depth and egg buoyancy may have evolved to reduce dispersal of the early life stages. For example, the spawning season of coastal cod (*Gadus morhua*) in a fjord coincides with frequent winds from the south so that cod eggs tend to be retained under down-welling conditions. Paris and Cowen (2004) indicate that larvae of coral reef fish have active swimming ability by which they can increase the chances of retention near the parental populations. These findings support

the view of common retention mechanisms of early life stages among marine species (Swearer *et al.* 2002).

Recruitment from fish larvae to juveniles/adults is not easily studied owing to the small size and high mortality rates of the larvae. Traditional mark-recapture experiments have not been able to identify natal origins of marine fishes. However, recent advances in tagging techniques, such as archival tags and natural geochemical signatures in otoliths (ear bones), have led to a wide consensus that marine fish species return to natal spawning grounds ('natal homing') (Thorrold *et al.* 2001; Svedäng *et al.* 2007). However, complete retention throughout the planktonic life stages is not necessary to guarantee genetic isolation of the adult populations, as long as older individuals have strong natal homing behaviour at any stage of their life (Svedäng *et al.* 2007). Petitgas *et al.* (2010) propose that learning behavior also is one of the determinants of life cycle closure. That is, once a fish has adopted an initial migration trajectory, population persistence could be reinforced by learning and social interaction because fish have sensory capabilities for communication. As for Atlantic herring (*Clupea harengus*), it is hypothesized that migration routes are learned and adopted by young and first-time spawning herring (i.e. recruit spawners) as they join older and more experienced spawning herring (i.e. repeat spawners) (McQuinn 1997). With its resident behaviour, Pacific cod (*Gadus macrocephalus*) lay a single batch of demersal eggs and the larvae remain near the bottom of the water column. Dispersal of the adult is limited by bathymetric and oceanographic (temperature or salinity) barriers (Cunningham *et al.* 2009), thus potentially all life history stages can be retained within geographically small areas. In dynamic ocean environment, these features of behavioural repertoires can be changed (Rose *et al.* 2011). Small pelagic capelin (*Mallotus villosus*) modify spawning locations and feeding areas in response to climate-driven changes in temperature (Huse and Ellingsen 2008).

A single fish species can occur over a wide area within which its distribution is essentially discontinuous. Subareas can have a characteristic population or set of local populations, either of which may be recognized as stocks (Iles and Sinclair 1982). Generally, stock is defined as an intraspecific group of randomly mating individuals with temporal or spatial integrity (Ihssen *et al.* 1981), and considered to be a management unit (Larkin 1972). In this thesis, the term 'stock' is only used when it refers to fisheries assessment entities, otherwise the term 'population' is used. A metapopulation concept has been embraced in conservation

biology and management (Hanski and Simberloff 1997). The metapopulation is broadly defined as a collection of local populations inhabiting discrete patches of suitable habitat, interacting through dispersal and persisting in a balance between stochastic extinctions and recolonizations (Hanski and Gilpin 1997). The present thesis addresses how egg buoyancy contributes to population separation during early life history stages and attempts to understand population structure in the context of the metapopulation.

Motivation and study objectives

Atlantic cod populations have diverged but intermingle during particular life history stages at spawning, nursery or feeding areas (Svedäng *et al.* 2010). In Norwegian waters, Northeast Arctic cod (NEAC) and Norwegian coastal cod (NCC) are managed as separate units. The two stocks can be distinguished from one another by differences in the structure of growth zones (Stransky *et al.* 2008), genetic structures (Sarvas and Fevolden 2005), and the number of vertebrae (Berg and Albert 2003). Also the two stocks have different life history characteristics. NEAC feeding areas are the Barents Sea and near Svalbard, but they perform a long distance of spawning migration from the feeding areas to the coast of Norway, partly co-existing in the same areas as NCC during spawning seasons. NCC inhabit coastal areas and fjords of Norway with short migration patterns, and spawn closer to inshore and in shallower regions than NEAC. Fjord-spawning NCC is more stationary than coastal-spawning NCC (Olsen *et al.* 2010). Recently, population genetic studies have shown that NCC may be structured into local populations on a surprisingly small scale (Pogson and Fevolden 2003; Dahle *et al.* 2006; Westgaard and Fevolden 2007; Knutsen *et al.* 2011). The spatial scale has been narrowed down to the level of individual fjords, so growing consensus is that there is a need to preserve spawning and nursery areas at a local scale in order to preserve genetic diversity and population resilience of the NCC (Jorde *et al.* 2007; Olsen *et al.* 2008).

Few studies have been done to elucidate spatially structured adaptive traits based on ecological and physiological differences among local populations of NCC. Local variation in total length and maturity at age has been found between cod from different fjords and coastal waters (Berg and Albert 2003) and along the Skagerrak coast (Olsen *et al.* 2004). Otterå *et al.* (2006, 2012) found different spawning time among four local populations

under identical environmental conditions. These findings may reflect genetic variations interacting across the local populations. Ciannelli *et al.* (2010) and Myksvoll *et al.* (2011) reported a retention mechanism of cod eggs within fjords in relation to fjord circulation patterns. In this sense, it is of interest to investigate whether local populations have hereditary characteristics in egg specific gravity selecting for vertical distributions that would contribute to local recruitment and separation of populations. Moreover, physiological features of egg specific gravity can provide basic knowledge for egg viability and realistic understanding of egg dispersal.

In this thesis, the main focus is on physiological and ecological significance of egg buoyancy to ultimately understand population structuring of Atlantic cod. Specifically it is first explored theoretically and experimentally what determines egg specific gravity of Atlantic cod (**Paper I**), and how egg buoyancy is associated with osmoregulatory ability using biochemical components (**Paper II**). At the population level, it is studied whether or not the phenotypes of egg specific gravity are different among local populations by undertaking laboratory examinations (**Paper III**). The measurements of egg specific gravity are applied to model egg dispersal patterns during spawning seasons along the coasts and fjords in the northern Norway with regard to population connectivity in real local environments (**Paper IV**). With these results from four papers, the general discussion focuses on egg buoyancy, egg vertical distribution, maternal effects on egg viability, and spatial population structure in a metapopulation context.

Summary of papers

Paper I

- This study examines what determines egg specific gravity of Atlantic cod (*Gadus morhua* L.) by measuring egg diameter, dry weight, water content, yolk volume, perivitelline space, chorion (eggshell) thickness, and egg specific gravity. Based on the physiological measurements, working hypotheses are (1) that egg specific gravity varies during embryonic development caused by changes in yolk volume and (2) that individual variability of initial egg specific gravity is determined by differences in chorion thickness and relative chorion volume fractions. For comprehensive understanding of the variability, the specific gravity of yolk plus embryo is calculated and discussed in detail.

- *Main findings*: in general terms, the specific gravity of fertilized eggs increases slightly until the completion of gastrulation and then gradually decreases, but with a sudden increase just prior to hatching. Yolk volume decreases as development progresses. Among individual egg batches, there is a significant relationship between egg specific gravity and chorion volume fraction, indicating that eggs with a relatively small fraction of chorion volume have low egg specific gravity, while eggs with a high fraction of chorion volume have high egg specific gravity. This study confirms that chorion material is a main contributor to the initial egg specific gravity. During development, it is expected that the simultaneous changes of volume increase and osmolarity decrease of the yolk plus embryo cause the eggs to be more buoyant at later stages.

Paper II

- Changes in egg specific gravity are partly due to impaired osmoregulation. This study examines universal cellular membrane “sodium-potassium ATPase pump” indirectly by relating ooplasmic content of adenylate nucleotides (ATP, ADP and AMP), coenzymes (FAD and NAD) and the resulting energy charge (EC) ($EC = ([ATP] + 0.5[ADP]) / ([ATP] + [ADP] + [AMP])$) with the corresponding egg specific gravity. Working hypotheses are (1) that adenylate nucleotide content increases and egg specific gravity decreases as the embryo develops and (2) that energy charge influences egg specific gravity.
- *Main findings*: egg diameter and egg dry weight decline during spawning season, while egg specific gravity is approximately constant between egg batches. Within each egg batch, the mean specific gravity at early stages decreases at later stages, accompanied by increased contents of ATP and ADP. The high content of ATP might reflect an improved ability for egg osmoregulation. Hence, a low level of energy charge is believed to have negative implications for egg buoyancy. This study demonstrates that egg buoyancy in Atlantic cod is a positive function of energy charge, egg size, and egg developmental stage. The eggs used in the study are regarded as good quality eggs, so cod eggs in nature are assumed to have similar trends in egg specific gravity and levels of nucleotides.

Paper III

- Based on the increasing evidence of small-scaled geographical differentiations of local cod populations, the study objective is to examine if there are differences in egg specific gravity among local cod populations. It is hypothesized (1) that maternal attributes affect egg specific gravity and (2) that the phenotypes of egg specific gravity are different among local cod populations.
- Main findings: the relationship between egg specific gravity and female total length is not significantly correlated, but a negative trend is noticed. The phenotypes of egg specific gravity are similar among local populations and the associated variability is greater at the individual level than at the population level. The noted gradual decrease in specific gravity during development seems to be a generic pattern for pelagic fish eggs. Due to no significant differences in egg specific gravity between Norwegian coastal cod and Northeast Arctic cod, the eggs from the two stocks should coexist locally with similar vertical distributions under the same environmental conditions. The study provides needed input to adequately understand and model fish egg dispersal.

Paper IV

- The Norwegian coast is populated by oceanic cod, coastal cod, and fjord cod. The aim of this study is to evaluate connectivity among different local cod populations in northern Norway, by analyzing dispersal patterns during egg stages. The working hypothesis is that physical environmental conditions influence egg dispersal. Moreover, the current results by a numerical ocean model are discussed in the context of metapopulation structure in Norwegian coastal cod.
- Main findings: the spawning areas are classified into three egg retention regimes: large retention in fjords, intermediate in coastal areas, and no retention in offshore areas. The high retention in fjord systems is associated with subsurface distribution of the eggs caused by local salinity profiles. The intermediate retention is associated with small-scale eddies and friction in between the various small islands. Offshore areas are highly influenced by the northward flowing Norwegian Coastal current. With the previous studies of spawning-site fidelity of adult cod, the high egg retention in fjords supports the idea of substructuring NCC in a metapopulation context.

General discussion

Egg buoyancy

Definition, determinant, and variability

Egg buoyancy is defined as the difference in specific gravity between the ambient water and the egg. Based on this principle, fish eggs can be described largely as pelagic, bathypelagic, and bottom eggs where their vertical distributions are determined by the balance between the buoyancy forces and the diffusive forces (Sundby 1991). Pelagic eggs have positive buoyancy due to lower specific gravity than ambient water of the upper mixed layer, and are consequently distributed with increasing concentration towards the surface. Bathypelagic eggs are intermediate in specific gravity between the upper mixed layer and the bottom layer, and are consequently distributed in the pycnocline with peak concentration at the depth of neutral egg buoyancy. Bottom eggs have negative buoyancy due to higher specific gravity than the bottom layer, and are consequently distributed with increasing concentration towards the sea bed (Page *et al.* 1989; Sundby 1983, 1991).

Although buoyancy measurements of marine fish eggs have been made on a very limited number of species, we know that the majority of marine fish eggs are positively buoyant with increasing egg numbers towards the surface (Sundby 1983). Examples of pelagic egg distribution are eggs of North Sea mackerel (*Trachurus trachurus*) (Iversen 1973), North Sea plaice (*Pleuronectes platessa*) (Pommeranz 1973; Coombs *et al.* 1990), Northeast Arctic cod (*Gadus morhua*) (Sundby 1983), sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) off the southern coast of England (Southward and Barrett 1983) and the Bay of Biscay (Coombs *et al.* 2004), and Benguela sardine (*Sardinops sagax*) (Stenevik *et al.* 2001). Examples of bathypelagic egg distribution are found among eggs of Atlantic halibut (*Hippoglossus hippoglossus*) in the Norwegian fjords (Haug *et al.* 1984, 1986), Baltic cod eggs (*Gadus morhua*) (Nissling and Westin 1991), and fjord spawning populations of Norwegian coastal cod (Myksvoll *et al.* 2011). Examples of bottom egg distribution are eggs of saffron cod (*Eliginus gracilis*) in the northeast Pacific (Dunn and Matarese 1987) and capelin (*Mallotus villosus*) in the Barents Sea (Bakke and Bjørke 1973). In addition to the above examples of egg distribution in vertical equilibrium, there are also marine eggs that do not have a stable vertical distribution but change from fertilization until hatching

over the wide range of water depths as for example Cape hake (*Merluccius capensis*) eggs in the Northern Benguela (Sundby *et al.* 2001) and blue whiting (*Micromesistius poutassou*) eggs in the west of the British Isles (Ådlandsvik *et al.* 2001). All these different types of vertical distributions are results of a wide range of adaptation to the ambient ecosystems in order to optimize offspring survival (Sundby *et al.* 2001; Myksvoll *et al.* 2011).

Egg specific gravity is determined during oocyte maturation processes inside maternal fish and finally by yolk hydration just prior to the egg release into the seawater (Craik and Harvey 1987; Kjesbu *et al.* 1992; Thorsen and Fyhn 1996). After the release, one additional change occurs due to the formation of perivitelline space between chorion and the vitelline membrane which is filled with ambient seawater (Davenport *et al.* 1981). Among the egg compositional materials, carbohydrates, proteins, free amino acids, and salts are denser than seawater and contribute to negative buoyancy. On the contrary, lipid and pure water content are the only two components less dense than seawater, and these components provide positive buoyancy (Craik and Harvey 1987). During the extensive hydrolysis of ovoplasmic proteins into free amino acids, a massive influx of water occurs and the swelled eggs contain water of 60-70 % of wet weight for demersal eggs and 90-92 % for pelagic eggs (Craik and Harvey 1987). The high water content is the principal cause of positive buoyancy in the eggs. Lipid makes little contribution to egg buoyancy in marine species (Thorsen *et al.* 1996; Riis-Vestergaard 2002). As a contributor to negative buoyancy, chorion material of fish eggs is made of proteins which harden when the eggs are fertilized (Kjørsvik and Lønning 1983). The specific gravity of wet chorion has been calculated as 1.20 g cm⁻³ for Atlantic cod (*Gadus morhua*) eggs (Kjesbu *et al.* 1992) and 1.18 g cm⁻³ for Atlantic halibut (*Hippoglossus hippoglossus*) eggs (Mangor-Jensen and Huse 1991). The relative fraction of chorion volume in a cod egg has been revealed as a main factor determining individual buoyancy differences (**Paper I**). As lipids do, carbohydrates make little contribution to egg buoyancy due to relatively little quantity in an egg (Craik and Harvey 1987).

During egg development, the specific gravity varies and has been observed for Atlantic cod eggs (Mangor-Jensen 1987; Nissling and Westin 1991; Anderson and deYoung 1994; **Paper I, II, III**), Atlantic halibut eggs (Mangor-Jensen and Waiwood 1995), Cape hake eggs (Sundby *et al.* 2001), and European anchovy eggs (Ospina-Álvarez *et al.* 2012). However, the reason why this specific gravity varies is much less understood. According to

Paper I, the simultaneous changes in the volume and specific gravity of yolk plus embryo govern significantly the developmental variability in total egg specific gravity. As for Atlantic cod, the fertilized eggs increase their specific gravity until gastrulation and then gradually decrease, but with a sudden rise just before hatching. Such temporal changes during development seem to be typical for pelagic marine eggs, and reflect a good function of egg osmoregulation (**Paper II**).

Atlantic cod (*Gadus morhua* L.)

Buoyancy-related egg vertical distribution

Egg vertical distribution is determined by interaction between biological (i.e. egg buoyancy, egg diameter) and physical (i.e. seawater density, seawater viscosity, turbulence) factors (Sundby 1991). As seen in Table 1, Atlantic cod (*Gadus morhua*) populations seem to have a surprisingly similar range of egg specific gravity and egg size, except for the Baltic cod. For this reason, different types of egg vertical distributions will be more explained by spatial and temporal variability in water characteristics than the egg buoyancy. For example, the Northern cod eggs are pelagic and concentrated above the pycnocline ($\sim 1.026 \text{ g cm}^{-3}$) at 50 m depth on the northeastern Newfoundland shelf in June, but the egg distribution prolongs deeper on inner shelf than outer shelf because the inner shelf is characterized by colder, lower salinity of water than that of the outer shelf (Anderson and deYoung 1995). In contrast, when the study period is extended (March to August) in a Newfoundland fjord, the cod eggs show both pelagic and bathypelagic distributions (Knickle and Rose 2010). That is, the eggs are broadly distributed near the surface area during spring due to weak thermal and pycnal stratification in the upper mixed layer (constant at $\sim 0 \text{ }^\circ\text{C}$ and $\sim 1.026 \text{ g cm}^{-3}$ from surface to 200 m depth). However, once the stratification gets stronger during summer due mainly to freshwater input, the eggs concentrate below the pycnocline at 10 m depth, and they are likely to be retained in the fjord.

Table 1 General range of specific gravity, equivalent salinity (ppt) of neutral buoyancy at the observed temperature (°C), and diameter (mm) for cod eggs from different local environments. The method of egg collection is different in the literature; N refers to ‘natural spawning in a tank’, P refers to ‘plankton ring net in the field’, S refers to ‘hand-stripped fertilization’, R refers to ‘recalculation from egg distributional depths together with water density and temperature, NI refers to ‘no information’.

Cod origin	Specific gravity	Salinity	Temperature	Diameter	
Norway	Porsangen fjord ^{1,N}	1.024 - 1.025	30 - 32	6	1.26 - 1.44
	Tysfjord ^{1,N}	1.023 - 1.025	29 - 32	6	1.29 - 1.40
	Helgeland ^{1,N}	1.023 - 1.025	30 - 32	6	1.25 - 1.36
	Øygarden ^{2,N}	1.024 - 1.026	31 - 33	6	1.27 - 1.39
	Tvedestrand fjord ^{3,P}	1.021 - 1.026	27 - 33	4	-
Barents Sea ^{4,N}	1.023 - 1.026	29 - 33	5	1.17 - 1.45	
Iceland ^{5,S}	1.023 - 1.026	29 - 33	6	1.24 - 1.58	
Newfoundland/Grand Banks ^{6,N+S}	1.022 - 1.026	28 - 33	8	1.20 - 1.60	
Northern Gulf of St. Lawrence ^{7,R}	1.024 - 1.026	30 - 33	2	1.35 - 1.44	
Georges Bank ^{8,NI}	1.025 - 1.026	32 - 33	4	1.50	
Baltic ^{9,S}	1.009 - 1.014	12 - 18	7	1.48 - 1.77	

1, **Paper III**; 2, Stenevik *et al.* 2008; 3, Ciannelli *et al.* 2010; 4, **Paper II**, 5, Marteinsdóttir and Begg 2002; 6, Anderson and deYoung 1994, 1995; 7, Ouellet 1997; 8, ICES 2005; 9, Nissling *et al.* 1994

Such retention phenomenon of cod eggs is also evident in Norwegian fjord systems. The fjords are characterized by shallow sills at the mouth and narrow entrances, functioning as sheltered areas (Asplin *et al.* 1999). Typically, fjords have three different water layers; a thin upper brackish layer, an intermediate layer, and a deep basin layer below the sill (Stigebrandt, 1981). Higher concentration of cod eggs occurs inside than outside fjords, and, in particular, fjords with shallow sills exhibit an abrupt reduction in egg number over the sill (Knutsen *et al.* 2007). **Paper IV** shows cod eggs spawned in fjords tend to be trapped inside and have low numbers of recruiting eggs from neighbouring coastal and fjord areas. The retention mechanism is caused by Norwegian coastal cod spawning near the head of fjords where the less saline brackish water is thick enough to let the eggs have subsurface distributions (Myksvoll *et al.* 2011). The majority of cod eggs will also have heavier

specific gravity than the upper brackish water. Under this condition, in-flowing intermediate water holds the eggs inside fjords (Stenevik *et al.* 2008; Myksvoll *et al.* 2011). Accordingly, spatial-temporal variations in estuarine systems play a key role in determining the egg distributions.

Icelandic cod seem to have adapted reproductive strategies associating with egg buoyancy. Main spawning grounds are south and southwest coasts of Iceland. Additional spawning occurs also within fjords of the west, north and east coasts (ICES 2005). In the main spawning grounds, it is known that large cod spawn closer to the shore and small cod spawn in deeper water along the continental edge and banks (Marteinsdóttir *et al.* 2000). The eggs from large cod are likely to have heavier specific gravity (1.025 to 1.027 g cm⁻³) than those of small cod (1.023 to 1.025 g cm⁻³) (Marteinsdóttir and Begg 2002). Thus, inshore large cod may have evolved heavy eggs for retention, and offshore small cod develop light eggs for transport to favourable nursery grounds.

The Baltic Sea is characterized by strong vertical stratification of salinity, temperature, and oxygen. A permanent halocline separates low saline water (6 to 8 ppt) on the surface from high saline deep water (10 to 20 ppt) (Fonselius and Valderrama 2003). Main spawning grounds of Baltic cod are the Bornholm Basin, the Gdansk Deep and the Gotland Deep in the eastern Baltic (ICES 2005). Optimal physical conditions for cod egg development in this region is salinity > 11 ppt, oxygen concentration > 2 mL L⁻¹, and temperature > 1.5 °C (Wieland *et al.* 1994). As a result, the Baltic cod eggs neutrally buoyant at 12~18 ppt concentrate within and below the halocline from 60 m depth to the bottom in the Bornholm Basin (Nissling 2004). However, since the level of dissolved oxygen fluctuates widely and acute hypoxia is common on the bottom, it is essential to achieve neutral egg buoyancy at depths above the critical oxygen levels (Nissling and Westin 1991).

Maternal effects on egg viability

Reproductive potential represents the ability of a fish stock to produce offspring that may recruit to the adult population or fishery (Trippel 1999). Traditionally, the assumption that spawner biomass is directly proportional to total egg production by fish stocks has underlain spawner-recruitment relationships (Trippel *et al.* 1997; Marshall *et al.* 1998). However,

during the past decade a number of studies have found the reproductive potential varies with age, size and condition of spawning fish and spawning experience (e.g. Chambers and Waiwood 1996; Solemdal 1997; Marteinsdóttir and Steinarsson 1998; Marteinsdóttir and Begg 2002; Carr and Kaufman 2009), giving rise to studies of maternal effects on offspring viability for more realistic assessment of spawning stock biomass.

Atlantic cod is a multiple batch-spawner, producing up to 20 egg batches within a single spawning season, and mean egg size becomes progressively smaller with increasing egg batches (Kjesbu 1989; Marteinsdóttir and Steinarsson 1998). Due to such batch sequence, the relationships between parents and offspring characteristics are difficult to detect in the Atlantic cod. In general, old fish produce larger eggs than young fish (Kjesbu *et al.* 1996). Recruitment of Baltic cod is significantly favoured by egg production from old fish since the old fish produce highly buoyant eggs with larger opportunities for survival (Vallin and Nissling 2000). Larger females produce larger eggs, and larger eggs hatch into larger larvae (Chambers and Waiwood 1996; Marteinsdóttir and Steinarsson 1998; Marteinsdóttir and Begg 2002). In the study of Icelandic cod, larvae from large eggs begin feeding earlier and experience a higher feeding success than those from small eggs (Marteinsdóttir and Steinarsson 1998). A similar study of Norwegian coastal cod shows that larvae given by early egg batches tend to have higher hatching success (Solemdal *et al.* 1995). Female condition is also positively linked to egg size (Chambers and Waiwood 1996; Marteinsdóttir and Steinarsson 1998). Ouellet *et al.* (2001) have found high hatching success in egg batches from high pre-spawning condition of females. Accordingly, all of these studies demonstrate a significant effect of maternal attributes on their egg viability.

In addition to female age, length and condition, reproductive history also influences the offspring viability. First-time spawners breed for a shorter period, produce fewer egg batches, exhibit lower fecundity, and produce smaller eggs with lower fertilization and hatching rates than do second-time spawners (Trippel 1998). The longer spawning period of repeat spawners may be highly advantageous because the protracted release of eggs into the variable environment could improve progeny survival matching up with the time of abundant prey for the larvae (Trippel 1998). Similarly, the variation in egg specific gravity from repeat spawners is greater than recruit spawners (Kjesbu *et al.* 1992; **Paper II**). It is likely that the eggs from repeat spawners could be spread more vertically and less detectable by predators. Moreover, egg mortality is lower for repeat spawners (Solemdal *et*

al. 1995). Therefore, it seems that old repeat spawners contribute to more viable eggs than young recruit spawners.

Regarding fishing pressure, cod populations across the entire north Atlantic have experienced declines in abundance and selective removal of older and larger spawners as a result of increased exploitation (Begg *et al.* 1999). According to Scott *et al.* (1999), when egg viability is considered, there could be up to a 3.3-fold difference in reproductive potential due only to differences in the age/size structure of the population. Presently, many scientists believe that we have to abandon the traditional use of spawning stock biomass and assess the functional relationships between female characteristics and egg viability (Trippel 1999; Marteinsdóttir and Begg 2002, Scott *et al.* 2006).

Spatial population structure in a metapopulation context

A metapopulation is a set of local populations between which limited immigration and emigration occur (Hanski and Simberloff, 1997). If populations are sustained by 100% of self-recruitment (i.e. closed population, recruitment from their own offspring) or by a high number of recruits from other populations (i.e. open population, recruitment from a common larval pool), the metapopulation concept is not appropriate. However, if populations exhibit not only self-recruitment that largely dictate their population fate but also replenishment from outside the populations that affects population structure to an extent that it cannot be ignored, metapopulation concept is relevant (Kritzer and Sale 2004). Thus, primary empirical and theoretical approaches are a question of how demographically connected local populations are.

Many marine species have great spatial dispersal during early life history, and hence dispersal and gene flow may be widespread. In this feature, they do not appear to fit metapopulations. However, we now know that traditionally recognized fish ‘stocks’ often possess metapopulation structure (Hanski & Gilpin, 1997). This concept has been applied to different marine fish species such as coral reef fishes (e.g. Kritzer and Sale 2006; Bay *et al.* 2008), plaice (*Pleuronectes platessa*) (Hunter *et al.* 2003), Atlantic herring (*Clupea harengus*) (e.g. McQuinn 1997; Johannessen *et al.* 2009), and weakfish (*Cynoscion regalis*) (Thorrold *et al.* 2001). Metapopulation structuring in Atlantic cod (*Gadus morhua*) has also

been indicated in the North Sea and west of Scotland (Wright *et al.* 2006a; Heath *et al.* 2008) as well as the Northwest Atlantic (Robichaud and Rose 2001; Smedbol and Wroblewski 2002; Rose *et al.* 2011), but this is still speculative because of the difficulty of estimating the exchange degree at all life history stages.

As a common method for estimating population connectivity, genetic studies have found sub-structuring Atlantic cod over the North Atlantic ocean (e.g. Imsland and Jonsdottir 2003), but genetic differentiation can be undetectable under low level of exchange (Palumbi 2003). Accordingly, non-genetic approaches are needed to understand connectivity for a metapopulation theory. Svedäng *et al.* (2010) reported cod stock separation in the Kattegat and Öresund (eastern North Sea) using three independent methods: genetic surveys, tagging experiments and otolith chemistry analysis. Even though the genetic surveys showed no stock differentiation, tagging and otolith methods for migratory patterns identified discrete spawning groups at close distances (<100 km). A similar phenomenon that cod remain resident to particular areas and return to the same spawning site year after year (termed ‘spawning site fidelity’) has been observed for sedentary cod populations inhabiting bay, coast and fjord areas (Robichaud and Rose 2004; Wright *et al.* 2006a, 2006b; Skjæraasen *et al.* 2011). The above mentioned studies illustrate that cod inhabiting these coastal areas do not move far offshore.

The spawning site fidelity alone cannot represent discrete local populations if there are extensive egg and larval drift resulting in a large degree of mixing cross the populations (Grimm *et al.* 2003). Egg development time varies, depending upon ambient temperature, from one week at 12 °C to five weeks at -1 °C (Page and Frank 1989). As such, cod eggs having a long dispersal period must be considered for a connectivity study. In **Paper IV** on biophysical modelling, eggs were released from a wide range of inshore and offshore areas and showed three types of drift patterns: (1) eggs spawned offshore were transported long distances (named no retention in the paper), (2) eggs spawned coastal inshore had a small degree of retention (medium retention) caused by meso-scale meanders/eddies and friction force between various small islands, and (3) eggs spawned inside fjords had a high degree of retention (large retention) caused by tides/eddies and a semi-closed topographic condition. The high egg retention in typical fjord systems was also found by studies of Knutsen *et al.* (2007), Ciannelli *et al.* (2010), Knickle and Rose (2010), and Myksvoll *et al.* (2011). In addition to the eggs, larval cod also have a long pelagic life span, but the larvae

exhibit active vertical movement that affects their drift trajectories and temperature exposure (Vikebø *et al.* 2007). Øresland and André (2008) found that cod larvae collected inside a fjord differed genetically from those outside the fjord. Larval vertical behaviour might facilitate retention inside the fjord, opposing out-flow currents. Sarvas and Fevolden (2005) observed that cod larvae dwelling in fjords with a narrow entrance for currents were genetically separated from neighbouring local populations. Therefore, it is likely that typical fjord systems provide both spawning areas and nursery areas for the early life history stages.

In addition to the movement during early life stages, it is also important to understand the connectivity between juvenile and spawning adult cod, because juvenile cod tend to exhibit a high degree of site fidelity within bays and sea lochs, but wide dispersal in open nursery areas (Lough *et al.* 1989). Gibb *et al.* (2007) examined the degree of isolation of juvenile cod in a region where a number of resident adult congregations have been found, indicating little or no inter-mixing juveniles between nursery patches in the North Sea and west of Scotland. In the same study areas, Wright *et al.* (2006b) demonstrated that coastal spawning cod tend to originate from nearby nursery areas. Taken together, the geographical isolation during juvenile stages and strong spawning site fidelity are evidence for localized resident populations. Moreover, there is growing evidence that cod return to natal spawning areas (termed 'natal homing', Robichaud and Rose 2001; Svedäng and Svenson 2006; Svedäng *et al.* 2007; Svedäng *et al.* 2010). If the natal homing is a true property of Atlantic cod, it can maintain population integrity in spite of shared habitat with other populations at any life history stages. In particular, this feature seems to be crucial for population-separation mechanism between long-distance migratory and resident cod at the same places (Svedäng *et al.* 2007).

Atlantic cod have diverse migratory behaviour from sedentary residents that exhibit year round site fidelity to dispersers that move and spawn in large geographical areas (Robichaud and Rose 2004). As discussed above, the evidence for spawning site fidelity, juvenile aggregation, and egg/larval retention characterizes metapopulation structuring, but has been observed mostly in resident coastal populations with a small home range. Probably, broadly dispersing cod populations such as the Grand Bank cod and the southern Gulf of St Lawrence cod may not be appropriate to metapopulation concepts. Regardless of whether Atlantic cod possess metapopulations, understanding spatial-temporal dynamics is an important first step for population conservation and management. As demonstrated by

Stephenson (1999), decline in Atlantic cod abundance may be attributable to the loss of spawning individuals and areas, therefore spawning areas and times must be preserved under a precautionary approach.

Future perspectives

Early life history stages have long been thought to be critical for successful recruitment to the parental population (e.g. Hjort 1914). The vertical and horizontal distributions of these early stages are commonly studied with numerical bio-physical models because direct observation in the field is limited by the feasibility of taking large numbers of sampling stations. The modelling also allows prediction of the spatial distribution under different environmental scenarios. With regard to dispersal variability of fish eggs, buoyancy is a key biological factor determining egg vertical positions (Sundby 1991). As demonstrated in this thesis, egg buoyancy of Atlantic cod changes during incubation, and the phenotypes of egg specific gravity vary greatly at the individual female level. This variability of egg buoyancy may be applicable to other pelagic fish eggs. For more realistic dispersal simulations, direct measurements of egg buoyancy must be performed to be added in the modelling studies. In addition, if it is clarified where is source (i.e. high contribution to overall egg production) and sink spawning regions (i.e. low contribution to overall egg production), the source local populations must be protected. This is because individuals strayed from the source can reinforce the abundance of the sink populations and prevent from the collapse.

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