REPRODUCTIVE POTENTIAL OF PACIFIC COD (*GADUS MACROCEPHALUS*) IN ALASKA

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Abstract

The reproductive potential of female fishes, which results from the number of eggs they produce and the quality of individual eggs, is a critical factor in fisheries biology. Reproductive potential is important to individuals because maternal fitness is the product of the number of offspring produced and how many offspring survive. The growth rate of populations and their capacity for supporting commercial fisheries also depend on the number of viable offspring that females produce. I studied the reproductive potential of female Pacific cod (*Gadus macrocephalus*) in the North Pacific Ocean. Pacific cod is an important ecological and economic resource, yet much of its reproductive biology remains unexplored. I used several different approaches to investigate whether egg number or egg size are more important in determining reproductive potential, and to evaluate factors that influence reproduction. An analysis of life history variation among Pacific cod in Canada and Alaska demonstrated that despite differences in life history strategies, females from different populations had similar lifetime reproductive success (a proxy for individual fitness). I also collected Pacific cod in the Gulf of Alaska, eastern Bering Sea, and western Aleutian Islands from 2002 to 2005. Biochemical analyses of Pacific cod eggs revealed that Pacific cod produce low-energy eggs that are adapted for rapid development on the seafloor. Larger females produce eggs with less arachidonic acid (a fatty acid that has been linked to egg quality) than smaller females, suggesting that they may sacrifice egg quality to maintain fecundity. Determination of fecundity and egg size in 590 females from different areas and years revealed that maternal length and weight are excellent predictors of fecundity, but that variability in egg size is not related to the age or size of females. The greatest difference in reproductive potential among years and areas was reduced egg size in the eastern Bering Sea in 2003, which may have been due to changes in ocean temperature or prey availability that impacted the ability of females to store energy. These results suggest that female Pacific cod maximize their fitness through increased egg production, not egg quality, and that their reproductive success is under strong environmental control.

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Dedication

This work is dedicated to the Pacific cod of Alaska, many of whom had to die for this research to succeed. Fish have the misfortune of lacking in charisma, and I've often wondered how things would be if fish were as cute as puppies. I can only hope that the work I do is ultimately to their benefit. May your larvae flourish, my barbel-chinned friends!

GENERAL INTRODUCTION

The reproductive potential of female fishes, defined as the number of viable offspring they produce, is a critical factor in fish population dynamics and ecology. In this dissertation, the term reproductive potential describes both the number of eggs females produce (fecundity) as well as the quality of individual eggs. Egg quality refers to the resources in eggs that affect their fertilization and hatching rates and the probability of larval survival (Brooks et al. 1997), thereby affecting offspring viability. Because larger eggs often produce larger larvae that have competitive advantages or are better able to escape predators (Wootton 1990), greater egg size is equated here with better egg quality.

Eggs are the first of a series of early life stages that ultimately lead to recruitment into adult populations, and for species that exhibit no parental care eggs are the only life history stage of offspring over which females have direct control. The fitness of female fishes is directly linked to their reproductive potential. The growth rate of populations and their capacity to support commercial fisheries also depends on the number of viable offspring that females produce. Fecundity and egg quality are important concepts in fisheries management because the establishment of fishing mortality rates that prevent overfishing relies on an accurate assessment of the reproductive potential of females within a stock (Marshall et al. 2003).

Two types of evolutionary compromises influence the reproductive potential of female fishes. First, maternal fitness is the product of the total number of offspring produced by a female during her lifetime and the fitness of individual offspring (Smith & Fretwell 1974, Wootton 1990). Energy devoted to increasing offspring fitness (i.e.

improving egg quality) must be balanced by producing fewer total offspring. The life history of female fishes, which includes factors such as longevity and reproductive behavior, influences how they allocate energy between offspring quantity and quality (Wootton 1990). Second, females also distribute energy among reproduction, growth, and survival (Roff 1983, Stearns 1992). As females mature and begin to put resources towards reproduction, less energy is put toward growth. Females also appear to trade off between the fitness value of reproducing in any one year and the probability of surviving to reproduce in future years (Roff 1983, Rideout et al. 2005).

Pacific cod *Gadus macrocephalus* is a large (average 70 cm total length, 4kg body mass), demersal fish that is found across the North Pacific from the northwestern United States to China. Pacific cod are thought to be derived from Atlantic cod *Gadus morhua* as the result of an invasion into the Pacific Ocean over 3 million years ago (Grant & Stahl 1988, Carr et al. 1999). The two species are morphologically and ecologically similar, but differ in some aspects of their reproductive biology. Pacific cod release all of their eggs in a single event (Hattori et al. 1992) and the eggs are demersal (Sakurai & Hattori 1996, Westrheim 1996). In contrast, Atlantic cod are batch spawners and produce pelagic eggs (Westrheim 1996). Populations of Pacific cod on the eastern and western sides of the North Pacific are genetically distinct, but population structure at smaller scales has yet to be described (Grant et al. 1987). In Alaska, Pacific cod in the eastern Bering Sea (EBS) and Aleutian Islands (AI) are managed as a single Bering Sea/ Aleutian Islands (BSAI) stock. Pacific cod inhabiting the Alaskan portion of the Gulf of Alaska (GOA) are similarly managed as one population.

Pacific cod is an important ecological and economic resource in the North Pacific Ocean. It has a diverse diet that includes fish, shrimp and crabs (Livingston & deReynier

1996, Yang 2004). Predation by Pacific cod has impacted populations of walleye pollock *Theragra chalcogramma* in the EBS (Wespestad et al. 1986) and may have accelerated the reorganization of the marine community in the GOA during the late 1970s and 1980s by increasing the mortality of forage fishes and shrimps (Anderson & Piatt 1999). Cod also serves as prey for other fish and marine mammals and is an important food item for the endangered western population of Steller sea lions *Eumetopias jubatus* (Sinclair & Zeppelin 2002). Pacific cod is the target of large commercial fisheries in several countries on both sides of the North Pacific. Cod are taken with a variety of fishing gears and are targeted by large- and small-scale fishery operations. The cod fishery in Alaska is the state's second largest groundfish fishery, with the 2005 catch exceeding 240,000 metric tons.

Despite the importance of Pacific cod in the North Pacific, many aspects of its reproductive biology and the factors that affect its reproductive potential remain unexplored. The goal of this dissertation is to investigate egg production and egg quality in Pacific cod and to determine how individual, population, and environmental factors influence reproductive potential. To achieve this goal, four projects were undertaken that consider reproductive potential from different perspectives and at a variety of scales.

Egg production at the population scale is the focus of Chapter 1. Using data combed from the literature, I compare life history strategies among four Pacific cod stocks in Canada and Alaska. I determine how life histories influence female lifetime reproductive success (R_0), which represents the total number of eggs produced by an average female over her lifetime and incorporates information on survival, maturation, and fecundity (Stearns 1992). Calculation of R_0 assumes that all eggs are of equal quality, so that the fitness of a female is equivalent to the total number of eggs she is likely to produce during her lifetime. I also use a simple population dynamics model to demonstrate how life histories influence the resilience of populations to commercial fishing.

In contrast, Chapters 2 and 3 are devoted to investigating egg quality at the cellular level. Marine fish eggs generally take one of two forms: an egg with large amounts of lipids, most of which are in the form of energy-rich neutral lipids contained in an oil globule within the egg; or a low-lipid egg, lacking an oil globule and containing primarily polar lipids (Wiegand 1996). The latter eggs tend to have less energy available for embryo growth (Rønnestad et al. 1999). In chapter 2, I describe the proximate and lipid-class composition of eggs. I use a comparison to the eggs of Atlantic cod to infer how Pacific cod egg composition matches energetic and ecological requirements, and provide an explanation for the differences in egg buoyancy between the two species. In chapter 3, I concentrate on the effects of geographic area and maternal attributes on the lipid-class and fatty-acid composition of Pacific cod eggs. Several important fatty acids are thought to influence egg quality (Tocher 2003). Within a species, the fatty acid composition of eggs can vary among females from different regions (Wiegand 1996) and with the age and size of females (Evans et al. 1996, Wiegand et al. 2004). In this chapter, I study how egg composition varies between the EBS and AI and among females in the AI.

Egg production and egg quality are brought together in Chapter 4, in which I investigate reproductive potential at the level of individuals and populations. As is typical for most commercially fished marine species, current management of Pacific cod assumes that reproductive potential is proportional to spawning biomass (SSB). Individual variation in reproductive potential may violate this assumption, so I compare fecundity, egg size, and other reproductive parameters among female Pacific cod from three areas in Alaska. Because the relationship between SSB and reproductive potential is also assumed to be constant over time, I repeat my analyses over multiple years to determine the effects of temporal variability. The results in chapter 4 are also used to demonstrate how Pacific cod allocate energy among egg production, egg size, growth, and survival.

Literature Cited

Anderson PJ, Piatt JF (1999) Community reorganization in the Gulf of Alaska following ocean climate regime shift. Mar Ecol Prog Ser 189:117-123

Brooks S, Tyler CR, Sumpter JP (1997) Egg quality in fish: what makes a good egg? Rev Fish Biol Fish 7:387-416

Carr SM, Kivlichan DS, Pepin P, Crutcher DC (1999) Molecular systematics of gadid fishes: implications for the biogeographic origins of Pacific species. Can J Zool 77:19-26

Evans RP, Parrish CC, Brown JA, Davis PJ (1996) Biochemical composition of eggs from repeat and first-time spawning captive Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 139:139-149

Grant WS, Stahl G (1988) Evolution of Atlantic and Pacific cod: Loss of genetic variation and gene expression in Pacific cod. Evolution 42:138-146

Grant WS, Zhang CI, Kobayashi T (1987) Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Can J Fish Aquat Sci 44:490-498

Hattori T, Sakurai Y, Shimazaki K (1992) Maturation and reproductive cycle of female Pacific cod in waters adjacent to the southern coast of Hokkaido, Japan. Nippon Suisan Gakkaishi 58:2245-2252

Livingston P, deReynier Y (1996) Groundfish food habits and predation on commercially important prey species in the eastern Bering Sea from 1990 to 1992. AFSC Processed Report 96-04, Alaska Fisheries Science Center, National Marine Fisheries Service

Marshall CT, O'Brien L, Tomkiewicz J, Köster FW, Kraus G, Marteinsdottir G, Morgan MJ, Saborido-Rey F, Blanchard JL, Secor DH, Wright PJ, Mukhina NV, Björnsson H

(2003) Developing alternative indices of reproductive potential for use in fisheries management: case studies for stocks spanning an information gradient. J. Northwest Atl Fish Sci 33:161-190

Rideout RM, Rose GA, Burton MPM (2005) Skipped spawning in female iteroparous fishes. Fish Fish 6:50-72

Roff DA (1983) An allocation model of growth and reproduction in fish. Can J Fish Aquat Sci 40:1395-1404

Rønnestad I, Thorsen A, Finn RN (1999) Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture 177:201-216

Sakurai Y, Hattori T (1996) Reproductive behavior of Pacific cod in captivity. Fish Sci 62:222-228

Sinclair EH, Zeppelin TK (2002) Seasonal and spatial differences in diets in the western stock of Steller sea lions (*Eumetopias jubatus*). J Mammal 83:973-990

Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. Am Nat 108:499-506

Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11:107-184

Wespestad VG, Shimada AM, June JA (1986) Recruitment variations in Pacific cod and its effects in the Bering Sea and Gulf of Alaska. Bull Int North Pac Fish Comm 45:295-305

Westrheim SJ (1996) On the Pacific cod (*Gadus macrocephalus*) in British Columbia waters, and a comparison with Pacific cod elsewhere, and Atlantic cod (*Gadus morhua*). Can Tech Rep Fish Aquat Sci 2092

Wiegand MD (1996) Composition, accumulation, and utilization of yolk lipids in teleost fish. Rev Fish Biol Fish 6:259-286

Wiegand MD, Johnston TA, Martin J, Leggett WC (2004) Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Can J Fish Aquat Sci 61:110-121

Wootton RJ (1990) Ecology of teleost fishes. Chapman & Hall, London

Yang M-S (2004) Diet changes of Pacific cod (*Gadus macrocephalus*) in Pavlof Bay associated with climate changes in the Gulf of Alaska between 1980 and 1995. Fish Bull 102:400-405

CHAPTER ONE

Cause and consequence of life history variation in North American Pacific cod stocks [1]

Abstract

We characterized the life history strategies of four Pacific cod (*Gadus macrocephalus*) stocks in the eastern North Pacific Ocean. Southern stocks grew and matured more quickly, but reached smaller sizes and had shorter lifespans than northern stocks. These tradeoffs resulted in equivalent lifetime reproductive success among all stocks. Growth was highly dependent on latitude but not on temperature, possibly as a result of differences in growing-season length. Comparisons to Atlantic cod (*Gadus morhua*) revealed similar latitude-growth relationships among Atlantic cod stocks grouped by geographic region (e.g., Europe). In Pacific cod, greater size and longevity in the north appeared to be adaptations to overcome environmental constraints on growth and maintain fitness. An egg production-per-recruit model suggested that the life history strategy of northern Pacific cod stocks made them less resilient to fishing activity and age truncation than southern stocks. Climate change may alter the life history strategies of northern Pacific cod and increase their resilience to fishing.

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[1] Ormseth, OA and Norcross, BL. 2007. Cause and consequence of life history variation in North American Pacific cod stocks. Submitted to ICES Journal of Marine Science.

Introduction

Marine fish exhibit a myriad of life history strategies among different taxonomic groupings (Beverton 1992; Winemiller & Rose 1992; King & McFarlane 2003) and spatially separated populations of the same species (Jennings & Beverton 1991; Fargo & Wilderbuer 2000; Salvanes et al. 2004). Understanding the basis for such variation is a key goal in ecology and is valuable for improving the management of commercially fished species (King & McFarlane 2003).

Three endpoint strategies are often used to describe extremes of life history variation in fish (Winemiller 1992; King & McFarlane 2003). “Opportunistic” strategists grow quickly and become sexually mature at young ages, but have high rates of mortality. “Periodic” strategists have slower growth and delayed maturation but greater longevity. Both of these strategists favor high fecundity with little energetic investment in individual eggs and little or no parental care. “Equilibrium” strategists tend to be long-lived and devote large amounts of energy to a much smaller number of progeny.

These strategies result from environmental and genetic influences on life history traits. The environment can shape strategies by influencing the expression of particular traits. Variation in growth and annual reproductive output among Atlantic herring (*Clupea harengus*) stocks depends on environmental temperatures (Jennings & Beverton 1991). In some species, genetic changes compensate for environmental influences. The growth rate of high-latitude Atlantic silversides (*Menidia menidia*) is greater than that of low-latitude conspecifics held in a common environment, suggesting that this species has evolved to compensate for the effects of reduced growing-season length (Conover & Present 1990).

Latitude influences environmental parameters. Temperature generally decreases with latitude in the Pacific and Atlantic Oceans. Seasonal patterns of photoperiod length change with latitude, with extreme seasonal differences at high latitudes. Latitude may also affect growing season length in several ways. Individual species or their prey may have a particular window of time during the year when temperatures are sufficiently high for growth (Conover & Present 1990). In addition, the total annual amount of thermal energy available to organisms changes with latitude (Niewiarowski 2001).

The environment may influence life histories by imposing temporal or spatial scales on their evolution. Tropical yellowfin tuna (*Thunnus albacares*) have a stable environment, experience low recruitment variability, and live approximately 8 years; northern Atlantic cod (*Gadus morhua*) live in unpredictable surroundings, have high recruitment variability, and may live up to 20 years (Longhurst 1998). The greater longevity of cod in this case is thought to be a “bet-hedging” strategy that increases the chance of at least one good reproductive year in a highly variable environment.

Strategies also are constrained by tradeoffs among traits. Tradeoffs may result from the allocation of fixed resources, so that energy devoted to growth or reproduction is not available for maintenance and repair (Partridge & Sibly 1991). Thus, fast growth may occur at the expense of increased mortality. Tradeoffs may be genetic constraints, such as the optimization of enzymes for high temperatures that makes them less effective in cold temperatures (Angilletta et al. 2003). Environmental conditions may also enhance expression of some traits at the expense of others, e.g., warm temperatures tend to increase growth rates but also increase maintenance costs and the activity of predators (Wootton 1990).

Evolutionary theory suggests that trade-offs among traits reflect evolutionary pressure to maximize lifetime reproductive success, i.e., the total egg production of a female over her lifetime (R_0), and maintain fitness (Stearns 1992). Traits that reduce R_0, e.g. delayed maturation, may be offset by others, e.g. greater longevity, that are selected through evolutionary time because they increase R_0. Thus, populations of the same species with different life history strategies may be expected to maintain equal R_0 through trade-offs in life history traits (Beverton 1987). To our knowledge, only a few studies have attempted to test this theory empirically in fishes (Leggett & Carscadden 1978; Beverton 1987; Jennings & Beverton 1991).

The goals of this study are to understand how and why life histories vary among geographically separate stocks of Pacific cod (*Gadus macrocephalus*) and use life history information to explore how these stocks might respond differently to fishing activity and environmental change. Pacific cod is a large demersal fish that inhabits the continental shelf throughout the northern North Pacific Ocean. It has important ecosystem roles as predator and prey and is the target of lucrative commercial fisheries in the U.S. and Canada. Its broad geographical distribution and close taxonomic relationship to Atlantic cod, an ecologically similar species (Grant & Stahl 1988), make it a good candidate for the use of inter-regional comparisons in the study of life history traits.

We synthesized a variety of data from four Pacific cod stocks off the northwest coast of North America to meet four specific objectives: 1) characterize life history trait variation in these stocks; 2) understand how such variation results in different life history strategies and affects evolutionary fitness; 3) investigate environmental influences on growth and how they interact with natural selection to cause variability in life history traits; and 4) use a simple egg production-per-recruit model to understand how life history strategies affect resilience to fishing.

Methods

Life history strategies and fitness

Four Pacific cod stocks were chosen for this study based on their geographical separation (Fig. 1.1) and the availability of detailed life history information (Table 1.1). Two stocks were located off the west coast of British Columbia (BC): West Vancouver Island (WVI) and Hecate Strait (HEC). The remaining two stocks were from Alaska (AK): Gulf of Alaska (GOA) and Bering Sea / Aleutian Islands (BSAI). The AK stocks are much bigger than the BC stocks. The 2001 age 3+ biomass was estimated at 1 091 000 t and 553 000 t for the BSAI and GOA, respectively (Thompson & Dorn 2004; Thompson et al. 2004). In the WVI, 2001 adult biomass was estimated at 4497 t (Starr et al. 2002), and the 2001 adult biomass estimate in the HEC was 2960 t (Sinclair et al. 2001). The area occupied by the Alaskan stocks is also larger. BSAI cod inhabit over 200 000 km^2 and GOA cod are distributed over more than 110 000 km^2. The WVI and HEC stocks occupy approximately 3500 and 14 000 km^2, respectively.

An approximate latitude was calculated for each stock by averaging the latitudinal boundaries of the stock's distribution (Table 1.1). Boundaries were determined using historical locations of commercial fisheries catch. The WVI stock ranges from 48°N to 49°N and the HEC stock is distributed from 52.5°N to 54.5°N (Westrheim 1996). The GOA stock exists from 54°N to 60°N, and the BSAI stock extends from 52°N to 60°N (Fritz et al. 1998).

We relied on a variety of data sources for this study (Table 1.1). Most of the data on life history traits were obtained from stock assessment documents (BC: Sinclair et al. 2001, Starr et al. 2002; AK: Thompson & Dorn 2004, Thompson et al. 2004) and a comprehensive review of Pacific cod information (Westrheim 1996). Parameters for predicted fecundity at length in the AK stocks were preliminary (O. Ormseth, unpublished data), and maturity information for the AK stocks was obtained from the U.S. National Marine Fisheries Service (NMFS; J. Stark, personal communication, 2005). Data from all sources were collected after 1977 with the exception of maturity information for the WVI stock that was gathered from 1973 to 1985 (Westrheim 1996).

We used common equations to describe traits and obtained equation parameters either from the literature or through our own calculations based on published data. Throughout this paper, length refers to total length in cm. For each stock there were several estimates of instantaneous natural mortality rate (M) available. All published values, with the exception of one extreme value for the Hecate Strait stock, were used to calculate an average M for each stock (Appendix 1.A). To describe growth, we used the growth rate parameter (k) and asymptotic length (L_∞) from the von Bertalanffy growth model (LVB; Quinn & Deriso 1999). For the BC stocks, maturity ogives were used to determine the parameters a and b of the logistic equation:

$$P = \frac{1}{1 + e^{a+bL}}$$

where P is the probability of being mature and L is length. Parameters of the logistic equation were available for the AK stocks (J. Stark, personal communication, 2005). This equation was used to estimate length at 50% maturity (L_{50}) for all stocks. L_{50} was used with the LVB to estimate age at 50% maturity (A_{50}). Fecundity was described using the parameters α and β of the power equation fecundity = αL^{β}.

Probabilities of survival and maturation, as well as predicted length and fecundity were calculated for ages 1-18 for each of the four stocks (Appendix 1.B). The probability of survival to age x (s_x) was determined by setting age-0 survival (s_0) to 1 and calculating $s_x = s_0 e^{-Mx}$. Predicted length at age (L_x) was determined using the LVB model. Probability of maturation at age (p_x) was estimated using L_x and the logistic equation obtained from the maturity ogives. Fecundity at age (f_x) was determined using L_x and the power equation.

To describe life history strategies for each stock we calculated the most probable reproductive output at each age, which we termed "age-specific reproduction" (AR), according to the equation $AR_x = s_x p_x f_x$. Thus, AR is equal to the probability of a female cod reaching a particular age multiplied by the probability of the cod being mature at that age multiplied by the estimated fecundity at that age (Appendix 1.B). AR values were then plotted against age to graphically depict life history strategies.

As a measure of evolutionary fitness, we integrated AR values for each stock to calculate lifetime reproductive success (R_0) using the equation:

$$R_0 = \sum_{x=0}^{18} s_x p_x f_x$$

This method differed from standard notation (e.g., Charnov 1993) in that it included the probability of maturation within the age-specific calculation and integrated over all ages, rather than treating maturation as a knife-edge event and integrating over only those ages in which individuals are considered to be mature.

To understand the influence of the environment on life histories, we compared latitude and temperature to individual traits. Average annual temperature at 75 m depth was determined using data from three locations that were within the geographic distribution of three of the stocks (Fig. 1.1). We used annual average temperature because we had no information on the seasonal pattern of growth in Pacific cod and it allowed us to compare our results to similar work on Atlantic cod. A depth of 75 m was chosen based on available data and because it is the approximate midpoint of the summer depth range of Pacific cod (30-100m; Westrheim 1996). For each location, all available data from 1995-2003 were used to calculate monthly averages, and annual averages were calculated from the monthly values. For the WVI, we used data from Station P1 of the Canadian Department of Fisheries and Oceans (DFO) P Line (www.pac.dfo-mpo.gc.ca/). Gaps in the P1 data required that we include values from the early 1980s to get averages for some months. We were unable to locate temperature data for the area of the HEC stock, so that stock was not included in the temperature analysis. Data from the GAK1 station of the University of Alaska's Seward Line (www.ims.uaf.edu/gak1/) were used for the GOA stock. Values for the BSAI were based on the M2 mooring of the Pacific Marine Environmental Laboratory, NOAA (www.beringclimate.noaa.gov/data/). Least-squares linear regression was used to assess latitude and temperature effects on life history traits.

To further explore the relationship between growth, latitude, and temperature we compared our findings on Pacific cod to the results of a study relating average ocean temperature to age-4 body weight in 17 Atlantic cod stocks (Brander 1994; Table 1.2 and Fig. 1.2). We calculated age-4 weights for the BC stocks using published length/weight relationships (Westrheim 1996) and obtained AK age-4 weight data from published stock assessments (Thompson & Dorn 2004; Thompson et al. 2004). We determined approximate latitudes for each Atlantic cod stock (Table 1.2) by reviewing stock descriptions on the websites of the DFO (http://www.dfo-mpo.gc.ca), North Atlantic Fisheries Organization (NAFO; http://www.nafo.int/), and International Council for the Exploration of the Sea (ICES; http://www.ices.dk). We separately plotted the natural log of age-4 weight against latitude and average annual temperature, and used linear regression to compare latitude and growth.

Fishing effects

An egg production-per-recruit (EPR) model was used to investigate the implications of life history differences for fisheries management (Quinn & Deriso 1999). To simplify the analysis of EPR, we limited it to the two stocks with the widest geographic separation, WVI and BSAI. Total egg production for a cohort of 1000 recruits over an 18-year period was calculated for different levels of fishing mortality (F). The total egg production for each level of F was divided by the egg production of an unfished cohort (F=0) to determine the amount by which a particular F would reduce EPR. $F_{E35\%}$, the level of F that reduces EPR to 35% of the unfished level, was used here as a gauge of the stocks' resilience to fishing effort. $F_{E35\%}$ is analogous to the more common reference point of $F_{35\%}$, which uses spawning biomass as a proxy for egg production. $F_{35\%}$ has been shown to be the upper limit to sustainable exploitation in fish stocks with a variety of life history strategies (Clark 1991).

Two age-specific parameters were used, fecundity and fishery selectivity, while natural mortality was held constant across all age classes (Appendix 1.C). Based on our analysis of life history traits, age at maturity was assumed to be knife-edged at 3 years in the WVI stock and 6 years in the BSAI. Fecundity was set to zero for pre-maturation ages. Survival of individuals from one age-class to the next was calculated using:

$$N_{a+1} = N_a \bullet e^{-(M+Fa)}$$

where *a* is age, N is cohort abundance, and F_a is the base F modified by age-specific selectivity parameters. Egg production for each age class was calculated using the equation:

$$N_{0,a} = f_a \bullet N_a \bullet e^{-((0.25*M)+(0.6*Fa))}$$

where $N_{0,a}$ is egg production and f_a is fecundity. The modifier 0.25*M was added because spawning in Pacific cod generally occurs in March (Westrheim 1996) and the modifier 0.6*F_a was added because approximately 60% of the annual catch occurs from January to March (catch data accessed from the NOAA Alaska regional office website, http://www.fakr.noaa.gov/default.htm.).

To test the importance of age structure in the two stocks we conducted the EPR analysis for three selectivity scenarios, "full recruitment", "trawl", and "longline". In the full recruitment scenario, fisheries were assumed to have knife-edged, full selectivity on the stocks beginning at the age of recruitment (age 2 in the WVI and age 3 in the BSAI; Appendix 1.C). In the trawl scenario, we assumed a selectivity ogive with low values for younger age classes and increased selectivity with age (Appendix 1.C). Selectivity values for this scenario were based on parameters used in a prediction model for the BSAI Pacific cod trawl fishery (J. Ianelli, NMFS, pers. comm. 2005). Because size at age was different between the two areas, we adjusted the selectivity for the WVI stock so that the same sizes of Pacific cod were selected equally in both areas. A third scenario used selectivity from the Pacific cod longline fishery, which has greater selectivity for smaller fish. Because only trawl gear is used in the WVI fishery, the longline scenario was not applied to that stock.

Results

Life history traits varied considerably among populations. Mortality was highest in the WVI stock and lowest in the BSAI stock (Table 1.1); survival was markedly reduced in the WVI stock relative to the other three stocks of Pacific cod (Fig. 1.3). While growth rates were higher in the Canadian stocks, asymptotic lengths were greater for the Alaskan stocks (Table 1.1). As a result, lengths at age were initially greater in the Canadian stocks but ultimately bigger in Alaska (Fig. 1.4). Canadian stocks matured more rapidly than Alaska stocks (Fig. 1.5). A_{50} and L_{50} were smallest in the WVI stock and largest in the BSAI, and the relative change in A_{50} was twice as high as the relative change in L_{50} (Table 1.1). The β parameters of the fecundity equation were greater in the Alaskan stocks (Table 1.1), as was age-specific fecundity (Fig. 1.6).

The trajectories of age-specific reproduction (AR) were substantially different among stocks (Fig. 1.7). In the WVI stock, AR peaked at age 3 and declined rapidly thereafter. AR in Hecate Strait peaked at age 6 and declined less quickly. In the GOA and BSAI, AR peaked at ages 7 and 8, respectively. In the Alaskan stocks, the reduction in AR after the peak was much more gradual than the reduction in AR in Canadian stocks. Lifetime reproductive success was equivalent among all stocks (Table 1.3).

Average latitude spanned 8.5° (Fig. 1.4A) and had a significantly inverse relationship with mortality ($n= 4$, $R^2 = 0.91$, $p = 0.044$), k ($n = 4$, $R^2 = 0.99$, $p = 0.002$; Fig. 1.8A) and ln (age-4 weight) ($n = 4$, $R^2 = 0.98$, $p = 0.007$). Annual average temperature at 75 m depth differed markedly among regions, but was not significantly related to latitude.

No significant influence of average annual temperature on k (Fig. 1.8B) or other life history traits was observed.

Latitude versus ln (age-4 wt.) comparisons for Atlantic cod revealed different complexes of stocks delineated by geographic area (Fig. 1.9). Atlantic cod stocks were grouped by geography as a Europe complex, which included Greenland, and southern and northern North America complexes separated at 45°40'N latitude; (Fig. 1.5 and Table 1.2). Growth was significantly and negatively related to latitude in the European ($n = 9$, $R^2 = 0.86$, $p < 0.0001$) and southern North American ($n = 3$, $R^2 = 1.0$, $p = 0.011$) stocks, but not in northern North America ($n = 5$, $p = 0.09$). Including Pacific cod data in a plot of the temperature/growth relationship for Atlantic cod stocks showed that growth in the WVI and BSAI had a similar relationship to temperature as Atlantic cod, but that GOA stocks had anomalously low growth (Fig. 1.10). The temperature/growth relationship was significant within the European complex ($n = 9$, $R^2 = 0.95$, $p < 0.0001$), but not in either the southern or northern North American Atlantic cod complexes.

In the egg production-per-recruit (EPR) model, the WVI stock was able to withstand higher fishing mortality. The full recruitment EPR model indicated that the WVI stock should be able to sustain an $F_{E35\%}$ of 0.33. The BSAI stock, on the other hand, could sustain an $F_{E35\%}$ of only 0.16. Under the trawl scenario, $F_{E35\%}$ increased to 1.00 in the WVI stock and 0.48 in the BSAI. In the longline scenario, $F_{E35\%}$ for the BSAI stock was 0.26.

Discussion

The Pacific cod stocks in this study display substantial variation in life history strategies but have equivalent lifetime reproductive success (R_0). Latitude has a strong negative influence on growth and sexual maturation, perhaps as a result of differences in growing season duration. Greater asymptotic length and possibly greater longevity may have evolved in northern stocks to compensate for this constraint and maintain fitness. Our results suggest that the strategy of northern Pacific cod stocks makes them less resilient to fishing. If climate warming affects growing season (Hassol 2004), it may alter the dynamics of northern Pacific cod and make them better able to withstand fishing pressure.

The four Pacific cod stocks examined here have different life history strategies, but trade-offs among traits preserve fitness. The large, sharp peak and subsequent rapid decline in age-specific reproduction (AR) in the West Vancouver Island (WVI) stock, and to a lesser extent in the Hecate Strait (HEC) stock, indicate that most of these fish grow and mature rapidly but attain a small maximum size and die off quickly. On a continuum between the opportunistic and periodic endpoint strategies (Winemiller and Rose 1992), this strategy is more opportunistic. Individuals in these stocks benefit by starting to reproduce at a young age, but their R_0 is reduced by a shorter life span and lower fecundity than northern females. Conversely the much more gradual rise and fall of AR in the Alaskan stocks indicates that those fish grow more slowly and mature at a later age, which would tend to reduce R_0. Potential reductions in R_0 in the Alaskan fish are offset by greater longevity and fecundity. The life history of the Alaskan stocks more closely resembles the periodic strategy.

Other fish species have demonstrated maintenance of fitness across separate populations. In Atlantic herring (Jennings & Beverton 1991) and walleye (*Sander vitreus*; Beverton 1987), there was considerable variation in life history traits among geographically separated stocks, but indices of R_0 were equivalent for most stocks. In contrast, northern stocks of American shad (*Alosa sapidissima*) preserved fitness by devoting greater energy reserves to migration and increasing the probability of offspring survival relative to southern stocks, which did not migrate and produced more eggs (Leggett & Carscadden 1978). Although northern females produced fewer progeny, the increased survival of those progeny preserved maternal fitness.

The equivalence of R_0 among Pacific cod stocks suggests that the processes regulating the survival of early life stages may be similar among the stocks, even though they exist in different environments. The value of R_0 (approximately one million) may also suggest a scale for the survival rate of cod eggs (i.e., one egg in a million survives to reproduce). This supposition is complicated by the necessary oversimplification of our analysis. We assumed that mortality was equal for all ages, but it is likely much greater for younger ages. While this analysis was valid for comparing R_0 among stocks, more detailed information would be required to calculate a "true" R_0 for Pacific cod.

Our results indicate that latitude has a much greater effect than temperature on growth of Pacific cod. Initially, we expected that growth rate in Pacific cod would be strongly influenced by temperature. Ambient water temperature has a strong effect on vital rates of fish and other ectotherms (Jobling 1993; Wootton 1990) and organisms reared at higher temperatures tend to have higher growth rates (Jobling 1997). In Atlantic cod, growth was highly dependent on average annual temperature in 17 different stocks (Brander 1994, 1995).

The close relationship between latitude and growth in Pacific cod may result from differences in growing season. The metabolic rate of ectotherms depends on the heat they gain from their environment, so growing season may be interpreted as the total annual amount of thermal energy an organism receives that it can devote to growth. For example, growth in a terrestrial ectotherm, the eastern fence lizard (*Sceloporus undulates*), was related to the heat energy available from its environment during the year (Niewiarowski 2001). This is the same concept that underlies the use of growing degree days (GDD), the integration of daily temperatures above a temperature threshold, to study growth in plants. GDD analysis has recently been applied to growth in fishes and crabs, where it appeared to be a consistently good predictor of size at age (Neuheimer & Taggart 2007).

Alternatively, growing season can indicate the window of time during the year when growth can occur. This window may depend on the availability of sufficiently high temperatures for growth: low-latitude populations of Atlantic silversides (*Menidia menidia*) experience a growing season that is 2.5 times as long as high-latitude populations (Conover & Present 1990). A growing season window may also result from the availability of adequate sunlight for primary production. In laboratory experiments, exposure to natural photoperiod appeared to maintain equivalent growth in two groups of Atlantic cod despite differences in ambient temperature between the groups (Levesque et al. 2005). This suggests that growth in Atlantic cod is adapted to seasonal patterns that would vary with latitude.

Several factors may have obscured our interpretation of the temperature/growth relationship in Pacific cod. Growth in the GOA stock appears anomalously low, and GOA and BSAI fish grow at similar rates despite a wide difference in ambient temperature. This similarity may be a result of incomplete separation between the two stocks. Tagged cod have been found to move between the GOA and the Bering Sea (Shimada & Kimura 1994; D. Urban, Alaska Dept. of Fish and Game, personal communication, 2003). This exchange between stocks may be sufficient to affect our estimation of growth rates.

It is also likely that our estimates of average annual temperature do not reflect the full range of ambient temperatures available to Pacific cod. We were limited to three single-point monitoring stations for our temperature values. While we feel that these offered a valid means of comparing temperatures among regions, fixed stations limit the ability to describe thermal habitats (Ottersen et al. 1998). Pacific cod may move substantial distances to maintain preferred temperatures. In Hecate Strait, cod changed their depth distribution among years to maintain equivalent ambient temperatures (Perry et al. 1994) and cod spawning migrations in the Bering Sea may be an attempt to avoid cold, shallow water during the winter (Shimada & Kimura 1994). It is likely that temperature does play a role in regulating the growth of Pacific cod, and that the limitations of our data obscured that role.

Although temperature has been linked to growth in Atlantic cod (e.g., Brander 1995), our study suggests that Atlantic cod growth may also be subject to latitudinal effects apart from temperature. The close relationship between latitude and growth that we observed for Pacific cod is mirrored in two of the three Atlantic cod complexes. This may be because the stocks in each complex occur within an area of common oceanographic influence. The southern North America Atlantic cod stocks are all likely influenced by the warm Gulf Stream current that flows north along the eastern edge of North America but turns east before reaching Canada (Schmitz and McCartney 1993). In contrast, the northern North America stocks are probably more influenced by the cold Labrador Current flowing south. The Europe stocks are found within the North Atlantic and Norwegian currents. The one exception to this is the Greenland stock, but there appears to be connectedness between these fish and Icelandic cod (Stein and Borovkov 2004).

The temperature/growth relationship in Atlantic cod largely breaks down when a comparison is made only within a stock complex; the effect of temperature was significant only in the European stocks. This suggests that while temperature is important, it is not the only factor affecting growth. This is supported by the observation that growth in an Atlantic cod stock in Placentia Bay, Newfoundland was not inhibited by cold temperatures as long as food was sufficient (Mello & Rose 2005).

The results of our Pacific and Atlantic cod analyses suggest that latitudinal factors other than temperature also affect growth. The latitudinal effect on growth likely influences the onset of sexual maturity. Both sexual maturity traits, L_{50} and A_{50}, differ among the stocks but the L_{50} values are less variable among the stocks than the A_{50} values. This observation suggests that females need to reach a certain minimum length before reproducing, either to enhance survival or ensure an adequate level of egg production. A threshold size for maturation was observed in walleye stocks that had a constant L_{50} despite geographic variation in other traits (Beverton 1987). A minimum body size for reproduction in Pacific cod is supported by the fact that fecundity at the maturation ages used in the EPR model is almost equal between the WVI and BSAI. The marginally significant relationship between A_{50} and latitude also suggests a delay of maturation related to increasing latitude.

We suggest that greater asymptotic length and reduced mortality may have evolved in northern stocks to compensate for slower growth and maturation, which would otherwise reduce fitness. Because there are physiological and ecological bases for an inherent trade-off between growth and mortality (Beverton 1992, Arendt 1997), it may be that greater L_{∞} is the principal adaptation to environmental constraints on growth in Pacific cod.

Greater maximum size appears to maintain R_0 in these Pacific cod stocks, but we had insufficient evidence to discern whether this is a plastic response or if there are genetic differences among stocks. Increased body size could be an adaptive reaction norm (Stearns 1989) that occurs in all Pacific cod stocks, so that greater L_∞ is a plastic response to slower growth rates (Partridge & Coyne 1997). Alternatively, selection for larger body size may have driven local adaptation in northern Pacific cod so that there are genetic differences in preferred L_∞ among the various stocks. Either scenario is consistent with our results. Similar variation in Atlantic herring was attributed to plastic responses, based on a lack of genetic difference among stocks (Jennings & Beverton 1991). Studies using allozymes showed no genetic differences among North American Pacific cod stocks (Grant et al. 1987; Westrheim 1996). However, more recent work using microsatellite DNA analysis shows that genetic distance among Pacific cod from different areas is correlated with geographic distance (M. Canino, AFSC, pers. comm.), suggesting the possibility of stock structure.

We believe that effects on growth and compensation in other traits is the most likely explanation for the variation observed in this study, but our results are also consistent with the hypothesis that life histories evolve to match scales of temporal and/or spatial variability in the environment. The periodic strategy observed in the northern Pacific cod stocks may be a form of "bet-hedging". Greater longevity may serve to maintain reproductive output over a greater number of years to bridge gaps between infrequent strong recruitment events (Longhurst 2002; Winemiller 2005). In the North Pacific Ocean, climate variability occurs on different time scales. At lower latitudes, the El Niño- Southern Oscillation (ENSO) affects climate on an interannual 3-7 year scale (McGowan et al. 1998). Because ENSO is a tropical phenomenon, its influence wanes at higher latitudes (Lluch-Cota et al. 2001) where decadal-scale variability, such as that induced by the Pacific Decadal Oscillation, is more dominant (Mantua & Hare 2002). Thus greater longevity in the northern, more periodic stocks may serve to ensure at least one good reproductive event in an area subject to decadal-scale variability. Conversely rapid growth and maturation should enable opportunistic species to rapidly take advantage of short-term changes in climate (Winemiller 2005), so the southern Pacific cod stocks may be better suited to ENSO variability.

The life history strategy of northern Pacific cod stocks makes them less resilient to fishing pressure and more dependent on maintenance of age structure than southern stocks. Our estimate of $F_{E35\%}$ in the BSAI stock is less than half that of the WVI under a full recruitment scenario, and the gap increases when fishing effort is disproportionately selective for older age classes. The northern stock of rock sole (*Lepidopsetta* spp.) in the Bering Sea is similarly less resilient than the southern stock in Hecate Strait (Fargo & Wilderbuer 2000). Our results support the argument that periodic strategists are less resilient to fishing effort pressure due to longer generation times (Jennings et al. 1998; King & McFarlane 2003). Because it impairs the bet-hedging strategy, removal of older age classes by fishing may also make periodic strategists more susceptible to recruitment failures (Longhurst 2002). Age truncation in Atlantic cod stocks may have made them more vulnerable to poor environmental conditions (Drinkwater 2002).

If slower growth in the northern stocks results from lower temperatures and reduced availability of heat energy, climate change may affect Pacific cod stocks by altering their life histories. Average temperature and growing season length (in terms of the availability of heat energy) are both expected to increase substantially at high latitudes in the next century (Hassol 2004). Therefore the life history strategies of northern Pacific cod may change to more closely resemble that of the southern stocks, particularly if traits are formed entirely by plastic responses. If so, the resiliency to fishing of the northern stocks might also increase. However, this effect would be separate, and possibly different, from changes to recruitment and distribution that are also likely to occur (Beamish 1993, Brander 1997, Welch et al. 1998, Bailey 2000).

Certain analytical limitations may have affected our conclusions. Information used in this study came from different sources that used different estimating techniques and spanned a number of years. Although we used only data from after a climate "regime shift" that occurred in the mid-1970s (Benson & Trites 2002), growth and other processes may have fluctuated widely during the last 25 years. In Atlantic cod, for example, maturity ogives varied substantially within one stock over a 12-year period (Marshall et al. 1998). Our analysis was also limited by small sample sizes, particularly for temperature effects. Despite these limitations we feel that our study provides an accurate assessment of variation in Pacific cod life history.

High levels of commercial fishery exploitation may confound the interpretation of life history traits. Extended periods of heavy fishing pressure can result in faster growth and earlier maturation in fish stocks (Law 2000), and both of the BC stocks may have been overfished in the past (Sinclair et al. 2001; Starr et al. 2002; Westrheim 1996). However, Pacific cod have not experienced either the severity or duration of fishing effort that has altered life history traits in other species (Law 2000; Westrheim 1996), and are therefore less likely to have experienced age truncation. Additionally, all stocks used in this study are subject to similar rates of fishery exploitation and any age truncation that has occurred has probably affected both BC and AK stocks.

This study demonstrates the power of a few key life history parameters to describe major ecological differences among populations, as well as the difficulty of determining how such differences arose. Pacific cod stocks appear to have successfully adapted to the limitations of the areas they inhabit. However, these adaptations make some Pacific cod stocks less resilient to commercial fishing. Understanding the variation described in this paper can help to alleviate negative effects of fishing. Warming of Earth's climate could have long-term effects on the dynamics of Pacific cod populations, but such changes may be beneficial for high-latitude fish stocks.

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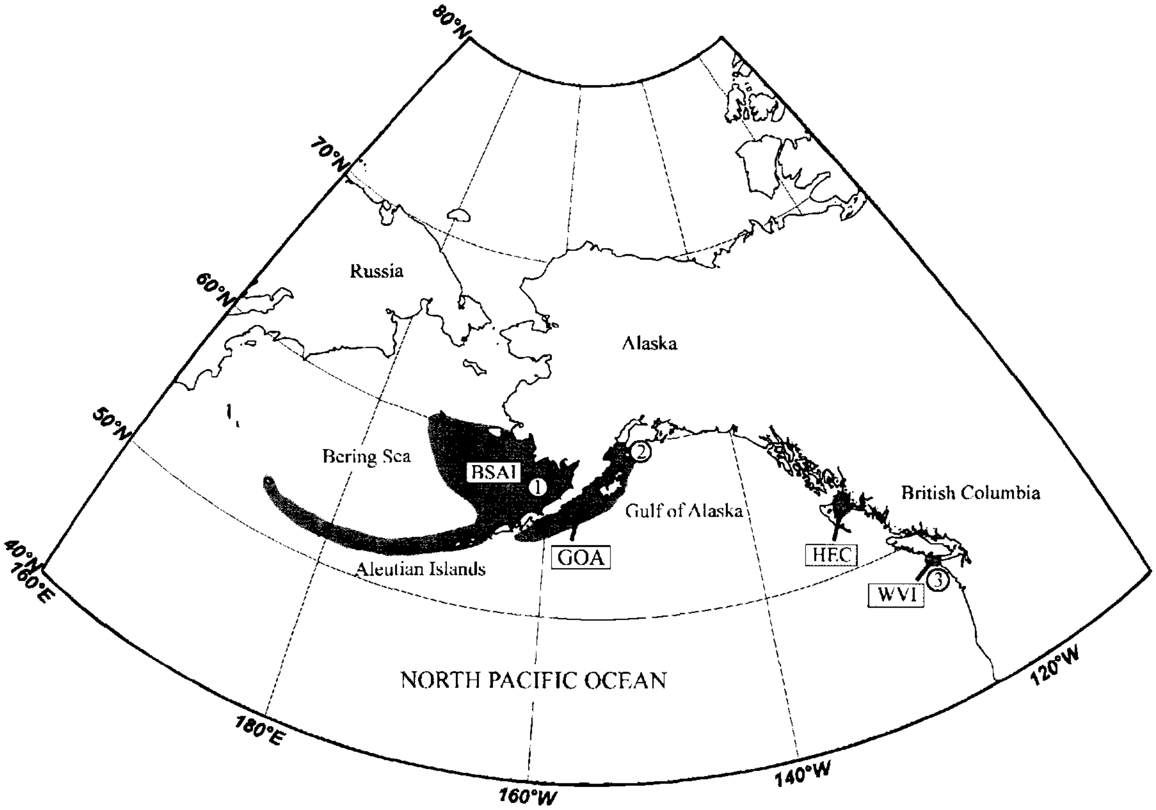

Figure 1.1. North Pacific Ocean with locations of Pacific cod stocks and temperature data sources. Gray shading indicates approximate extent of each labeled stock: BSAI, Bering sea/ Aleutian Islands; GOA, Gulf of Alaska; HEC, Hecate Strait; WVI, West Vancouver Island. Circled numbers indicate approximate locations of temperature data sources: 1) M2 mooring, 2) GAK1 station, 3) P1 station.

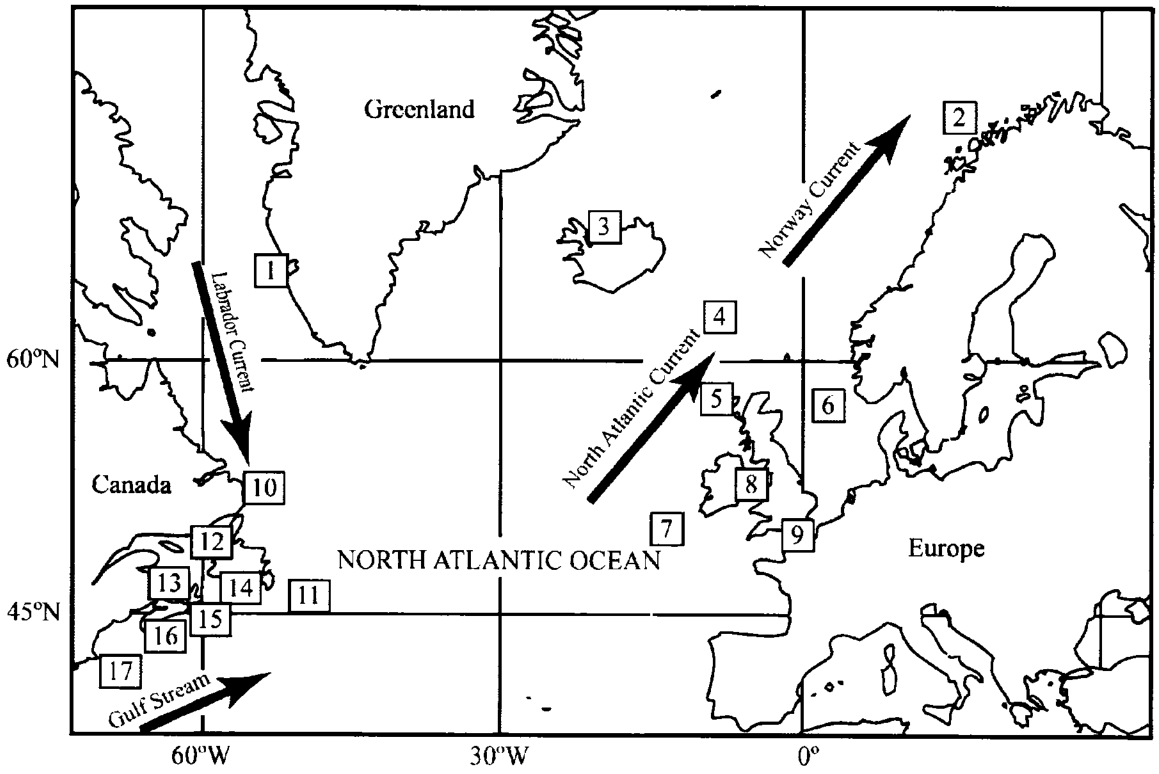

Figure 1.2. North Atlantic Ocean with approximate locations of 17 Atlantic cod stocks. Numbers correspond to the stocks listed in Table 1.2.

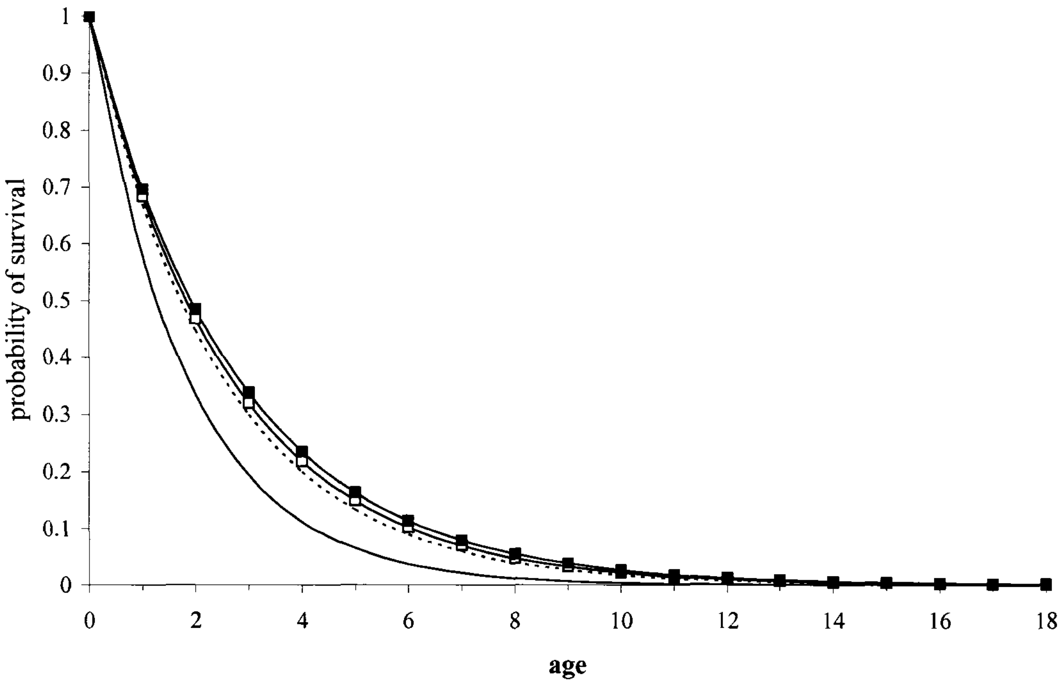

Figure 1.3. Survival at age in four Pacific cod stocks. Solid line with no symbols, WVI; broken line, HEC; line with open squares, GOA; line with solid squares, BSAI.

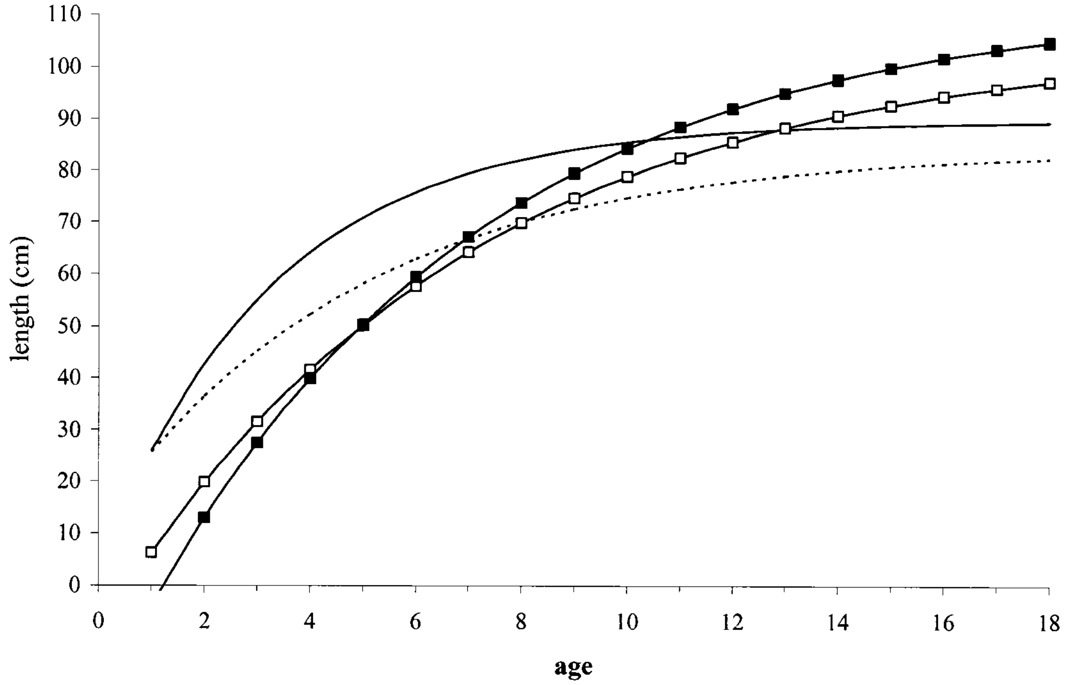

Figure 1.4. Length at age in four Pacific cod stocks. Solid line with no symbols, WVI; broken line, HEC; line with open squares, GOA; line with solid squares, BSAI.

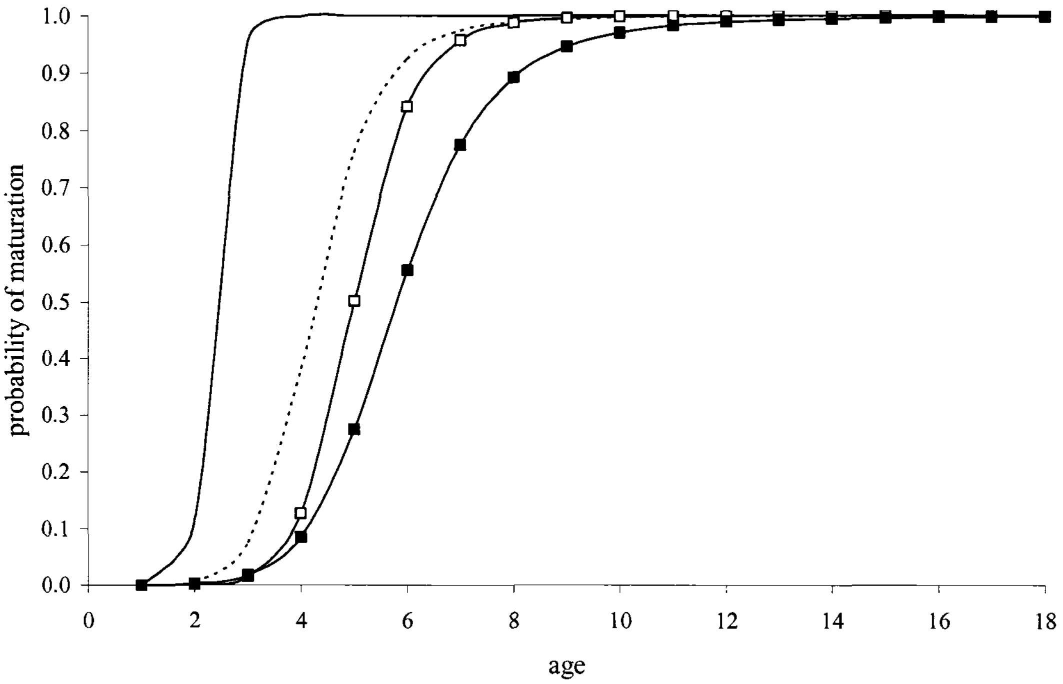

Figure 1.5. Proportion mature at age in four Pacific cod stocks. Solid line with no symbols, WVI; broken line, HEC; line with open squares, GOA; line with solid squares, BSAI.

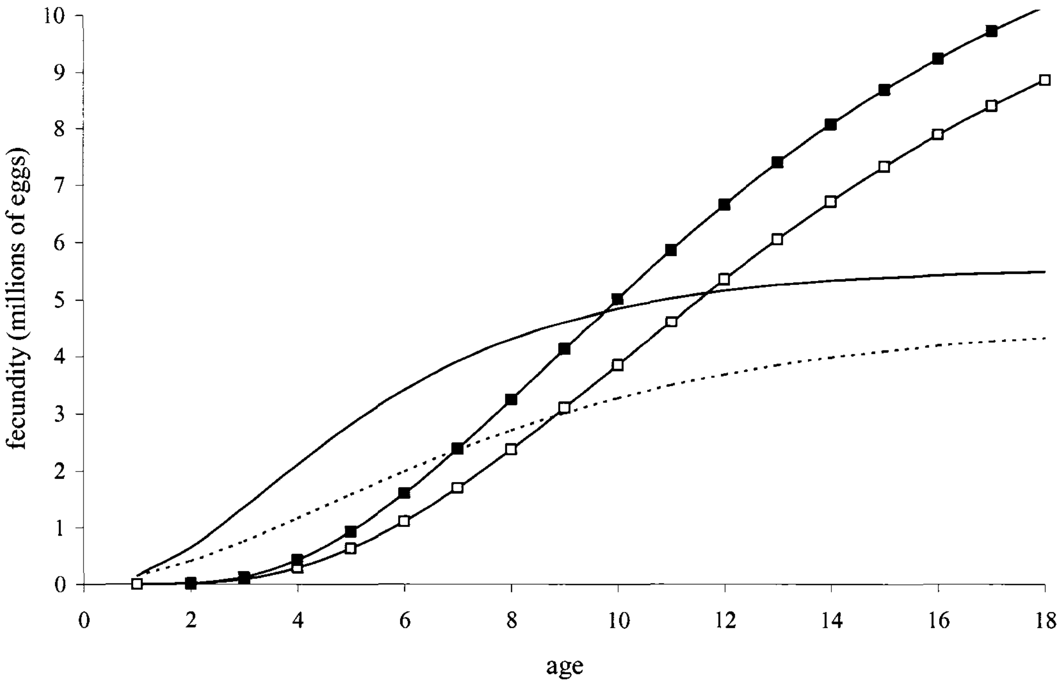

Figure 1.6. Fecundity at age in four Pacific cod stocks. Solid line with no symbols, WVI; broken line, HEC; line with open squares, GOA; line with solid squares, BSAI.

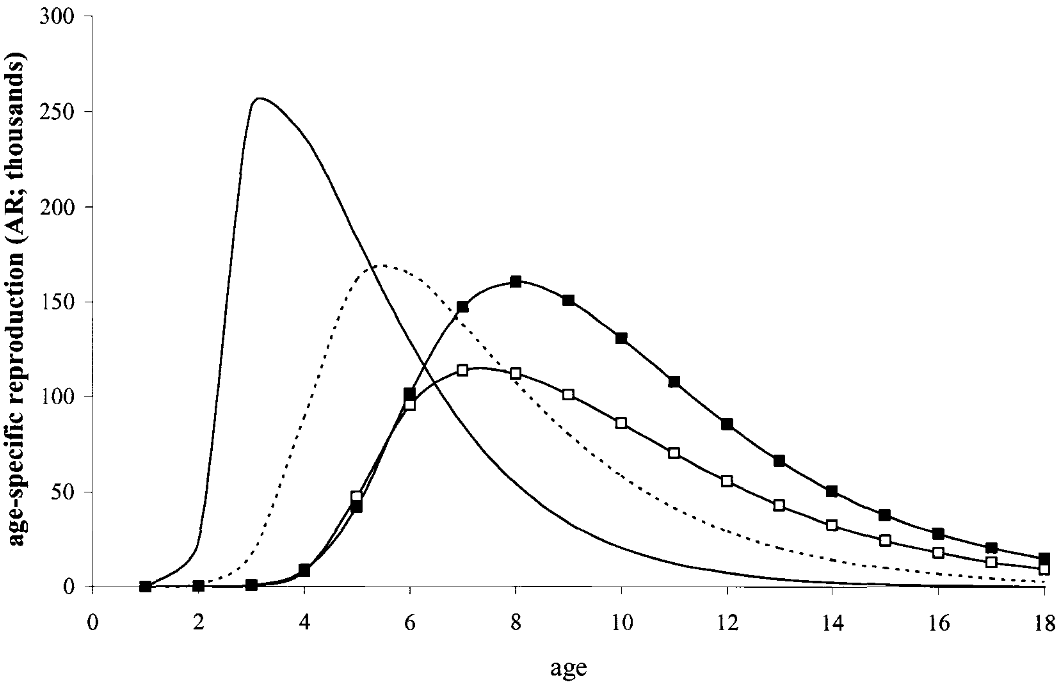

Figure 1.7. Age-specific reproduction (AR) for four Pacific cod stocks. Solid line with no symbols is WVI, broken line is HEC, line with open squares is GOA, line with solid squares is BSAI.

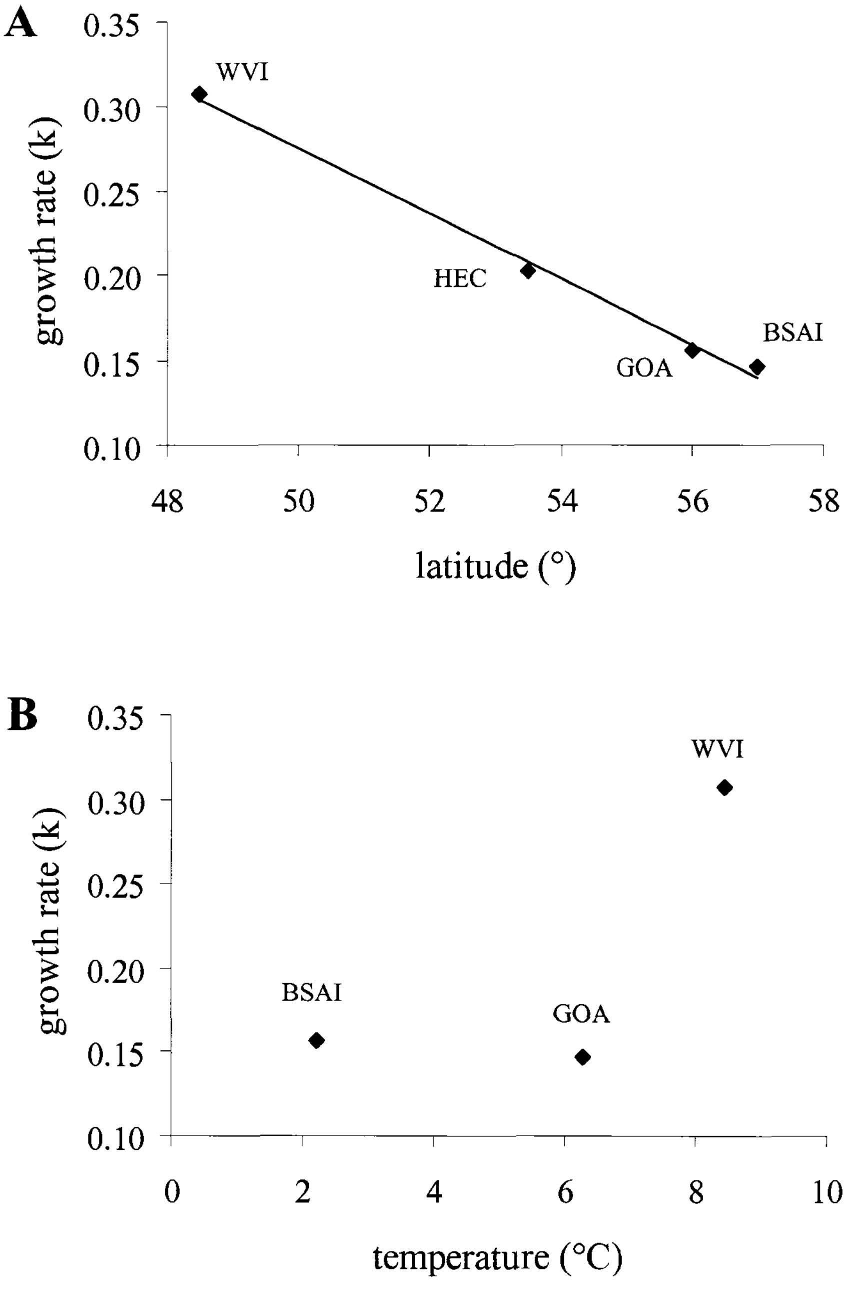

Figure 1.8. Latitude (A) and temperature (B) effects on growth rate in various Pacific cod stocks. Pacific cod stocks are named on figure. Least-squares regression line for latitude is indicated on the figure.

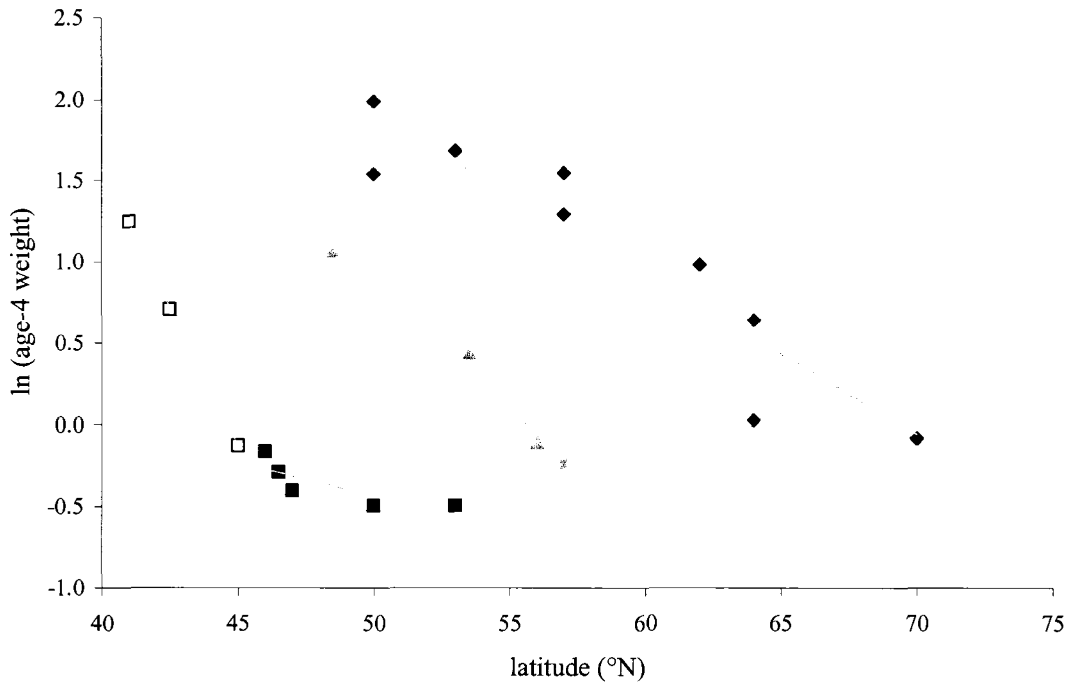

Figure 1.9. Latitude versus ln(age-4 wt.) for Atlantic and Pacific cod stocks. Atlantic cod stocks: black diamonds, Europe; open squares, southern North America; solid squares, northern North America. Grey triangles are Pacific cod. Least-squares regression lines for each group of stocks are indicated on the figure.

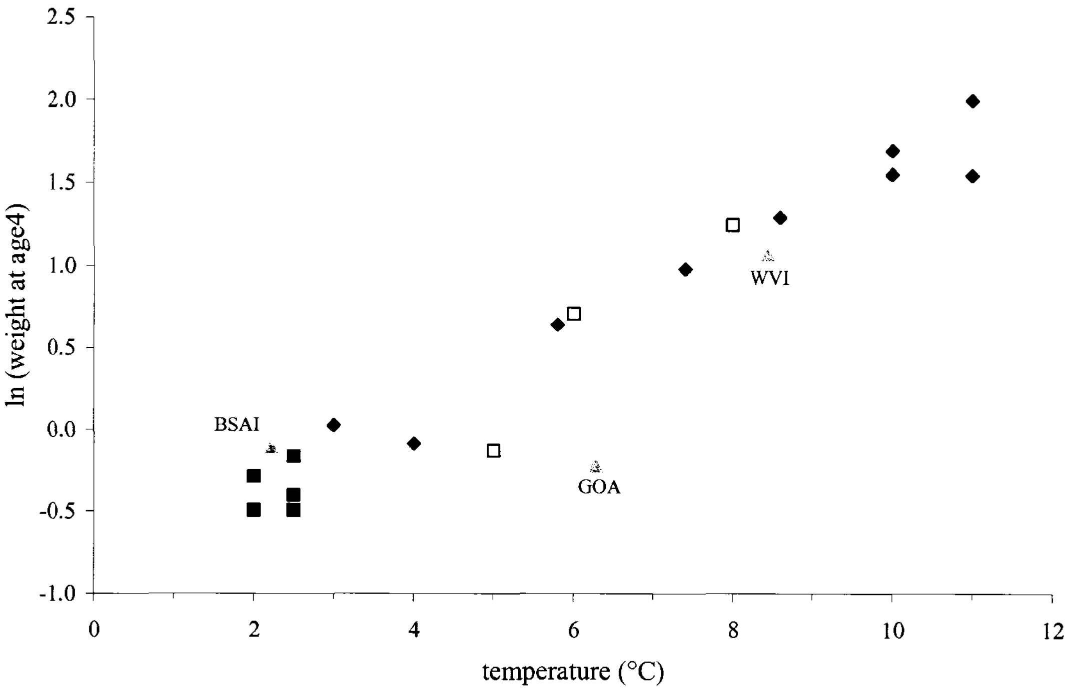

Figure 1.10. Temperature versus ln(age-4 wt.) for Atlantic and Pacific cod stocks. Atlantic cod stocks: black diamonds, Europe; open squares, southern North America; solid squares, northern North America. Grey triangles are Pacific cod.

Table 1.1. Life history parameters for four North American Pacific cod stocks. T, annual average temperature at 75 m; M, instantaneous natural mortality rate; k, growth parameter from the von Bertalanffy growth model (LVB); L_∞, asymptotic length from LVB; A_{50}, age at 50% maturity; L_{50}, length at 50% maturity; α and β, parameters of the power equation for fecundity. Sources for data are listed as footnotes.

	WVI	HEC	GOA	BSAI
Latitude (°N)	48.5	53.5	57	56
T (°C)	8.4	n/a	6.3	2.2
M	0.55[a,b,c]	0.40[a,b]	0.38[a,d]	0.36[a,e]
k	0.307[a]	0.203[a]	0.147[d]	0.157[e]
L_∞ (cm)	89[a]	84[a]	105[d]	113[e]
age-4 wt. (kg)	2.9[a,h]	1.5[a,h]	0.8[d]	0.9[e]
A_{50}	2.4[a,h]	4.3[a,h]	5.0[f]	5.8[f]
L_{50} (cm)	48[a,h]	54[a,h]	50[f]	58[f]
α	12.1[a]	12.1[a]	0.1[g]	2.5[g]
β	2.9[a]	2.9[a]	4.0[g]	3.3[g]

a) Westrheim (1996)
b) Sinclair et al. (2001)
c) Starr et al. (2002)
d) Thompson et al. (2004)
e) Thompson and Dorn (2004)
f) J. Stark, NOAA/NMFS, pers. comm.
g) O. Ormseth, unpubl. data
h) involved calculations by the authors

Table 1.2. Values for Atlantic cod stocks included in latitude/ temperature/ growth comparisons. T (average annual temperature) and age-4 weight are from Brander (1994). Latitudes were estimated from official descriptions of the various stocks.

	Stocks and complexes	Latitude (°N)	T (°C)	Age-4 weight (kg)
	Atlantic cod: Europe			
1)	Greenland	64	3.0	1.0
2)	NE Arctic	70	4.0	0.9
3)	Iceland	64	5.8	1.9
4)	Faroe	62	7.4	2.7
5)	W Scotland	57	10.0	4.7
6)	North Sea	57	8.6	3.6
7)	Celtic Sea	50	11.0	7.3
8)	Irish Sea	53	10.0	5.4
9)	Eastern Channel	50	11.0	4.7
	Atlantic cod: northern N. America			
10)	Labrador	53	2.0	0.6
11)	S Grand Bank	46	2.5	0.9
12)	N Gulf of St Lawrence	50	2.5	0.6
13)	S Gulf of St Lawrence	47	2.5	0.7
14)	St Pierre Bank	46.5	2.0	0.8
	Atlantic cod: southern N. America			
15)	E Scotian Shelf	45	5.0	0.9
16)	W Scotian Shelf	42.5	6.0	2.0
17)	Georges Bank	41	8.0	3.5

Table 1.3. Lifetime reproductive success (R_0) for individuals from four North American Pacific cod stocks.

Stock	R_0
West Vancouver Island	1.05×10^6
Hecate Strait	0.95×10^6
Gulf of Alaska	0.83×10^6
Bering Sea / Aleutian Islands	1.15×10^6

Literature Cited

Angilletta, M. J., Wilson, R. S., Navas, C. A., and James, R. S. 2003. Tradeoffs and the evolution of thermal reaction norms. Trends in Ecology and Evolution, 18: 234-240.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. Quarterly Reviews in Biology, 72: 149–177.

Bailey, K. M. 2000. Shifting control of recruitment of walleye pollock *Theragra chalcogramma* after a major climatic and ecosystem change. Marine Ecology Progress Series, 198: 215-224.

Bakkala, R.G., Thompson, G.G., and Shimada, A.M. 1986. Pacific cod. In Condition of groundfish resources of the eastern Bering Sea and Aleutian Islands Region in 1985, pp. 31-48. Ed. by R. G. Bakkala and L.-L. Low. NOAA Technical Memorandum NMFS F/NWC-104.

Beamish, R. J. 1993. Climate and exceptional fish production off the west coast of North America. Canadian Journal of Fisheries and Aquatic Sciences, 50: 2270-2291.

Benson, A. J., and Trites, A. W. 2002. Ecological effects of regime shifts in the Bering Sea and eastern North Pacific Ocean. Fish and Fisheries, 3: 95-113.

Beverton, R. J. H. 1987. Longevity in fish: some ecological and evolutionary perspectives. In Ageing processes in animals, pp. 161-186. Ed. by A. D. Woodhead, M. Witten, and K. Thompson. Little, Brown and Company, Boston.

Beverton, R. J. H. 1992. Patterns of reproductive strategy parameters in some marine teleost fishes. Journal of Fish Biology, 41 (Supplement B): 137-160.

Brander, K. M. 1994. Patterns of distribution, spawning, and growth in North Atlantic cod: the utility of inter-regional comparisons. ICES Marine Science Symposia 198: 406-413.

Brander, K. M. 1995. The effect of temperature on growth of Atlantic cod (*Gadus morhua* L.). ICES Journal of Marine Science, 52: 1-10.

Brander, K. M. 1997. Effects of climate change on cod (*Gadus morhua*) stocks. In Global Warming: Implications for freshwater and marine fish, pp. 255-278. Ed. by C. M. Wood and D. G. McDonald. Cambridge University Press, Cambridge.

Charnov, E. L. 1993. Life history invariants. Oxford University Press, New York.

Clark, W. G. 1991. Groundfish exploitation rates based on life history parameters. Canadian Journal of Fisheries and Aquatic Sciences, 48: 734-750.

Conover, D. O., and Present, T. M. C. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia, 83: 316-324.

Drinkwater, K. F. 2002. A review of the role of climate variability in the decline of northern cod. American Fisheries Society Symposium, 32: 113-130.

Fargo, J., and Wilderbuer, T. 2000. Population dynamics of rock sole (*Lepidopsetta bilineata*) in the North Pacific. Journal of Sea Research, 44: 123-144.

Foucher, R.P., and Tyler, A.V. 1988. Pacific cod. In Groundfish stock assessments for the west coast of Canada in 1987 and recommended yield options for 1988, pp. 45-74. Ed. by J. Fargo, M. W. Saunders, and A.V. Tyler. Canadian Technical Reports in Fisheries and Aquatic Sciences 1617.

Fritz, L. W., Greig, A., and Reuter, R. F. 1998. Catch-per-unit-effort, length, and depth distributions of major groundfish and bycatch species in the Bering Sea, Aleutian Islands, and Gulf of Alaska regions based on groundfish fishery observer data. NOAA Technical Memorandum NMFS-AFSC-88.

Grant, W. S., and Stahl, G. 1988. Evolution of Atlantic and Pacific cod: Loss of genetic variation and gene expression in Pacific cod. Evolution, 42: 138-146.

Grant, W. S., Zhang, C. I., and Kobayashi, T. 1987. Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Canadian Journal of Fisheries and Aquatic Sciences, 44: 490-498.

Hassol, S. J. 2004. Impacts of a warming Arctic: Arctic Climate Impact Assessment. Cambridge University Press, Cambridge.

Jennings, S., and Beverton, R. J. H. 1991. Intraspecific variation in the life history tactics of Atlantic herring (*Clupea harengus* L.) stocks. ICES Journal of Marine Science, 48: 117-125.

Jennings, S., Reynolds, J. D., and Mills, S. C. 1998. Life history correlates of responses to fisheries exploitation. Proceedings of the Royal Society of London B, 265: 333-339.

Jobling, M. 1993. Bioenergetics: feed intake and energy partitioning. In Fish Ecophysiology, pp. 1-44. Ed. by J. C. Rankin, and F. B. Jensen. Chapman & Hall, London.

Jobling, M. 1997. Temperature and growth: modulation of growth rate via temperature change. In Global Warming: Implications for freshwater and marine fish, pp. 225-254. Ed. by C. M. Wood, and D. G. McDonald. Camridge University Press, Cambridge.

King, J. R., and McFarlane, G. A. 2003. Marine fish life history strategies: applications to fishery management. Fisheries Management and Ecology, 10: 249-264.

Law, R. 2000. Fishing, selection, and phenotypic evolution. ICES Journal of Marine Science, 57: 659-668.

Leggett, W. C., and Carscadden, J. E. 1978. Latitudinal variation in reproductive characteristics of American shad *Alosa sapidissima*: evidence for population specific life history strategies in fish. Journal of the Fisheries Research Board of Canada, 35: 1469-1478.

Levesque, H. M., Short, C., Moon, T. W., Ballantyne, J. S., and Driedzic, W. R. 2005. Effects of seasonal temperature and photoperiod on Atlantic cod (*Gadus morhua*). I. Morphometric parameters and metabolites. Canadian Journal of Fisheries and Aquatic Sciences, 62: 2854-2863.

Lluch-Cota, D. B., Wooster, W. S., and Hare, S. R. 2001. Sea surface temperature variability in coastal areas of the Northeastern Pacific related to the El Nino-

Southern Oscillation and the Pacific Decadal Oscillation. Geophysical Research Letters, 28: 2029-2032.

Longhurst, A. 1998. Cod: perhaps if we all stood back a bit? Fisheries Research, 38: 101-108.

Longhurst, A. 2002. Murphy's law revisited: longevity as a factor in recruitment to fish populations. Fisheries Research, 56: 125-131.

Mantua, N. J., and Hare, S. R. 2002. The Pacific Decadal Oscillation. Journal of Oceanography, 58: 35-44.

Marshall, C. T., Kjesbu, O. S., Yaragina, N. A., Solemdal, P., and Ulltang, O. 1998. Is spawner biomass a sensitive measure of the reproductive and recruitment potential of Northeast Arctic cod? Canadian Journal of Fisheries and Aquatic Sciences, 55: 1766-1783.

McGowan, J. A., Cayan, D. R., and Dorman, L. M. 1998. Climate-ocean variability and ecosystem response in the northeast Pacific. Science, 281: 210-217.

Mello, L. G. S., and Rose, G. A. 2005. Seasonal growth of Atlantic cod: effects of temperature, feeding and reproduction. Journal of Fish Biology, 67: 149-170.

Neuheimer, A. B., and Taggart, C. T. 2007. The growing degree-day and fish size-at-age: the overlooked metric. Canadian Journal of Fisheries and Aquatic Sciences, 64: 375-385.

Niewiarowski, P. H. 2001. Energy budgets, growth rates, and thermal constraints: toward an integrative approach to the study of life-history variation. The American Naturalist, 157: 421-433.

Ottersen, G., Michalsen, K., and Nakken, O. 1998. Ambient temperature and distribution of north-east Arctic cod. ICES Journal of Marine Science, 55: 67-85.

Partridge, L., and Coyne, J. A. 1997. Bergmann's rule in ectotherms: is it adaptive? Evolution, 51: 632-635.

Partridge, L., and Sibly, R. 1991. Constraints in the evolution of life histories. Philosophical Transactions of the Royal Society of London B, 332: 3-13.

Perry, R. I., Stocker, M., and Fargo, J. 1994. Environmental effects on the distributions of groundfish in Hecate Strait, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences, 51: 1401-1409.

Quinn, T. J., and Deriso, R. B. 1999. Quantitative fish dynamics. Oxford University Press, New York.

Salvanes, A. G. V., Skjaeraasen, J. E., and Nilsen, T. 2004. Sub-populations of coastal cod with different behaviour and life-history strategies. Marine Ecology Progress Series, 267: 241-251.

Schmitz, W.J. Jr., and McCartney, M.S. 1993. On the North Atlantic circulation. Reviews of Geophysics, 31: 29-50

Shimada, A. M., and Kimura, D. K. 1994. Seasonal movements of Pacific cod, *Gadus macrocephalus*, in the eastern Bering Sea and adjacent waters based on tag-recapture data. Fishery Bulletin, 92: 800-816.

Sinclair, A., Martell, S., and Boutillier, J. 2001. Assessment of Pacific cod off the west coast of Vancouver Island and in Hecate Strait, November 2001. Department of Fisheries and Oceans Canada, Nanaimo, BC.

Starr, P. J., Sinclair, A. S., and Boutillier, J. 2002. West coast Vancouver Island Pacific cod assessment. Department of Fisheries and Oceans Canada, Nanaimo, BC.

Stein, M., and Borovkov, V.A. 2004. Greenland cod (*Gadus morhua*): modeling recruitment variation during the second half of the 20th Century. Fisheries Oceanography, 13: 111-120.

Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. BioScience, 39: 436-445.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford.

Thompson, G. G., and Dorn, M. W. 2004. Assessment of the Pacific cod stock in the eastern Bering Sea and Aleutian Islands area. In Stock assessment and fishery evaluation report for the groundfish resources of the Bering Sea/ Aleutian Islands regions, pp. 185-302. Ed. by Plan Team for Groundfish Fisheries of the Bering Sea and Aleutian Islands. North Pacific Fishery Management Council, Anchorage, AK.

Thompson, G.G., and Methot, R.D. 1993. Pacific cod. In Stock assessment and fishery evaluation report for the groundfish resources of the Bering Sea/ Aleutian Islands regions as projected for 1994, Chapter 2. Ed. by Plan team for the groundfish fisheries of the Bering Sea and Aleutian Islands. North Pacific Fishery Management Council, Anchorage, AK.

Thompson, G.G., and Shimada, A.M. 1988. Pacific cod. In Condition of groundfish resources of the eastern Bering Sea and Aleutian Islands Region in 1987, pp. 33-49. Ed. by R. G. Bakkala. NOAA Technical memorandum NMFS F/NWC-139.

Thompson, G.G., and Shimada, A.M. 1990. Pacific cod. In Condition of groundfish resources in the Bering Sea- Aleutian Islands Region as assessed in 1988, pp. 44-66. Ed. by L.-L. Low and R.E. Nerita, R.E.NOAA Technical memorandum NMFS F/NWC-178.

Thompson, G.G., and Zenger, H.H. 1995. Pacific cod. In Stock assessment and fishery evaluation report for the groundfish resources of the Gulf of Alaska as projected for 1996, Chapter 2. Ed. by Plan team for the groundfish fisheries of the Bering Sea and Aleutian Islands. North Pacific Fishery Management Council, Anchorage, AK.

Thompson, G. G., Zenger, H. H., and Dorn, M. W. 2004. Assessment of the Pacific cod stock in the Gulf of Alaska. In Stock assessment and fishery evaluation report for the groundfish resources of the Gulf of Alaska, pp. 131-232. Ed. by Plan Team for Groundfish Fisheries of the Gulf of Alaska. North Pacific Fishery Management Council, Anchorage, AK.

Welch, D. W., Ishida, Y., and Nagasawa, K. 1998. Thermal limits and ocean migrations of sockeye salmon (*Oncorhynchus nerka*): long term consequences of global warming. Canadian Journal of Fisheries and Aquatic Sciences, 55: 937-948.

Wespestad, V.G., Shimada, A.M., and June, J.A. 1986. Recruitment variations in Pacific cod and its effects in the Bering Sea and Gulf of Alaska. Bulletin of the International North Pacific Fisheries Commission, 45: 295-304.

Westrheim, S. J. 1996. On the Pacific cod (*Gadus macrocephalus*) in British Columbia waters, and a comparison with Pacific cod elsewhere, and Atlantic cod (*Gadus morhua*). Canadian Technical Report of Fisheries and Aquatic Sciences, No. 2092.

Westrheim, S.J., and Foucher, R.P. 1985. Pacific cod. In Groundfish stock assessments for the west coast of Canada in 1984 and recommended yield options for 1985, pp. 57-116. Ed. by A. V. Tyler and G. A. McFarlane. Canadian Manuscript Reports in Fisheries and Aquatic Sciences, No. 1813.

Winemiller, K. O. 1992. Life-history strategies and the effectiveness of sexual selection. Oikos, 63: 318-327.

Winemiller, K. O. 2005. Life history strategies, population regulation, and implications for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences, 62: 872-885.

Winemiller, K. O., and Rose, K. A. 1992. Patterns of life-history diversification in North American fishes: implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences, 49: 2196-2218.

Wootton, R. J. 1990. Ecology of teleost fishes. Chapman & Hall, London.

Zenger, H.H., and Thompson, G.G. 1993. Pacific cod. In Stock assessment and fishery evaluation report for the groundfish resources of the Gulf of Alaska as projected for 1994, Chapter 2. Ed. by Plan team for the groundfish fisheries of the Bering Sea and Aleutian Islands. North Pacific Fishery Management Council, Anchorage, AK.

Appendix 1.A. Values and data sources used in estimation of natural mortality rates (M). Subheadings under Sinclair et al. (2001) refer to alternative stock assessment models included in the document.

source	WVI	HEC	GOA	BSAI
Westrheim and Foucher (1985)	0.58	0.65		
Foucher and Tyler (1988)	0.69	0.61		
Sinclair et al. (2001):				
-all yr, no sea level	0.42	0.23		
-all yr, sea level		0.23		
-to 1995, sea level (HEC)	0.46	0.29		
Starr et al. (2002)	0.58			
Zenger and Thompson (1993)			0.27	
Thompson & Zenger (1995)			0.50	
Thompson et al. (2004)			0.37	
Bakkala et al. (1986)				0.44
Wespestad et al. (1986)				0.50
Thompson & Shimada (1988)				0.22
Thompson & Shimada (1990)				0.29
Thompson & Methot (1993)				0.35
Thompson and Dorn (2004)				0.37
Average	**0.55**	**0.40**	**0.38**	**0.36**

Appendix 1.B. Values used to calculate age-specific reproduction. Values were calculated for ages 1-18: s_x, survival rate to age x; p_x, probability of being mature at age x; f_x, estimated fecundity at age x in millions of eggs.

	s_x				p_x				f_x			
age	WVI	HEC	GOA	BSAI	WVI	HEC	GOA	BSAI	WVI	HEC	GOA	BSAI
1	0.579	0.669	0.684	0.697	0	0	0	0	1.5	1.5	1.6	-
2	0.336	0.448	0.468	0.485	0.11	0.01	0	0	6.5	4.1	1.5	1.1
3	0.194	0.299	0.320	0.338	0.96	0.08	0.02	0.02	1.4	7.6	9.8	1.3
4	0.113	0.200	0.219	0.235	1	0.38	0.13	0.09	2.1	1.2	3.0	4.3
5	0.065	0.134	0.150	0.164	1	0.76	0.50	0.27	2.8	1.6	6.4	9.3
6	0.038	0.090	0.102	0.114	1	0.93	0.84	0.56	3.4	2.0	1.1	1.6
7	0.022	0.060	0.070	0.080	1	0.97	0.96	0.78	3.9	2.4	1.7	2.4
8	0.013	0.040	0.048	0.055	1	0.99	0.99	0.89	4.3	2.7	2.4	3.2
9	0.007	0.027	0.033	0.039	1	0.99	1	0.95	4.6	3.0	3.1	4.1
10	0.004	0.018	0.022	0.027	1	1	1	0.97	4.8	3.3	3.9	5.0
11	0.002	0.012	0.015	0.019	1	1	1	0.98	5.0	3.5	4.6	5.9
12	0.001	0.008	0.010	0.013	1	1	1	0.99	5.2	3.7	5.3	6.7
13	0.001	0.005	0.007	0.009	1	1	1	0.99	5.2	3.8	6.0	7.4
14	0	0.004	0.005	0.006	1	1	1	0.99	5.3	4.0	6.7	8.1
15	0	0.002	0.003	0.004	1	1	1	1	5.4	4.1	7.3	8.7
16	0	0.002	0.002	0.003	1	1	1	1	5.4	4.2	7.9	9.2
17	0	0.001	0.002	0.002	1	1	1	1	5.4	4.3	8.4	9.7
18	0	0.001	0.001	0.001	1	1	1	1	5.5	4.3	8.8	1.0

Appendix 1.C. Values used in the egg production-per-recruit model. WVI, West Vancouver Island stock; BSAI, Bering Sea/Aleutian Islands stock. "Full recruit", "trawl", and "longline" refer to models with alternative patterns of fishery selectivity. Fecundity (in millions of eggs) was estimated from published values and preliminary data. Selectivity, age-specific fisheries selectivity based on stock assessments and an unpublished projection model for the BSAI trawl fishery. fec, fecundity; sel, selectivity; M, natural mortality rate.

	WVI				BSAI				
			full recruit	trawl			full recruit	trawl	longline
age	fec	M	sel	sel	fec	M	sel	sel	sel
1	0	0.55	0	0.017	0	0.36	0	0	0
2	0	0.55	1	0.067	0	0.36	0	0.003	0.006
3	1.4	0.55	1	0.387	0	0.36	1	0.017	0.088
4	2.1	0.55	1	0.581	0	0.36	1	0.067	0.396
5	2.8	0.55	1	0.655	0	0.36	1	0.193	0.740
6	3.4	0.55	1	0.729	1.6	0.36	1	0.387	0.888
7	3.9	0.55	1	0.827	2.4	0.36	1	0.581	0.877
8	4.3	0.55	1	0.888	3.2	0.36	1	0.729	0.808
9	4.6	0.55	1	0.924	4.1	0.36	1	0.827	0.742
10	4.8	0.55	1	0.953	5	0.36	1	0.888	0.698
11	5	0.55	1	0.953	5.9	0.36	1	0.924	0.672
12	5.2	0.55	1	0.953	6.7	0.36	1	0.953	0.650
13	5.2	0.55	1	0.953	7.4	0.36	1	0.953	0.650
14	5.3	0.55	1	0.953	8.1	0.36	1	0.953	0.650
15	5.4	0.55	1	0.953	8.7	0.36	1	0.953	0.650
16	5.4	0.55	1	0.953	9.2	0.36	1	0.953	0.650
17	5.4	0.55	1	0.953	9.7	0.36	1	0.953	0.650
18	5.5	0.55	1	0.953	10	0.36	1	0.953	0.650

CHAPTER TWO

Proximate, lipid class, and free amino acid composition of Pacific cod eggs[1]

Abstract

We investigated the proximate, lipid class, and free amino acid (FAA) composition of eggs from Pacific cod (*Gadus macrocephalus*), a common fish species in the North Pacific Ocean that is closely related to Atlantic cod (*Gadus morhua*). Pacific cod eggs contain 82.9% water. Of the egg dry mass, 10.9% is lipid and 76.3% is protein and FAAs. Polar lipids form 78.9% of the total lipids, and FAAs make up 12.3% of the total amino acid pool. These results indicate that the composition of Pacific cod eggs is typical of low-lipid marine fish eggs that lack oil globules. Pacific cod eggs are similar in many ways to eggs of Atlantic cod, but Pacific cod eggs are demersal while those from Atlantic cod are pelagic. The difference appears to be due to reduced amounts of FAAs in Pacific cod relative to Atlantic cod that result in reduced egg hydration and negative buoyancy.

Introduction

Egg survival is a critical determinant of reproductive success for all oviparous fishes. Females can influence egg survival through physiological means, such as the provisioning of the egg ovoplasm with adequate energetic and structural resources, and through behavioral adaptations like nest guarding or providing optimal habit for egg development (Wootton 1990; Brooks et al. 1997). Egg provisioning is the most direct

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[1] Ormseth OA, Buck CL, and Norcross BL. 2007. Proximate, lipid class, and free amino acid composition of Pacific cod eggs. Prepared for submission to Marine Biology.

way that females can control offspring survival, and determining the resource requirements of a species' eggs is important for understanding their reproductive success.

Eggs of marine fish can be divided into two groups based on the presence or absence of an oil globule (Wiegand 1996; Rønnestad et al. 1999; Riis-Vestergaard 2002). Eggs with oil globules are richer in neutral lipids, particularly triacylglycerols (TAGs), that are used primarily as energy substrates (Wiegand 1996). For embryos in these eggs, the amount of neutral lipid in the globule can be crucial for larval survival (Berkeley et al. 2004). Eggs without oil globules tend to have low total lipid that is composed mainly of polar lipids (Wiegand 1996). This type of egg generally has less energy available for embryos, and the embryos have a greater reliance on the catabolism of amino acids (Finn et al. 1995; Rønnestad et al. 1999; Riis-Vestergaard 2002).

Pacific cod (*Gadus macrocephalus*) is a large demersal fish species distributed throughout the North Pacific Ocean. It is a major commercial fishery target in several countries and also serves important ecosystem roles as predators and prey. Despite its importance in the North Pacific, the basic biology of Pacific cod eggs remains largely unexplored. Depending on their geographical location, Pacific cod spawn from December through March (Hattori et al. 1992; Westrheim 1996). Females release all of their eggs in a single event (Hattori et al. 1992) and the eggs are demersal (Sakurai and Hattori 1996; Westrheim 1996). While cod egg and larval development are not well understood, it appears that the eggs remain on the bottom for approximately two weeks until they hatch and the larvae ascend into the upper layers of the water column (Rugen and Matarese 1988). Pacific cod are thought to be derived from Atlantic cod (*Gadus* *morhua*) as the result of an invasion into the Pacific Ocean 3.0-3.5 million years ago (Grant and Stahl 1988; Carr et al. 1999). The two species are morphologically and ecologically similar, but Atlantic cod spawn pelagic eggs.

The goal of this study was to characterize the composition of Pacific cod eggs and to understand how it relates to their ecological requirements. Specifically, we examined the hypothesis that the composition of Pacific cod eggs will reflect adaptations for negative buoyancy and a relatively short period of dependence on endogenous energy reserves. We also expect that Pacific and Atlantic cod eggs will have similar compositions except for adaptations that affect egg buoyancy.

Methods

Eggs were collected from spawning female Pacific cod from two areas, approximately 1500 km apart, in different years (Fig. 2.1). March 20-24, 2004, eggs were collected from seven females in the eastern Bering Sea (EBS) in a 30 nm^2 area approximately 5 nm north of Unimak Island (54°46'N, 164°8'W). February 26 to March 4 of 2005, eggs were collected from 21 females in a 600 nm^2 area centered on the Near Islands at the western end of the Aleutian Islands (AI; approximate location 52°51'N, 173°11'E). Fish from the EBS were caught using pot gear during a research survey; AI samples were collected aboard a commercial factory trawler. Pacific cod were collected under federal Scientific Research Permit 2003-26, and this research was approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks (Project #04-61).

Females in spawning condition were randomly selected from the catch and selected for use in this study according to a schedule of length-bins (40-120 cm in 5-cm increments) designed to provide samples from across a spectrum of age and size. Females were considered to be in spawning condition if eggs were loose and could be freely extruded from the gonopore by pressing the abdomen. Eggs flowing from the gonopore were collected in 2 ml cryovials and immediately frozen in liquid nitrogen for free amino acid (FAA) and lipid analysis. Eggs were initially stored in liquid nitrogen and then transferred to a -80°C freezer upon return to the laboratory. Additional eggs were collected in plastic vials and frozen at -20°C for determination of water and total amino acid (free and protein amino acid) contents.

Water and total amino acid (TAA) content were analyzed at the University of Alaska Fairbanks. Weighed eggs (~20 g) were freeze-dried until the sample weight did not change over a 24-hour period (usually 4 days). Weight lost was used to calculate % water content. Eggs were homogenized using a mortar and pestle, and a subsample of the homogenate was used for analysis of total nitrogen (N) content by the combustion method (Sweeney and Rexroad 1987). A weighed subsample (~100 mg) was inserted into a Leco TruSpec nitrogen analyzer (Leco Corp., St. Joseph, MI, USA) and combusted to convert all N into NO_x gases. The NO_x gases were reduced to N_2, and the N_2 was measured using thermal conductivity. TAA content was calculated by multiplying the nitrogen content by the standard conversion factor 6.25 (AOAC 1995). Ash and carbohydrate contents were not measured separately and were calculated by subtracting the lipid and TAA contents from total dry mass.

Free amino acid analysis was conducted by Scientific Research Consortium (St. Paul, MN, USA; www.aminoacids.com). Frozen samples were homogenized and the liquefied sample was transferred to a 1.5 ml microfuge tube and weighed. To remove the protein amino acids, sulfosalicylic acid/ glucosaminic acid was added and the sample was vortexed thoroughly. The sample was centrifuged at 15,000 rpm, and the supernatant was removed and refrigerated at 4°C for one hour. The supernatant was diluted using sulfosalicylic acid/ glucosaminic acid and Beckman Li-S solution (Beckman Coulter, Inc., Fullerton, CA, USA). The sample was vortexed and centrifuged and the supernatant removed and refrigerated at 4°C for one hour. The samples were removed from the refrigerator and immediately injected into a Beckman Instruments Model 7300 HPLC amino acid analyzer (Beckman Coulter, Inc., Fullerton, CA, USA). Absorbance was measured at 440 and 570 nm following post-column color development by ninhydrin reagent at 131°C. Data acquisition and management was accomplished using Beckman System Gold 8.10 chromatography software (Beckman Coulter, Inc., Fullerton, CA, USA). Percentage of protein amino acid (PAA) in the TAAs was calculated by subtracting the percentage of FAAs from 100%.

Quantification of total lipids, polar lipids, TAGs, and free fatty acids (FFAs) was conducted by Lipid Technologies, LLC (Austin, MN, USA). Egg lipids were extracted using a 2:1 chloroform/methanol mixture according to the method of Bligh and Dyer (1959). Total mass of egg lipid was calculated and is presented as a percentage of wet mass; this value was combined with water content data for each female to calculate total lipid as a percentage of dry mass. Lipid extracts were maintained under nitrogen and kept frozen until further analyses were performed. Immediately prior to lipid class separation, lipid samples were dried under nitrogen and resuspended in chloroform. Lipid classes were separated on commercial silica gel G plates (AnalTech, Inc., Newark, DE, USA). The chromatographic plates were developed in a solvent system consisting of distilled petroleum ether: diethyl ether: acetic acid (80:20:1, v/v/v). Following development, silica gel plates were sprayed with a methanolic solution containing 0.5%

2,7-dichlorofluorescein and lipid classes were visualized under ultraviolet light. Desired lipid bands were then scraped into Teflon® screw-cap tubes. Samples were transesterified with boron trifluoride (10%) in excess methanol in an 80°C water bath for 90 minutes. Resulting fatty acid methyl esters were extracted with water and petroleum ether and stored frozen until gas chromatographic analysis was performed.

Fatty acid composition was determined using capillary gas chromatography. Methyl esters were dried under nitrogen, resuspended in hexane, and separated and quantified using a Shimadzu GC17capillary gas chromatograph (Shimadzu Corp., Kyoto, Japan) utilizing a 30m Restek free fatty acid phase coating (Restek Corp., Bellefonte, PA, USA). Chromatographic data were collected and processed with EZChrom software (Scientific Software, Inc., Pleasanton, CA, USA). Fatty acids were identified by comparison to authentic fatty acid standards and quantified with peak area and internal standard. Individual fatty acid values were totaled to obtain a total mass for each lipid class.

Energy density was calculated by multiplying the total lipid, PAA, and FAA fractions by their energy equivalents and summing the resulting values. We used energy equivalents determined to be specifically appropriate for fish egg components (Riis-Vestergaard 2002): 35.6 Joules/mg (J/mg) for lipids, 20.8 J/mg for FAAs and 18.2 J/mg for PAAs.

Because percentage values have a binomial distribution, all data were arcsine square root transformed prior to calculation of mean values (Zar 1999). Means were backtransformed for reporting purposes. To generate standard errors (SEs) for the backtransformed means, we calculated 95% confidence intervals (CIs) using the transformed data. We then backtransformed the CIs, averaged the upper and lower CI for each mean, and used the resulting value to calculate the SE using the formula (CI= $t_{\alpha/2}$ x SE) and the appropriate values for $t_{\alpha/2}$.

Results

Water and total amino acids were the largest components of Pacific cod eggs (Table 2.1). Water content of Pacific cod eggs averaged 82.9% and variability among eggs from different females was low. Total amino acids made up 76.3% of the egg dry mass, and 12.3% of this fraction was formed of FAAs. Total lipid formed 10.9% of the egg dry mass and 78.9% of this was in the form of polar lipid. Triacylglycerols constituted 48% of the neutral lipids. Egg energy density was 18.03 J/mg, and the protein amino acids were the largest energetic fraction in the egg (Table 2.1).

Six amino acids formed the greater part of the free amino acid fraction. Leucine and lysine were the predominant essential amino acids (EAA), and the largest non-essential amino acid (NEAA) components were alanine, serine, glutamine, and taurine (Fig. 2.2). Variability among eggs from different females was low, as indicated by the size of the standard errors (Fig. 2.2). Variability within NEAAs was greater than with EAAs.

Discussion

Eggs of Pacific cod are typical of marine fish eggs lacking oil globules, with low total lipid that is made up primarily of polar lipids. Low lipid and energy density in Pacific cod eggs may be due to short hatching times and reduced energy requirements for developing embryos. The compositions of Pacific and Atlantic cod eggs are similar in many ways. However, eggs of Pacific cod have a lower concentration of FAAs that likely reduces the degree of egg hydration and causes Pacific cod eggs to be less buoyant than eggs of Atlantic cod.

The total lipid and lipid class composition of Pacific cod eggs are similar to values observed in Atlantic cod (Table 2.1). Both species have low concentrations of total lipids, and polar lipids are the most abundant lipid class. Thus, eggs from Pacific and Atlantic cod are typical of marine fish eggs that lack oil globules (Wiegand 1996; Riis-Vestergaard 2002). Consistent with previous observations in Japan (Hattori et al. 1992), we did not find oil globules in Pacific cod eggs from Alaska. They are also lacking in Atlantic cod (Riis-Vestergaard 2002).

A possible explanation for the lipid composition of Pacific and Atlantic cod eggs, and the lack of an oil globule, could be the low energetic requirements of the eggs. Eggs that hatch rapidly result in larvae that begin feeding sooner, so embryos are less reliant on yolk energy reserves (Wootton 1990). Consequently, eggs with fast-growing embryos tend to have lower lipid levels than those with slower-growing embryos (Tocher and Sargent 1984). Pacific cod eggs held at 6°C hatch in 14.4-15.9 days depending on salinity (Alderdice and Forrester 1971), nearly identical to hatching times observed in Atlantic cod eggs held at the same temperature (Finn et al. 1995). In contrast, eggs of lumpsucker (*Cyclopterus lumpus*) hatch in 41-45 days at 5°C and contain 21-26% lipid (Lønning et al. 1988), and eggs of sand eel (*Ammodytes lancea*) hatch in approximately 27 days at 8°C and contain 20% lipid (Tocher and Sargent 1984).

The key compositional differences between Pacific and Atlantic cod eggs appear to be those components that affect egg buoyancy (Table 2.1). Pacific cod eggs have less water than Atlantic cod eggs, 83% vs. 93% (Riis-Vestergaard 2002) and also contain a much lower fraction of free amino acids, 12% vs. 37% (Finn et al. 1995).

These differences support the hypothesis that buoyancy is achieved in pelagic eggs through a high concentration of FAAs that increases the osmolality of the eggs and cause water to enter the egg, reducing its density (Thorsen and Fyhn 1996; Thorsen et al. 1996; Finn et al. 2002a; Finn et al. 2002b).

A possible mechanism for reduced FAA content in Pacific cod can be illustrated by comparing the FAA profiles of Pacific cod, Atlantic cod, and an assemblage of tropical fishes that produce demersal eggs. Free amino acids that increase the osmolality of fish eggs are thought to result from the cleavage of yolk proteins prior to egg hydration (Finn et al. 2002a). The post-hydration FAA profile reflects the addition of these FAAs, which change the relative concentrations of FAAs in the egg (Finn et al. 2002a). The FAA profile of Atlantic cod eggs (Fig. 2.2) is similar in many ways to that of Pacific cod (Fig. 2.2), with roughly equal amounts of EAA and NEAA and more variability in the NEAA fraction. However, Pacific cod eggs have altered levels of several NEAAs and their taurine content is conspicuously higher than in Atlantic cod. Demersal eggs of tropical fishes have a lower total FAA content (2.6%; Rønnestad et al. 1999) than either Pacific or Atlantic cod, and have an FAA profile that is dominated by the NEAAs in general and taurine in particular (Fig. 2.2; Rønnestad et al. 1999). Developing pelagic eggs also have a high level of taurine, which is diluted by the addition of yolk-derived FAAs during hydration (Finn et al. 2002a). Thus, we believe the FAA composition of Pacific cod eggs results from mechanisms similar to those that occur in Atlantic cod but to a lesser degree, resulting in a less-diluted version of the FAA profile of Atlantic cod as well as lower total FAA and water content.

The buoyancy of marine fish eggs depends mainly on their water content. Atlantic cod eggs in the brackish Baltic Sea have a density of 1.011 g cm^{-3} and are neutrally buoyant at a salinity of 14.3 ‰, while eggs of Atlantic cod in marine waters off Norway have a density of 1.026 g cm^{-3} and are neutrally buoyant at 33.0 ‰ (Thorsen et al. 1996). The difference in buoyancies among eggs from the two areas was attributed to differential water content of the eggs (96.6% and 92.7%, respectively; Thorsen et al. 1996). Lipid content of Pacific cod eggs does not appear to determine their buoyancy, as many demersal eggs have high lipid contents despite their negative buoyancy (Tocher and Sargent 1984; Lønning et al. 1988). Therefore, we conclude that reduced FAA and water content are the chief mechanisms of negative buoyancy in Pacific cod eggs.

The differential PAA content between Pacific and Atlantic cod might also be partially explained by differential thickness of the chorion, a proteinaceous envelope that surrounds the ovoplasm and later the developing embryo. Most demersal fish eggs have a thicker chorion than pelagic eggs do; the chorion is thought to protect the eggs from the substrate (Lønning et al. 1988). Although we did not assess this, it is possible that demersal Pacific cod eggs also have a thicker chorion than pelagic Atlantic cod eggs. The added PAAs from a thicker chorion might partially explain the reduced lipid and FAA fractions in Pacific cod eggs relative to Atlantic cod. Amino acids in the chorion would be unavailable for use as an energy source during development.

Our results allow us to infer patterns of energy utilization in Pacific cod eggs based on information from Atlantic cod. Prior to first feeding, FAAs are the primary energy source for developing embryos of Atlantic cod, with polar lipids second in importance and contributing much less to overall embryo metabolism (Finn et al. 1995). The energy density of Pacific cod eggs (18.0 J/mg) is similar to that of Atlantic cod eggs (18.6 J/mg; Riis-Vestergaard 2002), probably because the ratios of lipid to TAAs are similar in both species. Thus, it is likely that the relative use of lipids and amino acids in

Pacific cod eggs is similar to that in Atlantic cod. However, the lower FAA content in Pacific cod eggs may reduce the energy available to embryos. While the energy equivalents of PAA and FAA are similar (Riis-Vestergaard 2002), amino acids in proteins must first be liberated before they can be catabolized. This would reduce the amount of energy gained from the amino acids.

Our results also provide insight into the importance of freshwater influx into fish eggs. Hydration that results from increased FAA content provides buoyancy, but is also thought to be an essential mechanism that allowed teleosts to adapt to saltwater (Fyhn et al. 1999; Rønnestad et al. 1999). Because embryos lack the ability to osmoregulate, hydration is important for maintaining osmotic conditions within the egg in the hyperosmotic environment of seawater (Fyhn et al. 1999). However, our results and the composition of other pelagic and demersal eggs suggest that the amount of hydration can vary among species and populations in order to maintain optimal egg buoyancy (Thorsen et al. 1996; Finn et al. 2002b). Thus, the role of FAAs in maintaining osmolality may be secondary to their role in buoyancy. Similarly, the importance of FAAs as an energy source may depend on their availability.

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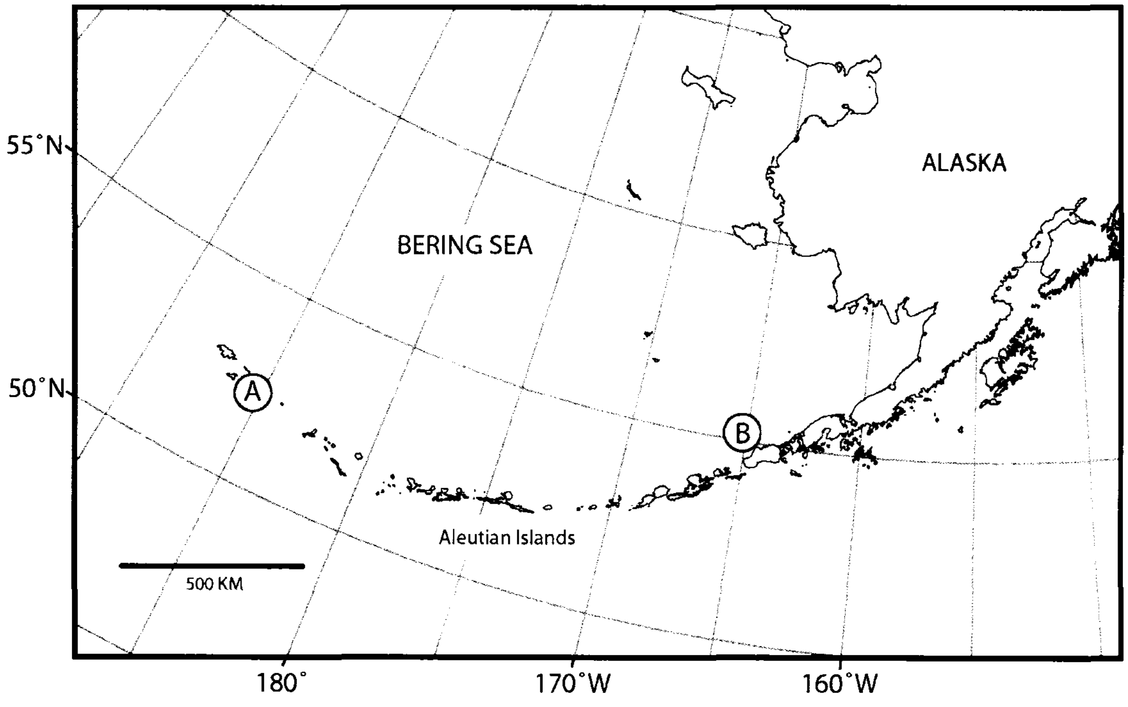

Figure 2.1. Approximate sampling locations for eggs from Pacific cod. Samples from the eastern Bering Sea were collected north of Unimak Island (A) in March 2004. Samples from the western Aleutian Islands were collected off the Near Islands (B) in March 2005.

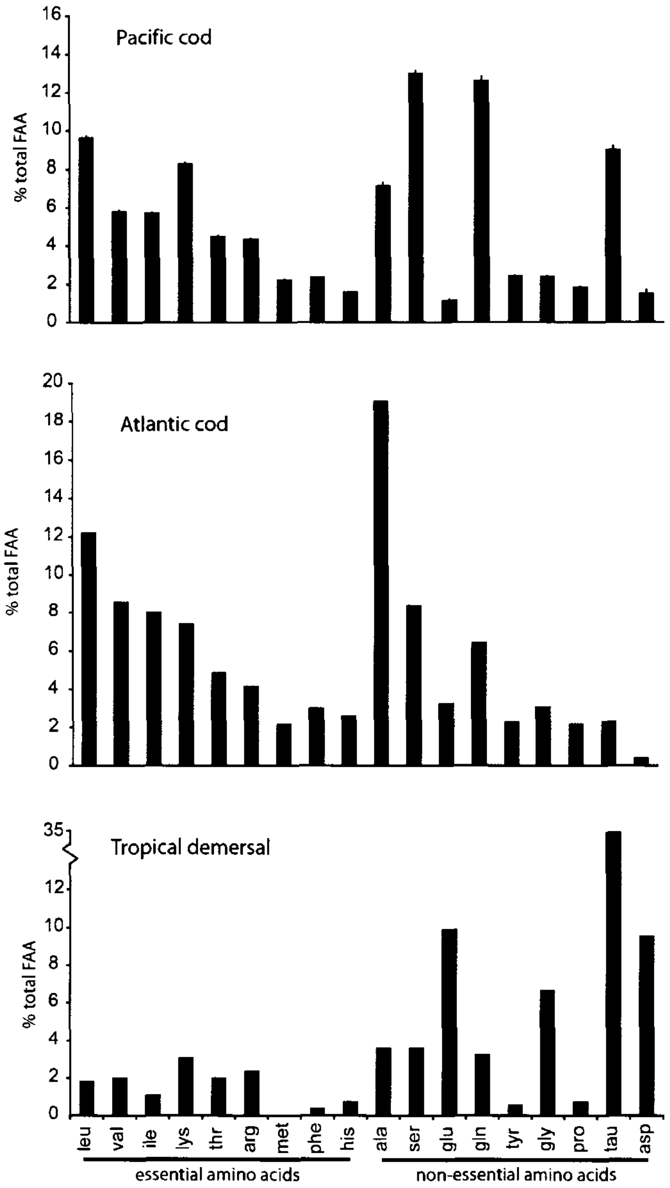

Figure 2.2. Free amino acid (FAA) composition of eggs from Pacific cod, Atlantic cod, and a tropical fish assemblage. Pacific cod data are from the current study; error bars = SE. Atlantic cod data are from Finn et al. 1995. Tropical assemblage data are the pooled egg FAA composition of 10 Panamanian fish species spawning demersal eggs; data from Rønnestad et al. (1999). All data are shown as percentage of total FAAs. Note break in axis in bottom plot. Otherwise, scales are identical among all three plots. Abbreviations for essential amino acids are: leu, leucine; val, valine; ile, isoleucine; lys, lysine; thr, threonine; arg, arginine; met, methionine; phe, phenylalanine; his, histidine. Abbreviations for non-essential amino acids are: ala, alanine; ser, serine; glu, glutamic acid; gln, glutamine; tyr, tyrosine; gly, glycine; pro, proline; tau, taurine; asp, aspartic acid.

Table 2.1. Proximate composition and energy density of eggs from Pacific cod and Atlantic cod. Pacific cod values are from the current study; source for Atlantic cod data are indicated in the footnotes. TAA = total amino acids (protein + free amino acids), PAA = protein amino acids, FAA = free amino acids. SE = standard error. J/mg = Joules/milligram. Pacific cod N = 28.

	Pacific cod		Atlantic cod
component	mean	SE	
water (% wet mass)	82.9	0.3	93.0[1]
dry mass (% wet mass)	17.1	0.3	7.0[1]
TAA (% dry mass)	76.3	0.2	73.7[2]
total lipid (% dry mass)	10.9	0.1	10-12[3], 12.9[2]
ash & carbohydrate (% dry mass)	12.7	0.2	13.5[2]
PAA (% dry mass)	66.9	0.2	46.4[2]
FAA (% dry mass)	9.4	0.1	27.3[2]
FAA/TAA	12.3	0.2	37.4[2]
polar lipids (% total lipids)	78.9	0.8	71.7[3]
neutral lipids (%total lipids)	21.1	0.8	28.3[3]
triacylglycerol (% neutral lipids)	48.0	2.0	44.2[3]
free fatty acids (% neutral lipids)	11.2	0.8	19.1[3]
lipid energy density (J/mg)	3.89	0.05	-
FAA energy density (J/mg)	1.96	0.03	-
PAA energy density (J/mg)	12.18	0.04	-
total egg energy density (J/mg)	18.03	0.06	18.62[1]

1) Riis-Vestergaard 2002
2) Finn et al. 1995
3) Tocher and Sargent 1984

Literature Cited

Alderdice DF, Forrester CR (1971) Effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*). Journal of the Fisheries Research Board of Canada 28: 883-902

AOAC (1995) Official methods of analysis of the Association of Official Analytical Chemists International. Washington D.C.

Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85: 1258-1264

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917

Brooks S, Tyler CR, Sumpter JP (1997) Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7: 387-416

Carr SM, Kivlichan DS, Pepin P, Crutcher DC (1999) Molecular systematics of gadid fishes: implications for the biogeographic origins of Pacific species. Canadian Journal of Zoology 77: 19-26

Finn RN, Fyhn HJ, Evjen MS (1995) Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. Marine Biology 124: 355-369

Finn RN, Oestby GC, Norberg B, Fyhn HJ (2002a) In vivo oocyte hydration in Atlantic halibut (*Hippoglossus hippoglossus*); proteolytic liberation of free amino acids, and ion transport, are driving forces for osmotic water influx. The Journal of Experimental Biology 205: 211-224

Finn RN, Wamboldt M, Fyhn HJ (2002b) Differential processing of yolk proteins during oocyte hydration in marine fishes (Labridae) that spawn benthic and pelagic eggs. Marine Ecology Progress Series 237: 217-226

Fyhn HJ, Finn RN, Reith M, Norberg B (1999) Yolk protein hydrolysis and oocyte free amino acids as key features in the adaptive evolution of teleost fishes to seawater. Sarsia 84: 451-456

Grant WS, Stahl G (1988) Evolution of Atlantic and Pacific cod: Loss of genetic variation and gene expression in Pacific cod. Evolution 42: 138-146

Hattori T, Sakurai Y, Shimazaki K (1992) Maturation and reproductive cycle of female Pacific cod in waters adjacent to the southern coast of Hokkaido, Japan. Nippon Suisan Gakkaishi 58: 2245-2252

Lønning S, Kjørsvik E, Falk-Petersen I-B (1988) A comparative study of pelagic and demersal eggs from common marine fishes in northern Norway. Sarsia 73: 49-60

Riis-Vestergaard J (2002) Energy density of marine pelagic fish eggs. Journal of Fish Biology 60: 1511-1528

Rønnestad I, Thorsen A, Finn RN (1999) Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture 177: 201-216

Rugen WC, Matarese AC (1988) Spatial and temporal distribution and relative abundance of Pacific cod (*Gadus macrocephalus*) larvae in the western Gulf of Alaska. Northwest and Alaska Fisheries Center, National Marine Fisheries Service

Sakurai Y, Hattori T (1996) Reproductive behavior of Pacific cod in captivity. Fisheries Science 62: 222-228

Sweeney RA, Rexroad PR (1987) Comparison of LECO FP-228 "Nitrogen Determinator" with AOAC copper catalyst Kjeldahl method for crude protein. Journal of AOAC International 70: 1028-1030

Thorsen A, Fyhn HJ (1996) Final oocyte maturation in vivo and in vitro in marine fishes with pelagic eggs: yolk protein hydrolysis and free amino acid content. Journal of Fish Biology 48: 1195-1209

Thorsen A, Kjesbu OS, Fyhn HJ, Solemdal P (1996) Physiological mechanisms of buoyancy in eggs from brackish water cod. Journal of Fish Biology 48: 457-477

Tocher DR, Sargent JR (1984) Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. Lipids 19: 492-499

Westrheim SJ (1996) On the Pacific cod (*Gadus macrocephalus*) in British Columbia waters, and a comparison with Pacific cod elsewhere, and Atlantic cod (*Gadus morhua*), Canadian Technical Report of Fisheries and Aquatic Sciences No. 2092

Wiegand MD (1996) Composition, accumulation, and utilization of yolk lipids in teleost fish. Reviews in Fish Biology and Fisheries 6: 259-286

Wootton RJ (1990) Ecology of teleost fishes. Chapman & Hall, London

Zar JH (1999) Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey

CHAPTER THREE

Influence of geographic area and maternal attributes on the lipid and fatty acid composition of Pacific cod eggs[1]

Abstract

We investigated the lipid and fatty acid (FA) composition of eggs from Pacific cod *Gadus macrocephalus*, an important commercial fish species in the North Pacific Ocean, and examined how geographic area and maternal attributes influence egg quality. Egg samples were collected in the eastern Bering Sea and the western Aleutian Islands. These areas are widely separated geographically but Pacific cod from both areas are managed as a single stock. Palmitic, oleic, docosahexaenoic, and eicosapentaenoic (EPA) acids were the main FAs in the polar lipids and triacylglycerols of Pacific cod eggs. Principal component analysis of FA composition separated eggs from the two areas into distinct groups. This may be due to differences in diet or may result from local adaptation, which would suggest that stock structure exists within the single management unit. The concentration of arachidonic acid (AA) decreased with maternal age and size, while linoleic acid content and EPA/AA ratios increased. This suggests that eggs from older females may be of slightly lower quality. Effects of geographic area on FA composition were more dramatic than maternal effects and may be more important determinants of egg quality.

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[1] Ormseth OA, Buck CL, and Norcross BL. 2007. Influence of geographic area and maternal attributes on the lipid and fatty acid composition of Pacific cod eggs. Prepared for submission to Canadian Journal of Fisheries and Aquatic Sciences.

Introduction

The reproductive potential of fishes depends on how many of their eggs hatch and survive to older life stages. The term “egg quality” has been used to describe a suite of factors that influence egg survival, including the quantity and composition of chemical resources in eggs (Brooks et al. 1997). Egg quality is important from the standpoint of fisheries management because assessing the reproductive potential of fish stocks is central to the assignment of harvest levels that prevent overfishing (Marshall et al. 2003). In this paper, we focus on egg lipids and use egg quality to denote the lipid resources in eggs that influence the probability of their being fertilized, hatching and developing into viable larvae.

In general, fish egg lipids are used as energy reserves, structural components, and precursors for essential biomolecules. Egg lipids occur in two main groups: polar lipids that tend to have structural and precursor roles, and neutral lipids, which are mainly used as a source of energy. Eggs with low levels of total lipid typically have a higher proportion of polar vs. neutral lipids. In eggs with high lipid content the additional lipid is usually in the form of neutral lipids, particularly triacylglycerols (TAGs), that form an oil globule separate from the egg yolk (Wiegand 1996).

Fatty acid (FA) composition of egg lipids can influence their quality, and several FAs are thought to be especially important to fish embryos (Tocher 2003). Three highly unsaturated fatty acids (HUFAs) are thought to play crucial structural and precursor roles in fish eggs (Sargent 1995, Wiegand 1996, Tocher 2003). Arachidonic acid (AA), 20:4(n-6), usually occurs in small amounts in fish eggs and serves a precursor for eicosanoids. Eicosanoids are hormone-like molecules involved in the stress response in animals and AA content may affect how embryos and larvae react to stressors. Eicosapentaenoic acid (EPA), 20:5(n-3), is important structurally and is also involved in eicosanoid production and competes with AA for binding sites. Docosahexaenoic acid (DHA), 22:6(n-3), is an important FA in the formation of structural membranes, particularly in neural tissue.

Pacific cod *Gadus macrocephalus* is a large demersal fish species distributed throughout the northern North Pacific Ocean. It is the target of major commercial fisheries in several countries with the 2005 catch in Alaska exceeding 240,000 t (Thompson et al. 2006). Pacific cod also serves important ecosystem roles as predators and prey. Despite the importance of Pacific cod, the biochemistry of its eggs and factors that affect egg quality are largely unknown. Management of Pacific cod stocks requires a thorough understanding of how reproductive potential, which is dependent on egg quality, varies among individual females. The goal of this study is to investigate factors that influence the egg quality of Pacific cod.

Egg composition, and therefore quality, may be influenced by the geographic origin of female fish as well as by maternal attributes such as age and size. Pacific cod in the Bering Sea and Aleutian Islands (BSAI) are managed as one stock, and genetic separation within this stock has yet to be demonstrated (Grant et al. 1987). However, this stock inhabits a huge region and ocean conditions in the eastern Bering Sea (EBS) and the western Aleutian Islands (AI) are different (Ladd et al. 2005). The species composition of fishes differs between these two areas (Logerwell et al. 2005), as do Pacific cod diets (Thompson et al. 2006). Diet and environmental conditions can alter FA composition (Wiegand et al. 2004). In addition, maternal age and size can influence egg composition and quality. For example, first-time Atlantic halibut *Hippoglossus*

hippoglossus spawners produce eggs with lower fertilization rates and reduced concentrations of essential FAs (Evans et al. 1996).

In our study, we hypothesized that the composition of Pacific cod eggs would vary with geographic area and that egg quality would improve with the age and size of females. We collected eggs from wild Pacific cod and analyzed their lipid composition to meet the following objectives: 1) describe the fatty acid composition of polar and neutral lipids in eggs and 2) investigate the effects of geographic area and maternal age and length on egg quality.

We examined the composition of a number of FAs in Pacific cod egg lipids, but focused our investigations on a subset of FAs we considered to have the potential to affect egg quality. Palmitic acid, 16:0, palmitoleic acid, 16:1(n-7), and oleic acid, 18:1(n-9), serve as precursors for more complex FAs and are abundant in the fish egg lipids of many species. Linoleic acid, 18:2(n-6), and α-linolenic acid, 18:3(n-3), serve as precursors for AA, EPA, and DHA, which were also included in the subset along with the ratios EPA/AA and DHA/EPA (Sargent 1995, Wiegand 1996, Arts et al. 2001, Tocher 2003). Because their relative proportions have been linked to egg quality (Wiegand 1996, Tocher 2003), included in this subset were the larger categories used to describe FA structure, including saturated FAs (SFAs), monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), HUFAs, and the total amount of (n-3) and (n-6) FAs.

Methods

Eggs were collected from spawning female Pacific cod from two areas, approximately 1500 km apart, in different years (Fig. 3.1). During March 20-24, 2004, eggs were collected from seven females in the EBS in a 30 nm^2 area approximately 5 nm north of Unimak Island (54°46'N, 164°8'W). From February 26 to March 4 of 2005, eggs were collected from 21 females in a 600 nm^2 area centered on the Near Islands at the western end of the Aleutian Island chain (approximate location 52°51'N, 173°11'E). Fish from the EBS were caught using pot gear during a research survey; AI samples were collected aboard a commercial factory trawler. Pacific cod were collected under federal Scientific Research Permit 2003-26, and this research was approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks (Project #04-61).

Females in spawning condition were randomly selected from the catch and chosen for use in this study according to a schedule of length-bins (40-120 cm in 5-cm increments) designed to provide samples from across a spectrum of age and size. Females were determined to be in spawning condition if eggs were loose and could be freely extruded from the gonopore by pressing the abdomen. For the lipid analyses, eggs flowing from the gonopore were collected in 2 ml cryovials and immediately frozen in liquid nitrogen. Eggs were initially stored in liquid nitrogen and then transferred to a -80°C freezer upon return to the laboratory. Additional eggs were collected in plastic vials and frozen at -20°C for determination of water content.

Age was determined by the Age and Growth Laboratory at the Alaska Fisheries Science Center (National Marine Fisheries Service, Seattle, WA) using otoliths. Total length of fish was measured to the nearest cm. To determine water content of the eggs, weighed portions of eggs (~20 g) were freeze-dried until the sample weight (measured to 0.001 g) did not change over a 24-hour period (usually 4 days). The weight lost was used to calculate % water content.

Lipid class and fatty acid (FA) composition analyses were conducted by Lipid Technologies, LLC (Austin, MN, USA). Egg lipids were extracted using a 2:1 chloroform/methanol mixture according to the method of Bligh and Dyer (1959). Total egg lipid was calculated as percent wet mass; this value was combined with water content data for each female to calculate total lipid as a percentage of dry mass. The lipid extracts were maintained under nitrogen and kept frozen prior to additional processing. Immediately prior to lipid class separation, lipid samples were dried under nitrogen and resuspended in chloroform. Lipid classes were separated on commercial silica gel G plates (AnalTech, Inc., Newark, DE, USA). The chromatographic plates were developed in a solvent system consisting of distilled petroleum ether: diethyl ether: acetic acid (80:20:1, v/v/v). Following development, the silica gel plates were sprayed with a methanolic solution containing 0.5% 2,7-dichlorofluorescein and lipid classes were visualized under ultraviolet light. Desired corresponding lipid bands were then scraped into Teflon® screw-cap tubes. The samples were transesterified with boron trifluoride (10%) in excess methanol in an 80°C water bath for 90 minutes. Resulting fatty acid methyl esters were extracted with water and petroleum ether and stored frozen until gas chromatographic analysis was performed.

Fatty acid composition was determined using capillary gas chromatography. The methyl esters were dried under nitrogen, resuspended in hexane, and separated and quantified using a Shimadzu GC17capillary gas chromatograph (Shimadzu Corp., Kyoto, Japan) utilizing a 30m Restek free fatty acid phase coating (Restek Corp., Bellefonte, PA, USA). Chromatographic data were collected and processed with EZChrom software (Scientific Software, Inc., Pleasanton, CA, USA). Fatty acids were identified by comparison to authentic fatty acid standards and quantified with peak area and internal standard. Individual fatty acid values were totaled to obtain a total mass for each lipid class.

Because values expressed as percentages have a binomial distribution, all data were arcsine square root transformed prior to analysis (Zar 1999). All statistical analyses were performed using S-Plus software (Insightful, Inc., Seattle, WA, USA). Means were backtransformed for reporting purposes. To generate standard errors (SEs) for the backtransformed means, we calculated 95% confidence intervals (CIs) using the transformed data. We then backtransformed the CIs, averaged the upper and lower CI for each mean, and used the resulting value to calculate the SE using the formula (CI= $_{t\alpha/2}$ x SE) and the appropriate values for $t_{\alpha/2}$.

Variation in lipid class and FA composition of eggs between the EBS and AI was examined in two ways. Analysis of covariance (ANCOVA) using length as a covariate was used to test differences in mean values between areas. Principal component analysis (PCA) was used to determine grouping of the FA data using two sets of FA data. To investigate grouping by overall FA composition, we used all measured polyunsaturated fatty acids (PUFAs) as well as any other FA that constituted more than 1% of the total FA content. A second PCA was performed to determine how the data were grouped within the subset of FAs that we determined to be of potential importance to egg quality.

Maternal effects were examined for the subset of important FAs and FA ratios. Effects due to maternal age were tested using analysis of variance (ANOVA), and Tukey multiple comparison tests were used to determine significant differences among ages. Regression analysis was used to examine the influence of maternal length. Because the EBS dataset was small and contained only a limited range of ages and sizes, maternal effects were examined only in the AI dataset. In addition, the age analysis was limited to ages 7-11 because a single age-6 value in the AI dataset compromised the ANOVA.

Results

Although there were large, significant differences in the age and length of females from the two areas (Table 3.1), the ranges of both age and length overlapped between the two areas (Table 3.1). EBS females averaged 5.7 years of age, while the age of AI females averaged 8.6 years. Mean length was 66.6 cm in the EBS and 93.6 cm in the AI.

Lipid and lipid-class content were related to area but not maternal attributes (Table 3.1). Water content was similar between the EBS and AI yet total lipid as percentage of wet mass was significantly greater in the AI eggs. There was no difference between areas in total lipid as percentage of dry mass. Polar lipids were higher in the AI. In the EBS, neutral lipids contained a higher fraction of TAGs, and the percentage of FFAs in the neutral lipids did not differ between areas. Maternal age and length were not related to either total lipid content or lipid class composition.

Fatty acid (FA) composition was dominated by a small number of fatty acids. Palmitic, 16:0, and oleic, 18:1(n-9), acids, as well as DHA, 22:6(n-3), and EPA, 20:5(n-3), were the primary constituents of the polar lipids and TAGs (Table 3.2). Palmitic acid and EPA content were higher in the polar lipids, and oleic acid was higher in the TAGs. DHA in polar lipids was almost three times as high as the DHA in TAGs. The amount of AA in the eggs overall was low, but was higher in the polar lipids. PUFA made up 50.9% of the FAs in the polar lipids, but only 31.6 % of the TAGs. MUFA content was much higher in the TAGs than in the polar lipids.

The FA composition of the polar lipids differed significantly between the EBS and AI (Table 3.3). The proportions of EPA and palmitic and linoleic acids were higher in the EBS than the AI, and AA content was more than twice as high in the EBS. Eggs from the AI contained more linoleic acid. AI eggs had more total (n-3) FAs and less total (n-6) FAs than the EBS eggs. EPA/AA, DHA/EPA, and (n-3)/(n-6) ratios were higher in the AI eggs.

Area had significant effects on the FA composition of the TAGs (Table 3.4) that were partially different from effects observed in the polar lipids. Palmitic, palmitoleic, oleic and arachidonic acid contents were all higher in the EBS, while linoleic and α-linolenic acids were greater in the AI. EPA was not different between areas. Saturated fatty acids were greater in the EBS. The EPA/AA ratio was the only one that differed between areas.

In the polar lipids and TAGS, PCA using the full set of FAs showed a strong separation of the data into two groups that corresponded to area. The first PCs accounted for approximately ¾ of the total variability in the FA composition of polar lipids (Fig. 3.2) and TAGs (Fig. 3.3). In both lipid classes, the separation into area groups occurred along the axis of the first PC. The AI samples were more closely grouped than those from the EBS: the 2nd PC in both classes spread the EBS samples more widely than the AI samples. For the polar lipids, the FAs that contributed most to the PCs were 18:1(n-7), 20:1(n-7), ss:1(n-9), oleic acid, AA, and EPA (Fig. 3.2). Separation in the TAG PCA was due to the same (n-7) and (n-9) FAs and oleic acid, but DHA replaced AA and EPA (Fig. 3.3). Sample #19 from the AI grouped closely to the EBS samples in the polar lipids PCA (Fig. 3.2). Inter-quartile range tests on several FAs indicated that this sample was an extreme outlier in the AI dataset, and it was eliminated from statistical tests between areas.

Principal component analyses for only the subset of important FAs also separated the data into area groups, but the results of the PCAs were more different between polar lipids and TAGs than in the full-set PCAs. The first PCs accounted for less of the overall variability (54-65 %) than the PCs in the full-set PCA but separated the data into the same area groups for both lipid classes. In the polar lipids PCA (Fig. 3.4), the data were separated mainly by AA, EPA, DHA, and oleic and linoleic acids. The most influential FAs in the PCA for the TAGs were palmitic and oleic acids, and the composition of the PCs was different from the polar lipids (Fig. 3.5). Sample 19 was an outlier in the polar lipids but not the TAGs.

Fatty acid composition of AI eggs was significantly related to the age and total length of females. The polar lipids of age-10 and -11 eggs contained more linoleic and α-linolenic acid than those of age-7 eggs (Table 3.5). Total length was positively associated with the polar-lipid proportions of linoleic (Fig. 3.6 and Table 3.5) and α-linolenic acid (Table 3.5), as well as the EPA/AA ratio (Fig. 3.7 and Table 3.5), and negatively related to polar-lipid AA content (Fig. 3.8 and Table 3.5). Length had a positive relationship with TAG linoleic acid (Fig. 3.6 and Table 3.5) and (n-6) FA content (Table 3.5), and was negatively related to the percentages of palmitic and oleic acids and the ratio of (n-3) to (n-6) FAs in the TAGs (Table 3.5).

Discussion

Total lipid and lipid class composition may not be important determinants of egg quality, at least within the range of values observed in this study. We observed no maternal effects on lipid composition, and the analysis of area effects on lipids was inconclusive. Results of the total lipid analysis were contradictory and depended on whether wet or dry mass was used in the calculation. Because the water content of eggs was not different between the EBS and AI, this result is hard to explain. We conclude that total lipid differences between the areas are likely not biologically relevant. There were consistent differences in lipid class composition between the two areas, but the mean differences and the F-values indicate that these differences were small. These results suggest that either the quality of Pacific cod eggs in this study was equal among females, or that egg quality depends on other factors. The latter interpretation is supported by observations in other species. Maternal size did not affect lipid content in eggs of walleye *Sander vitreus* (Wiegand et al. 2004). First-time Atlantic halibut spawners produced eggs with less lipid than eggs from repeat spawners, but this difference could largely be explained by differences in egg size (Evans et al. 1996). In Atlantic cod *Gadus morhua* (Salze et al. 2005) and Atlantic halibut (Bruce et al. 1993), lipid content and lipid class composition of eggs did not affect their hatching or larval survival rates.

Pacific cod eggs have low lipid levels, but the lipids they possess have a high proportion of long-chain fatty acids that have been associated with egg quality. For example, the polar lipids of Pacific cod contain large amounts of PUFAs known to be important for structural membranes and as building blocks for hormones and other biomolecules (Tocher 2003). Pacific cod TAGs have more MUFAs than PUFAs, which is consistent with the role of TAGs as an energy source as MUFAs are largely used for energy (Tocher 2003). Pacific cod eggs are particularly high in DHA, which has important functions in neural cell membranes (Watanabe 1993, Sargent 1995) and EPA, which is important structurally and as a precursor for eicosanoids (Tocher 2003). Pacific cod have only a small amount of AA, as do other marine fishes (Falk-Petersen et al. 1986; Salze et al 2005). AA is used almost exclusively as an eicosanoid precursor, so it may only be needed in small amounts (Tocher 2003). Lipid class and fatty acid profiles of Pacific cod are similar to those of several gadid fishes from the Atlantic Ocean (Tocher and Sargent 1984), suggesting that there is high conservation of FAs in the family Gadidae and that the requirements for complex, long-chain FAs are similar among marine fishes.

There were substantial differences in FA composition between the EBS and the AI. Principal component analysis revealed a strong separation into area groups in both lipid classes and in both variable sets. In the polar lipids, which formed the majority of lipids in eggs from both areas, many of the FAs and FA ratios that we deemed important for egg quality were different between the EBS and AI. Area differences in total (n-3) and (n-6) and fatty acid ratios reflected the variability in individual fatty acid contents. The most dramatic difference between the two areas was the fraction of AA in the polar lipids, which was more than twice as high in the EBS eggs. Similar within-species differences in egg FA composition have been observed among geographically separated populations of Atlantic cod (Pickova et al. 1997), walleye (Wiegand et al. 2004), and lobster *Homarus americanus* (Castell et al. 1994), respectively.

The FA composition of Pacific cod eggs may be influenced by differences in diets of females between the EBS and AI. Pacific cod in the EBS have a more diverse diet, with adult walleye pollock (*Theragra chalcogramma*) as the single largest component (26%) and shrimp and crab species also important (Thompson et al. 2006). In the AI, just three species or species groups make up almost 50% of the diet: Atka mackerel

(*Pleurogrammus monopterygius*), non-pandalid shrimps (families of the infraorder Caridea), and sculpins (superfamily Cottoidea; Thompson et al. 2006). In general, diets of fishes in the EBS have a greater proportion of benthic and demersal species relative to the AI, where diets are more pelagic-based (Aydin et al., in review). In Prince William Sound (PWS) in the Gulf of Alaska, whole-body FA composition of fishes and invertebrates appears to differ between pelagic and demersal environments (Iverson et al. 2002). For example, the fraction of AA appears to be higher in demersal species, which is consistent with the higher AA content we observed in Pacific cod eggs in the EBS. However, PWS sculpins and greenlings (family Hexagrammidae, of which Atka mackerel is a member) also have high concentrations of AA (Iverson et al. 2002), and these species are important in Pacific cod diets in the AI. It is also unclear whether the PWS data are applicable to the EBS and AI, which are very different ecosystems from PWS. Understanding the influence of diet on the FA composition of Pacific cod eggs will require area-specific FA data for Pacific cod prey species.

Eggs from older and larger Pacific cod had a different FA composition than those from younger fish. Maternal age and size had the greatest influence on linoleic acid and AA content and on EPA/AA ratios, which were driven by variability in AA content. Decreased AA content with female size in Pacific cod is the opposite of what has been observed in other species. Eggs of repeat Atlantic halibut spawners had higher AA content and enhanced survival relative to first-time spawners (Evans et al. 1996). In walleye AA increased with maternal length (Wiegand et al. 2004).

Interpretation of differences in FA composition in general and AA content in particular is made difficult by conflicting evidence regarding the connection between AA content and egg quality. Hatching success has been positively related to AA content in

Atlantic cod (Pickova et al. 1997, Salze et al. 2005) and in Atlantic halibut (Evans et al. 1996). Enhanced survival of larval walleye was positively associated with the abundance of AA relative to EPA (Czesny et al. 1999). However, AA content has also been found to be unrelated to fertilization and hatching rates in Atlantic cod, although the relevant study may have been hampered by low lipid recovery (Penney et al. 2006). In spotted wolf-fish *Anarhichas minor*, AA content is negatively associated with egg survival and hatching rates (Tveiten et al. 2004). Because there is good evidence that AA positively influences egg quality in Atlantic cod, reduced AA content in eggs from larger Pacific cod and in Pacific cod from the AI may be indicative of lower egg quality.

Differences in FA composition of eggs from the EBS and AI may result from local adaptation to environmental conditions. Eggs from the EBS have more AA, but AI eggs have higher DHA/EPA ratios, which have been linked to egg quality (Watanabe 1993, Izquierdo 1996). In addition, competitive interactions among DHA, EPA, and AA obscure the roles they play in egg quality (Sargent 1995, Tocher 2003). The apparent contradictions could be resolved if differential FA composition reflects adaptation rather than differences in quality. The difference in AA content between areas is very large, and it is interesting to note that a similar two-fold increase has been observed among eggs from different stocks of Atlantic cod (Pickova et al. 1997) and lobster (Castell et al. 1994). In lobster this difference was attributed to terrestrial and freshwater influences, as the inshore lobster stock had the higher AA content. In Atlantic cod, however, it was the brackish-water Baltic stock that had a lower AA value. Because of its connection with eicosanoid production, increased AA has been linked with a more robust stress response in fish larvae (Sargent 1995). While high AA content has usually been considered maladaptive there may be conditions, such as greater larval density or different prey availability, where a stronger response to stress is beneficial.

The concept of local adaptation in Pacific cod eggs is supported by evidence from other species that the FA composition of fish polar lipids is highly conserved. Diet appears to mainly influence the FA composition of neutral lipids (Mourente and Odriozola 1990, Pickova et al. 1997, Furuita et al. 2002), and has less of an effect on the composition of polar lipids. Differences in the FA composition of polar lipids of eggs from two different Atlantic cod stocks persisted even after females from those stocks were held in captivity and fed a common diet for 27 months (Pickova et al. 1997). Similarly, differences in the FA composition of polar lipids of heart tissue of two Atlantic cod stocks remained after they were held for more than three years under identical conditions (Joenson et al. 2000). Domesticated Atlantic salmon *Salmo salar* maintained a different egg polar lipid FA profile from other captive stocks, even after several generations (Pickova et al. 1999). In cases where diet has been shown to affect polar lipid FA composition, the diets used have been extremely low or high in essential FAs (Furuita et al. 2000, Furuita et al. 2002). Differential regulation of the FA composition of polar lipids versus TAGs is supported in our study by the contrasting PCA results for each lipid class, particularly for the PCAs using the subset of important FAs.

If local adaptation is present, it suggests that Pacific cod in the EBS and AI may form substocks of the larger BSAI population. Use of FA composition in delineating stock structure has been proposed in lobster egg lipids (Castell et al. 1994) and demonstrated in heart tissue of Atlantic cod (Joenson et al. 2000) and redfish *Sebastes mentella* (Joenson and Grahl-Nielsen 2004). Results of our study will be a useful complement to ongoing research into the genetics and movement of Pacific cod within the BSAI.

Adaptation seems to be a less likely explanation for maternal length effects on FA composition. AA decreases with maternal length along a continuum, and a gradual adaptive response with increasing size seems improbable. Environmental or population influences, which might promote adaptation, should not be that different among fish of different sizes, particularly as all fish in the regression analysis were age 6 or older.

Larger females may have a lower AA content because egg production outpaces the availability of AA. As in many fishes, fecundity in Pacific cod increases approximately with the cube of the length (Westrheim 1996). While we have no information on how the availability of AA increases with size, it may be that larger females are as limited as smaller females in their ability to sequester AA. If so, they would have less AA available to devote to each individual egg. This idea is supported by the observation that Japanese flounder *Paralichthys olivaceus* fed on diets high in (n-3) fatty acids had increased egg production but reduced AA content in individual eggs (Furuita et al. 2002).

The inverse relationship between linoleic acid and AA in Pacific cod eggs is of interest because in most vertebrates, linoleic acid serves as a precursor for AA (Tocher 2003). It is possible that larger Pacific cod, unable to supply their eggs with sufficient AA, substitute linoleic acid in the eggs that is later elongated to AA. Marine fish are less capable of converting linoleic acid than freshwater fish due to a lack of the necessary enzymes (Sargent 1995), but this may result from the greater availability of AA in marine vs. freshwater fish diets, reducing the need for marine fish to produce it. Marine fish appear to possess the genes necessary for producing the enzymes needed to elongate precursor molecules (Tocher 2003), and Pacific cod may possess the ability to produce AA from linoleic acid if it is necessary to do so.

Our analysis of area effects on FA composition is complicated by the difference in age and size of females from the EBS and AI. For example, EBS eggs have higher AA content, smaller females have higher AA content, and the EBS females we collected were on average smaller, so it is possible that area-related variability in AA is the result of area-related size differences. In the case of linoleic acid, it is difficult to tell whether differences are related to area or maternal influences: while values for the EBS are lower, they may also simply fit the length-linoleic acid relationship observed for the AI eggs (Fig. 6). Our ability to distinguish length effects from other factors was also hindered by the limited range of sizes in each group. Cod greater than 100 cm total length are often found in the EBS (Thompson et al. 2006), whereas our largest EBS sample was 78 cm. Similarly, our sample set from the AI did not include any fish smaller than 70 cm whereas cod as small as 50 cm are common in the AI.

Despite age and size differences between the EBS and AI and the incomplete representation of EBS and AI cod populations, we believe that area differences in FA composition are due to either diet or adaptation and not maternal size. The best evidence for this conclusion is the separation of samples by the various PCAs. Separation into area groups is very distinct, and despite overlap in age and size between the two areas (Table 3.1) there is no overlap in the two areas in the PCA. Furthermore, replacing the individual sample numbers in one of the polar lipid PCAs with the corresponding lengths (Fig. 3.9) demonstrates that very little of the separation in the PCA is length-related. While there is some tendency for the AI samples to segregate by length, the relationship is weak, especially relative to the separation due to area. Comparing the length-AA relationships between areas suggests differences in AA are due to area rather than length (Fig. 3.7). The range of age- and size-related variability in

AA within the AI samples is also much smaller than the difference in AA between areas. Finally, several of the FAs that differed between areas (e.g. oleic acid) were not related to maternal length.

Samples from each area were collected in different years and at slightly different times of year, but there are several reasons why we believe this did not influence our results. All the eggs we collected were loose within the female, and Pacific cod eggs at this stage are fully developed and hydrated (Hattori et al. 1992). Water content of the eggs was the same between areas, supporting our assumption that all eggs were at the same stage of development. Ambient temperature influences the FA composition of membranes (Tocher 2003), but this would not account for differences in AA that is not incorporated into membranes. There was no interannual variation in the FA composition of eggs of Atlantic cod collected in the Baltic Sea cod over 3 successive years (Pickova et al. 1997).

Our observation that area has a greater influence on egg composition than do maternal attributes is similar to results from other species. The FA composition of walleye eggs varied among populations and with maternal size, but population differences were larger and more consistent than maternal effects (Wiegand et al. 2004). Substantial interpopulation variability in egg composition has also been observed in Atlantic cod (Pickova et al. 1997) and lobster (Castell et al. 1994). In our study, maternal effects were present but they were much smaller than the differences between the EBS and AI. These results suggest that population and perhaps environmental influences are bigger factors in the composition and quality of fish eggs than differences among females within a population.

Our study is the first to investigate the FA composition of Pacific cod eggs. While we generated some intriguing results, more work needs to be done to understand their implication for management of Pacific cod. In particular, FA analysis of eggs from the full size range of Pacific cod in the EBS and AI needs to be performed. Manipulating the diets of captive cod would allow determination of the influence of prey FAs on the FA composition of eggs and whether the FA composition of polar lipids is conserved under different environmental conditions.

Acknowledgments

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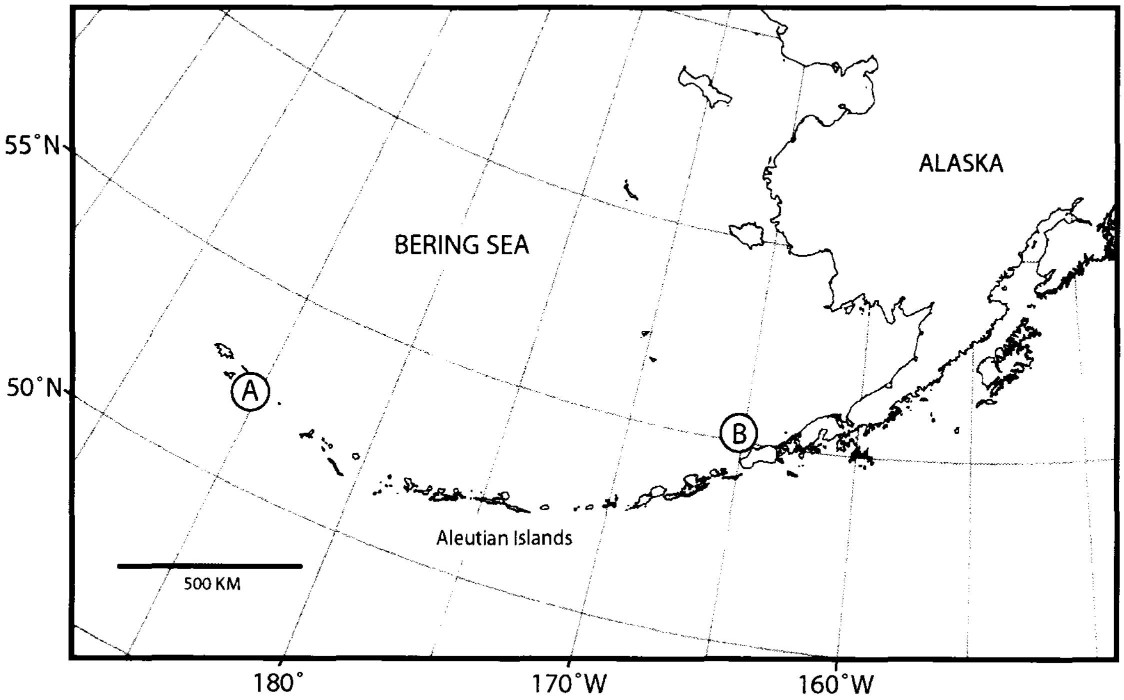

Figure 3.1. Sampling locations for Pacific cod eggs. Samples from the eastern Bering Sea were collected north of Unimak Island (A) in March 2004. Samples from the western Aleutian Islands (AI) were collected off the Near Islands (B) in March 2005.

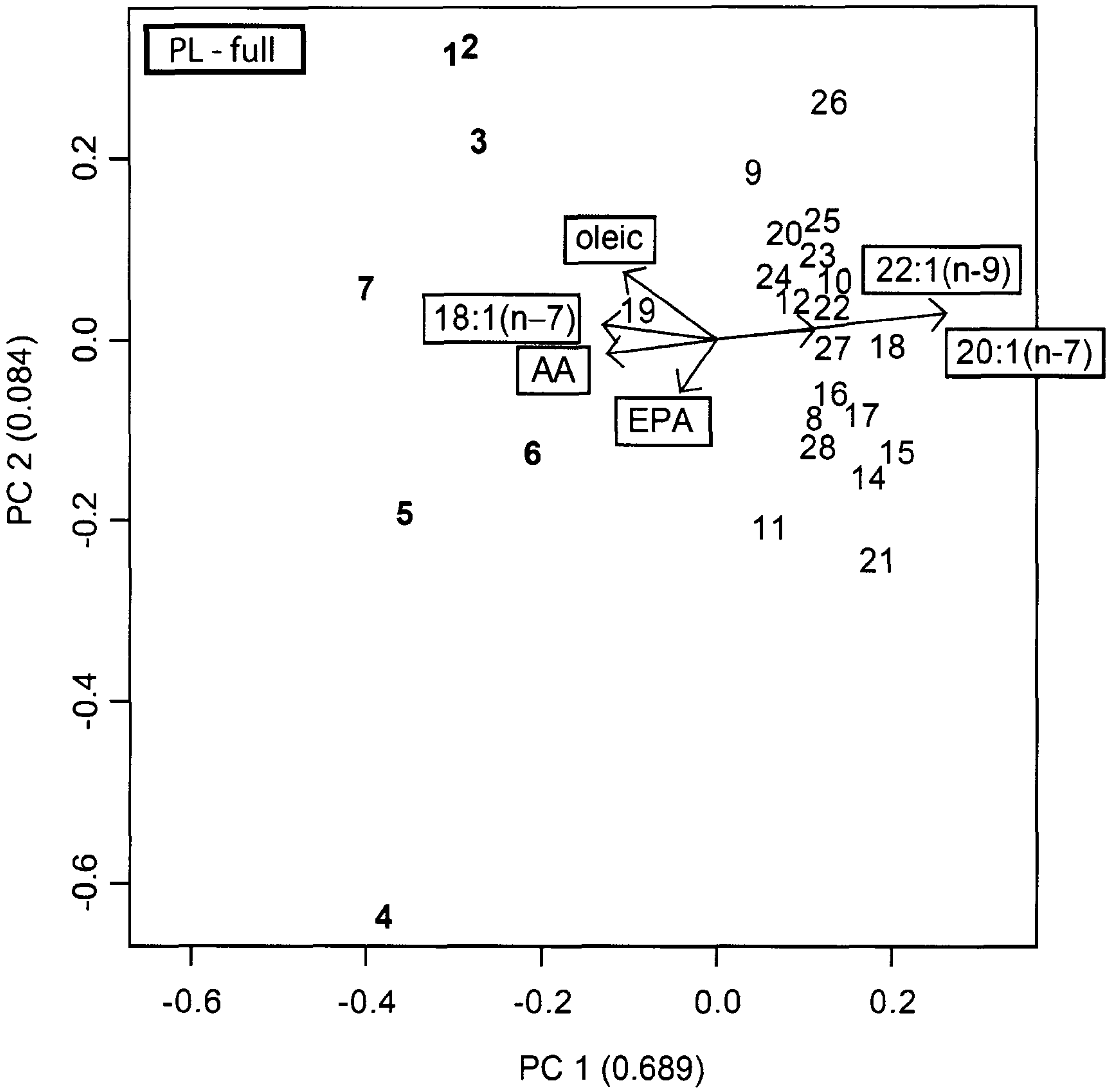

Figure 3.2. Principal component (PC) analysis for fatty acid (FA) composition in the polar lipids (PL) of Pacific cod eggs, using all polyunsaturated FAs (PUFAs) and any FA contributing more than 1% of the total FA pool ("full"). Values following each axis label are the proportions of variability in the dataset explained by each of the first 2 two PCs. Numbers 1-7 (bold) are EBS samples; numbers 8–28 are AI samples. Text boxes indicate the four most important FAs in the first PC and the two most important FAs in the second PC. Length of arrows indicates the relative contribution of that FA in forming the PCs. Direction of arrows indicates the relative contribution of that FA to each of the two PCs. AA = arachidonic acid, EPA = eicosapentaenoic acid.

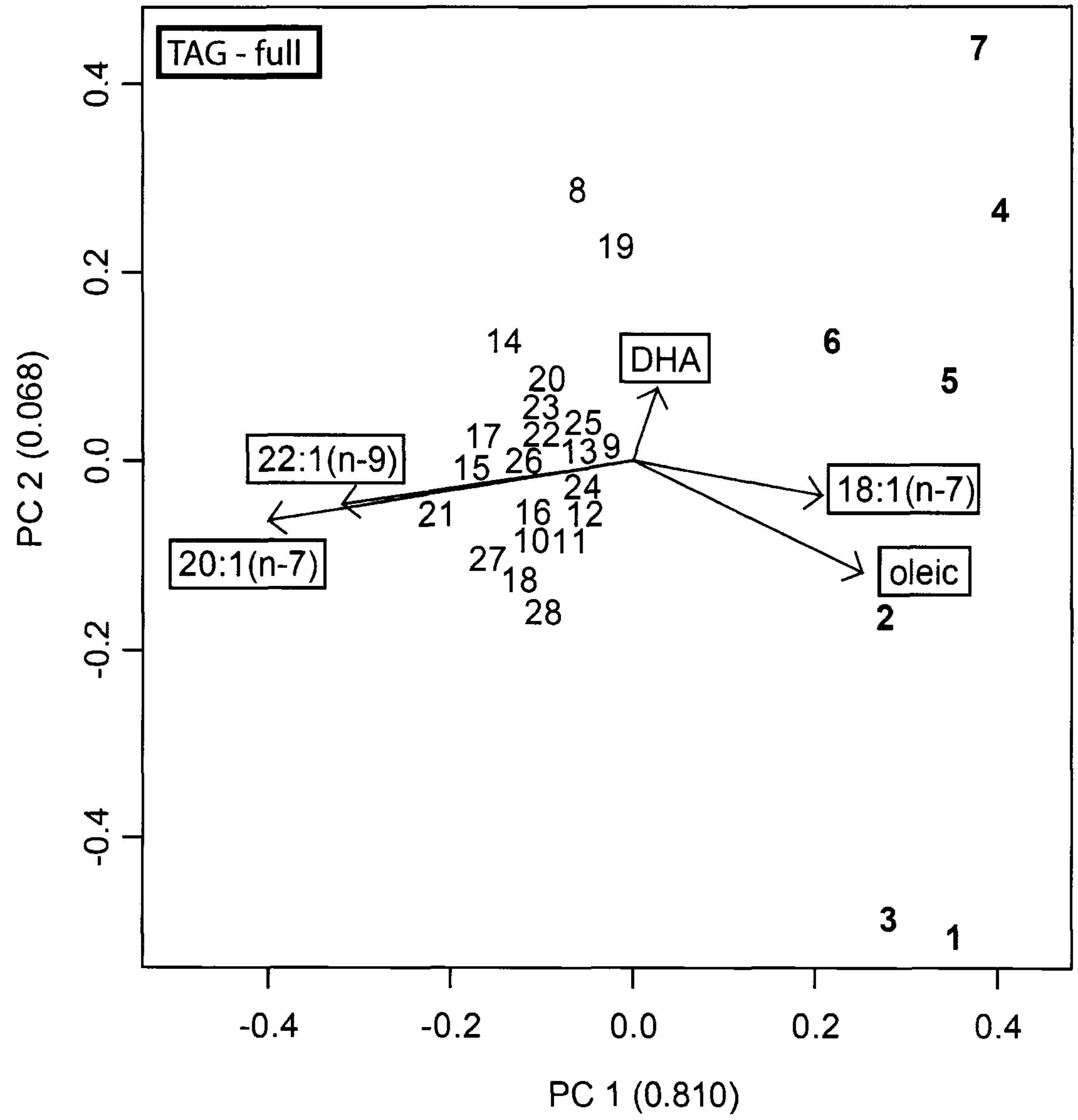

Figure 3.3. Principal component (PC) analysis for fatty acid (FA) composition in the triacylglycerols (TAG) of Pacific cod eggs, using all polyunsaturated FAs (PUFAs) and any FA contributing more than 1% of the total FA pool ("full"). Values following each axis label are the proportions of variability in the dataset explained by each of the first 2 two PCs. Numbers 1-7 (bold) are EBS samples; numbers 8–28 are AI samples. Text boxes indicate the four most important FAs in the first PC and the two most important FAs in the second PC. Length of arrows indicates the relative contribution of that FA in forming the PCs. Direction of arrows indicates the relative contribution of that FA to each of the two PCs. DHA = docosahexaenoic acid.

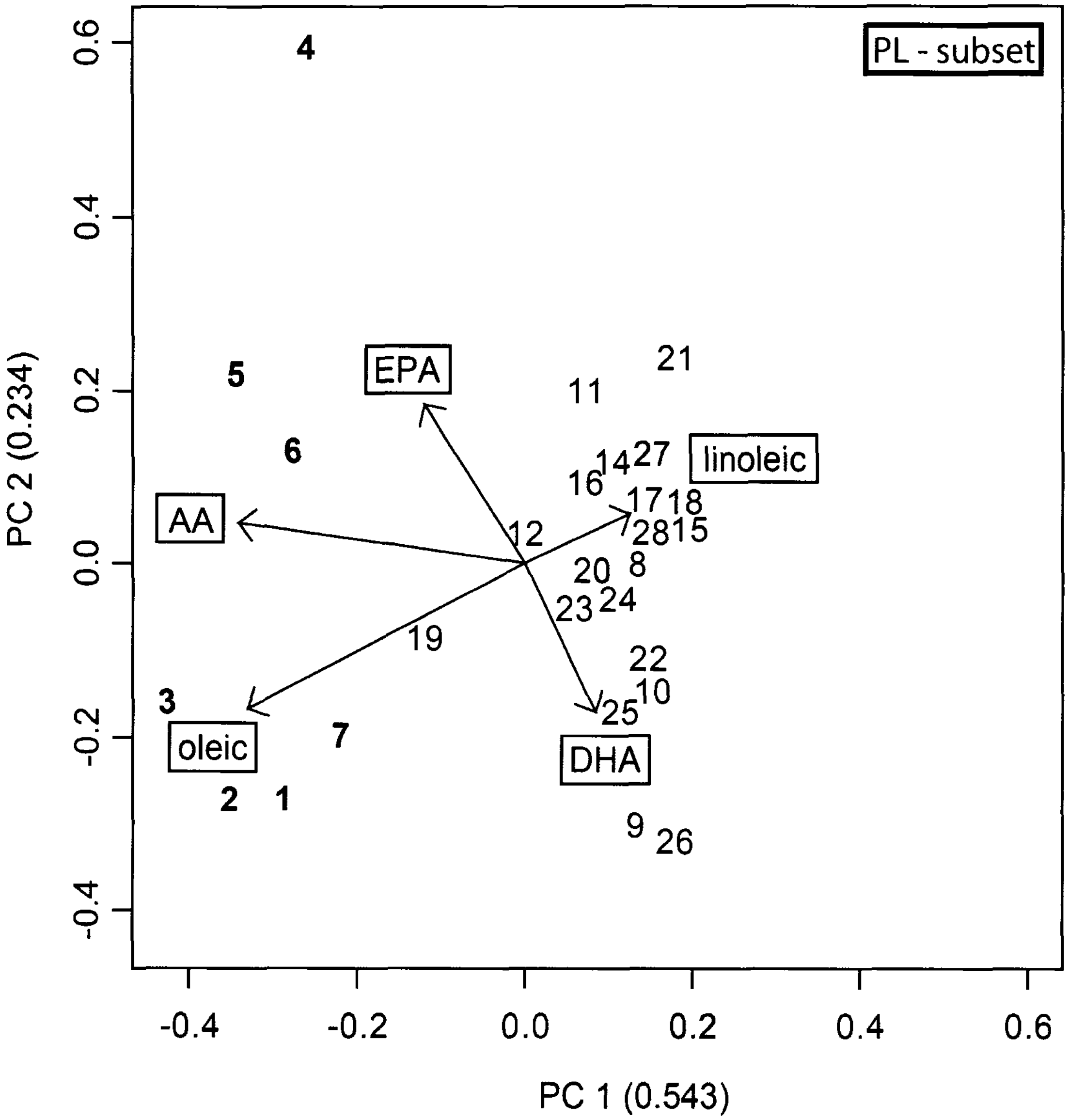

Figure 3.4. Principal component (PC) analysis for fatty acid (FA) composition in the polar lipids (PL) of Pacific cod eggs, using only those FAs of potential importance to egg quality ("subset"). Values following each axis label are the proportions of variability in the dataset explained by each of the first 2 two PCs. Numbers 1-7 (bold) are EBS samples; numbers 8–28 are AI samples. Text boxes indicate the four most important FAs in the first PC and the two most important FAs in the second PC. Length of arrows indicates the relative contribution of that FA in forming the PCs. Direction of arrows indicates the relative contribution of that FA to each of the two PCs. AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

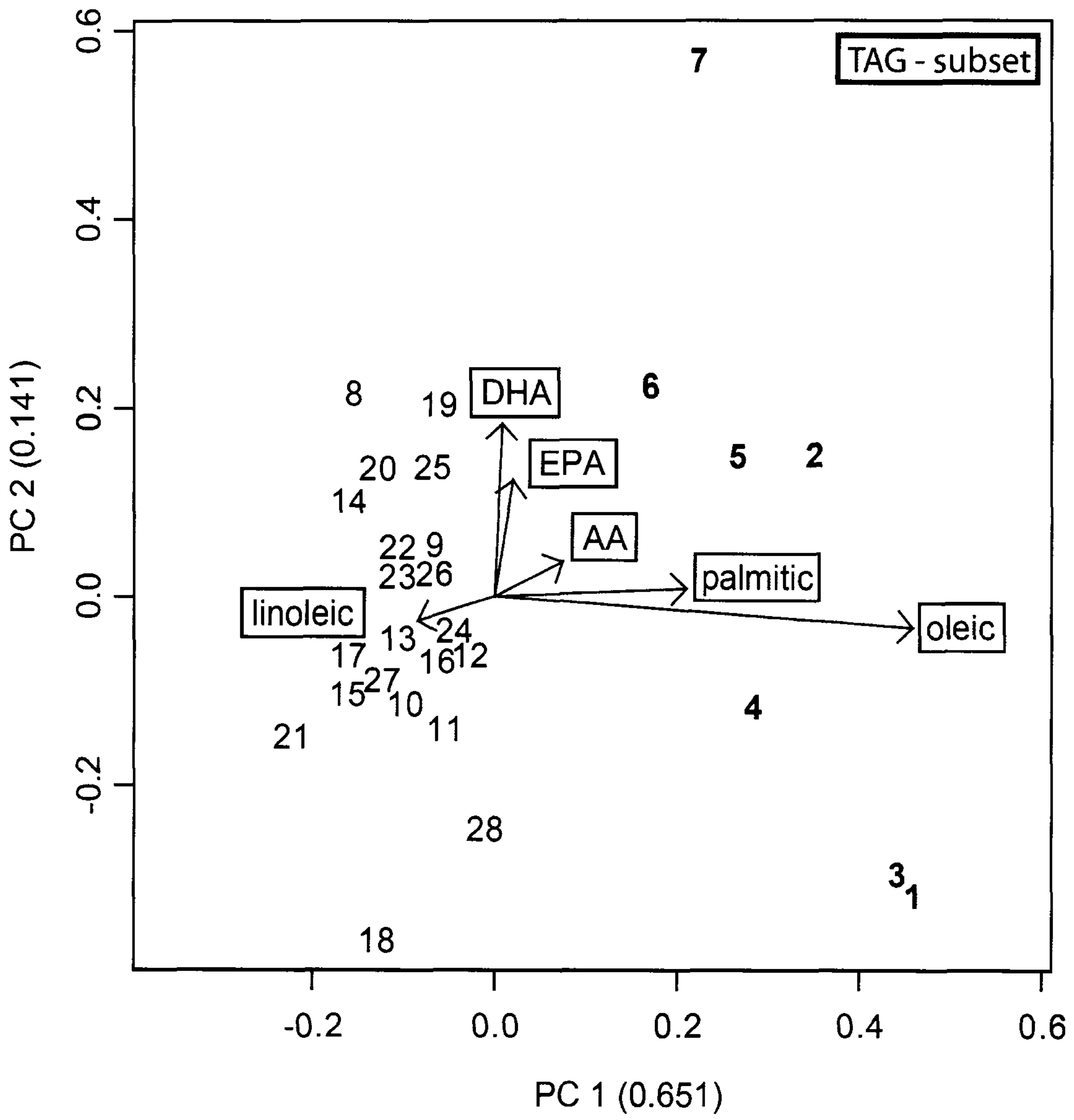

Figure 3.5. Principal component (PC) analysis for fatty acid (FA) composition in triacylglycerols (TAG) of Pacific cod eggs, using only those FAs of potential importance to egg quality ("subset"). Values following each axis label are the proportions of variability in the dataset explained by each of the first 2 two PCs. Numbers 1-7 (bold) are EBS samples; numbers 8–28 are AI samples. Text boxes indicate the four most important FAs in the first PC and the two most important FAs in the second PC. Length of arrows indicates the relative contribution of that FA in forming the PCs. Direction of arrows indicates the relative contribution of that FA to each of the two PCs. AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

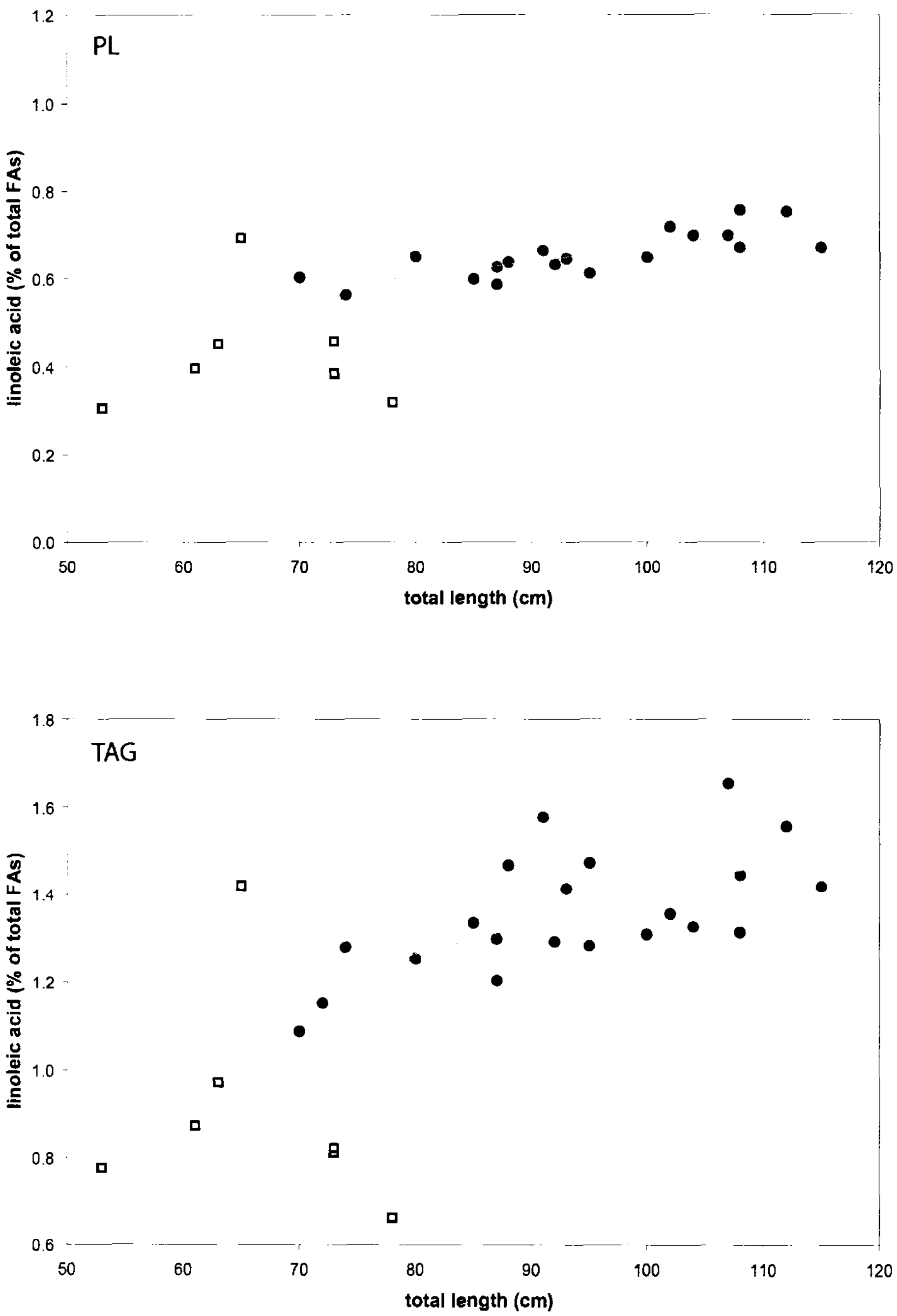

Figure 3.6. Maternal total length versus linoleic acid, 18:2(n-6), content of polar lipids (PL; upper panel) and triacylglycerols (TAG; lower panel) from Pacific cod eggs. Data are shown as % of total fatty acid pool in that lipid class. Open squares, EBS; solid circles, AI. Lines are results of least-squares linear regression (Table 3.7).

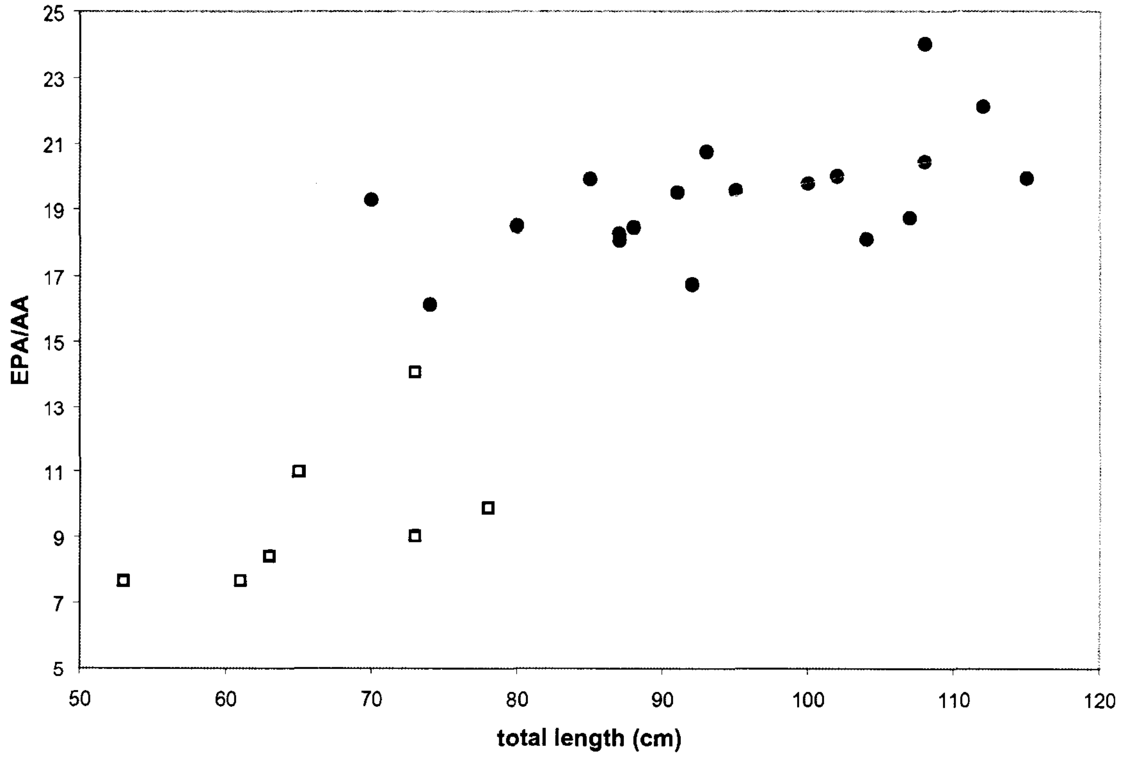

Figure 3.7. Maternal total length versus arachidonic acid (AA), 20:4(n-6), content of polar lipids from Pacific cod eggs. Data are shown as % of total fatty acid pool in that lipid class. Open squares, EBS; solid circles, AI. Line is the result of least-squares linear regression (Table 3.7).

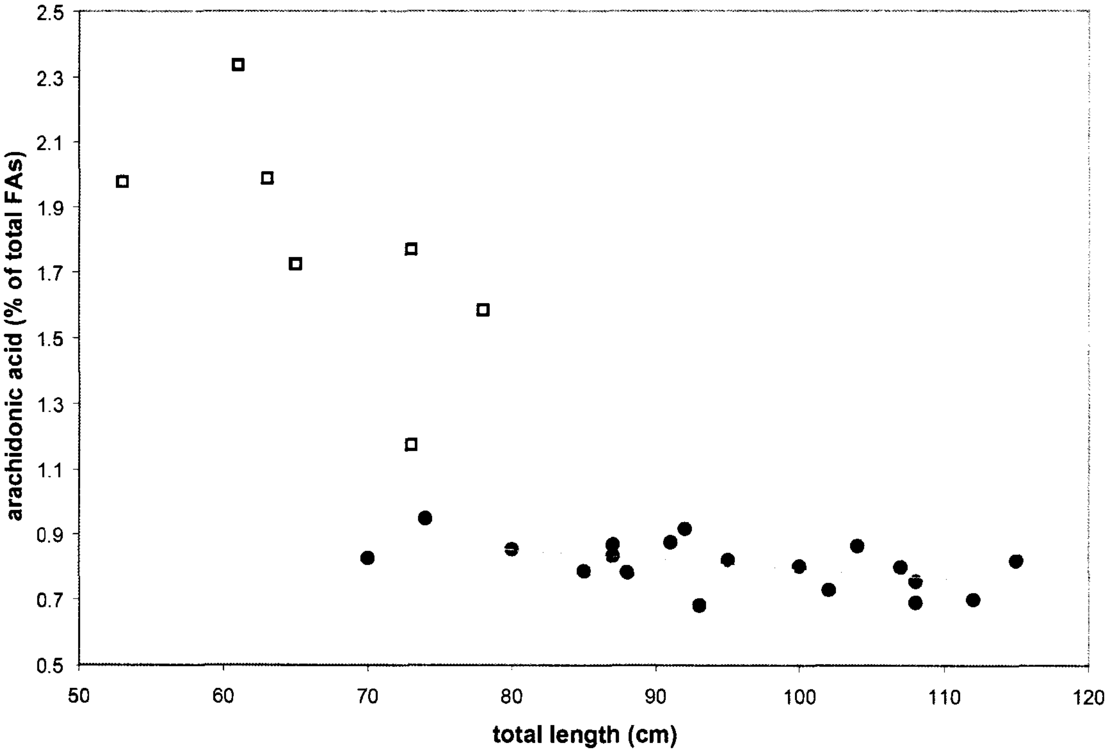

Figure 3.8. Maternal total length versus EPA/AA ratio in polar lipids from Pacific cod eggs. Open squares, EBS; solid circles, AI. Line is the result of least-squares linear regression (Table 3.7).

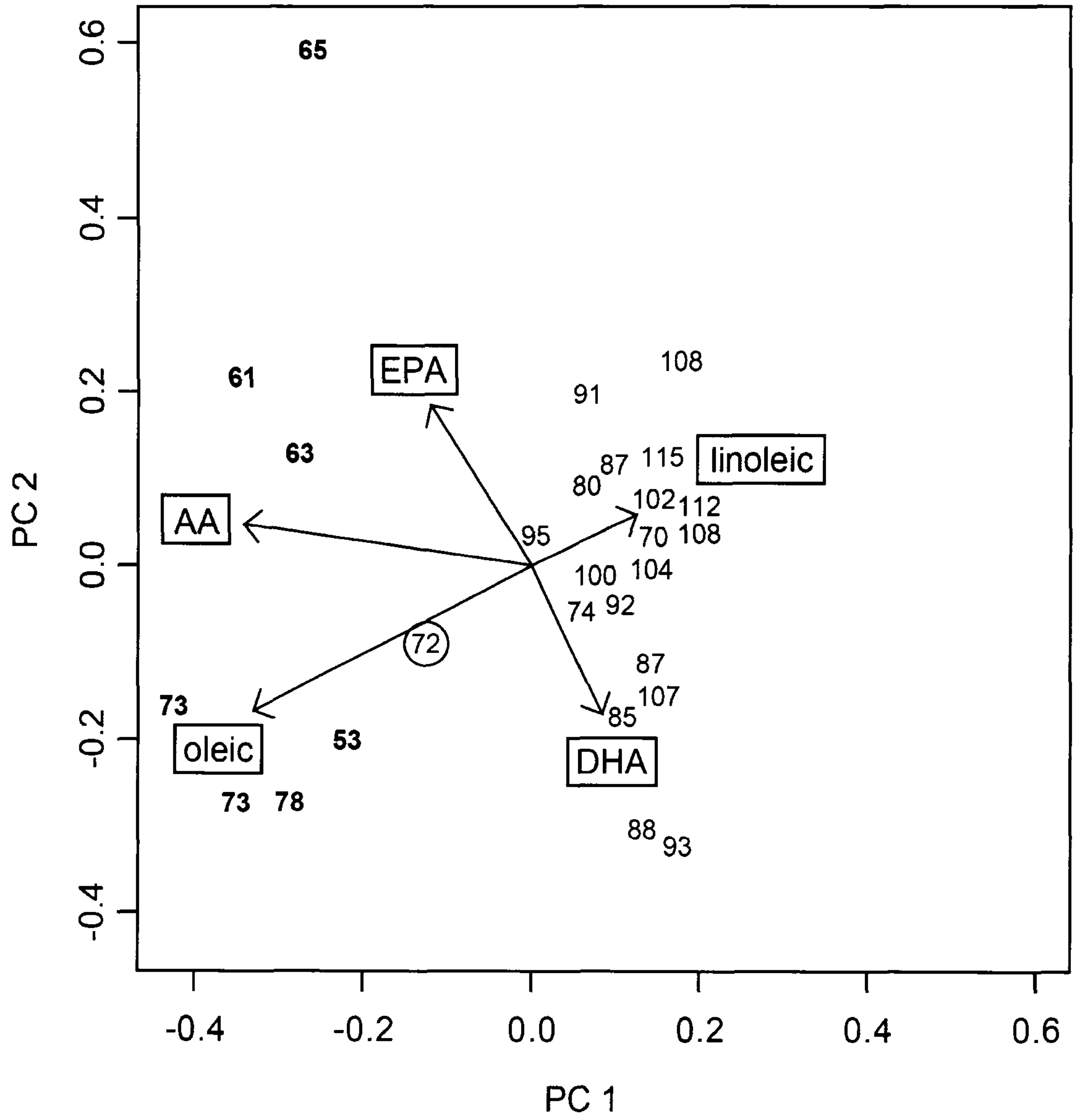

Figure 3.9. Length data for PCA plot of important FAs in polar lipids (Fig. 3.4). Individual sample numbers have been replaced with total length values (cm) corresponding to specific individuals. Circled datapoint indicates outlier in the AI dataset.

Table 3.1. Age and size of female Pacific cod from the eastern Bering Sea (EBS) and Aleutian Islands (AI), and the lipid composition of their eggs. F and p values are the results of ANOVAs between the two areas. Significant differences ($p<0.05$) are listed in bold. SE = standard error. EBS, N = 7; AI, N = 21.

	EBS		AI			
range in ages	4-7		6-11			
range in total length	53-78		70-115			
	mean	SE	mean	SE	F	p-value
age	**5.67**	**0.42**	**8.65**	**0.33**	**22.98**	**<0.0001**
total length (cm)	**66.6**	**3.3**	**93.5**	**2.99**	**28.73**	**<0.0001**
somatic weight (kg)	**3.38**	**0.85**	**9.97**	**1.09**	**8.474**	**0.0086**
water (% wet mass)	83.4	1.4	82.8	0.2	0.708	0.4080
total lipid (% dry mass)	10.4	0.5	11.1	0.1	3.606	0.0692
total lipid (% wet mass)	**1.72**	**0.09**	**1.91**	**0.02**	**7.97**	**0.0090**
polar lipids (% TL)	**76.1**	**1.8**	**79.9**	**0.7**	**5.552**	**0.0266**
neutral lipids (% TL)	**23.9**	**1.8**	**20.1**	**0.7**	**5.552**	**0.0266**
triacylglycerols (% NL)	**41.2**	**2.5**	**50.3**	**2.3**	**4.711**	**0.0397**
free fatty acids (% NL)	10.0	1.3	11.7	1.0	0.868	0.3602

Table 3.2. Fatty acid composition of polar lipids and triacylglycerols in eggs of Pacific cod. Values shown are for all polyunsaturated fatty acids (FAs), any other FAs that constituted more than 1% of the total fatty acids for any lipid class, and FA ratios. Components listed in italics are those in the subset considered to be of importance relative to egg quality. SE = standard error. For total lipids and triacylglycerol, N = 28; for polar lipids, N = 27. SFA= saturated FAs; MUFA= monounsaturated FAs; PUFA= polyunsaturated FAs; HUFA= highly unsaturated FAs.

		polar lipids		triacylglycerols	
FA component	name	mean	SE	mean	SE
14:0		1.8	0.1	4.1	0.2
16:0	palmitic	18.8	0.2	10.1	0.4
16:1(n-7)	palmitoleic	3.8	0.1	7.4	0.1
18:0		1.4	0.0	1.9	0.1
18:1(n-9)	oleic	9.3	0.3	15.1	0.7
18:1(n-7)		3.4	0.2	5.1	0.3
18:2(n-6)	linoleic	0.6	0.0	1.2	0.1
18:3(n-6)		0.0	0.0	0.1	0.0
18:3(n-3)	α-linolenic	0.2	0.0	0.5	0.0
18:4(n-3)		0.3	0.0	1.3	0.1
20:1(n-7)		3.7	0.3	11.5	1.0
20:2(n-6)		0.2	0.0	0.2	0.0
20:3(n-9)		0.0	0.0	0.0	0.0
20:3(n-6)		0.1	0.0	0.1	0.0
20:4(n-6)	AA	1.0	0.1	0.4	0.0
20:3(n-3)		0.1	0.0	0.2	0.0
20:4(n-3)		0.4	0.0	0.9	0.0
20:5(n-3)	EPA	15.9	0.2	12.3	0.3
22:1(n-9)		0.6	0.1	5.6	0.6
22:4(n-6)		0.2	0.0	0.7	0.0
22:5(n-6)		0.1	0.0	0.0	0.0
22:5(n-3)		2.2	0.1	2.5	0.1
22:6(n-3)	DHA	29.4	0.2	10.9	0.3
SFA		22.5	0.2	16.7	0.4
MUFA		22.7	0.3	47.3	0.7
PUFA		50.9	0.2	31.6	0.5
HUFA		50.1	0.2	30.1	0.5
(n-3)		48.6	0.2	28.7	0.5
(n-6)		2.3	0.1	2.9	0.1
AA+EPA		16.9	0.3	12.8	0.3
EPA/AA		16.00	1.09	30.24	1.75
DHA/EPA		1.86	0.04	0.89	0.03
(n-3)/(n-6)		21.03	0.79	10.06	0.19

Table 3.3. Fatty acid composition and ratios of the polar lipids in eggs of Pacific cod by area. P-values are the results of ANCOVAs for area effects on the listed components. Components where significant area differences ($p<0.05$) were observed are listed in bold. SE = standard error. EBS = eastern Bering Sea, AI = Aleutian Islands. EBS, N = 7; AI, N = 19. SFA= saturated fatty acids (FAs); MUFA= monounsaturated FAs; PUFA= polyunsaturated FAs; HUFA= highly unsaturated FAs. AA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid.

	EBS		AI			
	mean	SE	mean	SE	F	p-value
palmitic	**19.46**	**0.46**	**18.56**	**0.15**	**5.651**	**0.0266**
palmitoleic	3.76	0.19	3.75	0.04	0.011	0.916
oleic	**10.97**	**0.61**	**8.66**	**0.12**	**55.17**	**< 0.0001**
linoleic	**0.42**	**0.05**	**0.65**	**0.01**	**47.55**	**< 0.0001**
α-linolenic	0.22	0.02	0.24	0.01	1.801	0.1933
AA	**1.78**	**0.14**	**0.81**	**0.02**	**240.3**	**< 0.0001**
EPA	**16.68**	**0.5**	**15.57**	**0.17**	**7.053**	**0.0144**
DHA	28.82	0.57	29.65	0.24	2.399	0.1356
SFA	22.63	0.52	22.43	0.17	0.221	0.643
MUFA	22.81	0.94	22.71	0.18	0.039	0.8457
PUFA	51.01	0.48	50.91	0.19	0.077	0.7834
HUFA	50.38	0.46	50.05	0.19	0.808	0.3783
(n-3)	**47.99**	**0.36**	**48.78**	**0.19**	**4.628**	**0.0427**
(n-6)	**2.95**	**0.19**	**2.12**	**0.04**	**60.36**	**< 0.0001**
AA+EPA	**18.47**	**0.54**	**16.37**	**0.17**	**22.85**	**< 0.0001**
EPA/AA	**9.48**	**0.79**	**19.32**	**0.40**	**205.7**	**< 0.0001**
DHA/EPA	**1.73**	**0.08**	**1.91**	**0.03**	**5.35**	**0.0304**
(n-3)/(n-6)	**16.37**	**1.09**	**23.05**	**0.45**	**56.53**	**< 0.0001**

Table 3.4. Fatty acid composition and ratios of triacylglycerols in Pacific cod eggs by area. P-values are the results of ANCOVAs for area effects on the listed components. Components where significant area differences ($p<0.05$) were observed are listed in bold. SE = standard error. EBS = eastern Bering Sea, AI = Aleutian Islands. EBS, N = 7; AI, N = 20. SFA= saturated fatty acids (FAs); MUFA= monounsaturated FAs; PUFA= polyunsaturated FAs; HUFA= highly unsaturated FAs. AA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid.

	EBS		AI			
	mean	SE	mean	SE	F	p-value
palmitic	**12.86**	**0.58**	**9.18**	**0.19**	**74.28**	**< 0.0001**
palmitoleic	**7.91**	**0.34**	**7.22**	**0.10**	**6.99**	**0.0142**
oleic	**21.57**	**1.39**	**13.12**	**0.22**	**267**	**< 0.0001**
linoleic	**0.89**	**0.09**	**1.35**	**0.03**	**49.07**	**< 0.0001**
α-linolenic	**0.28**	**0.10**	**0.55**	**0.03**	**11.7**	**0.0022**
AA	**0.66**	**0.05**	**0.35**	**0.02**	**58.25**	**< 0.0001**
EPA	12.76	0.76	12.20	0.31	0.712	0.4071
DHA	11.43	1.12	10.72	0.18	1.612	0.2163
SFA	**18.38**	**0.83**	**16.10**	**0.34**	**10.28**	**0.0038**
MUFA	46.12	2.34	47.72	0.46	2.632	0.1178
PUFA	31.06	1.55	31.76	0.48	0.526	0.4752
HUFA	29.86	1.50	30.18	0.48	0.112	0.7411
(n-3)	28.24	1.46	28.86	0.46	0.461	0.5036
(n-6)	2.79	0.18	2.88	0.04	0.555	0.4634
AA+EPA	13.42	0.78	12.55	0.32	1.616	0.1734
EPA/AA	**19.59**	**1.83**	**34.90**	**1.11**	**57.2**	**< 0.0001**
DHA/EPA	0.91	0.10	0.88	0.02	0.132	0.7198
(n-3)/(n-6)	10.13	0.61	10.03	0.18	0.047	0.8307

Table 3.5. Maternal age and length effects on fatty acid (FA) content of Pacific cod eggs from the Aleutian Islands. Only those FAs and FA ratios for which significant effects were observed are listed. For age, "AD" refers to statistically significant relationships among ages. For length, "sign" refers to the relationship between independent and dependant variables: "+" = positive relationship, "-" = negative relationship. Polar lipids, N = 19; triacylglycerols, N = 20. AA= arachidonic acid, 20:4(n-6); EPA= eicosapentaenoic acid, 20:5(n-3).

age		F	p-value	AD
polar lipids	linoleic	4.847	0.0130	7<10, 7<11
	α-linolenic	4.470	0.0172	7<10, 7<12

length		R^2	F	p-value	sign
polar lipids	linoleic	0.63	28.59	0.0001	+
	α-linolenic	0.38	10.35	0.0051	+
	AA	0.26	6.121	0.0242	-
	EPA/AA	0.31	7.764	0.0127	+
triacylglycerols	palmitic	0.21	4.851	0.0409	-
	oleic	0.23	5.507	0.0306	-
	linoleic	0.35	9.793	0.0058	+
	(n-6)	0.2	4.63	0.0452	+
	(n-3)/(n-6)	0.28	6.979	0.0166	-

Literature Cited

Arts, M. T., R. G. Ackman, and B. J. Holub. 2001. "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Canadian Journal of Fisheries and Aquatic Sciences 58:122-137.

Aydin, K., Gaichas, S., Ortiz, I., Kinzey, D., and Friday, N. In review. A comparison of the Bering Sea, Gulf of Alaska, and Aleutian Islands large marine ecosystems through food web modeling. NOAA NMFS Tech Memo. 233 p.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Brooks, S., C. R. Tyler, and J. P. Sumpter. 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7:387-416.

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

Castell, J. D., L. D. Boston, R. J. Miller, and T. Kenchington. 1994. The potential identification of the geographic origin of lobster eggs from various wild stocks based on fatty acid composition. Canadian Journal of Fisheries and Aquatic Sciences 52:1135-1140.

Czesny, S., S. Kolkovski, K. Dabrowski, and D. Culver. 1999. Growth, survival, and quality of juvenile walleye *Stizostedion vitreum* as influenced by n-3 HUFA enriched *Artemia* nauplii. Aquaculture 178:103-115.

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

Falk-Petersen, S., Falk-Petersen, I.-B., Sargent, J. R., and Haug, T. 1986. Lipid class and fatty acid composition of eggs from the Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 52:207-211.

Furuita, H., H. Tanaka, T. Yamamoto, M. Shiraishi, and T. Takeuchi. 2000. Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. Aquaculture 187:387-398.

Furuita, H., H. Tanaka, T. Yamamoto, N. Suzuki, and T. Takeuchi. 2002. Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus*. Aquaculture 210:323-333.

Grant, W. S., C. I. Zhang, and T. Kobayashi. 1987. Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Canadian Journal of Fisheries and Aquatic Sciences 44:490-498.

Hattori, T., Y. Sakurai, and K. Shimazaki. 1992. Maturation and reproductive cycle of female Pacific cod in waters adjacent to the southern coast of Hokkaido, Japan. Nippon Suisan Gakkaishi 58:2245-2252.

Iverson, S.J., Frost, K.J., and Lang, S.L.C. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series 241:161-181

Izquierdo, M. S. 1996. Essential fatty acid requirements of cultured marine fish larvae. Aquaculture Nutrition 2:183-191.

Joenson, H., and O. Grahl-Nielsen. 2004. Stock structure of *Sebastes mentella* in the North Atlantic revealed by chemometry of the fatty acid profile in the heart tissue. ICES Journal of Marine Science 61:113-126.

Joenson, H., P. Steingrund, I. Fjallstein, and O. Grahl-Nielsen. 2000. Discrimination between two reared stocks of cod (*Gadus morhua*) from the Faroe Islands by chemometry of the fatty acid composition in the heart tissue. Marine Biology 136:573-580.

Ladd, C., G. L. Hunt, C. W. Mordy, S. A. Salo, and P. J. Stabeno. 2005. Marine environment of the eastern and central Aleutian Islands. Fisheries Oceanography 14:22-38.

Logerwell, E. A., K. Aydin, S. Barbeaux, E. Brown, M. E. Conners, S. Lowe, J. W. Orr, I. Ortiz, R. Reuter, and P. Spencer. 2005. Geographic patterns in the demersal ichthyofauna of the Aleutian Islands. Fisheries Oceanography 14:93-112.

Marshall, C. T., L. O'Brien, J. Tomkiewicz, F. W. Köster, G. Kraus, G. Marteinsdottir, M. J. Morgan, F. Saborido-Rey, J. L. Blanchard, D. H. Secor, P. J. Wright, N. V. Mukhina, and H. Björnsson. 2003. Developing alternative indices of reproductive potential for use in fisheries management: case studies for stocks spanning an information gradient. Journal of Northwest Atlantic Fisheries Science 33:161-190.

Mourente, G., and J. M. Odriozola. 1990. Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). Fish Physiology and Biochemistry 8:93-101.

Penney, R. W., P. L. Lush, J. Wade, J. A. Brown, C. C. Parrish, and M. P. M. Burton. 2006. Comparative utility of egg blastomere morphology and lipid biochemistry for prediction of hatching success in Atlantic cod, *Gadus morhua* L. Aquaculture Research 37:272-283.

Pickova, J., P. C. Dutta, P.-O. Larsson, and A. Kiessling. 1997. Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (*Gadus morhua*) stocks. Canadian Journal of Fisheries and Aquatic Sciences 54:2410-2416.

Pickova, J., A. Kiessling, A. Pettersson, and P. C. Dutta. 1999. Fatty acid and carotenoid composition of eggs from two nonanadromous Atlantic salmon stocks of cultured and wild origin. Fish Physiology and Biochemistry 21:147-156.

Salze, G., D. R. Tocher, W. J. Roy, and D. A. Robertson. 2005. Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. Aquaculture Research 36:1488-1499.

Sargent, J. R. 1995. Origins and functions of egg lipids: nutritional implications. In N. R. Bromage and R. J. Roberts, editors. Broodstock management and egg and larval quality. Blackwell Science Ltd., Oxford. Pages 353-372

Thompson, G.G., Dorn, M. W., Gaichas, S., and Aydin, K. 2006. Assessment of the Pacific cod stock in the Eastern Bering Sea and Aleutian Islands Area. In Plan Team for Groundfish Fisheries of the Bering Sea/Aleutian Islands (editors), Stock assessment and fishery evaluation report for the groundfish resources of the Bering Sea/Aleutian Islands regions. North Pacific Fishery Management Council, Anchorage, AK, pp. 237-340.

Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11:107-184.

Tocher, D. R., and J. R. Sargent. 1984. Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. Lipids 19:492-499.

Tveiten, H., M. Jobling, and I. Andreassen. 2004. Influence of egg lipids and fatty acids on egg viability, and their utilization during embryonic development of spotted wolf-fish, *Anarhichas minor* Olafsen. Aquaculture Research 35:152-161.

Watanabe, T. 1993. Importance of docosahexaenoic acid in marine larval fish. Journal of the World Aquaculture Society 24:152-161.

Westrheim, S. J. 1996. On the Pacific cod (*Gadus macrocephalus*) in British Columbia waters, and a comparison with Pacific cod elsewhere, and Atlantic cod (*Gadus morhua*). Canadian Technical Report of Fisheries and Aquatic Sciences 2092.

Wiegand, M. D. 1996. Composition, accumulation, and utilization of yolk lipids in teleost fish. Reviews in Fish Biology and Fisheries 6:259-286.

Wiegand, M. D., T. A. Johnston, J. Martin, and W. C. Leggett. 2004. Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Canadian Journal of Fisheries and Aquatic Sciences 61:110-121.

Zar, J. H. 1999. Biostatistical analysis, Fourth edition. Prentice Hall, Upper Saddle River, New Jersey.

CHAPTER FOUR

Reproductive potential of Pacific cod in Alaska: maternal, year, and area effects[1]

Abstract

Reproductive potential (fecundity and egg size) of Pacific cod (*Gadus macrocephalus*) in the Gulf of Alaska, eastern Bering Sea and the western Aleutian Islands was examined over a four-year period. Fecundity was positively related to female age, but at any particular age there was a wide range in fecundity values. In contrast, female length and somatic weight were excellent predictors of fecundity. Condition added only slightly, though significantly, to the explanatory power of length in predicting fecundity. Relative fecundity (eggs per g body weight) increased slightly with female size. Egg size was highly variable and was not related to maternal age, size or condition. Differences in reproductive potential among years and areas were mainly due to variation in egg size. The most dramatic difference was reduced egg size of Pacific cod in the eastern Bering Sea in 2003, which may have been due to changes in ocean temperature that impacted the ability of females to store energy. Our results suggest that females of a given size have a strong tendency to maintain consistent levels of fecundity, and that larger females increase their reproductive potential by maximizing fecundity at the expense of egg size. There is also evidence of energetic tradeoffs among reproduction, growth, and survival in Pacific cod.

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[1] Ormseth OA and Norcross BL. 2007. Reproductive potential of Pacific cod in Alaska: maternal, year, and area effects. Prepared for submission to Marine Ecology Progress Series.

Introduction

Fish populations depend on the production of viable offspring. Because eggs are the first of a series of early life stages that ultimately lead to recruitment into adult populations, the term “reproductive potential” is often used to describe the number of viable eggs produced by either individual females or populations (Marshall et al. 1998, Trippel 1999, Marteinsdottir & Begg 2002). In this paper, we use reproductive potential as a measurable quantity that describes the number of eggs produced by individuals (fecundity) as well as the resources in those eggs that affect the survival of embryos and larvae.

The reproductive potential of a female fish influences fitness and results from trade-offs in energy allocation. Maternal fitness is the product of the number of offspring produced as well as individual offspring fitness (Smith & Fretwell 1974, Wootton 1990). Because energy resources are usually limited, an increase in energy devoted to maximizing offspring fitness (e.g., by producing larger eggs) must be balanced by producing fewer offspring. Females allocate resources according to their life history strategy and the environment their offspring will experience (Wootton 1990, Bernardo 1996). Larger eggs often produce larger larvae that are more resistant to starvation, have a competitive advantage, or are better able to avoid predators than those from smaller eggs (Marshall 1953, Blaxter and Hempel 1963, Wootton 1990, Berkeley et al. 2004). If these advantages outweigh the cost of producing fewer eggs, females are expected to produce larger eggs.

More broadly, females distribute energy resources between reproduction, growth, and survival (Roff 1983, Stearns 1992). Growth typically slows as females mature and energy is shifted to reproduction. Females also appear to trade between the fitness value of reproducing in the present year and the probability of survival and reproducing in future years, when they will be larger and produce more eggs (Roff 1983). The latter phenomenon occurs over evolutionary time scales, so that reproductive investment is related to longevity (Gunderson 1997), but also occurs on an annual basis within individuals (Rideout et al. 2005).

The reproductive potential of females is important for fisheries management. The establishment of acceptable fishing mortality rates is usually based on maintaining a level of reproductive potential that will allow a population to persist while simultaneously maximizing the yield to fisheries (Quinn & Deriso 1999). Because body weight and fecundity are related to changes in volume, they are generally expected to increase isometrically as fish grow as a function of the cube of fish length (Ricker 1978). Thus, fecundity is usually proportional to fish weight and most biological reference points for achieving maximum sustained yield (MSY) use spawning stock biomass (SSB) as a proxy for reproductive potential. As a result B_{MSY}, the SSB at which MSY is achieved, is considered the lower limit of acceptable stock size in many U.S. marine fisheries (Mace 2001).

Over the last decade, it has become apparent that SSB may not be an adequate measure of reproductive potential for some species. If size-specific fecundity or egg viability vary among females of a particular species, then two populations of equal biomass could differ in the total number of eggs produced depending on the composition of the population. For example, if the relative fecundity (eggs per gram body weight) of age-5 fish is half that of age-10 fish, then a population of older fish would produce twice as many eggs as a population of the same biomass made up of younger fish. Relative fecundity increases slightly with maternal age and size in some populations of Atlantic cod (Marshall et al. 1998, Marteinsdottir & Begg 2002) and is influenced by the level of energy reserves in females (Marteinsdottir & Begg 2002). Increases in egg size or survival with female age and size have been observed in Atlantic herring (Blaxter and Hempel 1963), Atlantic cod (Kjesbu et al. 1996, Marteinsdottir & Steinarsson 1998, Trippel 1998, Marteinsdottir & Begg 2002), plaice *Pleuronectes platessa* (Kennedy et al. 2007), brown trout *Salmo trutta* (Einum & Fleming 1999), walleye *Sander vitreus* (Johnston & Leggett 2002), capelin *Mallotus villosus* (Chambers et al. 1989), and black rockfish *Sebastes melanops* (Berkeley et al. 2004). In black rockfish, enhanced survival resulted from the increased size of an oil globule in the eggs containing energy-rich neutral lipids (Berkeley et al. 2004).

If reproductive potential is greater in older and larger fish, the age and size structure of a population need to be considered in attempting to understand SSB-recruitment relationships and estimating biological reference points. Inclusion of maternal effects on egg viability in recruitment models of Atlantic cod resulted in a substantial decrease in the estimated number of potential recruits, and this effect was magnified under increasingly higher levels of fishing mortality (Scott et al. 1999). Ignoring the effects of differential larval viability can also lead to the establishment of harvest rates that result in overfishing (Murawski et al. 2001, O'Farrell & Botsford 2006).

The relationship between SSB and reproductive potential is also typically assumed to be constant over time. Temporal variation in fecundity or egg viability could lead to over- or under-estimation of reproductive potential in some years. Within the same species, fecundity at length can vary interannually and among populations as a result of environmental influences or changes in female nutritional status (Paulson & Smith 1977, Bromage et al. 1990, DeMartini 1991, Kraus et al. 2000). A possible mechanism may be the relationship between egg production and liver energy content, which is an indicator of the energy reserves available for reproduction (Marshall et al. 1999, Marshall et al. 2000).

The goal of this study is to understand factors that influence the reproductive potential of female Pacific cod *Gadus macrocephalus*. Pacific cod is a demersal fish species distributed throughout the northern North Pacific Ocean. It is the target of major commercial fisheries in Canada, the United States, Russia, and Japan. It is also an important predator of fishes and benthic invertebrates (Livingston & deReynier 1996, Yang 2004) and serves as prey for other fishes and marine mammals (Westrheim 1996, Sinclair & Zeppelin 2002). At present, stock assessment models for Pacific cod in Alaska assume that SSB is directly proportional to reproductive potential and that this relationship does not change among years (Thompson et al. 2006). The veracity of this assumption is critical for effective management of Pacific cod.

We hypothesized that egg production and egg size in Pacific cod would increase with maternal age, size and condition. We gathered samples of Pacific cod from the Gulf of Alaska (GOA), eastern Bering Sea (EBS) and western Aleutian Islands (AI) over four years to meet the following objectives: 1) explore potential trade-offs between egg number and egg size, 2) determine maternal age and size effects on egg production (quantity and size), 3) quantify variability in reproductive potential among years and geographical areas, and 4) look for evidence of compromises among reproduction, growth, and survival.

Methods

Sample collection and measurement

Female Pacific cod from the GOA were obtained from commercial processing plants and during a single research cruise. Samples were collected during the winters (January-March) of 2002, 2003, and 2004. Fish sampled at plants in Kodiak, AK (57°47'N, 152°23'W) were caught using a variety of gears throughout the central GOA (U.S. National Marine Fisheries Service statistical areas 620 & 630; Fig. 4.1). Fishery observers determined sex and collected females according to a schedule of length bins based on total length (<45 cm, 45-105 cm in 5-cm increments, and >105 cm). Samples were double-wrapped in plastic bags and frozen at -20°C. Additional samples were collected during a short dedicated research cruise in March 2003. Fish were caught using jig gear at a site approximately 10 nm southeast of Kodiak (Fig. 4.1). Sample selection and preservation were identical to the observer collections.

In June of each year, samples were thawed. Sexual maturity was assessed macroscopically based on ovary wall color, relative ovary size, egg color and opacity, and the consistency of the egg mass according to an adapted 5-point maturity scale: 1-immature ; 2- developing; 3- pre-spawning; 4-spawning; and 5-spent (Appendix 4.A). Total length (to the nearest cm), total body weight (nearest 0.01 kg), gonad weight (GW; nearest g), and liver weight (LW; nearest g) were measured, and one ovary from each fish was frozen in doubled plastic bags at -20°C for further analysis. Stomach contents were removed and weighed to the nearest g. Otoliths were removed and placed in plastic vials for subsequent determination of age, which was conducted by the Age and Growth Laboratory at the Alaska Fisheries Science Center (AFSC) of the U.S. National Marine Fisheries Service (NMFS).

Female Pacific cod from the eastern Bering Sea (EBS) were collected using pot gear during winter research cruises conducted by the Fisheries Interaction Team at the AFSC. All samples were collected in a 30-nm^2 study area approximately 5 nm north of Unimak Island (54°46'N, 164°8'W; Fig. 4.1). Samples were collected during February and March of 2003 and during March of 2004 and 2005. Fish were randomly selected from the catch and females were collected for analysis according to the length schedule above. Measurements and tissues were taken as for the GOA samples except that fish were dissected immediately upon capture, and all weights were measured using motion-compensated balances (Model S182, Marel ehf., Gardabaer, Iceland; resolution = 1 g).

Samples were collected in the central and western Aleutian Islands (AI) aboard a commercial factory trawler. Samples were collected in February and March 2005 as the vessel traversed the islands from Adak Island (51°52'N, 176°39'W) to the Near Islands (52°51'N, 173°11'E) and back; the majority of samples were collected in the vicinity of Kiska Island (51°58'N, 177°29'E) and the Near Islands (Fig. 4.1). Selection, collection, and measurement were conducted as for the EBS samples.

Pacific cod were collected under federal Scientific Research Permit 2003-26, and this research was approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks (Project #04-61).

Because a variety of metrics are used to describe condition and reproductive potential, it is important to define which quantities are used in an analysis. Somatic weight (SW) was determined by subtracting GW and the weight of the stomach contents from the total body weight. Condition factor, which relates observed body weight to expected body weight, has been shown to be a good indicator of the energy reserves available to fish (Lambert & Dutil 1997a). To analyze condition effects on reproduction within a particular area and year, relative condition factor within each dataset (K_w) was calculated according to the formula K_w= SW/SW_{pw}, where SW_{pw} is the predicted SW using the formula SW=a * (total length)^b and the a and b parameters were estimated using only the data within each dataset. To compare among datasets from different areas and years, relative condition factor across datasets (K_a) was calculated according to the formula K_a= SW/SW_{pa}, where SW_{pa} is the predicted weight using all data in the entire study to estimate the parameters (Bolger & Connolly 1989, Froese 2006). Hepatosomatic index (HSI) was calculated using HSI=LW/SW.

HSI values were not calculated for the GOA datasets because livers had deteriorated sufficiently in many cases that accurate weights could not be determined. This was attributed to the fact that GOA collections came from commercial catches and may have been insufficiently refrigerated prior to being sampled. This was not a problem in the EBS and AI where samples were dissected immediately after being caught.

Several metrics were used to describe reproduction. We considered total GW to be an index of reproductive potential as it incorporates fecundity and egg size. Egg dry weight (EDW) was used to compare egg size. The gonadosomatic index (GSI) relates GW to body weight and was used to describe reproductive investment. GSI was calculated using GSI=GW/SW, where GW is the weight of both ovaries.

To ensure that we compared reproductive potential among females at equivalent stages of ovarian development, our analyses were limited to females with ovaries of maturity stage 3 (pre-spawning). Stage 4 (spawning) eggs would have been the most appropriate for comparison of egg size, but because stage-4 eggs are loose in the female and are extruded upon capture it is impossible to accurately measure total gonad weights and fecundity from spawning females. We assumed that eggs from all stage-3 females had reached a consistent stage of development where the eggs contained most of the energetic resources they would receive from the female. In fish eggs the bulk of growth in size is due to the process of vitellogenesis, in which proteins, lipids, and other molecules are deposited in the developing oocyte to form yolk (Tyler & Sumpter 1996). When vitellogenesis is complete, the eggs are hydrated and are ready to be released.

We tested the validity of our assumption in several ways. Based on microscopic assessment of stage-3 eggs and the GSI of stage-3 females in our study, we deduced that our stage 3 corresponds to the "pre-maturation" phase described in histological studies of Pacific cod ovaries in Japan (Hattori et al. 1992). This phase is immediately followed by egg hydration and ovulation. Preliminary analyses conducted by AFSC scientists indicate a high level of agreement between macroscopic assessment and histological determination of maturity stage in eggs collected from the eastern Bering Sea (S. Neidetcher, AFSC, pers. comm.). In addition, mean GSI for stage-3 females in our study is highly significantly different from all other stages, and the increase in weight between stage 3 and stage 4 ovaries can be mostly accounted for through egg hydration (Appendix 4.B). This implies that by the time the ovaries reach stage 3, females have transferred to the eggs most of the resources necessary for their survival.

Fecundity and egg size analyses

Thawed gonad samples were used for analyzing potential fecundity (PF) via gravimetry. PF is a measure of the eggs in the ovary available for spawning and is in contrast to absolute fecundity, which indicates the number of eggs actually released into the environment. Eggs per gram ovary tissue was calculated and multiplied by the total weight of both ovaries to obtain an estimate of PF. PF was determined in triplicate (~0.1

g subsamples) from each end and the middle of the ovary. Preliminary analyses indicated that while egg size was variable within some ovaries, it did not vary consistently with location in the ovary. To free the eggs from surrounding connective tissue, subsamples were transferred to glass tubes containing 2 mg of collagenase D (F. Hoffmann- La Roche Ltd., Nutley, NJ, USA) dissolved in 2 ml of 0.1M TRIS/ 5 mM $CaCl_2$ buffer (Marteinsdottir & Begg 2002). After subsamples were shaken rapidly for one hour at room temperature using a vortex mixer (Vortex-Genie 2, Scientific Industries Inc., Bohemia, NY, USA), the freed eggs were washed three times with TRIS/ $CaCl_2$ buffer. Eggs in fresh buffer were immediately transferred to a glass plate, where they were spread out in a thin layer of buffer. The glass plate was placed on a flatbed computer scanner and a high-resolution scan (600 dpi) was made of the plate.

Eggs were counted using a free image analysis program (ImageTool; University of Texas, San Antonio, TX, USA; http://ddsdx.uthscsa.edu/dig/itdesc.html). Initial counts were performed using ImageTool's automatic particle counter and were based on optical density. Any uncounted eggs were tallied manually using the "count and tag" feature of ImageTool. Two subsamples were counted initially and averaged; if the CV of the average was greater than 10% the third subsample was counted and all three estimates were averaged. Relative fecundity (RF) was calculated using the equation RF=PF/SW.

Egg dry weight was determined using data on egg water content and egg weight estimates from the fecundity analysis. Weighed single portions of eggs (~20 g) were freeze-dried until the sample weight did not change over a 24-hour period (usually 4 days). The weight lost was used to calculate % dry mass. Mean egg wet weight was calculated from the fecundity analysis and was multiplied by the % dry mass to estimate average egg dry weight for each female.

Environmental data

To examine how changes in reproductive potential in the EBS corresponded to changes in the ocean environment, we obtained environmental data for the EBS for the years 2002-2005 from the website of the Pacific Marine Environmental Laboratory (PMEL) of the U.S. National Oceanic and Atmospheric Administration (http://www.beringclimate.noaa.gov/data/). Data on annual averages and anomalies were obtained for 1) bottom temperature on the EBS shelf during the summer, 2) May sea surface temperature (SST) at a mooring in the southern EBS maintained by PMEL (M2; 56.8°N, 164°W), and 3) winter (January-April) SST at M2.

Statistical analyses

Several approaches were used to examine the influence of age, size, and condition on reproductive potential, as well as for comparing results among the six datasets. Age effects on GW, PF, EDW, GSI, and RF were analyzed using ordered ANOVA in S-Plus (Insightful, Inc., Seattle, WA, USA). Maternal size and condition effects were analyzed in two ways. Within each dataset GW, PF, length, SW, K_w and HSI were log-normalized and multiple linear regression was used to determine the variability in GW and PF explained by length only, SW only, and length plus K_w and HSI. K_w and HSI were eliminated from the multiple regression if the p-value for each variable exceeded 0.05. For comparisons among datasets, the relationships between size and GW and PF were modeled using a power function. Parameters were estimated using nonlinear least squares performed with the Solver tool in Excel (Microsoft, Inc., Redmond, WA, USA) and F values were calculated using established methods (Zar 1999). Differences in size-GW and size-PF power models among datasets were examined using F-tests (Zar 1999). Size and condition effects on EDW, GSI, and RF were analyzed via linear regression. Comparisons of mean values for K, HSI, EDW, GSI, and RF among datasets were accomplished using ANCOVA with female length as a covariate and multiple comparisons. For all tests full F- and p-values are reported and $p < 0.05$ was used as the level of significance, except in the comparison of size-GW and size-PF relationships among the EBS datasets where a Bonferroni-adjusted p-value of 0.017 was used.

Results

The number of samples collected for each dataset varied, as did the age and size of females in each dataset. Sample sizes of maturity stage-3 females available for analyses of GW ranged from 47 in the EBS in 2005 to 142 in the AI in 2005 (Table 4.1). Sample sizes for the more detailed analyses of PF and EDW (Table 4.1) were reduced by the loss of a substantial number of samples from the 2003 GOA and EBS collections due to freezer failure (N = 98) and the loss of samples from analysis errors (N = 24). Age ranges were similar among datasets, though the youngest females were sampled in the GOA and the oldest females in the AI (Table 4.1). Mean age was highest in the AI (Table 4.1). Mean length and length ranges were similar among all areas and years except the AI in 2005, where mean as well as minimum and maximum lengths were greater (Table 4.1). Similarly, mean somatic weight and the range of somatic weights were higher in the AI in 2005 than in the other datasets (Table 4.1).

Maternal effects

Maternal age influenced some of the reproductive parameters measured in this study. Age had a highly significant, positive effect on GW (Fig. 4.2 & Table 4.2) and PF (Fig. 4.3 & Table 4.2) in all areas and years. Age had a negative association with EDW in the EBS in 2005 (Table 4.2). In the GOA in 2003 and the AI in 2005, GSI increased with age (Table 4.2). RF increased with age in the GOA in 2002, the EBS in 2005, and the AI in 2005 (Table 4.2).

Female length and somatic weight were positively related to GW. In the GOA in 2002 and 2003, and the EBS in 2003 and 2005, length explained a greater percentage of the variability in GW than did somatic weight, although differences in R^2 values were small (Table 4.3). HSI was not significant in any of the multiple regressions. In the EBS in 2004 and the AI in 2005, somatic weight explained slightly more of the variability in GW than did length, and K_w made a significant contribution to the length-GW regression (Table 4.3). For datasets where K_w was significant, the R^2 values for the multiple regressions on GW were almost identical to the R^2 values for the simple somatic weight-GW regressions (Table 4.3).

Potential fecundity increased with maternal condition and size. K_w was a significant variable in the multiple regressions in all areas and years except the EBS in 2005 (Table 4.3), while HSI was not significant in any of the datasets. The additional variability explained by the inclusion of K_w in the length-PF relationship ranged from 1.3% to 3.9% (Table 4.3). The multiple regressions on PF had identical R^2 values to the somatic weight-PF relationships in the EBS in 2003 and 2004, and similar values to the somatic weight-PF models in the GOA in 2003 and AI in 2005 (Table 4.3). In the GOA in 2002, length and SW explained equivalent proportions of the variability in PF and the inclusion of K_w only slightly improved the length-PF fit (Table 4.3). In the EBS in 2005, the length-PF model had a slightly lower R^2 than the somatic weight-PF model.

The relationships between female size and EDW and GSI varied among areas and years, while RF consistently increased with female length and somatic weight. Maternal length (Fig. 4.4) and somatic weight were negatively related to EDW in the GOA in 2003 and EBS in 2005, but had no relationship to EDW in the other areas and years (Table 4.4). GSI was positively related to length in the GOA in 2003, the EBS in 2004 and 2005, and the AI in 2005, and to somatic weight in the EBS in 2004 and AI in 2005 (Table 4.4). The amount of variability in GSI explained by size was low (Table 4.4). RF increased with length in all areas and years (Fig. 4.5 & Table 4.4) and with somatic weight in all cases except for the GOA in 2002 (Table 4.4). R^2 values for the size-RF relationships were generally low but were highest in the EBS in 2005 and the AI in 2005 (Table 4.4).

Condition had a slight negative influence on EDW, GSI, and RF in some but not all areas and years. The relationship between K_w and EDW was significant only in the GOA in 2003 (Table 4.5). K_w was inversely related to GSI in the GOA in 2002 and 2003, and in the EBS in 2003 and 2005 (Table 4.5). RF was negatively associated with K_w in the GOA in 2002 and EBS in 2005 (Table 4.5).

The effects of HSI varied among areas and years. HSI was moderately correlated with maternal length in the EBS in 2003 ($r = 0.46$), 2004 ($r = 0.58$), and 2005 ($r = 0.55$), and weakly correlated with length in the AI in 2005 ($r = 0.16$). HSI was negatively associated with EDW and GSI in the EBS in 2003, but was positively related to GSI in the EBS in 2005 (Table 4.5). HSI positively affected RF in the EBS datasets (Table 4.5).

Year effects

In the GOA, reproduction and condition varied between 2002 and 2003. Gonad weight at length (Fig. 4.6A) and somatic weight (Fig. 4.6B) were greater in 2003 (Table 4.6). Potential fecundity (Fig. 4.6C & Table 4.6) increased more rapidly with total length in 2003, but there was no difference in the PF-somatic weight relationship among years (Fig. 4.6D & Table 4.6). Egg dry weight, GSI, and RF were not different between years, while K_a was higher in 2002 (Table 4.6).

In the EBS, reproductive potential was much different in 2003 relative to 2004 and 2005, but condition was similar among all years. Gonad weight at length was different among all years and was much lower in 2003 than in 2004 and 2005 (Fig. 4.7A and Table 4.6). Potential fecundity at length was also lowest in 2003, but there was no difference in the length-PF relationship between 2004 and 2005 (Fig. 4.7B and Table 4.6). Similarly, the increase in GW with somatic weight was different among all years and substantially lower in 2003 than in the other two years (Fig. 4.8A & Table 4.6). The increase in PF with somatic weight was lower in 2003 relative to the other years and was not different between 2004 and 2005 (Fig. 4.8B & Table 4.6). Egg dry weight and GSI were different among all three years, with the lowest values in 2003 and the highest values in 2005 (Table 4.6). Mean EDW and GSI in 2003 were approximately 2/3 of the 2005 values (Table 4.6). RF was highest in 2004 and lowest in 2003, and only the difference between those two years was significant (Table 4.6). K_a and HSI were not different among years (Table 4.6).

Area effects

Substantial differences in reproduction and condition existed between the GOA and EBS in 2003. GW at length was greater in the GOA (Fig. 4.9A & Table 4.7), as was GW at somatic weight (Fig. 4.9B & Table 4.7). Potential fecundity at length was not different between the two areas (Fig. 4.9C & Table 4.7), but PF increased faster with somatic weight in the GOA (Fig. 4.9D & Table 4.7). Egg dry weight, GSI, and RF were lower in the EBS (Table 4.7), but K_a was higher in the EBS (Table 4.7).

The comparison between the EBS and AI in 2005 produced contrasting results. Whereas length-GW (Fig. 4.10A) and somatic weight-GW (Fig. 4.10B) relationships were not different between the areas (Table 4.7), the AI had slightly reduced PF at length (Fig. 4.10C & Table 4.7) and somatic weight (Fig. 4.10D & Table 4.7). Egg dry weight was slightly higher in the AI (Table 4.7). GSI was greater in the AI but RF was not different between the two areas (Table 4.7). K_a and HSI were greater in the AI (Table 4.7).

Environmental data

Temperature differences in the EBS from 2002-2005 were small and all years had positive summer bottom temperature anomalies (Table 4.8). Summer bottom temperature and May SST were lowest in 2002, and winter SST was highest in 2003 (Table 4.8).

Discussion

Pacific cod appear to increase their reproductive potential by maximizing the number, and not the size, of eggs they produce. The strong maternal effect on potential fecundity, and the lack of such an effect on egg size, indicates that older and larger females devote the additional volume and energy they possess to increasing their fecundity. The slight increase in relative fecundity with female size also supports this conclusion. The consistency in potential fecundity at size among females and among areas and years suggests that, with some exceptions, females closely regulate their egg production. Fecundity is also the main determinant of gonad weight, which we used as an index of reproductive potential.

There is a strong tendency to maintain egg production in Pacific cod. Size is an excellent predictor of fecundity in Pacific cod, particularly in comparison with other species where R^2 values for size-fecundity relationships can range between 50% and 80% (e.g., Bromage et al. 1990, Chigbu & Sibley 1994, Gundersen et al. 1999, Kraus et al. 2000). This suggests that in Pacific cod, fecundity at a given size is closely regulated. The variability in gonad weight at size among years in the EBS appeared to be mostly due to differences in egg dry weight. A similar result was observed in the comparison between the EBS and GOA. Variability in GSI among all areas and years was associated with differences in EDW (Fig 4.11), suggesting that females that devote less energy to reproduction do so by reducing egg size rather than fecundity. The observation that egg size was equal between years in the GOA while the length-potential fecundity relationship was different seems to contradict this conclusion, but overall differences in reproductive potential between the two years were slight. In 2005, EDW and fecundity at size differed between the EBS and the Aleutian Islands (AI), while size-gonad weight relationships were equal. This may be evidence of different reproductive strategies between the areas. Alternatively, because the differences in EDW and potential fecundity are small, they may be statistically significant but not biologically relevant.

Maternal effects on egg size are apparently not universal among fishes. An increase in egg size with female size is sufficiently widespread among different species that it has been described as a general relationship in fishes (Kamler 2005). However, the strength of the relationship is questionable. Maternal effects on egg size are often much weaker than the relationship between female size and fecundity (Trippel 1998, Johnston & Leggett 2002, Marteinsdottir & Begg 2002, Kennedy et al. 2007), and at least one study of Atlantic cod reported no female size effects on eggs (Chambers & Waiwood 1996). In our study, the presence of a maternal effect on egg size in only two out of six area/year combinations, as well as the low observed R^2 values, makes it doubtful that this is a biologically relevant result for Pacific cod. Moreover, the relationships that were significant were negative, with older females producing smaller eggs. There is no relationship between female size and egg size in Alaskan populations of walleye pollock *Theragra chalcogramma* (Hinckley 1990), and Pacific cod and walleye pollock share ancestry with Atlantic cod (Carr et al. 1999). Therefore, it is possible that maximizing fecundity instead of egg size is a common strategy that developed among North Pacific gadids.

The absence of maternal effects on egg size in Pacific cod may also be the result of a limited history of exploitation by commercial fisheries. The historically heavy fishing pressure on Atlantic cod has resulted in females maturing at earlier ages and smaller sizes (Law 2000, Olsen et al. 2004). It is possible that the maternal effects on egg size observed in Atlantic cod are due to lower reproductive ability of young spawners that would not have matured in an unfished population (Trippel 1995). This is supported by the observation that it is first-time Atlantic cod spawners that have particularly poor reproduction (Trippel 1998). Similarly, correlations between maternal size and egg size in haddock *Melanogrammus aeglefinus* were mainly due to reduced egg size in precocious 2-year-old spawners (Hislop 1988). Although Pacific cod have been commercially fished since the late 1800s, fishing activity has been low and inconsistent until recent decades (Westrheim 1996). Therefore, Pacific cod may not have experienced the change in maturation and the reduced reproductive ability of younger spawners observed in Atlantic cod.

Reliance on maintaining egg production in Pacific cod may represent a strategy to maximize fitness through the optimal allocation of resources. Evolutionary fitness of females relies on a trade-off between the number of offspring they produce and the fitness of those offspring (Smith & Fretwell 1974, Wootton 1990). Theoretical and empirical evidence suggest that egg size evolves through selection on maternal, rather than offspring, fitness (Smith & Fretwell 1974, Einum & Fleming 1999, 2000). If this is the case, females will not produce larger eggs unless the benefits of doing so outweigh the cost of reducing the number of eggs produced. In Pacific cod, females may not be able to substantially increase offspring fitness by increasing egg size. The advantages that are thought to come from larger egg size (enhanced larval competitive ability and predator avoidance) may not be as important for Pacific cod larvae as they are in other species. Alternatively, the survival of offspring may be under such strong environmental control, e.g., the timing and availability of phytoplankton prey (Cushing 1975), that the female is unable to enhance the fitness of offspring by supplying them with additional resources. The lack of maternal effects on egg size in walleye pollock suggests that this may be a North Pacific Ocean phenomenon.

Our results validate the use of spawning biomass as a proxy for reproductive potential in the management of Pacific cod. Because egg size does not vary with age or size, it is likely that offspring viability is similar among ages and that the number of offspring produced by cod is a good indicator of reproductive potential. However, age structure may affect other aspects of spawning. Older Atlantic cod spawn for longer periods of time than younger Atlantic cod (Hutchings & Myers 1993, Lawson and Rose 2000), and delayed spawning of Atlantic cod in the Baltic Sea during the 1990s was partially due to the prevalence of younger spawners in the population (Wieland et al. 2000). Modeling of Atlantic cod populations suggested that truncation of age structure shortens the total spawning time and may impact stock reproductive potential (Scott et al. 2006). In addition, because relative fecundity increases with female size in Pacific cod, a population biomass made up of larger fish would potentially produce a greater number of eggs than one made up of smaller fish. Maternal effects on relative fecundity could be accounted for by introducing the proper scaling factor into the individual size-fecundity relationships in assessment models.

Our results support approaches to fisheries management that restrict fishing to a level that limits truncation of age and size structure of fished populations. Pacific cod are managed under a strict system that limits catches to levels determined appropriate by fishery scientists (Thompson et al. 2006). The trend towards earlier maturation observed in Atlantic cod does not appear to be present in Pacific cod, although data are sparse. Maternal size effects on reproductive potential in Atlantic cod may be due to early maturation of young fish that produce less viable eggs (Olsen et al. 2004, Trippel 1998). If so, the lack of such effects in Pacific cod supports current management practices, which may have helped to maintain age at maturity above a threshold level where reproductive potential is reduced.

Interannual variability in reproduction presents a problem for stock assessment. Because the relationship between spawning biomass and reproductive potential is assumed to be constant over time, the reduced gonad weight and potential fecundity observed in the EBS during 2003 may have led to an overestimation of reproductive potential in that year. Unfortunately, we were unable to identify easily measured metrics, such as changes in body condition that could be used to detect symptoms of reproductive impairment. A concerted monitoring effort, such as a program to annually assess size-gonad weight relationships, would provide the ability to detect changes in reproduction and incorporate that information into assessment models.

Somatic weight may integrate the effects of length and condition on fecundity. The somatic weight of Pacific cod was a better predictor of fecundity than was length, although the difference was slight, and similar results were observed in Atlantic cod (Koops et al. 2004, Trippel & Neil 2004, Skæraasen et al. 2006) and walleye pollock (Hinckley 1987). Weight may best predict fecundity because it integrates size and condition information (Koops et al. 2004), which is supported in our study by the observation that somatic weight explained approximately the same amount of variability in fecundity as did the combination of length and condition. Alternatively, fecundity may depend on weight because weight is a gauge of the volume of space available for egg production. Thus, condition may be an important factor simply because it adjusts length values to account for weight. While the results of our study cannot resolve this question, there does seem to be an interaction between length, somatic weight, and condition.

It is possible that condition influences reproductive potential only when energy reserves are severely depleted. The small amount of additional variance in potential fecundity explained by condition in Pacific cod contrasts with observations in Atlantic cod, where condition had a substantial influence on fecundity (Kjesbu et al. 1991, Marteinsdottir & Steinarsson 1998, Lambert & Dutil 2000, Marteinsdottir & Begg 2002). Reduced fecundity in Atlantic cod was related to major changes in ocean temperature and food availability that heavily impacted condition (Kjesbu et al. 1998). Laboratory experiments that demonstrated condition effects on reproduction in Atlantic cod used females that varied widely in weight at length (Lambert & Dutil 2000). Length-weight relationships in our study were very tight, so the range in condition among the females we used may simply have been too small to observe effects on reproduction.

Total liver energy, which is related to liver size and thus HSI, has been suggested as a proxy for egg production in Atlantic cod (Marshall et al. 1999, Marshall et al. 2000). Pacific cod also rely on energy stored in the liver during gonadal development and spawning (Smith et al. 1990). Therefore, the lack of a relationship between HSI and fecundity found here was unexpected. There are several possible reasons why we did not observe HSI effects. HSI and female length were positively correlated, so it may have been impossible to separate their effects. Alternatively, we may have measured liver size during the wrong season. Atlantic cod fecundity is related to the liver lipid energy they possess 3-4 months before the start of spawning, while liver energy at the time of spawning has a much weaker relationship with fecundity (Skæraasen et al. 2006). This agrees with the hypothesis that reproductive output depends on an assessment of female energy reserves at the start of vitellogenesis (Kjesbu et al. 1991). Another possibility is that, as with condition, the range of HSI values observed in our study was too limited to detect impacts on reproduction. In Atlantic cod HSI has an asymptotic relationship with total liver energy, and the increase in liver energy with liver size is much slower at HSI values above 5% (Lambert & Dutil 1997a). Mean HSI was greater than 6% in all areas and years in the current study, so there may not have been a large difference in total liver energy.

Variation in reproductive investment among female Pacific cod may represent differential allocation of energy towards growth and reproduction. The distribution of energy in fish changes as they grow older (Roff 1983, Stearns 1992). Immature fish devote energy mainly to growth, while older females put more energy into reproduction. In the present study, GSI values increased slightly with age and length in most cases, suggesting that younger females invest slightly less of their energy in reproduction relative to older females. This is consistent with theory that predicts that at transition ages, when females first begin to spawn, they are still dividing energetic resources between growth and reproduction (Heino & Kaitala 1996, Charnov et al. 2001).

Our results suggest that there is an energetic trade-off between reproduction and survival in Pacific cod. Mature female Atlantic cod appear not to spawn in some years (Burton et al. 1997, Rideout et al. 2000), apparently due to low nutritional status (Rideout et al. 2000). Fish in poor condition face an increased risk of mortality (Lambert & Dutil 1997b). Thus, there appears to be an evolutionary trade-off between the fitness value of reproduction in the current year and the probability of survival and future reproduction (Fiksen & MacKenzie 2001, Rideout et al. 2005). While our study was not designed to examine the possibility of non-spawning in mature females, we did observe an apparent compromise between condition and reproductive investment in the EBS. Condition and HSI were similar among years but mean GSI differed, suggesting that females were defending energy stores at the expense of reproduction. The opposite trade-off was observed in the GOA, where condition was different between 2002 and 2003 but GSI was not, and the contrast in results between the two areas is difficult to explain. While it is possible that environmental or demographic factors differ sufficiently between the EBS and the GOA to alter the selective forces that affect fitness, it seems unlikely that two adjacent populations of the same species would have opposite behaviors. Data from a greater number of years may be necessary to fully evaluate this phenomenon.

Compromises between reproduction and the maintenance of energy stores are less clear in the comparisons among areas. In 2003, females appeared to be trading off between condition and GSI between the EBS and GOA. However, females from the two areas did not maintain a common level of either condition or reproductive investment. There may be differences in growth or morphology among the two populations that hinder a direct comparison, and analysis of area effects in 2003 is complicated by the extremely low reproductive potential in the EBS. The high HSI value observed in the AI in 2005 relative to the EBS, which was the only parameter that was substantially different between the two areas, may not be a meaningful result if HSI values were universally high among all areas and years.

Female reproduction in the EBS in 2003 may have been negatively affected by ocean temperature. Because fish are ectotherms, metabolic rates and energetic costs increase with ambient temperature (Jobling 1994). Pacific cod store energy during the summer when feed is abundant and temperatures are high, and transfer those energy stores to their eggs in the fall and winter (Smith et al. 1990, Westrheim 1996). During the summer of 2002, when Pacific cod that spawned in 2003 were storing energy, bottom temperatures and May sea surface temperatures in the EBS were lower than they were in 2003 and 2004. Conversely, 2003 winter sea surface temperatures were the highest of any year. Thus females spawning in the winter of 2003 had experienced lower temperatures in summer, when they were storing energy, and higher temperatures in the winter when they were putting energy towards eggs. The higher winter temperatures may have increased overall energy expenditure, reducing energy available for reproduction in females that already had lower energy reserves due to a colder summer.

Acknowledgments

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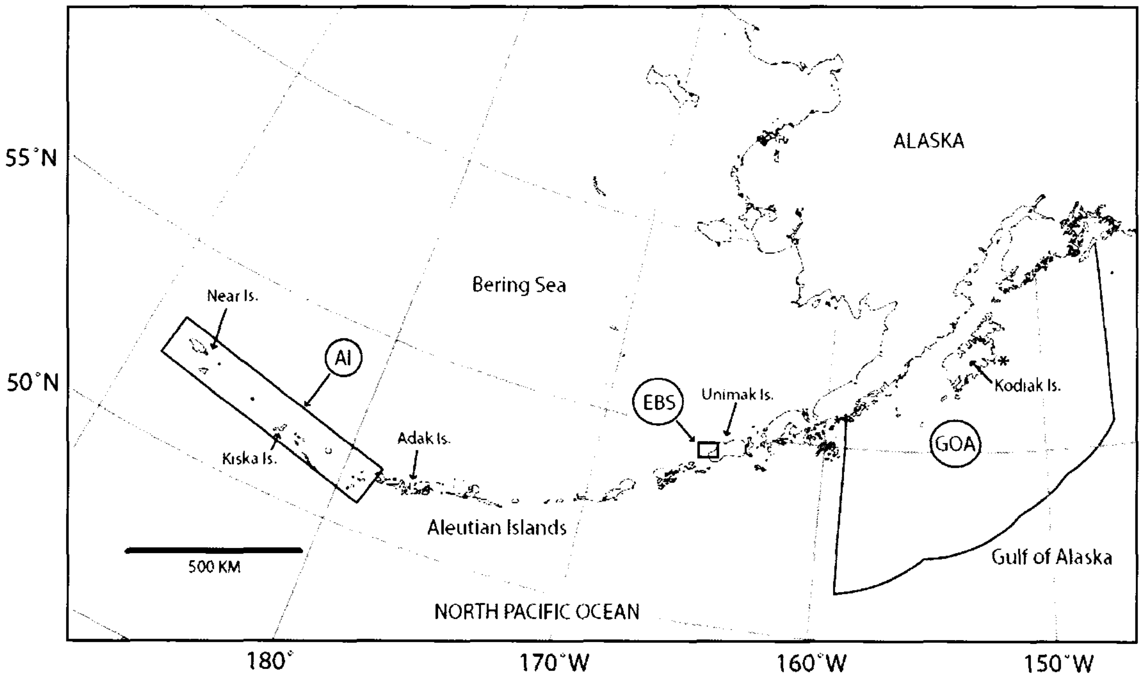

Figure 4.1. Map of the North Pacific Ocean showing locations where Pacific cod samples were collected. Circled letters indicate area: Gulf of Alaska (GOA), eastern Bering Sea (EBS), and western Aleutian Islands (AI). For the AI and EBS, boxes indicate approximate spatial extent of sample collection. For the GOA, polygon marks the U.S. National Marine Fisheries Service statistical areas (620 & 630) where samples collected in processing plants on Kodiak Island were caught in commercial fisheries. The asterisk indicates approximate location of research cruise near Kodiak, AK.

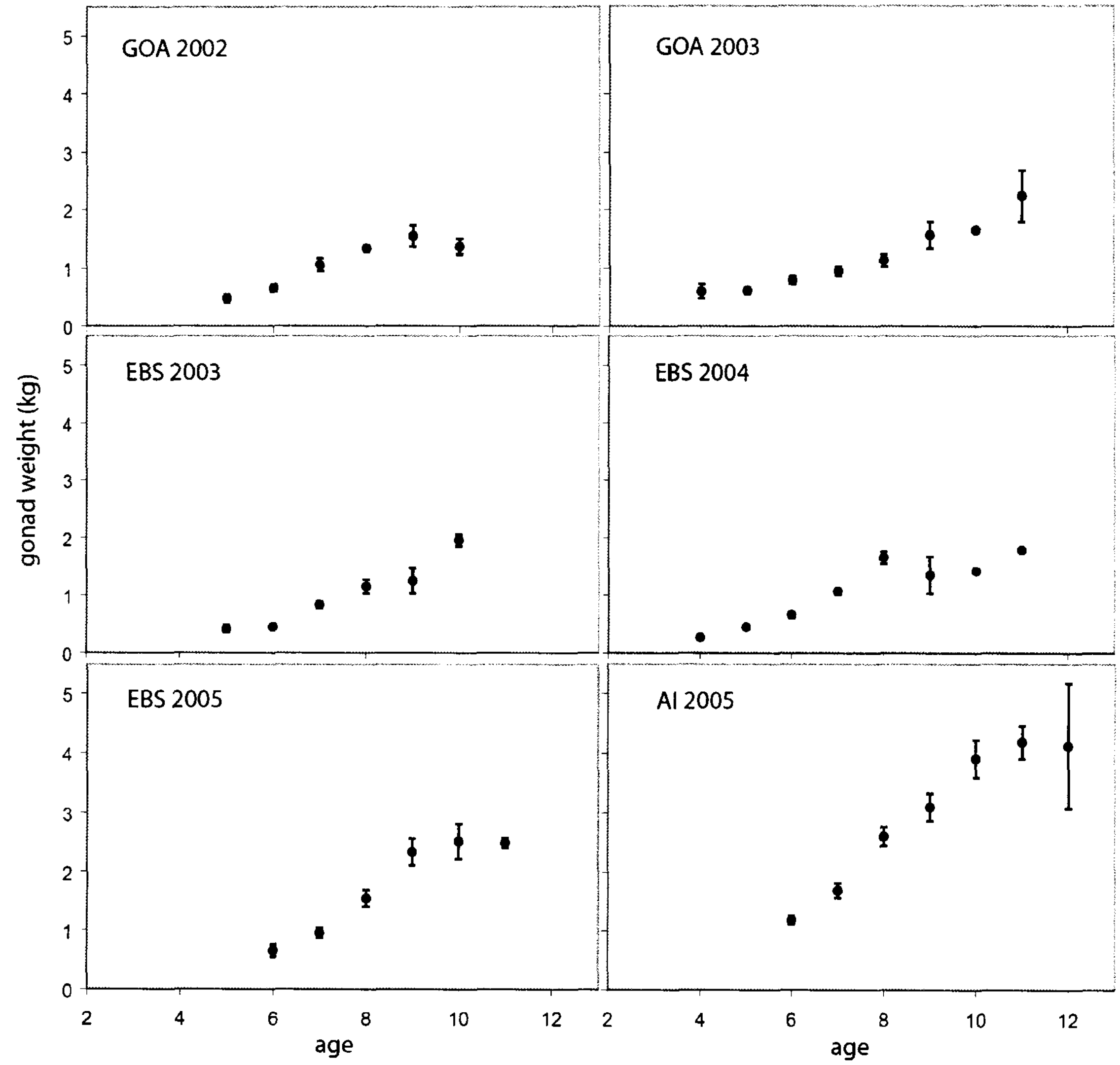

Figure 4.2. Relationships between gonad weight and age for female Pacific cod collected in the Gulf of Alaska (GOA) in 2002 and 2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. Values shown are means ± standard error.

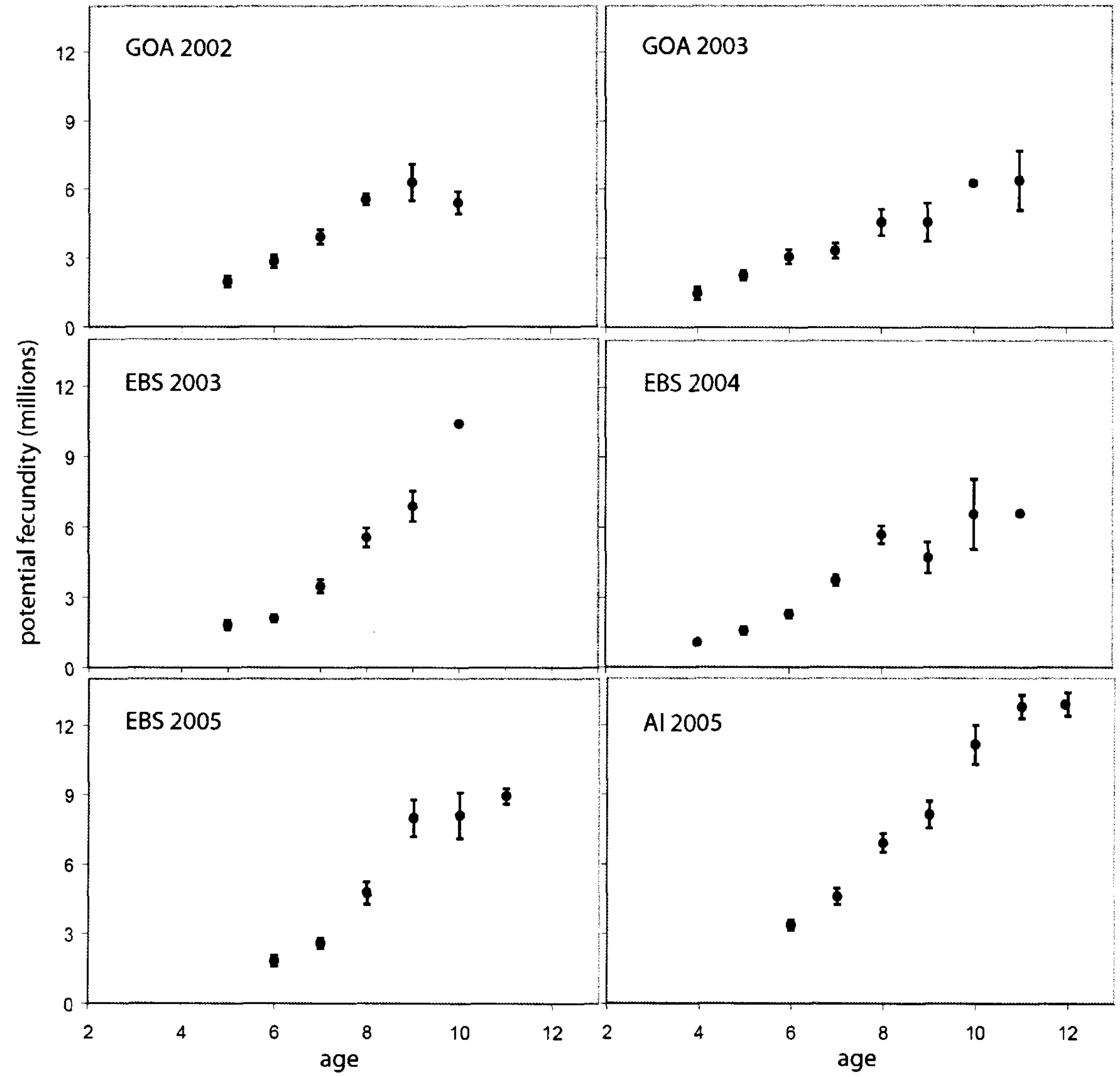

Figure 4.3. Relationships between potential fecundity and age for female Pacific cod collected in the Gulf of Alaska (GOA) in 2002 and 2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. Values shown are means ± standard error.

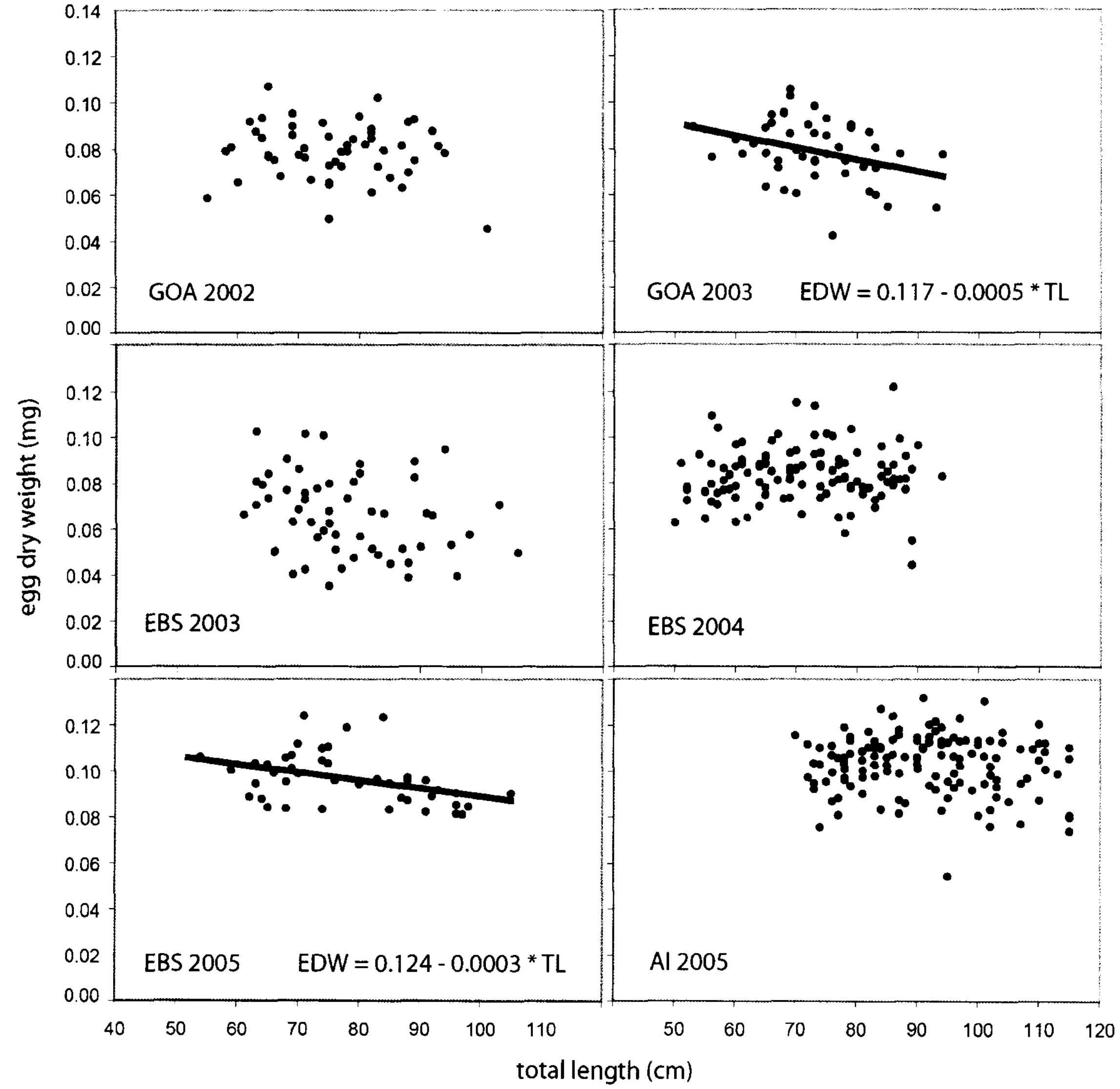

Figure 4.4. Relationships between egg dry weight (EDW) and total length (TL) for female Pacific cod collected in the Gulf of Alaska (GOA) in 2002 and 2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. Lines are predicted values from linear regression for cases where the model was significant at the $p < 0.05$ level.

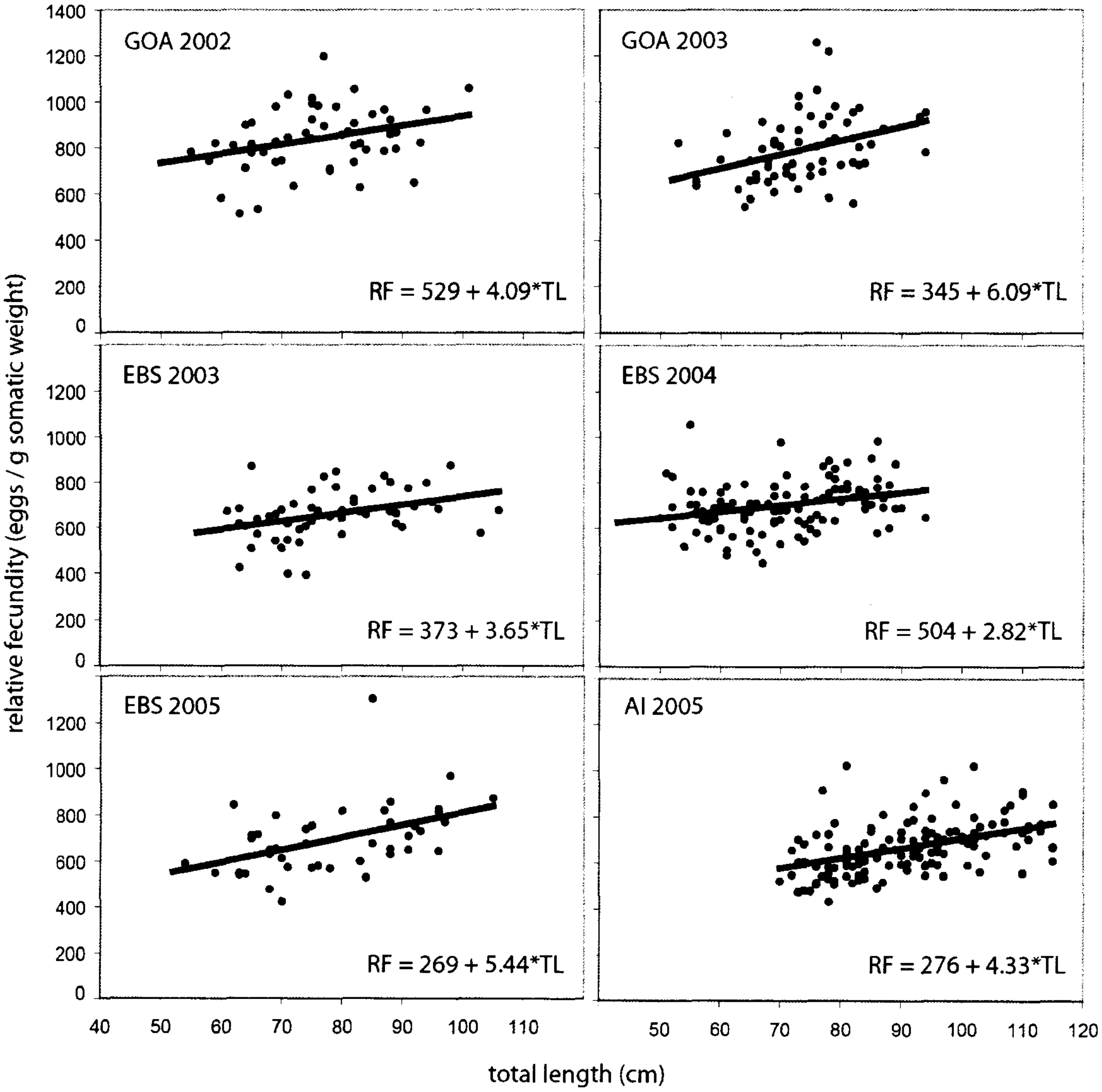

Figure 4.5. Relationships between relative fecundity (RF) and total length (TL) for female Pacific cod collected in the Gulf of Alaska (GOA) in 2002 and 2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. Lines are predicted values from linear regression and for cases where the model was significant at the $p < 0.05$ level.

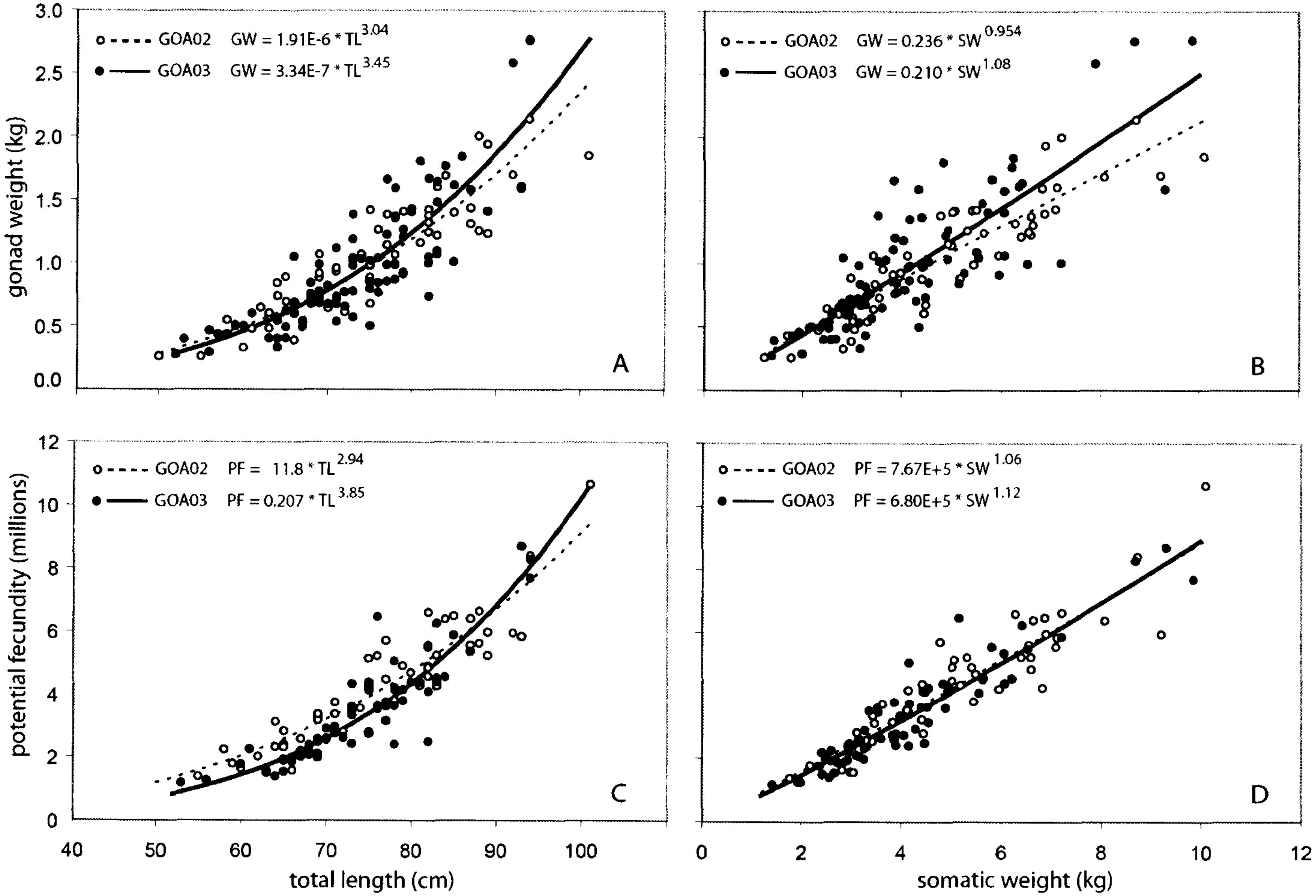

Figure 4.6. Maternal size effects on reproductive potential for Pacific cod collected in the Gulf of Alaska in 2002 and 2003 with respect to (A) gonad weight (GW) vs. total length (TL), (B) GW vs. somatic weight (SW), (C) potential fecundity (PF) vs. TL , and (D) PF vs. SW. Circles indicate observed values for 2002 (open) and 2003 (filled). Lines are nonlinear regressions for 2002 (broken) and 2003 (solid).

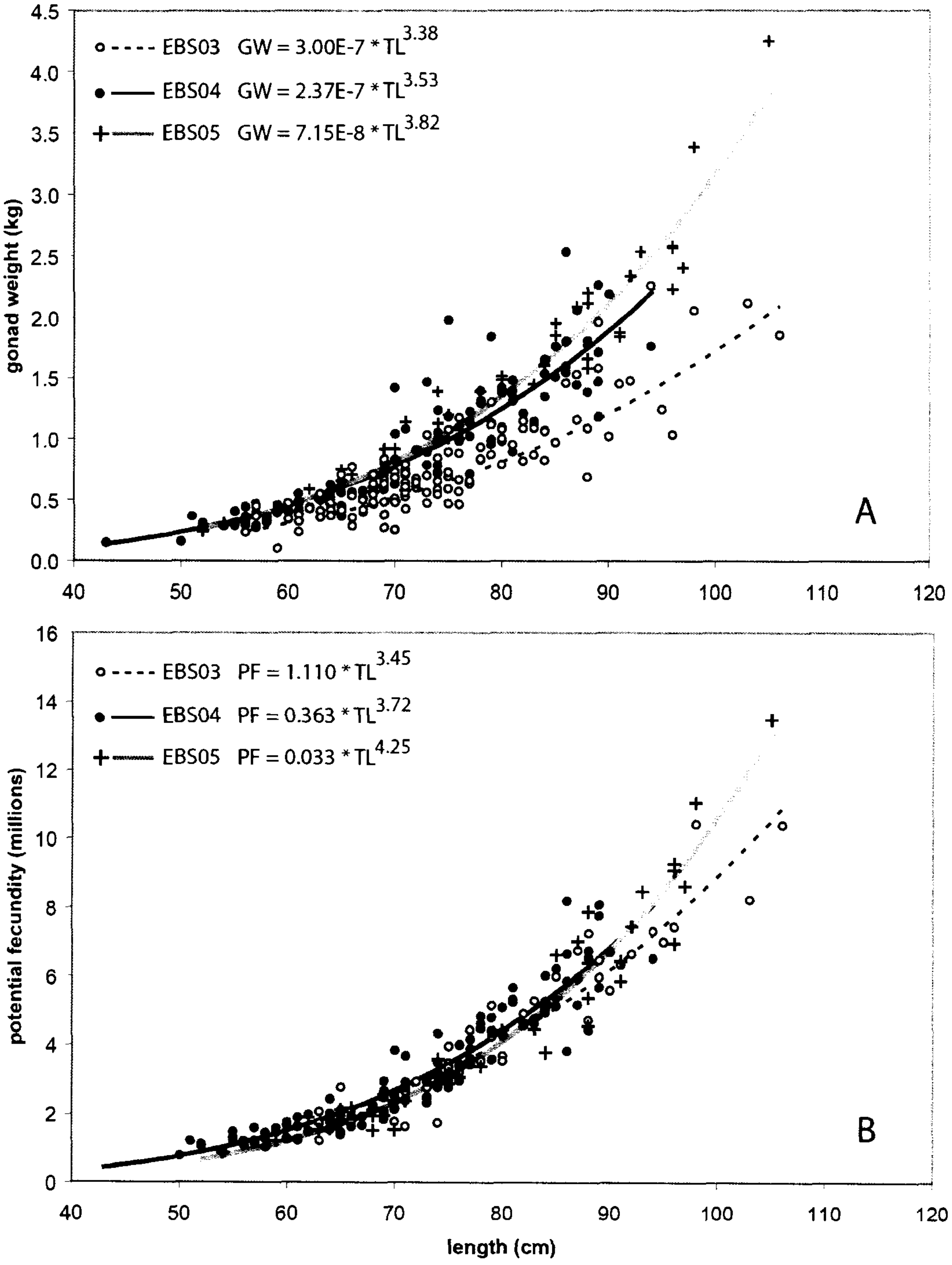

Figure 4.7. Maternal length effects on reproductive potential for Pacific cod collected in the eastern Bering Sea in 2003, 2004, and 2005. (A) gonad weight (GW) vs. total length (TL) and (B) potential fecundity (PF) vs. TL. Observed values are shown for 2003 (open circles), 2004 (filled circles), and 2005 (crosses). Lines are predicted values from nonlinear regression for 2003 (dashed), 2004 (solid black), and 2005 (solid gray).

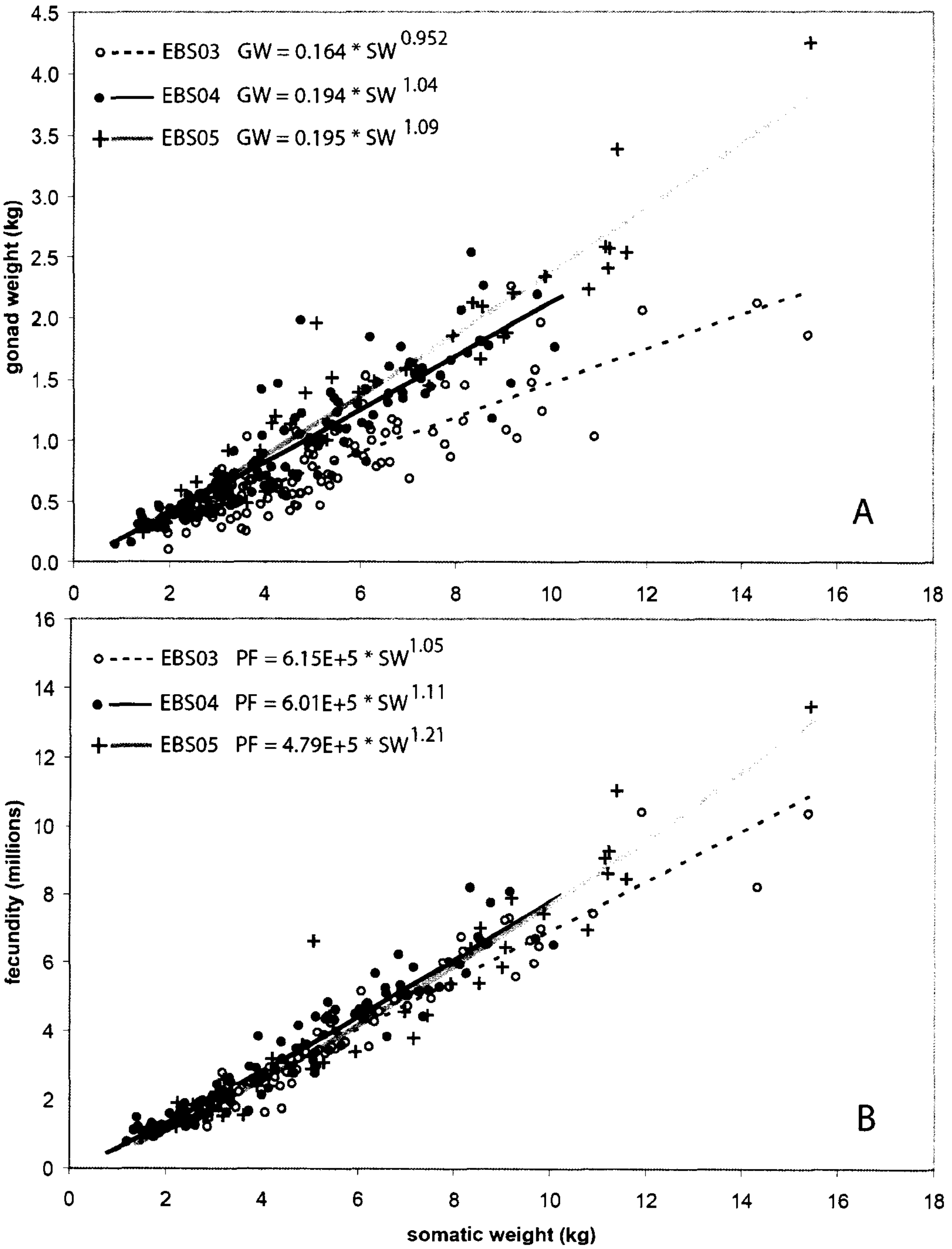

Figure 4.8. Effects of maternal somatic weight on reproductive potential for Pacific cod collected in the eastern Bering Sea in 2003, 2004, and 2005. (A) gonad weight (GW) vs. somatic weight (SW) and (B) potential fecundity (PF) vs. SW. Observed values are shown for 2003 (open circles), 2004 (filled circles), and 2005 (crosses). Lines are predicted values from nonlinear regression for 2003 (dashed), 2004 (solid black), and 2005 (solid gray).

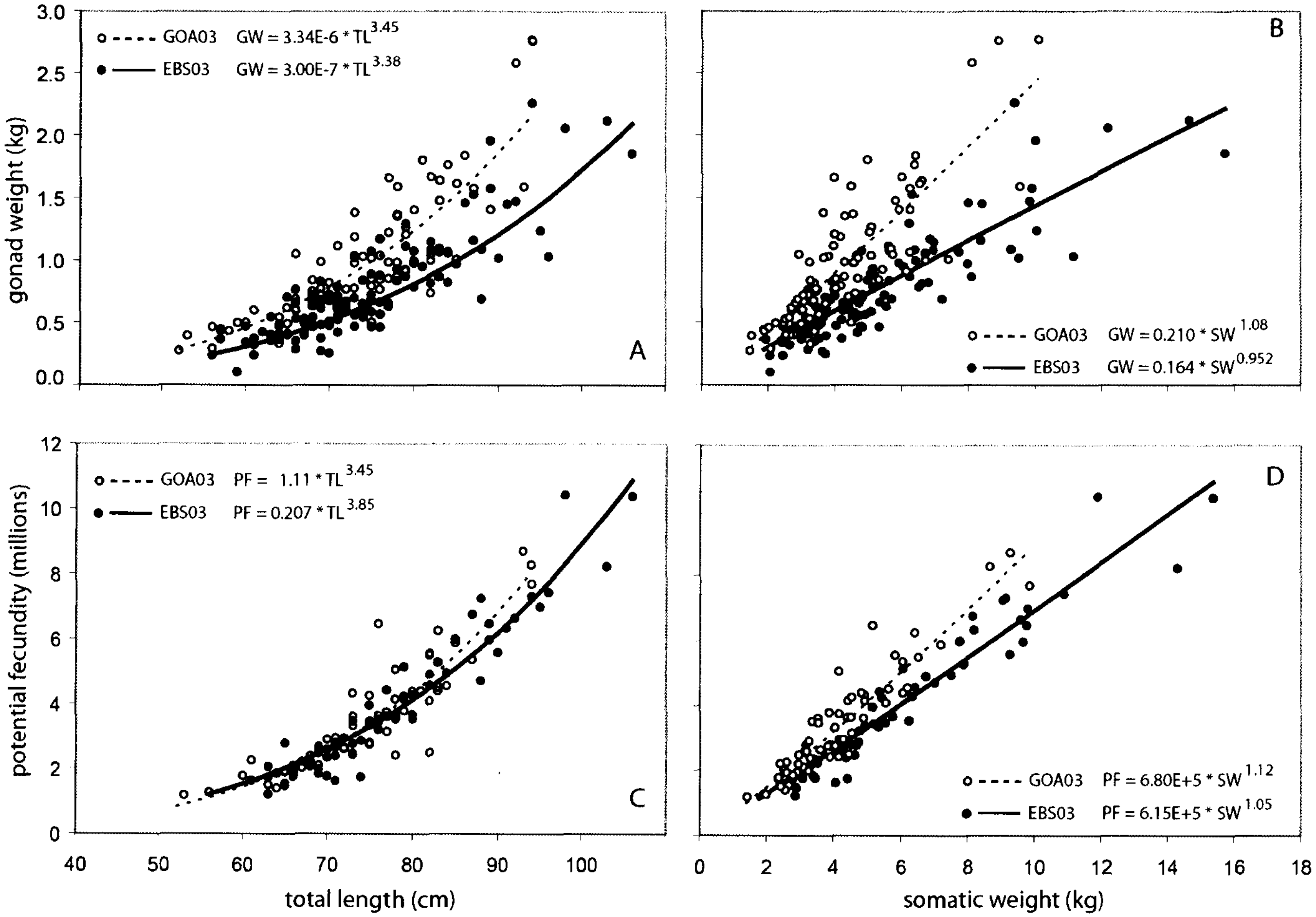

Figure 4.9. Comparison of reproductive relationships between female Pacific cod collected in the Gulf of Alaska (GOA) and the eastern Bering Sea (EBS) in 2003. (A) gonad weight (GW) vs. total length (TL), (B) GW vs. somatic weight (SW), (C) potential fecundity (PF) vs. TL, (D) PF vs. SW. Observed values are shown for the GOA (open circles) and EBS (filled circles). Lines are predicted values from nonlinear regression for the GOA (dashed) and EBS (solid).

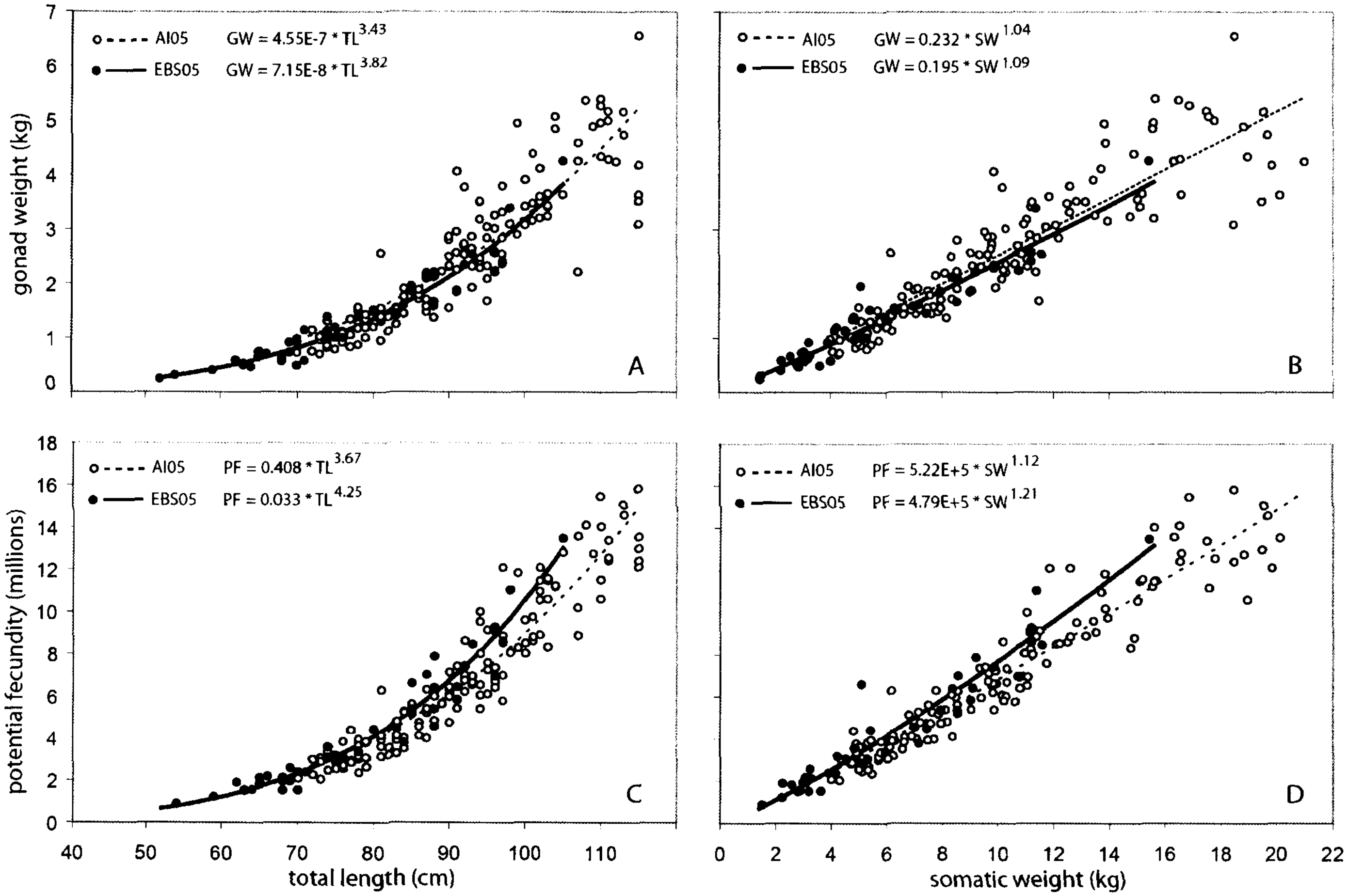

Figure 4.10. Comparison of reproductive relationships between female Pacific cod collected in the Aleutian Islands (AI) and eastern Bering Sea (EBS) in 2005. (A) gonad weight (GW) vs. total length (TL), (B) GW vs. somatic weight (SW), (C) potential fecundity (PF) vs. TL, (D) PF vs. SW. Observed values are shown for the AI (open circles) and EBS (filled circles). Lines are predicted values from nonlinear regression for the AI (dashed) and EBS (solid).

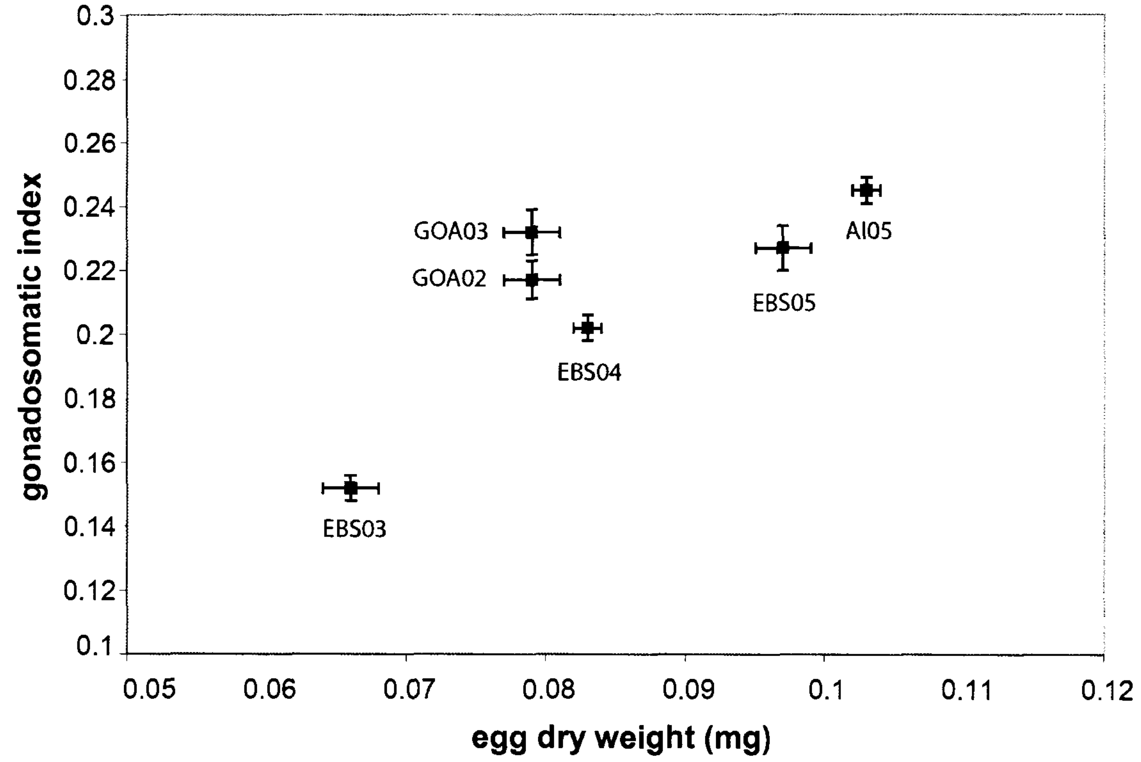

Figure 4.11. Mean gonadosomatic index vs. mean egg dry weight for sampled Pacific cod from six area/year combinations corresponding to the Gulf of Alaska (GOA, Aleutian Islands (AI), and eastern Bering Sea (EBS) in 2002 (02) through 2005 (05). Means are shown ± SE.

Table 4.1. Descriptive statistics of female Pacific cod collected in the Gulf of Alaska (GOA) in 2002-2003, eastern Bering Sea (EBS) in 2002-2005, and Aleutian Islands (AI) in 2005. SW = somatic weight. N_1 refers to sample size available for analyses of gonad weight and gonadosomatic index. N_2 is the sample size available for analyses of fecundity, egg dry weight, and relative fecundity. F and p values are the results of ANOVAs and multiple comparisons; means with common subscripts are not significantly different.

dataset		age	length	SW	N_1	N_2
GOA 2002	mean	7.1^a	74.4^a	4.77^a	58	52
	SE	0.2	1.5	0.26		
	min.	3	50	1.24		
	max.	10	101	10.10		
GOA 2003	mean	6.5^a	74.0^a	4.33^a	88	63
	SE	0.2	1.3	0.22		
	min.	4	52	1.38		
	max.	11	94	9.29		
EBS 2003	mean	6.9^a	73.9^a	4.94^a	119	56
	SE	0.1	0.9	0.23		
	min.	5	56	1.79		
	max.	10	106	15.37		
EBS 2004	mean	6.5^a	70.4^a	4.24^a	132	112
	SE	0.1	1.0	0.19		
	min.	4	43	0.87		
	max.	11	94	10.07		
EBS 2005	mean	7.7^b	77.7^a	6.00^b	47	44
	SE	0.2	1.9	0.49		
	min.	5	52	1.45		
	max.	12	105	15.43		
AI 2005	mean	8.1^b	91.1^b	10.03^c	142	137
	SE	0.1	1.0	0.37		
	min.	5	70	3.99		
	max.	14	115	20.98		
	F	18.91	59.85	72.99		
	p	<0.0001	<0.0001	<0.0001		

Table 4.2. F-statistics and p-values for ANOVA tests for female age effects on reproductive parameters of Pacific cod collected in the Gulf of Alaska (GOA) in 2002-2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. GW = gonad weight, PF = potential fecundity, EDW = egg dry weight, GSI = gonadosomatic index, RF = relative fecundity. Bold indicates statistical significance at the p<0.05 level. See Table 4.1 for sample sizes.

dataset		GW	PF	EDW	GSI	RF
GOA 2002	F	**15.70**	**13.35**	0.61	0.44	**2.83**
	p	**<0.0001**	**<0.0001**	0.6930	0.8195	**0.0280**
GOA 2003	F	**12.77**	**6.29**	1.94	**2.90**	1.95
	p	**<0.0001**	**<0.0001**	0.0898	**0.0095**	0.0786
EBS 2003	F	**19.60**	**31.45**	1.03	0.89	**5.42**
	p	**<0.0001**	**<0.0001**	0.4091	0.4893	**0.0005**
EBS 2004	F	**32.86**	**31.26**	1.25	1.51	1.46
	p	**<0.0001**	**<0.0001**	0.2813	0.1710	0.1913
EBS 2005	F	**21.83**	**27.74**	**3.60**	0.45	1.64
	p	**<0.0001**	**<0.0001**	**0.0093**	0.8073	0.1746
AI 2005	F	**22.28**	**30.22**	1.20	**2.79**	**3.34**
	p	**<0.0001**	**<0.0001**	0.3088	**0.0139**	**0.0043**

Table 4.3. Regression results for female size effects on gonad weight and potential fecundity of Pacific cod collected in the Gulf of Alaska (GOA) in 2002-2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. L = length, Kw = relative condition factor within datasets, SW = somatic weight. β_0 is the intercept and β_1 and β_2 are the coefficients of the variables included in the model. ΔR^2 is the change in the R^2 value for that variable set relative to the R^2 for the length-only regression. Dashes indicate that K_w was not significant in that dataset at the $p<0.05$ level. For sample sizes see Table 4.1.

GONAD WEIGHT

dataset	variable(s)	β_0	β_1	β_2	R^2	F	p	ΔR^2
GOA 2002	ln L	-5.24	1.38		0.84	294.9	<0.0001	
	ln L + ln K_w	-	-	-	-	-	-	-
	ln SW	-0.01	0.47		0.828	268.9	<0.0001	-0.012
GOA 2003	ln L	-6.04	1.56		0.718	219.3	<0.0001	
	ln L + ln K_w	-	-	-	-	-	-	-
	ln SW	-0.2	0.5		0.684	186.5	<0.0001	-0.034
EBS 2003	ln L	-5.54	1.41		0.752	354.4	<0.0001	
	ln L + ln K_w	-	-	-	-	-	-	-
	ln SW	-0.09	0.42		0.744	340	<0.0001	-0.008
EBS 2004	ln L	-5.79	1.51		0.841	687.2	<0.0001	
	ln L + ln K_w	-6.34	1.51	0.78	0.856	382.1	<0.0001	0.015
	ln SW	0.01	0.45		0.855	766.2	<0.0001	0.014
EBS 2005	ln L	-7.73	1.97		0.922	531.3	<0.0001	
	ln L + ln K_w	-	-	-	-	-	-	-
	ln SW	-0.1	0.56		0.91	452.5	<0.0001	-0.012
AI 2005	ln L	-10.55	2.61		0.857	849.6	<0.0001	
	ln L + ln K_w	-11.38	2.6	1.26	0.875	487.6	<0.0001	0.018
	ln SW	-0.53	0.78		0.874	974.7	<0.0001	0.017

Table 4.3 continued.

POTENTIAL FECUNDITY

dataset	variable(s)	β_0	β_1	β_2	R^2	F	p	ΔR^2
GOA 2002	ln L	1.86	3.08		0.869	332.2	<0.0001	
	ln L + ln K_w	0.77	3.16	1.04	0.882	184	<0.0001	0.013
	ln SW	13.46	1.11		0.866	323.3	<0.0001	-0.003
GOA 2003	ln L	-0.6	3.62		0.839	317.5	<0.0001	
	ln L + ln K_w	-1.25	3.49	1.75	0.878	216.4	<0.0001	0.039
	ln SW	13.35	1.16		0.872	415.1	<0.0001	0.033
EBS 2003	ln L	-1.23	3.75		0.893	450.8	<0.0001	
	ln L + ln K_w	-3.08	3.81	2.25	0.914	281.2	<0.0001	0.021
	ln SW	13.14	1.14		0.914	572.9	<0.0001	0.021
EBS 2004	ln L	-0.66	3.64		0.924	1343	<0.0001	
	ln L + ln K_w	-1.8	3.61	1.82	0.939	844.4	<0.0001	0.015
	ln SW	13.35	1.08		0.939	1686	<0.0001	0.015
EBS 2005	ln L	-2.6	4.06		0.946	737.3	<0.0001	
	ln L + ln K_w	-	-	-	-	-	-	-
	ln SW	13.19	1.14		0.926	523.5	<0.0001	-0.02
AI 2005	ln L	-2.17	3.95		0.912	1412	<0.0001	
	ln L + ln K_w	-3.31	3.94	1.7	0.927	851.5	<0.0001	0.015
	ln SW	13.02	1.17		0.924	1647	<0.0001	0.012

Table 4.4. Regression results for female size effects on reproductive parameters of Pacific cod collected in the Gulf of Alaska (GOA) in 2002-2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. EDW = egg dry weight, GSI = gonadosomatic index, RF = relative fecundity. Values are the results of simple linear regression; β_1 indicates the coefficient on the independent variable. Results listed in bold are significant at the $p<0.05$ level. See Table 4.1 for sample sizes.

		LENGTH			SOMATIC WEIGHT		
dataset		EDW	GSI	RF	EDW	GSI	RF
GOA 2002	β_1			**4.09**			
	R^2			**0.09**			
	F	0.58	0.74	**5.23**	0.60	0.05	2.19
	p	0.4511	0.3933	**0.0265**	0.4417	0.8205	0.1455
GOA 2003	β_1	**-0.0005**	**0.002**	**6.09**	**-0.003**		**25.94**
	R^2	**0.12**	**0.05**	**0.13**	**0.18**		**0.09**
	F	**5.72**	**4.78**	**9.14**	**9.93**	0.91	**6.05**
	p	**0.0211**	**0.0315**	**0.0037**	**0.0029**	0.3420	**0.0168**
EBS 2003	β_1			**3.65**			**12.1**
	R^2			**0.14**			**0.11**
	F	3.94	<0.00	**8.68**	3.63	0.15	**6.69**
	p	0.0523	0.9629	**0.0047**	0.0621	0.6951	**0.0124**
EBS 2004	β_1		**0.001**	**2.82**		**0**	**15.01**
	R^2		**0.05**	**0.08**		**0.04**	**0.1**
	F	0.06	**6.84**	**9.97**	0.03225	**4.77**	**11.66**
	p	0.8012	**0.0010**	**0.0021**	0.8578	**0.0308**	**0.0009**
EBS 2005	β_1	**-0.0003**	**0.001**	**5.44**	**-0.001**		**17.01**
	R^2	**0.16**	**0.09**	**0.21**	**0.17**		**0.15**
	F	**8.05**	**4.42**	**11.13**	**8.38**	7.85	**7.24**
	p	**0.0070**	**0.0412**	**0.0018**	**0.0060**	0.1807	**0.0102**
AI 2005	β_1		**0.001**	**4.33**		**0.003**	**10.59**
	R^2		**0.1**	**0.21**		**0.07**	**0.17**
	F	1.22	**16.1600**	**35.4000**	1.44	**10.88**	**27.37**
	p	0.2706	**<0.0001**	**<0.0001**	0.2323	**0.0012**	**<0.0001**

Table 4.5. Regression results for female condition effects on reproductive parameters of Pacific cod collected in the Gulf of Alaska (GOA) in 2002-2003, eastern Bering Sea (EBS) in 2003-2005, and Aleutian Islands (AI) in 2005. K_w = relative condition factor within datasets, HSI = hepatosomatic index, EDW = egg dry weight, GSI = gonadosomatic index, RF = relative fecundity. Values are the results of simple linear regression; β_1 indicates the coefficient on the independent variable. Results listed in bold are significant at the $p<0.05$ level. See Table 4.1 for sample sizes.

		K_w			HSI		
dataset		EDW	GSI	RF	EDW	GSI	RF
GOA 2002	β_1		**-0.102**	**-425**			
	R^2		**0.07**	**0.11**			
	F	0.37	**4.1**	**6.03**			
	p	0.5453	**0.0476**	**0.0175**			
GOA 2003	β_1	**-0.043**	**-0.164**				
	R^2	**0.14**	**0.12**				
	F	**7.18**	**12.01**	0.02			
	p	**0.0103**	**0.0008**	0.8834			
EBS 2003	β_1		**-0.093**		**-0.366**	**-0.566**	**1855**
	R^2		**0.04**		**0.12**	**0.06**	**0.08**
	F	0.73	**4.63**	<0.00	**7.2**	**7.43**	**4.82**
	p	0.398	**0.0334**	0.9759	**0.0096**	**0.0074**	**0.0324**
EBS 2004	β_1						**1907**
	R^2						**0.08**
	F	0.06	2.27	0.08	2.88	0.06	**9.15**
	p	0.8005	0.1346	0.7842	0.0924	0.7998	**0.0031**
EBS 2005	β_1		**-0.238**	**-721**		**0.815**	**4022**
	R^2		**0.21**	**0.18**		**0.09**	**0.19**
	F	0.98	**12.09**	**9.19**	1.57	**4.34**	**9.81**
	p	0.3267	**0.0011**	**0.0042**	0.2168	**0.0431**	**0.0032**
AI 2005	β_1						
	R^2						
	F	1.09	0.29	0.91	0.16	0.22	0.74
	p	0.2992	0.5881	0.3423	0.6899	0.6375	0.39

Table 4.6. Comparison of maternal effects, reproductive parameters, and body condition among years within the Gulf of Alaska (GOA) in 2002-2003 and eastern Bering Sea (EBS) in 2003-2005. Left columns contain results of F-tests on models relating length and somatic weight (SW) to gonad weight (GW) and potential fecundity (PF). Right columns contain results of ANCOVAs on mean values of relative condition factor across datasets condition factor (K_a), hepatosomatic index (HSI), egg dry weight in mg (EDW), gonadosomatic index (GSI), and relative fecundity (RF) using length as a covariate. Results listed in bold are significant at the p<0.05 level except for the EBS model comparisons, where a Bonferroni-adjusted p-value of 0.017 was used. For EBS samples, asterisks indicate significant differences among datasets. See Table 4.1 for sample sizes.

model comparisons

GOA: 2002 vs 2003	F	p
length x GW	**4.62**	**0.0114**
SW x GW	**3.40**	**0.0363**
length x PF	**7.42**	**0.0009**
SW X PF	0.47	0.627

EBS: 2003 vs 2004	F	p
length x GW	**49.05**	**<0.0001**
SW x GW	**66.29**	**<0.0001**
length x PF	**6.01**	**0.003**
SW X PF	**10.10**	**0.0001**
EBS: 2003 vs 2005	**F**	**p**
length x GW	**94.71**	**<0.0001**
SW x GW	**103.25**	**<0.0001**
length x PF	**10.23**	**0.0001**
SW X PF	**6.82**	**0.0017**
EBS: 2004 vs 2005	**F**	**p**
length x GW	**5.66**	**0.0041**
SW x GW	**5.29**	**0.0059**
length x PF	3.54	0.0314
SW X PF	2.01	0.1372

ANCOVAs for mean values

		K_a		EDW	GSI	RF
GOA 2002	mean	**0.99**		0.079	0.217	840
	SE	**0.02**		0.002	0.006	19
GOA 2003	mean	**0.91**		0.079	0.232	793
	SE	**0.01**		0.002	0.007	19
	F	**17.27**		<0.01	2.67	3.28
	p	**<0.0001**		0.964	0.1047	0.0727
EBS 2003	mean	1.02	0.067	**0.066***	**0.152***	**657***
	SE	0.01	0.002	**0.002**	**0.004**	**14**
EBS 2004	mean	1.01	0.064	**0.083***	**0.202***	**704***
	SE	0.01	0.001	**0.001**	**0.004**	**10**
EBS 2005	mean	1.02	0.064	**0.097***	**0.227***	**695**
	SE	0.02	0.002	**0.002**	**0.007**	**23**
	F	0.78	2.04	**67.23**	**72.89**	**3.49**
	p	0.4611	0.132	**<0.0001**	**<0.0001**	**0.0323**

Table 4.7. Comparison of maternal effects and reproduction between the Gulf of Alaska (GOA) and eastern Bering Sea (EBS) in 2003 and between the EBS and Aleutian Islands (AI) in 2005. Left columns contain results of F-tests on models relating length and somatic weight (SW) to gonad weight (GW) and potential fecundity (PF). Right columns contain results of ANCOVAs on mean values of relative condition factor across datasets condition factor (K_a), hepatosomatic index (HSI), egg dry weight in mg (EDW), gonadosomatic index (GSI), and relative fecundity (RF) using length as a covariate. Results listed in bold are significant at the $p<0.05$ level. See Table 4.1 for sample sizes.

model comparisons

2003: GOA vs EBS	F	p
length x GW	**34.86**	**<0.0001**
SW x GW	**71.29**	**<0.0001**
length x PF	3.07	0.0503
SW X PF	**25.06**	**<0.0001**

2005: EBS vs AI	F	p
length x GW	0.71	0.4918
SW x GW	0.79	0.4533
length x PF	**8.50**	**0.0003**
SW X PF	**8.58**	**0.0003**

ANCOVAs for mean values

		K_a	EDW	GSI	RF
GOA 2003	mean	**0.91**	**0.079**	**0.232**	**793**
	SE	**0.01**	**0.002**	**0.007**	**19**
EBS 2003	mean	**1.02**	**0.066**	**0.152**	**657**
	SE	**0.01**	**0.002**	**0.004**	**14**
	F	**63.54**	**17.90**	**132.17**	**36.88**
	p	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

		K_a	HSI	EDW	GSI	RF
EBS 2005	mean	**1.02**	**0.064**	**0.097**	**0.227**	695
	SE	**0.01**	**0.002**	**0.002**	**0.007**	23
AI 2005	mean	**1.06**	**0.087**	**0.103**	**0.245**	671
	SE	**0.01**	**0.001**	**0.001**	**0.004**	10
	F	**10.00**	**81.47**	**10.87**	**5.10**	1.60
	p	**<0.0001**	**<0.0001**	**0.0012**	**0.0251**	0.2078

Table 4.8. Environmental information for the eastern Bering Sea. Data were obtained from the website of the Pacific Marine Environmental Laboratory (PMEL) of the U.S. National Oceanic and Atmospheric Administration (http://www.beringclimate.noaa.gov/data/). SST = sea surface temperature, JFMA = January-April, M2 = mooring maintained by PMEL at 56.8°N, 164°W. Anomalies are departures from long-term means standardized by the standard deviations of the means; years used to calculate means are indicated for each variable.

	2002	2003	2004	2005
Average summer EBS shelf bottom temperature				
value (°C)	3.29	3.8	3.39	-
anomaly (1982-2002)	1.18	1.96	1.33	-
EBS shelf May SST				
value (°C)	2.59	3.5	3.29	-
anomaly (1970-2000)	0.35	1.55	1.27	-
Average winter (JFMA) EBS Shelf SST at M2				
value (°C)	-	0.75	-1.45	0.15
anomaly (1959-1999)	-	1.25	0.61	1.07

Literature Cited

Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258-1264

Bernardo J (1996) The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. Am Zool 36:216-236

Blaxter JHS and Hempel G (1963) The influence of egg size on herring larvae (*Clupea harengus* L.). J Cons Int Expl Mer 28: 211-240

Bolger T, Connolly PL (1989) The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol 34:171-182

Bromage NR, Hardiman P, Jones J, Springate J, Bye N (1990) Fecundity, egg size and total egg volume differences in 12 stocks of rainbow trout, *Oncoryhnchus mykiss* Richardson. Aquac Fish Manage 21:269-284

Burton MPM, Penney RM, Biddiscombe S (1997) Time course of gametogenesis in Northwest Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 54:122-131

Carr SM, Kivlichan DS, Pepin P, Crutcher DC (1999) Molecular systematics of gadid fishes: implications for the biogeographic origins of Pacific species. Can J Zool 77:19-26

Chambers RC, Leggett WC, Brown JA (1989) Egg size, female effects, and the correlations between early life history traits of capelin, *Mallotus villosus*: an appraisal at the individual level. Fish Bull 87:515-523

Chambers RC, Waiwood KG (1996) Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua.* Can J Fish Aquat Sci 53:1986-2003

Charnov EL, Turner TF, Winemiller KO (2001) Reproductive constraints and the evolution of life histories with indeterminate growth. Proc Nat Acad Sci 98:9460-9464

Chigbu P, Sibley TH (1994) Relationship between abundance, growth, egg size and fecundity in a landlocked population of longfin smelt, *Spirinchus thaleichthys*. J Fish Biol 45:1-15

Cushing DH (1975) Marine ecology and fisheries. Cambridge University Press, Cambridge, England

DeMartini EE (1991) Annual variations in fecundity, egg size, and the gonadal and somatic condition of queenfish *Seriphus politus* (Sciaenidae). Fish Bull 89:9-18

Einum S, Fleming IA (1999) Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. Proc Roy Soc London B 266:2095-2100

Einum S, Fleming IA (2000) Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565-567

Fiksen Ø, MacKenzie BR (2001) Optimal state-dependent allocation to growth or reproduction in cod. ICES CM 2001 V:12

Froese R (2006) Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J Appl Ichthyol 22:241-253

Gundersen AC, Kjesbu OS, Nedreaas KH (1999) Fecundity of Northeast Arctic Greenland halibut (*Reinhardtius hippoglossoides*). J Northwest Atl Fish Sci 25:29-36

Gunderson DR (1997) Trade-off between reproductive effort and adult survival in oviparous and viviparous fishes. Can J Fish Aquat Sci 54:990-998

Hattori T, Sakurai Y, Shimazaki K (1992) Maturation and reproductive cycle of female Pacific cod in waters adjacent to the southern coast of Hokkaido, Japan. Nippon Suisan Gakkaishi 58:2245-2252

Heino M, Kaitala V (1996) Optimal resource allocation between growth and reproduction in clams: why does indeterminate growth exist? Funct Ecol 10:245-251

Hinckley S (1987) The reproductive biology of walleye pollock, *Theragra chalcogramma*, in the Bering Sea, with reference to spawning stock structure. Fish Bull 85:481-498

Hinckley S (1990) Variation of egg size of walleye pollock *Theragra chalcogramma* with a preliminary examination of the effect of egg size on larval size. Fish Bull 88:471-483

Hislop JRG (1988) The influence of maternal length and age on the size and weight of eggs and the relative fecundity of haddock, *Melanogrammus aeglefinus* in British waters. J Fish Biol 32:923-930

Hutchings JA, Myers RA (1993) Effect of age on the seasonality of maturation and spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic. Can J Fish Aquat Sci 50:2468-2474

Jobling M (1994) Fish Bioenergetics. Chapman and Hall, London

Johnston TA, Leggett WC (2002) Maternal and environmental gradients in the egg size of an iteroparous fish. Ecology 83:1777-1791

Kamler E (2005) Parent-egg-progeny relationships in teleost fishes: an energetics perspective. Rev Fish Biol Fish 15:399-421

Kennedy J, Geffen AJ, Nash RDM (2007) Maternal influence on egg and larval characteristics of plaice (*Pleuronectes platessa* L.). J Sea Res 58:65-77

Kjesbu OS, Klungsøyr J, Kryvi H, Witthames PR, Walker MG (1991) Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. Can J Fish Aquat Sci 48:2333-2343

Kjesbu OS, Solemdal P, Bratland P, Fonn M (1996) Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 53:610-620

Kjesbu OS, Witthames PR, Solemdal P, Walker MG (1998) Temporal variations in the fecundity of Arcto-Norwegian cod (*Gadus morhua*) in response to natural changes in food and temperature. J Sea Res 40:303-321

Koops MA, Hutchings JA, McIntyre TM (2004) Testing hypotheses about fecundity, body size and maternal condition in fishes. Fish Fish 5:120-130

Kraus G, Müller A, Trella K, Köster FW (2000) Fecundity of Baltic cod: temporal and spatial variation. J Fish Biol 56:1327-1341

Lambert Y, Dutil J-D (1997a) Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? Can J Fish Aquat Sci 54:104-112

Lambert Y, Dutil J-D (1997b) Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Can J Fish Aquat Sci 54:2388-2400

Lambert Y, Dutil J-D (2000) Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. Can J Fish Aquat Sci 57:815-825

Law R (2000) Fishing, selection, and phenotypic evolution. ICES J Mar Sci 57:659-668

Lawson GL and Rose GA (2000) Small-scale spatial and temporal patterns in spawning of Atlantic cod (*Gadus morhua*) in coastal Newfoundland waters. Can J Fish Aquat Sci 57:1011-1024

Livingston P, deReynier Y (1996) Groundfish food habits and predation on commercially important prey species in the eastern Bering Sea from 1990 to 1992. AFSC Processed Report 96-04, Alaska Fisheries Science Center, National Marine Fisheries Service

Mace PM (2001) A new role for MSY in single-species and ecosystem approaches to fisheries stock assessment and management. Fish and Fisheries 2:2-32

Marshall CT, Kjesbu OS, Yaragina NA, Solemdal P, Ulltang O (1998) Is spawner biomass a sensitive measure of the reproductive and recruitment potential of Northeast Arctic cod? Can J Fish Aquat Sci 55:1766-1783

Marshall CT, Yaragina NA, Adlandsvik B, Dolgov AV (2000) Reconstructing the stock-recruit relationship for Northeast Arctic cod using a bioenergetic index of reproductive potential. Can J Fish Aquat Sci 57:2433-2442

Marshall CT, Yaragina NA, Lambert Y, Kjesbu OS (1999) Total lipid energy as a proxy for total egg production by fish stocks. Nature 402:288-290

Marshall NB (1953) Egg size in Arctic, Antarctic and deep-sea fishes. Evolution 7:328-341.

Marteinsdottir G, Begg GA (2002) Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser 235:235-256

Marteinsdottir G, Steinarsson A (1998) Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. J Fish Biol 52:1241-1258

Murawski SA, Rago PJ, Trippel EA (2001) Impacts of demographic variation in spawning characteristics on reference points for fishery management. ICES J Mar Sci 58:1002-1014

O'Farrell MR, Botsford LW (2006) The fisheries management implications of maternal-age-dependent larval survival. Can J Fish Aquat Sci 63:2249-2258

Olsen EM, Heino M, Lilly GR, Morgan MJ, Brattey J, Ernande B, Dieckmann U (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature 428:932-935

Paulson AC, Smith RL (1977) Latitudinal variation of Pacific herring fecundity. Trans Am Fish Soc 106:244-247

Quinn TJ, Deriso RB (1999) Quantitative fish dynamics. Oxford University Press, New York

Ricker WE (1978) Computation and interpretation of biological statistics of fish populations. Bull Fish Res Board Can 191: 382 p.

Rideout RM, Burton MPM, Rose GA (2000) Observations on mass atresia and skipped spawning in northern Atlantic cod, from Smith Sound, Newfoundland. J Fish Biol 57:1429-1440

Rideout RM, Rose GA, Burton MPM (2005) Skipped spawning in female iteroparous fishes. Fish and Fisheries 6:50-72

Roff DA (1983) An allocation model of growth and reproduction in fish. Can J Fish Aquat Sci 40:1395-1404

Scott BE, Marteinsdottir G, Wright P (1999) Potential effects of maternal factors on spawning stock-recruitment relationships under varying fishing pressure. Can J Fish Aquat Sci 56:1882-1890

Scott BE, Marteinsdottir G, Begg GA, Wright PJ, Kjesbu OS (2006) Effects of population size/age structure, condition and temporal dynamics of spawning on reproductive output in Atlantic cod (*Gadus morhua*). Ecol Model 191:383-415

Sinclair EH, Zeppelin TK (2002) Seasonal and spatial differences in diets in the western stock of Steller sea lions (*Eumetopias jubatus*). J Mammal 83:973-990

Skæraasen JE, Nilsen T, Kjesbu OS (2006) Timing and determination of potential fecundity in Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 63:310-320

Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. Am Nat 108:499-506

Smith RL, Paul AJ, Paul JM (1990) Seasonal changes in energy and the energy cost of spawning in Gulf of Alaska Pacific cod. J Fish Biol 36:307-316

Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford

Thompson GG, Dorn MW, Gaichas S, and Aydin K (2006) Assessment of the Pacific cod stock in the eastern Bering Sea and Aleutian Islands area. In: Plan Team for Groundfish Fisheries of the Bering Sea and Aleutian Islands (ed) Stock assessment and fishery evaluation report for the groundfish resources of the Bering Sea/ Aleutian Islands regions. North Pacific Fishery Management Council, Anchorage, AK, p 219-330

Trippel EA (1995) Age at maturity as a stress indicator in fisheries. Bioscience 45:759-771

Trippel EA (1998) Egg size and viability and seasonal offspring production of young Atlantic cod. Trans Am Fish Soc 127:339-359

Trippel EA (1999) Estimation of stock reproductive potential: history and challenges for Canadian Atlantic gadoid stock assessments. J Northwest Atl Fish Sci 25:61-81

Trippel EA, Neil SRE (2004) Maternal and seasonal differences in egg sizes and spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*) in relation to body size and condition. Can J Fish Aquat Sci 61:2097-2110

Tyler AV (1995) Warmwater and cool-water stocks of Pacific cod (*Gadus macrocephalus*): a comparative study of reproductive biology and stock dynamics. In: Beamish RJ (ed) Climate change and northern fish populations, Can Spec Publ Fish Aquat Sci 121:537-545

Tyler CR, Sumpter JP (1996) Oocyte growth and development in teleosts. Rev Fish Biol Fish 6:287-318

Westrheim SJ (1996) On the Pacific cod (*Gadus macrocephalus*) in British Columbia waters, and a comparison with Pacific cod elsewhere, and Atlantic cod (*Gadus morhua*). Can Tech Rep Fish Aquat Sci 2092

Wieland K, Jarre-Teichmann A, and Horbowa K (2000) Changes in the timing of spawning of Baltic cod: possible causes and implications for recruitment. ICES J Mar Sci 57:452-464

Wootton RJ (1990) Ecology of teleost fishes. Chapman & Hall, London

Yang M-S (2004) Diet changes of Pacific cod (*Gadus macrocephalus*) in Pavlof Bay associated with climate changes in the Gulf of Alaska between 1980 and 1995. Fish Bull 102:400-405

Zar JH (1999) Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey

Appendix 4.A. Macroscopic maturity index for Pacific cod. This index was created by the authors in cooperation with AFSC personnel, and is based on the maturity index described by Tyler (1995).

Maturity stage	Distinguishing characteristics
1	*Immature*: Ovaries small (<10 cm); ovary wall transparent, white or pink; obvious lack of developing egg mass within the ovary.
2	*Developing*: Ovary length less than ½ of body cavity; ovaries have well-developed blood vessels; oocytes are bright orange, opaque, and highly adhesive.
3	*Pre-spawning*: Ovary length greater than 1/2 of body cavity; blood vessels dark, less obvious, may be absent; eggs are cream-colored and translucent; eggs are very distinct and less adhesive; distinctive characteristic is loose consistency similar to Cream of Wheat.
4	*Spawning*: Eggs are larger, clear with a yellow cast and completely loose in the ovary; eggs flow freely from the gonopore with slight pressure on abdomen.
5	*Spent*: Gonads still long, but flaccid and obviously empty. Fluid and tissue may remain within the ovary.

Appendix 4.B. Validation of consistency in egg development among female Pacific cod of maturity stage 3.

Overview

We confined our analysis of egg size to female Pacific cod with ovaries in stage 3 (pre-spawning) condition. We assumed that eggs from stage-3 females would be at similar stages of development and that they had largely completed the process of vitellogenesis. This appendix describes the two approaches we used to validate our assumptions.

Approach/ Methods

Gonadosomatic index (GSI; ovary weight/ somatic weight) is a good indicator of the developmental status of Pacific cod eggs (Hattori et al. 1992), so we analyzed the amount of variability in GSI of females assigned to different maturity stages. If maturity stage-3 eggs from different females were at similar stages of development, variability in GSI within the stage-3 female group should be small relative to GSI variability among maturity stages. Therefore, GSI values for stage-3 females should be significantly different from all other maturity stages and substantially higher than stage-2 eggs. We used ANOVA and Tukey multiple comparisons to test for differences in GSI among maturity stages.

We also compared the water content of stage-3 eggs to the water content of stage-4 (spawning) eggs to determine whether the increase in gonad weight (and therefore GSI) between stages could be attributed solely to an increase in water weight through egg hydration. If the increase in gonad weight between stage 3 and stage 4 is mainly due to increased water content, it is likely that stage-3 eggs have largely completed vitellogenesis and that there will be little further increase in their energetic resources. We compared ovaries from two hypothetical 100-g females so that we could easily convert mean GSI values to ovary weights.

Results

The mean GSI of stage-3 females (20.8%; $N = 568$) was highly significantly different ($F = 313.2$, $p << 0.0001$) from that of stage-1 (0.7%; $N = 268$) and stage-2 (9.8%; $N = 366$) females, and was also different from the stage-4 females for which we were able to reliably calculate GSI (38.1%; $N = 33$; Figure 4.A.1). The eggs of these stage-4 females were not completely loose, which allowed us to measure the entire weight of the ovaries.

Mean water content was 71.0% for stage-3 eggs and 82.9% for stage-4 eggs. Because we calculated GSI using somatic weight, the mean GSI values indicate that the mean ovary weight for a stage-3 female weighing 100 g would be 16.5 g and the mean ovary weight for a 100-g stage-4 female would be 23.6 g (Table 4.A.1). The dry mass of the ovaries would be 4.78 g for the stage-3 female and 4.03 g for the stage-4 female (Table 4.A.1).

Conclusions

The GSI of stage-3 females was very different from earlier development stages. Therefore, our assumption of consistent development among stage-3 females appears to be validated. Some of the variability in egg size is still very likely to originate from small differences in developmental stage, but the large sample size in our study should help to overcome this observation error and increase the precision of egg-size estimates. In addition, there is no reason to believe that variability due to development stage creates bias in the estimates of egg size.

The dry weight of the hypothetical stage-3 female is higher than that of the stage-4 female, which is biologically unrealistic. This result may be due to the compounding of errors during the calculations. In addition, the water-content value used for the stage-4 eggs was determined on fully loose and running eggs. The stage-4 eggs we used in the analysis of GSI presented here were not completely free, and it may be that they were not fully hydrated. If these eggs did have less water then their ovary dry weight would be greater. While these results are inconclusive, they do suggest that there are minimal differences in egg dry weight between stage-3 and stage-4 females. This supports our conclusion that the process of vitellogenesis has been completed in stage-3 eggs.

Literature Cited

Hattori T, Sakurai Y, Shimazaki K (1992) Maturation and reproductive cycle of female Pacific cod in waters adjacent to the southern coast of Hokkaido, Japan. Nippon Suisan Gakkaishi 58:2245-2252

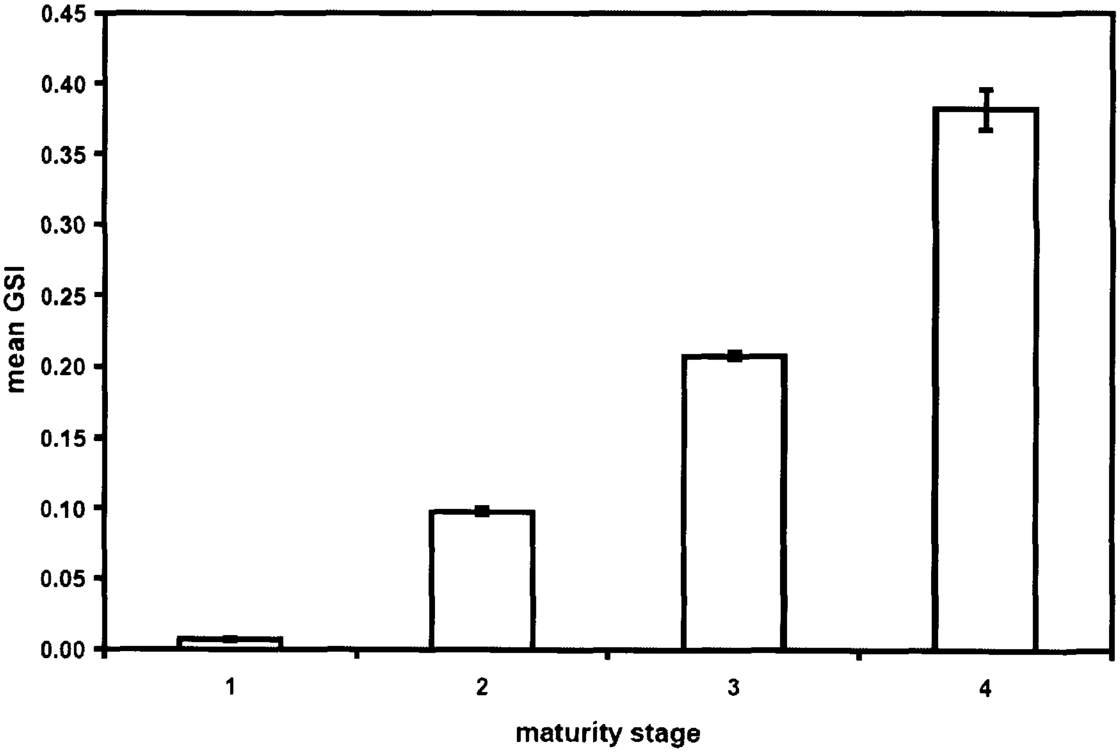

Figure 4.B.1. Mean GSI for female Pacific cod of different maturity stages. Error bars indicate ± SE.

Table 4.B.1. Estimated ovary weights for hypothetical female Pacific cod weighing 100 g. All weights in grams.

maturity stage	GSI	total weight	somatic weight	ovary weight	% H_2O	ovary dry weight
3	0.208	100	79.2	16.47	0.710	4.78
4	0.381	100	61.9	23.58	0.829	4.03

GENERAL CONCLUSIONS

Female Pacific cod appear to maximize their reproductive potential by maximizing the number, but not quality, of the eggs they produce. The analysis of Pacific cod life histories (Chapter 1) demonstrates that females among different stocks have similar lifetime reproductive success, suggesting that the maintenance of egg production levels is the key to increasing fitness. The negative influence of maternal length on a fatty acid linked to egg quality (Chapter 3) indicates that larger females do not invest in higher-quality eggs and that they might sacrifice egg quality to maintain egg production. Egg size is not related to maternal attributes, and Pacific cod appear to defend length- and weight-specific levels of fecundity at the expense of egg size (Chapter 4).

Lifetime reproductive success is preserved among Pacific cod females with different life history strategies. Pacific cod from Alaska grow and mature more slowly than individuals from Canadian stocks to the south, but live longer and reach a greater maximum size. Despite these different patterns, lifetime reproductive success is equivalent among stocks and centers on a value of one million eggs. I conclude that the growth of northern Pacific cod is limited by temperature and growing season length, and that greater longevity and larger maximum size evolved in the north to maintain fitness. The similarity in lifetime reproductive success among these different stocks supports the conclusion that it is the number of eggs produced by a female that determines her fitness.

Maximizing egg quality does not appear to be a priority for Pacific cod. Total lipid content and composition do not vary with female age or length. Eggs from older and larger females have slightly lower amounts of arachidonic acid (AA), a fatty acid that has been positively linked to egg quality (Tocher 2003). While the connection between AA content and increased egg and larval viability has not been firmly established, the results of Chapter 3 suggest that larger females may sacrifice egg quality to maintain egg production.

Fecundity is the most important element of Pacific cod reproductive potential. There is low variability in fecundity at female size, while egg size is highly variable and not related to the maternal attributes I studied. Variation in reproductive potential among geographic areas and years appears to be largely due to variation in egg size. Relative fecundity (the number of eggs produced per gram body weight) increases slightly with female size. Taken together, these results suggest that there is a strong tendency for female Pacific cod to maintain female size-dependent levels of fecundity, even at the expense of egg size.

The importance of egg production rather than egg size or quality in determining female reproductive potential is consistent with Pacific cod life history. Pacific cod are typical of "opportunistic" life history strategists, which are characterized by high fecundity, low survival of early life history stages, and no parental care of offspring (Winemiller 2005). Thus, it is advantageous for Pacific cod females to maximize the number of offspring they produce rather than increase the fitness of individual offspring. In contrast, an "equilibrium" life history strategist benefits from maximizing the investment in each offspring and the low number of offspring produced is balanced by their high survival rate (Winemiller 2005). Opportunistic strategists are also thought to be more susceptible to changes in the environment (Winemiller 2005). Producing as many eggs as possible makes sense if the survival of offspring is under such strong environmental control that it is unaffected by additional egg resources supplied by the female.

The composition of Pacific cod eggs differs from that of other species where strong maternal effects on egg survival have been observed. Pacific cod eggs are typical of marine fish eggs that are low in total lipid, have a high fraction of polar lipids, and lack oil globules rich in energy-producing neutral lipids (Chapter 2). The increase in larval survival rates with maternal age in black rockfish *Sebastes mentella* results from an increase in the amount of triacylglycerols available in oil globules in its eggs (Berkeley et al. 2004a). Similarly, greater larval survival in capelin *Mallotus villosus* is linked to an increase in oil globule size (Chambers et al. 1989). The absence of oil globules and low triacylglycerol content in Pacific cod eggs are consistent with the conclusion that maximizing egg and larval survival through egg provisioning is not a priority for Pacific cod.

The management of Pacific cod stocks will benefit from the results in this dissertation. Chapter 1 demonstrates how the life history of Pacific cod stocks in Alaska makes them more vulnerable to fishing activity than stocks further to south. The increased growing-season length and temperature that are expected to occur as a result of global climate change may alter the life histories of northern Pacific cod stocks, making them more similar to that of the southern stocks and more resistant to fishing pressure. The possibility of local adaptation in the fatty acid composition of Pacific cod eggs suggests that there may be substocks within the larger BSAI population. Differences between the EBS and AI in age, size, condition, and reproductive parameters support this conclusion. The analysis of maternal effects provides assurance that there are no differences in egg quality among female Pacific cod, but also suggests that relative fecundity and temporal variability in reproductive potential are factors that need to be considered in stock assessment.

My results support approaches to fisheries management that restrict fishing to a level that limits truncation of age and size structure in fished populations. Pacific cod are managed under a strict system that limits catches to levels determined appropriate by fishery scientists (Thompson et al. 2006). The trend towards earlier maturation observed in Atlantic cod does not appear to be present in Pacific cod, although data are sparse. Maternal size effects on reproductive potential in Atlantic cod may be due to early maturation of young fish that produce less viable eggs (Olsen et al. 2004, Trippel 1998). If so, the lack of such effects in Pacific cod supports current management practices, which may have helped to maintain age at maturity above a threshold level where reproductive potential is reduced.

The information presented here suggests that fishing effects on fitness should be considered in fisheries management. Current management operates at the population level and is designed to sustain populations at an optimal biomass. However, it ignores how fishing might affect individual fitness. The analysis in Chapter 1 demonstrates the importance of natural mortality in shaping life history strategies. If fishing mortality is included as an additional source of mortality in the calculation of lifetime egg production (R_0), fitness will decline. Species may be able to adapt to fisheries-induced increases in mortality rate to maintain R_0, e.g. by maturing at early ages as has been seen in Atlantic cod (Olsen et al. 2004). However, some species may not be able to adapt. For example, if growth rates are under environmental control, then species may not be able to compensate for increased mortality and will be less fit as a result of fishing. In addition, life history strategies have evolved under a certain set of environmental conditions.

Introducing fishing mortality into that same environment, particularly in size-selective fisheries, may cause some life history traits to become maladaptive (Walsh et al. 2006).

Disrupting life history strategies may also make species more vulnerable to environmental variability. Longevity in fishes (the corollary of low mortality) is thought to be an adaptation to temporal variability in climate that regulates the occurrence of years when conditions are especially favorable for offspring survival (Longhurst 2002, Winemiller 2005). Fish longevity may be matched to the scale of environmental variability to ensure that females experience at least one good reproductive event in their lifetime. If, as our analysis of reproductive potential suggests, the survival of Pacific cod offspring is under strong environmental control, then years with good conditions may be especially important for the reproductive success of female Pacific cod. Climate variability in the North Pacific occurs on decadal scales as the result of atmospheric phenomena such as the Pacific Decadal Oscillation (Mantua & Hare 2002), and the longevity of Pacific cod may be matched to this scale of temporal variation. Harvest rates that result in truncated age structure and reduced longevity may make Pacific cod more vulnerable to poor environmental conditions as has been suggested for Atlantic cod (Drinkwater 2002).

The maintenance of age structure in Pacific cod populations is also important because it helps to preserve the total egg production of populations. In species such as black rockfish, where older females produce eggs that are more likely to survive, maintaining an age structure that contains a sufficient number of older females may be necessary to maintain the reproductive output of populations and sustain yield to fisheries (Berkeley et al. 2004b). In Pacific cod, no such maternal effects were observed. However, the analysis of life histories in this dissertation demonstrated that the larger size of older females and increased longevity are critical for maintaining lifetime reproductive success, particularly in Alaskan stocks. The same thing is likely true from a population perspective: the total egg production of a population depends on a sufficient number of females surviving to older ages where their large size greatly increases fecundity.

Geographic area is an important factor to consider when evaluating the reproductive potential of Pacific cod. The analysis of life histories demonstrates that growth and reproduction can vary widely among populations of a single species. In addition, it appears that Pacific cod stocks have successfully adapted their life history strategy to local conditions. Adaptation may be present in the fatty acid composition of Pacific cod eggs. There may also be variation in reproductive potential and in the way that females allocate energetic resources among geographic areas in Alaska.

The results of this dissertation offer insights into the relationship between Pacific cod and the closely related Atlantic cod. The eggs of these two species have compositions that are in many ways similar, but the lower content of free amino acids in Pacific cod eggs results in reduced egg hydration. This explains the negative buoyancy of Pacific cod eggs, which is different from the pelagic Atlantic cod egg. The lack of a maternal effect on the egg size of Pacific cod is in contrast to observations in Atlantic cod, but is similar to observations in walleye pollock, a species that also evolved from Atlantic cod (Hinckley 1990). Therefore, a reliance on egg production vs. egg size may be a phenomenon that developed among North Pacific gadids. Maternal effects on egg size in Atlantic cod may also be the result of earlier maturation of young fish due to heavy fishing pressure (Trippel 1995, Law 2000). Although Pacific cod have been commercially fished since the late 1800s, fishing activity has been low and inconsistent until recent decades (Westrheim 1996). Therefore, Pacific cod may not have experienced the change in maturation and the reduced reproductive ability of younger spawners observed in Atlantic cod.

There are a number of ways that the information provided in this dissertation could be improved and expanded. Equivalent lifetime reproductive success among Pacific cod stocks is an interesting result that merits further exploration. Including more Pacific cod stocks, especially from the western side of the North Pacific, would allow us to determine the broader significance of the results. It would also be interesting to conduct similar analyses in other species, particularly the Atlantic cod stocks included in Chapter 1. Analysis of the fatty acid composition of eggs from the EBS and AI needs to be enhanced by a greater sample size to examine local adaptation more closely. A coordinated effort that investigates how fatty acid profiles, genetic signatures, and otolith microchemistry compare among these samples would be invaluable. Maternal effects on egg size should be further explored by using spawned eggs, as this might reduce the variability in egg size due to eggs being in slightly different stages of development. Additional years of data would help us understand the scope of variability in reproductive potential among areas and years in Alaska.

The work presented here examines the reproductive potential of Pacific cod from a number of different perspectives. It simultaneously supplies evidence for evolutionary adaptation and provides important information for the management of Pacific cod. Each chapter emphasizes the role of the environment in the reproductive success of Pacific cod.

Literature Cited

Berkeley SA, Chapman C, Sogard SM (2004a) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258-1264

Berkeley SA, Hixon MA, Larson RJ, Love MS (2004b) Fisheries sustainability via protection of age structure and spatial distribution of fish populations. Fisheries 29:23-32

Chambers RC, Leggett WC, Brown JA (1989) Egg size, female effects, and the correlations between early life history traits of capelin, *Mallotus villosus*: an appraisal at the individual level. Fishery Bulletin 87:515-523

Drinkwater KF (2002) A review of the role of climate variability in the decline of northern cod. American Fisheries Society Symposium 32:113-130

Hinckley S (1990) Variation of egg size of walleye pollock *Theragra chalcogramma* with a preliminary examination of the effect of egg size on larval size. Fishery Bulletin 88:471-483

Law R (2000) Fishing, selection, and phenotypic evolution. ICES Journal of Marine Science 57:659-668

Longhurst A (2002) Murphy's law revisited: longevity as a factor in recruitment to fish populations. Fisheries Research 56:125-131

Mantua NJ, Hare SR (2002) The Pacific Decadal Oscillation. Journal of Oceanography 58:35-44

Olsen EM, Heino M, Lilly GR, Morgan MJ, Brattey J, Ernande B, Dieckmann U (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature 428:932-935

Thompson GG, Dorn MW, Gaichas S, and Aydin K (2006) Assessment of the Pacific cod stock in the eastern Bering Sea and Aleutian Islands area. In: Plan Team for Groundfish Fisheries of the Bering Sea and Aleutian Islands (ed) Stock assessment and fishery evaluation report for the groundfish resources of the Bering Sea/ Aleutian Islands regions. North Pacific Fishery Management Council, Anchorage, AK, p 219-330

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11:107-184

Trippel EA (1995) Age at maturity as a stress indicator in fisheries. Bioscience 45:759-771

Trippel EA (1998) Egg size and viability and seasonal offspring production of young Atlantic cod. Transactions of the American Fisheries Society127:339-359

Walsh MR, Munch SB, Chiba S, and Conover DO (2006) Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecological Letters 9:142-148

Westrheim SJ (1996) On the Pacific cod (*Gadus macrocephalus*) in British Columbia waters, and a comparison with Pacific cod elsewhere, and Atlantic cod (*Gadus morhua*). Canadian Technical Reports in Fisheries and Aquatic Sciences, No. 2092

Winemiller KO (2005) Life history strategies, population regulation, and implications for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences 62:872-885