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Winter Flounder (*Pseudopleuronectes americanus*)

Reproductive Success 19 pp

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(NOAA□NMFS)

Narragansett Bay Estuary Project

WINTER FLOUNDER (*Pseudopleuronectes americanus*) REPRODUCTIVE SUCCESS

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FOREWORD

The United States Congress created the National Estuary Program in 1984, citing its concern for the "health and ecological integrity" of the nation's estuaries and estuarine resources. Narragansett Bay was selected for inclusion in the National Estuary Program in 1984 , and the Narragansett Bay Project (NBP) was established in 1985. Narragansett Bay was designated an "estuary of national significance" in 1988. Under the joint sponsorship of the U.S. Environmental Protection Agency and the Rhode Island Department of Environmental Management, the NBP's mandate is to direct a program of research and planning focussed on managing Narragansett Bay and its resources for future generations.

The NBP will develop a draft Comprehensive Conservation and Management Plan (CCMP) by December, 1991, which will recommend actions to improve and protect the Bay and its natural resources.

The NBP has established the following seven priority issues for Narragansett Bay:

- management of fisheries
- nutrients and potential for eutrophication
- impacts of toxic contaminants
- health and abundance of living resources
- health risk to consumers of contaminated seafood
- land-based impacts on water quality
- recreational uses

The NBP is taking an ecosystem/watershed approach to address these problems and has funded research that will help to improve our understanding of various aspects of these priority problems. The Project is also working to expand and coordinate existing programs among federal, state and local agencies, as well as with academic researchers, in order to apply research findings to the practical needs of managing the Bay and improving the environmental quality of its watershed.

This report represents the technical results of an investigation funded in part by monies provided by the Narragansett Bay Project under an Interagency Agreement #DW13931613-0104 between the United States Environmental Protection Agency and the National Oceanic and Atmospheric Administration. The results and conclusions contained herein are those of the author(s), and do not necessarily represent the views or recommendations of the NBP.

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Winter Flounder Reproductive Success

Executive Summary

The winter flounder is an important resource species in Narragansett Bay. Unlike many other species, its abundance in Narragansett Bay is dependent upon successful reproduction in the Bay. This study was designed to determine the effects of female size, spawning time, and spawning location on the number and viability of eggs and larvae produced. Winter flounder, collected at selected times and locations, were spawned in the laboratory. Viability of embryos and larvae were determined under controlled conditions. No difference was observed in the size and viability of eggs and larvae produced in Upper Narragansett Bay, Lower Narragansett Bay and Eastern Long Island Sound in 1988. Among Narragansett Bay fish, female size affected most of the reproductive parameters examined including both absolute and relative measures of total reproductive output (reproductive rate and gonadosomatic index), egg size, fecundity and viability. Spawning time was found to affect egg size, fecundity, and viability, but not reproductive rate or gonadosomatic index. Egg size increased with increasing female size and decreased as the spawning season progressed. Spawning time and female size explained 61% of the observed variability in egg size among females. Female size explained 95% of the variability in reproductive rate and 90% of the variability in fecundity. Female size and spawning time combined explained 94% of the variability in fecundity.

The effect of female size and spawning time on both fertility and hatch were non-additive. Embryos produced earlier in the spawning season appeared to have a survival advantage over those produced later in the spawning season. Embryos produced by small, late spawning fish appeared to be at a pronounced disadvantage.

These data point to the importance of older larger females in successful reproduction of the species. Variability, between years, in the size and viability of eggs and larvae reared under controlled conditions in the laboratory suggests their utility to a monitoring program, particularly if a correlation can be established between performance in the laboratory and in the field. Further studies should focus on *in situ* growth and survival of winter flounder larvae.

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Winter flounder *Pseudopleuronectes americanus* reproductive success. I. Among-location variability in size and survival of larvae reared in the laboratory

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ABSTRACT: Winter flounder *Pseudopleuronectes americanus* collected at selected locations in Long Island Sound (LIS), New York, and Narragansett Bay (NB), Rhode Island, USA, were spawned in the laboratory and the larvae reared for a month after hatching. In 1987 the average size of yolk-sac larvae varied widely among locations. Moreover, a direct correlation was observed between size of yolk-sac larvae and survival for the first month of life. Fish from NB produced the smallest larvae with the lowest survival rate. The Madison site in LIS produced the largest yolk-sac larvae with the highest survival rate. Size and biochemical composition (μg larva^{-1}) of yolk-sac larvae were correlated. Dry weight and RNA content were the best predictors of survival potential among the variables considered (protein, DNA, lipid content, and RNA/DNA ratio). In 1988 little difference was observed in viable hatch or weight of yolk-sac larvae among locations. While no significant difference in larval survival was observed between NB and LIS fish, survival was higher in the Madison group than the Morris Cove group from LIS. These data suggest that when differences in size among newly hatched larvae are sufficiently large, survival potential can be affected.

INTRODUCTION

Temperate marine fishes typically produce large numbers (thousands to millions) of small eggs (micrograms to milligrams dry weight). Survival through the embryonic and larval periods is low, frequently on the order of a few percent or less. Small changes in mortality rates during the early life stages can result in large and unpredictable changes in fish population abundance (Cushing 1975, Hunter 1981, Houde 1987).

Two potential contributors to differences in survival potential of individual eggs and larvae are size and biochemical composition. The embryos of most oviparous fishes are dependent upon material deposited in the developing oocyte to supply substrates for energy production and growth during the period from ovulation to initiation of feeding. Since a spawning fish has a finite amount of energy and metabolites to devote to reproduction, a balance must be achieved between size (mass) of an individual egg and the total number of eggs produced (Tanasichuk & Ware 1987). While it is generally believed that larger larvae have a survival advantage over smaller larvae, direct experimental evidence supporting this assumption is limited (von Westernhagen 1988).

The winter flounder *Pseudopleuronectes americanus* is an important resource species found off the northeast coast of North America. The population consists of numerous local stocks that spawn demersal, adhesive eggs in the different estuaries, bays and offshore banks along the coast (Perlmutter 1947, Saila 1961). Spawning extends from late winter through early spring. After spawning, adults may move offshore to deeper, cooler water but return to the spawning estuary in the fall with a high degree of consistency.

This study was undertaken to examine the variability in size, composition, and survival potential of winter flounder *Pseudopleuronectes americanus* larvae from different spawning sites, and the relations among these variables. Collection locations were selected to include

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a wide range of urbanization and anthropogenic contamination (Nelson et al. 1991). We determined the size (standard length and dry weight) and chemical composition of winter flounder larvae just prior to feeding initiation and related these data to their survival for the first month of life under standard rearing conditions. In a companion study (Buckley et al. 1991) we examined the factors affecting egg size and fecundity of winter flounder spawning at a single location over the spawning season. This work is part of a larger study on the effects of environmental and parental factors on the size, biochemical composition, and survival potential of winter flounder eggs and larvae.

METHODS

Adult winter flounder *Pseudopleuronectes americanus* from Long Island Sound (LIS), New York, USA, were collected with an otter trawl in February 1987 and again in February 1988, and transported live to the NMFS Milford Laboratory, Milford, Connecticut (Nelson et al. 1991). Collection sites were Hempstead, New York; Shoreham, New York; Morris Cove, Connecticut; and Madison, Connecticut (Fig. 1). Fish were held for

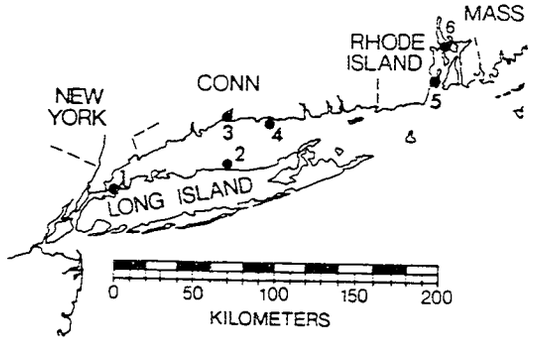

Fig. 1. Collection sites in Long Island Sound and Narragansett Bay, USA. (1) Hempstead, (2) Shoreham, (3) Morris Cove, (4) Madison, (5) lower West Passage Narragansett Bay, (6) upper Narragansett Bay

up to 4 wk in running seawater at ambient temperature (1.0 to 4.7 °C) until ripe. Fish were spawned between 23 February and 20 March 1987 (ambient temperature 1.7 to 4.7 °C) and between 9 February and 12 March 1988 (ambient temperature 1.7 to 4.4 °C). Eggs were stripped, fertilized, and coated with diatomaceous earth according to techniques developed by Smigielski & Arnold (1972). Fertilized eggs were transferred to Nitex* mesh baskets and incubated in flowing seawater at ambient temperature until transported to the NMF's Narragansett Laboratory usually within 2 d of spawning. Upon arrival at the Narragansett Laboratory, embryos were acclimated to either 7 °C (1987) or 5 °C (1988) at the rate of 1 °C per 6 h.

Adult *Pseudopleuronectes americanus*, caught in the lower West Passage of Narragansett Bay (NB), Rhode Island, on 9 March 1987, spawned between 10 and 20 March 1987. Fish caught at this site on 11 March 1988 spawned between 12 and 17 March. In 1988 several collections of adult winter flounder were made in upper NB between January and March (Buckley et al. 1991). Only upper NB fish spawning between 13 February and 18 March were used for comparison with other locations, since these dates encompassed the spawning dates for the other areas. Fish were transported to the Narragansett Laboratory and held in flowing seawater at ambient temperature (< 8.0 °C). Most fish spawned within 4 wk of capture. Eggs were stripped and handled as described for LIS fish except that embryos were incubated in a constant-temperature room at 7 °C in 1987 and 5 °C in 1988. During the 1988 spawning season fertilization and hatch rates were determined as described in Buckley et al. (1991).

Within 3 d after hatching, duplicate groups of 100 larvae each were transferred to 36 l glass tanks covered on 4 sides with black plastic and set in a constant-temperature room maintained at 7 °C. Tanks contained filtered seawater to which 1 l of a dense culture of the unicellular alga *Tetraselmis* sp. had been added. Tanks were gently aerated and salinities maintained between 28 and 30.5 ‰. When the larvae were first judged capable of feeding, generally on Day 3, any dead larvae were replaced.

In 1987 larvae were fed cultured rotifers (*Brachionus plicatilis*) and wild plankton at concentrations of 500 rotifers and 500 wild plankters l^{-1}. Rotifers were mass cultured on the alga *Tetraselmis* sp. Zooplankters were collected in the Narragansett Bay area using 55 and 110 µm mesh plankton nets. Only the portion passing through a 210 µm mesh was used. This fraction consisted of copepod nauplii, copepodites, and adults in addition to rotifers. Plankton densities were adjusted 6 d wk^{-1} back up to 500 rotifers and 500 wild plankters l^{-1}. Counts of prey items were made on duplicate 50 ml samples concentrated with a 55 µm Nitex screen prior to counting.

In 1988 larvae were fed cultured rotifers (*Brachionus plicatilis*) at the rate of 2000 rotifers l^{-1} d^{-1}. After establishing the density of a rotifer culture, a volume corresponding to 72 000 rotifers was concentrated on a sieve and added to each tank 6 d wk^{-1}. No wild plankton was added and prey counts were not made on the tanks holding the larvae in 1988.

The feeding regime was changed from the earlier protocol in an attempt to raise overall survival rates and

* Reference to trade names does not imply endorsement by the United States Government

Table 1. *Pseudopleuronectes americanus*. Dry weight and biochemical composition of yolk-sac winter flounder larvae produced by adults collected in Narragansett Bay and Long Island Sound, USA, during the 1987 spawning season. Values are means ± 1 SD. In each row, values with a letter in common are not significantly different (ANOVA, Tukey test, $p \leq 0.05$, SAS Institute Inc. 1985). See Fig. 1 for site locations

	Site				
	Madison	Hempstead	Shoreham	Morris Cove	Narrangansett Bay
n	6	9	5	7	6
Weight (µg)	31.9 ± 2.7 a	29.4 ± 3.2 a, b	24.8 ± 4.5 b, c	26.7 ± 4.1 a, b	20.2 ± 3.5 c
RNA (µg larva^{-1})	1.46 ± 0.15 a	1.37 ± 0.19 a, b	1.27 ± 0.15 a, b	1.35 ± 0.19 a, b	1.11 ± 0.13 b
DNA (µg larva^{-1})	0.44 ± 0.04 a	0.42 ± 0.04 a, b	0.40 ± 0.04 a, b	0.42 ± 0.05 a, b	0.36 ± 0.02 b
Protein (µg larva^{-1})	17.9 ± 2.4 a	18.4 ± 2.6 a	14.2 ± 2.9 a	16.6 ± 3.0 a	14.0 ± 2.0 a
Lipid (µg larva^{-1})	4.29 ± 0.63 a	4.56 ± 0.51 a	3.95 ± 0.53 a, b	4.60 ± 0.77 a, b	3.95 ± 0.53 b
RNA/DNA	3.35 ± 0.17 a	3.26 ± 0.19 a	3.20 ± 0.11 a	3.20 ± 0.23 a	3.05 ± 0.28 a

to facilitate work with larvae from the large number of fish spawned during the 1988 season. In 1987 the wild plankton was observed feeding on the rotifers and there was some concern that survival of the youngest larvae may have been limited by the availability of sufficiently small prey items. Larval survival rates, however, were very similar between years. Because of the changes in the feeding regime and differences in the spawning schedule, no direct comparisons of growth and survival rates were made between years.

After 28 d the tanks were drained and the survivors counted, measured, and weighed. Any physical abnormalities were noted at this time.

Initial samples for determination of standard length, dry weight and chemical analysis were taken 3 d after hatching from stock tanks from which the experimental larvae were removed. Standard lengths were measured on live unpreserved specimens with a filar micrometer in a dissecting microscope. Larvae were rinsed in distilled water, pipetted onto a plastic petri dish, freeze dried and weighed to the nearest 0.1 µg on a Cahn automatic electrobalance. During the 1987 spawning season, 3 groups of 50 yolk-sac larvae each were homogenized in 2.0 ml of ice-cold distilled water using an STD Tissumizer mechanical high-frequency homogenizer. Subsamples of 1.4, 0.075 and 0.4 ml of homogenate were used for analysis of nucleic acids, protein and lipid content, respectively. Nucleic acids and protein were determined as described in Buckley (1979). Total lipid content was determined using the sulphophosphovanillin method (Barnes & Blackstone 1973). Chemical analysis was not performed on larvae from the 1988 spawning season.

Data analysis was done using SAS System software for personal computers (SAS Institute Inc. 1985). Square root transformation was applied to percent survival values [(survival+0.5)$^{1/2}$] and arcsine transformation applied to fertilization and viable hatch rates [arcsine(%/100)$^{1/2}$] prior to analysis of variance and regression analysis (Steel & Torrie 1960).

RESULTS

1987 spawning season

Eggs were obtained from 7 females from lower NB (Site 5) and 29 females from 4 locations (Sites 1 to 4) in LIS (Fig. 1). Fertilization and hatch rates of eggs stripped from LIS fish were variable, ranging from 78 to 93% and from 45 to 84%, respectively (Nelson et al. 1991). Similar data are not available for Narragansett Bay fish in 1987. Significant differences (ANOVA, $p \leq 0.05$) were observed in the size and chemical composition of newly hatched winter flounder larvae produced by fish collected at the different locations (Table 1). Lower NB fish produced the smallest yolk-sac larvae, while fish collected at the Madison site in LIS produced the largest.

Survival of *Pseudopleuronectes americanus* from all locations for the first month was low (mean 3%, range 0 to 18%) compared to other species of temperate marine fish reared in the laboratory (Buckley et al. 1987). Of the 5 locations studied, survival was lowest for fish from NB, where only 1 larva in 1400 survived for the 28 d duration of the experiment (Table 2). Among

Table 2. *Pseudopleuronectes americanus*. Survival of winter flounder for the first month of life during the 1987 spawning season. Values indicate number of females spawned (n), mean larval survival, and mean rank (Wilcoxon score, SAS Institute Inc. 1985) for a given site. Mean ranks with a letter in common are not significantly different ($p \leq 0.05$, Kruskal-Wallis test, SAS Institute Inc. 1985). See Fig. 1 for site locations

Site	n	Survival (%)	Mean rank
Madison	6	6.00	25.4 a, b
Hempstead	9	4.83	26.9 a
Morris Cove	9	2.28	14.5 b, c
Shoreham	5	1.50	15.2 a, b, c
Narragansett	7	0.07	9.2 c

LIS fish, those from Madison produced the highest percentage of surviving larvae (6.0 %), while fish from Shoreham produced the lowest (1.5 %). Analysis of variance of rank scores indicated significant differences ($p \leq 0.05$) in survival between NB fish and certain of the LIS groups and between the Hempstead and Morris Cove sites in Long Island Sound (Table 2). Daily counts and removal of dead larvae, while not strictly quantitative, suggested that most of the mortality occurred during the second and third weeks. This corresponds to the time of completion of yolk absorption and initiation of feeding at 7 °C (Buckley 1982). The percentages of surviving larvae that were bent or otherwise malformed were: Hempstead 27 %, Shoreham 20 %, Madison 8 %, and Morris Cove 2 %. Surviving larvae from Madison (6.85 mm standard length) were the largest after 1 mo of feeding (Table 3),

Table 3. *Pseudopleuronectes americanus*. Mean length of survivors from the 1987 spawning season. Values indicate number of larvae measured (n) and mean standard length ± 1 SD. Mean lengths with a letter in common are not significantly different (ANOVA, Tukey test, $p \leq 0.05$, SAS Institute Inc. 1985). Bent or deformed larvae were not included in the analysis. See Fig. 1 for site locations

Site	n	Standard length (mm)
Madison	66	6.85 ± 0.94 a
Hempstead	63	6.14 ± 0.90 a, b
Shoreham	12	6.06 ± 0.75 b
Morns Cove	40	5.98 ± 1.48 b

significantly larger than larvae from Shoreham and Morris Cove (ANOVA, $p \leq 0.05$). No evidence of compensatory growth was observd, as the rank order of larval size among spawning locations remained relatively unchanged between hatching and 1 mo of life.

A plot of the mean survival of larvae from a given site against the mean weight of yolk-sac larvae from the same site showed a strong positive relation between these 2 variables (Fig. 2). When data from all locations were combined, significant correlations were observed among percentage survival (S%), dry weight, and chemical content of larvae shortly after hatching (Table 4). Dry weight of yolk-sac larvae was more highly

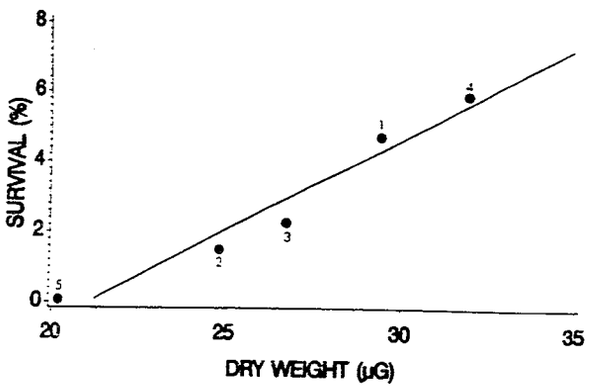

Fig. 2. *Pseudopleuronectes americanus*. Relation between mean dry weight of yolk-sac larvae and survival for the 1987 spawning season. Points are means for collection sites. (1) Hempstead, (2) Shoreham, (3) Morris Cove, (4) Madison, (5) lower West Passage Narragansett Bay

correlated with survival than was any single class of biomolecules. The relation between S% and dry weight (µg) of yolk-sac larvae from individual females was described by the equation:

$$(S\% + 0.5)^{1/3} = (0.116 \times \text{weight}) - 1.566,\ n = 31,\ r = 0.62$$

Addition of the content of any single class of biomolecules to the regression model as a second independent variable removed very little of the unexplained variation in survival.

1988 spawning season

Embryos from a total of 23 fish caught at 2 locations in LIS (Madison and Morris Cove) were transported

Table 4. *Pseudopleuronectes americanus*. Correlations among survival for first 4 wk of life, dry weight, and biochemical content (µg larva^{-1}) of winter flounder within 3 d of hatching. Values are correlation coefficients (r) for the 1987 spawning season. n = 30. See Fig. 1 for site locations

	Survival	Weight	RNA	DNA	Protein	Lipid
Survival						
Weight	0.56***					
RNA	0.43*	0.74***				
DNA	0.37*	0.73***	0.91***			
Protein	0.40*	0.72***	0.86***	0.77***		
Lipid	0.34	0.74***	0.83***	0.75***	0.85***	
RNA/DNA	0.32	0.46**	0.70***	0.35	0.64***	0.60***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 5. *Pseudopleuronectes americanus*. Size of yolk-sac larvae and viability of winter flounder spawned by fish from Narragansett Bay (NB) and Long Island Sound (LIS), USA, in 1988. Values are means ± 1 SD. In each column, values with a letter in common are not statistically different (ANOVA, $p \leq 0.05$)

Location	No. of females	Female length (mm)	Spawning date (Julian day)	Initial larval weight (g)	Fertility (%)	Viable hatch (%)	Survival[1] (%)
Upper NB[2]	16	319 ± 40	51 ± 3	29.7 ± 3.4 a	92.5 ± 10.6 a, b	71.7 ± 21.1 a	2.6 ± 2.8 a, b
Lower NB	9	336 ± 41	74 ± 1	25.6 ± 4.4 a	93.1 ± 11.8 a	84.0 ± 7.0 a	2.9 ± 2.2 a, b
Madison, LIS	9	288 ± 48	68 ± 3	27.0 ± 4.8 a	83.2 ± 8.2 b, c	75.8 ± 11.9 a	4.8 ± 4.3 a
Morris Cove, LIS	14	315 ± 42	51 ± 3	29.5 ± 4.5 a	79.4 ± 13.0 c	68.8 ± 17.2 a	1.4 ± 2.2 b

[1] Analysis of variance of rank scores
[2] Only females spawning between Julian Day 44 and 78 were used in this analysis

from the Milford Laboratory to the Narragansett Laboratory within several days of spawning. Embryos were obtained from 9 females from lower NB. To minimize any effect of spawning time on the results, a subset (n = 16) of the fish spawned from upper NB was used for purposes of comparison with fish from lower NB and LIS. Only fish spawned between Julian Day 44 and 78 were selected, corresponding to the range of spawning dates for the other locations.

No significant difference ($p \geq 0.05$) among sites was observed in viable hatch rates or the weight of yolk-sac larvae (Table 5). Fertilization rate was highest in fish from NB. While no significant difference ($p \geq 0.05$) in larval survival was observed between NB and LIS fish, survival was significantly higher in the Madison group than the Morris Cove group from LIS (Table 5). No significant correlation was observed between size (standard length or dry weight) of yolk-sac larvae and survival for the first month of life.

DISCUSSION

The daily mortality rate for winter flounder *Pseudopleuronectes americanus* larvae in our study averaged 13 % d^{-1} for the first month after hatching. Black et al. (1988) reported a value of 4 % d^{-1} for winter flounder larvae reared for a period of 2 mo under similar conditions. Laurence (1977) found that prey density had a strong influence on survival of winter flounder to metamorphosis in the laboratory. He reported daily mortality rates of 9 and 7 % d^{-1} at prey densities of 500 and 1000 plankters ml^{-1}, feeding levels similar to those maintained in our study. The higher mortality rate observed in the present study may have been due in part to the shorter rearing period that was chosen to encompass the period of high mortality shortly after yolk absorption. Estimates of natural mortality of winter flounder larvae in a small Connecticut estuary were high for the first month of life (20 % d^{-1}), decreasing to 9 % d^{-1} during the second month (Pearcy 1962). These estimates of natural mortality included mortality due to starvation and predation, but were corrected for transport of larvae out of the estuary.

For the 1987 spawning season our data show clear differences in size and survival of winter flounder larvae produced by adults collected in different locations in LIS and NB and a correlation between size of yolk-sac larvae and survival for the first month of life. The range in size of yolk-sac larvae produced during the 1988 spawning season was smaller, and no significant correlation was observed between larval size and survival for the first month of life. While lower NB produced the smallest larvae in both years, the differences in size among groups were not significant in 1988. Some lower NB fish produced extremely small winter flounder larvae in 1987 compared to other years and locations. Black et al. (1988) found differences in size of newly hatched winter flounder between locations in Narragansett Bay and Buzzard Bay, but no significant difference between locations in survival for the first 2 mo of larval life. The differences in size of yolk-sac larvae from different locations observed in our study in 1987 were considerably greater than those reported by Black et al. (1988) and may explain the difference in results.

Correlations between egg size and larval size have been observed in several fishes, including trout (Gray 1928), herring (Blaxter & Hempel 1963) and winter flounder *Pseudopleuronectes americanus* (Buckley et al. 1991). Generally, larger eggs produce larger larvae (Miller et al. 1988). Blaxter (1969) stated that 'larger larvae may be expected to be stronger, better swimmers, less susceptible to damage, and less liable to predation'. It is also expected that larger larvae are better able to capture and assimilate food. Blaxter & Hempel (1966) found that larger Atlantic herring larvae survived longer without food than those hatched from smaller eggs. Seasonal and regional differences in egg size have been reported for many species (Blaxter &

Hempel 1963, Cushing 1967, Bagenal 1971, Southward & Demir 1974, Ware 1975, Tanasichuk & Ware 1987). While positive correlations have been reported between larval size and numerous attributes potentially contributing to increased survival, including days to irreversible starvation, swimming speed, and mouth gape (Knutsen & Tilseth 1985, Miller et al. 1988), the relation between larval size at first feeding and survival is not well documented, particularly within species. Our data from the 1987 spawning season are among the few published reports showing a direct correlation between larval size and survival for the first month of life. Rosenberg & Haugen (1982) found evidence of size-selective mortality of larval turbot *Scophthalmus maximus* during the first month of life in predator-free enclosures. Their estimates of the mean size of survivors were higher than those for the overall population during the first week of life.

Several factors have been proposed as possible causes of intraspecific differences in egg or larval size. Many of these same factors can also contribute to differential larval mortality. Biological factors that affect larval size and mortality include genetic variability between and within stocks, and size, age or nutritional condition of the spawning female (Brown 1957, Hoar 1957). Environmental factors known to affect larval size and mortality are water temperature during gametogenesis and embryonic development (Blaxter & Hempel 1963, Bagenal 1971, Southward & Demir 1974, Ware 1975, Tanasichuk & Ware 1987, Buckley et al. 1990), dissolved oxygen levels, and exposure to environmental contaminants, including PCBs, pesticides, and heavy metals (Rosenthal & Alderdice 1976, Black et al. 1988).

The difference in mean dry weight of yolk-sac larvae, observed in 1987, between the largest and smallest groups (Madison in LIS and lower NB) was large, exceeding 50%. Since *Pseudopleuronectes americanus* populations consist of discrete spawning stocks (Perlmutter 1947, Saila 1961), genetic factors may have contributed to the observed variability in size and survival between locations. The much smaller differences between locations observed in 1988, however, suggest that genetic factors may not be dominant. Winter flounder fed reduced rations in the laboratory showed a reduction in fecundity but not egg size compared to well-fed fish (Tyler & Dunn 1976), suggesting that maternal nutrition is not a dominant factor in determining egg and larval size in winter flounder. Female age has been shown to affect egg size in winter flounder (Topp 1968), age 3 females producing smaller eggs than age 4 or 5 females. Our work with winter flounder spawning in Narragansett Bay (Buckley et al. 1991) suggested that female size can play a significant role in determining egg and larval size. In the present study no large difference in female size was apparent between locations. In 1987 lower Narragansett Bay fish were the last group collected and spawned, and they produced the smallest larvae. Among spring spawning fish there is a tendency for egg size to decrease with increasing water temperature. The observed 50% difference in size of yolk-sac larvae between locations is considerably greater than the differences observed among winter flounder larvae produced in the laboratory over a wide range of water temperatures by adults collected at a single location (Buckley et al. 1990). This suggests that while water temperature may have been a contributing factor, it was not the dominant factor affecting larval size in the present study. Our data on winter flounder spawning in Narragansett Bay indicated that spawning time can play a significant role in egg and larval size (Buckley et al. 1991).

Of our 6 collection sites, the Morris Cove, Hempstead, and upper Narragansett Bay sites are impacted by a variety of contaminants, including trace metals and organics (Greig et al. 1977, Pruell & Quinn 1985). The Madison, Shoreham and lower Narragansett Bay sites are considerably less impacted by contaminants (Greig et al. 1977, Pruell & Quinn 1985, Black et al. 1988, Nelson et al. 1991). The observed trends in size and survival of winter flounder with location were not entirely consistent with those expected on the basis of contaminant loadings. However, among sites in Long Island Sound, Madison stood out in 1987 as producing the largest larvae at yolk-sac stage and after 1 mo of feeding, and as having the highest survival rate and a low percentage of abnormal survivors. In both 1987 and 1988 survival was higher in larvae from Madison than from Morris Cove. This is consistent with observations of embryonic development suggesting that reproduction of winter flounder at the Morris Cove site has been compromised by high contaminant levels (Nelson et al. in press). Black et al. (1988) reported an 18% difference in weight of yolk-sac winter flounder larvae produced by adults taken from lower Narragansett Bay and New Bedford Harbor, Massachusetts. Larvae from New Bedford Harbor fish were smaller and their eggs contained significantly higher levels of PCBs. A significant inverse relation was observed between larval size and PCB content.

No single class of biomolecules appared elevated in groups of larvae with high dry weight or high survival. Contents of all classes of biomolecules measured were highly correlated with each other and with larval dry weight (Table 4). In 1987 dry weight of yolk-sac larvae was more highly correlated with survival than was any single class of biomolecules. The RNA/DNA ratio has been used as an index of growth and condition in fish (Buckley 1984, Bulow 1987). No significant correlation was observed between the RNA/DNA ratio of yolk-sac

winter flounder larvae and survival for the first month of life (Table 4). This was apparently due to the high correlation between RNA and DNA content, and the unique situation of larvae prior to feeding initiation, when they rely on endogenous energy reserves of maternal origin. The RNA content or simply the dry weight of yolk-sac larvae appear to be useful indicators of the survival potential of winter flounder through the critical first month of life.

In 1987 survival of *Pseudopleuronectes americanus* for the first month of the larval period was highly correlated with both size and chemical composition of larvae shortly after hatching. The correlation between size and survival of winter flounder larvae may have been, in part, due to a wider size spectrum of prey items available to larger larvae in our experimental systems. Larger larvae, because of their wider mouth gape, effectively experience a higher level of available food. This factor may be important for both laboratory-reared and wild larvae. It is possible that the lower viability of small larvae may be offset by increased fecundity (Buckley et al. 1991).

While both biological and environmental factors may have contributed to the observed differences in size and survival among larvae produced by Long Island Sound and Narragansett Bay winter flounder, we could not identify a single dominant factor. Our data on winter flounder spawning in Long Island Sound and Narragansett Bay (Buckley et al. 1991) suggest that female size and spawning time can have important effects on egg and larval size, fecundity, and spawning survival potential.

LITERATURE CITED

Bagenal, T. L. (1971). The interrelation of the size of fish eggs, the date of spawning and the production cycle. J. Fish Biol. 3: 207–219

Barnes, H., Blackstone, J. (1973). Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for 'total' lipids. J. exp. mar. Biol. Ecol. 12: 103–118

Black, D. E., Phelps, D. K., Lapan, R. L. (1988). The effects of inherited contamination on egg and larval winter flounder, *Pseudopleuronectes americanus*. Mar. environ. Res. 25: 45–62

Blaxter, J. H. S. (1969). Development: eggs and larvae. In: Hoar, W. S., Randall, D. J. (eds.) Fish physiology. Academic Press, New York, p. 177–252

Blaxter, J. H. S., Hempel, G. (1963). The influence of egg size on herring larvae (*Clupea harengus* L.). J. Cons. perm. int. Explor. Mer 28: 211–240

Blaxter, J. H. S., Hempel, G. (1966). Utilization of yolk by herring larvae. J. mar. Biol. Ass. U.K. 46: 219–234

Brown, M. E. (1957). Experimental studies on growth Chap. IX. In: Brown, M. E. (ed.) The physiology of fishes. Academic Press, New York, p. 361–400

Buckley, L. J. (1979). Relations between RNA-DNA ratio, prey density, and growth rate in Atlantic cod (*Gadus morhua*) larvae. J. Fish. Res. Bd Can. 36: 1497–1502

Buckley, L. J. (1982). Effects of temperature on growth and biochemical composition of larval winter flounder *Pseudopleuronectes americanus*. Mar. Ecol. Prog. Ser. 8: 181–186

Buckley, L. J. (1984). RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80: 291–298

Buckley, L. J., Halavik, T. A., Smigielski, A. S., Laurence, G. C. (1987). Growth and survival of the larvae of three species of temperate marine fishes reared at discrete prey densities. Am. Fish. Soc. Symp. 2: 82–92

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

Buckley, L. J., Smigielski, A. S., Halavik, T. A., Laurence, G. C. (1990). Effects of water temperature on size and biochemical composition of winter flounder *Pseudopleuronectes americanus* at hatching and feeding initiation. Fish. Bull. U.S. 88: 419–428

Bulow, F. J. (1987). RNA-DNA ratios as indicators of growth in fish: a review. In: Summerfelt, R. C., Hall, G. E. (eds.) Age and growth of fish. Iowa State University Press, Ames, p. 45–64

Cushing, D. H. (1967). The grouping of herring populations. J. mar. biol. Ass. U.K. 47: 193–208

Cushing, D. H. (1975). Marine ecology and fisheries. Cambridge University Press, Cambridge

Gray, J. (1928). The growth of fish. II. The growth-rate of the embryo of *Salmo fario*. J. exp. Biol. 6: 110–124

Greig, R. A., Reid, R., Wenzloff, D. (1977). Trace metal concentrations in sediments of Long Island Sound. Mar. Pollut. Bull. 8: 183–188

Hoar, W. S. (1957). The gonads and reproduction, Chap. VII. In: Brown, M. E. (ed.) The physiology of fishes. Academic Press, New York, p. 287–321

Houde, E. D. (1987). Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. 2: 17–29

Hunter, J. R. (1981). Feeding ecology and predation of marine fish larvae. In: Lasker, R. (ed.) Marine fish larvae: morphology, ecology and relation to fisheries. Washington Sea Grant Program. Univ. of Washington Press, Seattle, p. 33–37

Knutsen, G. M., Tilseth, S. (1985). Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. Trans. Am. Fish. Soc. 114: 507–511

Laurence, G. C. (1977). A bioenergetic model for the analysis of the feeding and survival potential of winter flounder, *Pseudopleuronectes americanus*, larvae during the period from hatching to metamorphosis. Fish. Bull. U.S. 75: 529–546

Miller, T. J., Crowder, L. B., Rice, J. A., Marschall, E. A. (1988). Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. 45: 1657–1670

Nelson, D. A., Miller, J. E., Rusanowsky, D., Greig, R. A., Sennefelder, G. R., Mercaldo-Allen, R., Kuropat, C., Gould, E., Thurberg, F. P., Calabrese, A. (1991). Comparative reproductive success of winter flounder in Long Island Sound and Boston Harbor: a 3-year study (biology, biochemistry, and chemistry). Estuaries

Pearcy, W. G. (1962). Ecology of an estuarine population of winter flounder phytoplankton (Walbaum). Bull. Bingham Oceanogr. Coll. 18: 5–78

Perlmutter, A. (1947). The black back flounder and its fishery in New England and New York. Bull. Bingham oceanogr. Coll. 11: 1–92

Pruell, R. J., Quinn, J. G. (1985). Geochemistry of organic contaminants in Narragansett Bay sediments. Estuar. coast. shelf Sci. 21: 195–312

Rosenberg, A. A., Haugen, A. S. (1982). Individual growth and size-selective mortality of larval turbot (*Scophthalmus maximus*) reared in enclosures. Mar. Biol. 72: 73–77

Rosenthal, H., Alderdice, D. F. (1976). Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Bd Can. 33: 2047–2065

Saila, S. B. (1961). A study of winter flounder movements. Limnol. Oceanogr. 6: 292–298

SAS Institute Inc. (1985). SAS/STAT guide for personal computers. Version 6 edn. SAS Institute, Inc., Cary, North Carolina

Smigielski, A. S., Arnold, C. R. (1972). Separating and incubating winter flounder eggs. Progve Fish Cult. 34: 113

Southward, A. J., Demir, N. (1974). Seasonal changes in dimensions and viability of the developing eggs of the cornish pilchard (*Sardinia pilchardus walbaum*) off Plymouth. In: Blaxter, J. H. S. (ed.) The early life history of fish. Springer-Verlag, New York. p. 53–68

This article was presented by K. Sherman, Narragansett, Rhode Island, USA

Steel, R. D. G., Torrie, J. H. (1960). Principles and procedures of statistics. McGraw-Hill, New York

Tanasichuk, R. W., Ware, D. M. (1987). Influence of interannual variations in water temperature on fecundity and egg size in Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 44: 1485–1495

Topp, R. W. (1968). An estimate of the fecundity of winter flounder, *Pseudopleuronectes americanus*. J. Fish. Res. Bd Can. 25: 1299–1302

Tyler, A. V., Dunn, R. S. (1976). Ration, growth, and measures of somatic and organ condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypotheses regarding population homeostasis. J. Fish. Res. Bd Can. 33: 63–75

von Westernhagen, H. (1988). Sublethal effects of pollutants on fish eggs and larvae. In: Hoar, W. S., Randall, D. J. (eds.) Fish physiology. Vol. XI. The physiology of developing fish, Part A. Eggs and larvae. Academic Press, San Diego, p. 253–346

Ware, D. M. (1975). Relation between egg size, growth, and natural mortality of larval fish. J. Fish. Res. Bd Can. 32: 2503–2512

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Winter flounder *Pseudopleuronectes americanus* reproductive success. II. Effects of spawning time and female size on size, composition and viability of eggs and larvae

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ABSTRACT: Vital statistics and embryo and larval viability were determined for winter flounder *Pseudopleuronectes americanus* spawning in Narragansett Bay, Rhode Island, USA, over the course of the spawning season. Fish approaching spawning condition were collected throughout the spawning season and hand-stripped in the laboratory. Larvae were reared through the first month of life. Female size affected most of the reproductive parameters examined, including both absolute and relative measures of total reproductive output (reproductive rate and gonadosomatic index), egg size, fecundity, and viability. Spawning time was found to affect egg size, fecundity, and viability, but not reproductive rate or gonadosomatic index. Egg size increased with increasing female size and decreased as the spawning season progressed. Spawning time and female size explained 61 % of the observed variability in egg size among females. Female size explained 95 % of the variability in reproductive rate and 90 % of the variability in fecundity. Female size and spawning time combined explained 94 % of the variability in fecundity. The effects of female size and spawning time on both fertility and hatch rate were non-additive. Embryos produced earlier in the spawning season appeared to have a survival advantage over those produced later in the spawning season. Embryos produced by small, late-spawning fish appeared to be at a pronounced disadvantage.

INTRODUCTION

High fecundity and high mortality during early life stages are characteristic of temperate marine fishes. Individuals that survive to reproduce are the rare exceptions. Much effort has recently been devoted to determining the role of the environment in the success or failure of larval cohorts and individuals. Less effort has been directed towards study of the role of intrinsic or innate biotic factors related to parental investment in gametes and the trade-off between the number and size of eggs produced. The existing body of literature suggests that egg size varies considerably among species, stocks and individuals (Hempel & Blaxter 1967, Bagenal 1971, Miller et al. 1988). Both spawning time (Hempel & Blaxter 1967, Bagenal 1971) and female size (Gall 1974, Kazakov 1981, Rogers & Westin 1981, Zastrow et al. 1988) have been implicated as factors affecting egg size within a species. The combined effects of spawning time and female size have received little attention. While spawning time has been recognized as affecting egg size, its affect on fecundity has not been considered for most species.

In this paper we examine the effects of the size and age of spawning female winter flounder *Pseudopleuronectes americanus*, and of spawning time, on reproductive rate, fecundity, egg size, egg composition, and viability of eggs and larvae. The relations among these variables are also examined. This effort was prompted by our earlier observations of large differences in size of yolk-sac larvae among winter flounder spawning at different locations and of a relationship between size of yolk-sac larvae and survival for the first month of life (Buckley et al. 1991).

We describe a practical approach to determining reproductive rate, gonadosomatic index (GSI), egg size, and fecundity of individual fish while studying egg and larval viability on the same individuals.

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METHODS

A site in upper Narragansett Bay north of Prudence Island, Rhode Island, USA, was chosen for intensive study of fish spawning over the duration of the spawning season. The site has been the subject of a major tagging program (State of Rhode Island, Department of Environmental Management) providing information on seasonal movements and maturation, and ready access to the large numbers of live fish necessary to obtain hydrating females over the entire spawning season. Mature winter flounder *Pseudopleuronectes americanus* were collected from January through March 1988. Females showing signs of hydration were selected from the catch. One collection of fish was also made in the lower West Passage of Narragansett Bay on 11 March 1988. Additional collections were made at the upper Narragansett Bay site in 1990.

Females were measured and weighed immediately before and after hand-stripping of eggs. The females were sacrificed after the second weighing and frozen. Subsamples of unfertilized eggs, taken for determination of dry weight and biochemical composition, were frozen at −70 °C. Egg dry weight was determined by freezing eggs in a monolayer on the walls of a glass lyophilization vial. After freeze-drying, the individual eggs were easily separated and 20 intact eggs from each female were weighed individually to the nearest 0.1 µg on a Cahn* electro-balance. Larval dry weights (20 larvae, 3 d after hatch) and egg RNA, lipid and protein content were determined as described in Buckley et al. (1991). Because of the low concentration of DNA in unfertilized *Pseudopleuronectes americanus* eggs (Buckley 1980), this component was not measured. The ratio of dry weight to wet weight of unfertilized eggs was determined by weighing a group of several hundred eggs from each spawn before and after freeze-drying.

The remainder of the eggs were fertilized with the sperm from 3 males and incubated at 5 °C. Larvae were reared as described in Buckley et al. (1991) using the feeding regime described for 1988. Duplicate groups of 200 larvae each from individual females were reared for 28 d in 36 l tanks. After initial inoculation with 1 l of a dense culture of algae *Tetraselmis* sp., larvae were fed cultured rotifers *Brachionus plicatilis* at the rate of 2000 rotifers l^{-1} d^{-1}. A volume of culture corresponding to 72000 rotifers was concentrated on a sieve and added to each tank 6 d wk^{-1}. After completion of the 28 d rearing period, tanks were drained and survivors were counted, measured and weighed.

Fertilization rate was estimated 24 h after spawning by selecting 100 eggs at random and counting, under a dissecting microscope, the number showing signs of cleavage. A group of 100 cleaving embryos were incubated in a 200 ml tube fitted with a Nitex mesh bottom set in a 36 l tank. Two days after hatching was completed the number of larvae and dead eggs was counted. Hatch rate was defined as the total number of larvae counted, and viable hatch as the number of active, free-swimming larvae showing no obvious abnormalities. Abnormalities observed included lordosis, scoliosis and larvae with their heads still inside the chorion.

Otolith and scale samples were taken from the female winter flounder after partial thawing. Fish ages were determined by counting the number of annual rings on several representative scales. The ovaries were removed, refrozen, freeze-dried and weighed to the nearest milligram. In 1990 several winter flounder were hand-stripped and weighed live, as above, and then returned to the holding tanks. These fish rehydrated and spawned 1 or 2 additional times. When the females appeared to be completely spent they were sacrificed and the ovaries treated as described above.

Data analysis was done using SAS System software for personal computers (SAS Institute Inc. 1985). Square root transformation was applied to percent survival values [$(\text{survival}+0.5)^{1/2}$] and arcsine transformation applied to fertilization and viable hatch rates [$\text{arcsine}(\%/100)^{1/2}$] prior to analysis of variance and regression analysis. For analysis of variance, fish were assigned to size and spawning date groups. Females were assigned to 1 of 3 size groups as follows: small (total length < 295 mm); medium (total length 295 to 326 mm); large (total length > 326 mm). Females were assigned to 1 of 2 spawning date groups depending upon when they spawned in relation to peak spawning, which was estimated to have occurred in upper Narragansett Bay the first week of March 1988 (J. Christopher Powell pers. comm.). Fish that spawned prior to 7 March were assigned to the early spawning group; fish that spawned after that date were assigned to the late group.

RESULTS

Forty *Pseudopleuronectes americanus* caught in Narragansett Bay were spawned by hand-stripping between 1 February and 11 April 1988. The spawning females ranged in size from 250 to 396 mm total length and from 240 to 1016 g in weight (Table 1). The relation between total length and wet weight just prior to spawning is given in Table 2. Scale analysis indicated at least 4 yr classes, with 3, 4, and 5 yr old fish predominating. No trend over time was observed in the size or age of fish spawned during the study. No signifi-

* Reference to trade names does not imply endorsement by the United States Government

cant correlation was observed between female size or age and spawning date. No significant difference was observed in either the mean spawning dates of small, medium and large females or the mean size of early- and late-spawning fish.

Reproductive rate and GSI

The wet weight of eggs obtained by a single hand-stripping ranged from a low of 36 g to a high of 144 g. On average, 1 hand-stripping produced 69 g of eggs. Dissection of females after hand-stripping revealed that the ovaries of most fish still contained a large number of mature eggs. The total wet weight of the ovaries just prior to spawning was estimated from the weight of eggs obtained plus the weight of the remaining ovary after hand-stripping. This number when expressed on a yearly basis (g yr^{-1}) is the reproductive rate (Ware 1985). The weight of eggs spawned, the remaining ovarian weight and the total ovarian weight all increased with female size (Fig. 1) and were unaffected by spawning

Table 1. *Pseudopleuronectes americanus*. Size and reproductive data on winter flounder from Narragansett Bay, Rhode Island, USA. TL: total length. Ovary wet weight: estimated wet weight of the ovary just before spawning

TL (mm)	Wet weight (g)	Spawning date (Julian day)	Ovary wet wt (g)	Egg dry wt (ug)	Fecundity[a] (no. eggs)
Upper Narragansett Bay					
294	430	32	118.4	50.2	–
266	263	32	77.5	43.8	–
295	352	32	91.3	46.2	–
321	532	33	157.8	54.4	–
372	669	45	207.8	47.3	–
284	315	49	107.9	–	–
381	774	49	281.6	54.4	–
261	260	50	65.2	39.7	–
292	286	50	68.5	43.4	–
315	373	51	82.5	54.0	192 240
374	802	52	335.6	54.2	1 035 653
250	240	53	66.9	45.9	222 491
351	580	56	188.3	43.8	–
290	351	57	105.9	41.0	–
298	397	84	103.8	38.9	–
334	597	85	188.0	40.7	832 196
396	1016	85	368.1	48.7	1 390 495
299	363	85	95.0	43.3	–
282	306	87	100.2	–	–
359	628	88	190.8	41.2	896 455
279	381	102	71.9	34.9	–
320	440	41	116.8	51.1	369 831
314	461	47	146.8	47.3	568 533
324	430	49	108.6	–	–
290	340	50	93.0	55.9	–
318	465	53	144.0	–	–
334	580	55	210.7	–	–
350	573	56	191.0	–	–
330	454	87	134.4	36.6	616 280
317	474	90	152.0	42.0	–
300	427	90	139.4	38.2	614 823
Lower West Passage					
390	930	72	322.7	47.9	1 131 404
327	489	73	152.6	46.9	679 486
330	592	73	202.0	45.0	753 385
360	727	74	230.6	46.0	822 999
305	416	74	114.1	35.7	619 677
266	266	74	82.9	40.3	364 602
356	642	75	237.6	50.8	902 800
385	946	75	386.7	52.9	–
304	406	77	135.4	–	–

[a] Fecundity estimates are given only for fish for which spent ovary dry weight was estimated

Table 2. *Pseudopleuronectes americanus*. Equations describing the relations among female size, spawning date, and reproductive effort of winter flounder from Narragansett Bay. [a] GSI: gonadosomatic index

Dependent variable (y)	Equation	n	r^2	$p > F$
Female weight (g)	ln y = 3.07 ln Length − 11.57	40	0.94	0.0001
Ovarine weight (g)	y = 0.420 Weight − 50.5	40	0.95	0.0001
	y = 2.034 Length − 492.4	40	0.83	0.0001
	y = 46.84 Age − 54.5	39	0.60	0.0001
GSI	y = 0.0164 Weight + 22.5	40	0.46	0.0001
Egg weight (mg)	y = −0.00016 Spawn date + 0.0560	33	0.31	0.0008
	y = 0.000011 Weight + 0.0397	33	0.16	0.02
	y = −0.00020 Spawn date + 0.000016 Weight + 0.0503	33	0.61	0.001
Fecundity (eggs female^{-1})	y = 1397 Weight − 73 780	18	0.90	0.0001
	y = 1324 Weight + 4539 Spawn date − 356 002	18	0.94	0.0001

[a] No significant difference was observed in the regression lines for upper and lower bay fish (female weight, ovarian weight, GSI). All regression lines are for upper and lower Narragansett Bay fish combined

date. Female weight at spawning explained 95 % of the variation in the reproductive rate (Table 2). The GSI [= (wet weight of eggs + wet weight of stripped ovary)/(wet weight of female prior to spawning)] just prior to spawning also increased with increasing female size and was unaffected by spawning date (Table 2). The eggs obtained by a single hand-stripping represented between 22 and 84 % of the total ovarian weight. The fraction of eggs spawned by a single hand-stripping decreased with increasing female size.

In 1990, 6 fish were spawned as described above but returned to holding tanks rather than sacrificed. These fish usually rehydrated and released eggs into the holding tank, or were hand-stripped 1 or 2 more times within several days of first spawning. The interval between spawns was typically 1 to 3 d. When it appeared that the fish were completely spent, they were weighed, and the ovaries removed and processed as described above. These spent ovaries contained very few mature eggs. The dry weight of the spent ovary represented on average 10.6 % of the estimated dry weight of the ovary just prior to first spawning. The dry weight of the ovary just prior to spawning was estimated by multiplying the difference between the wet weight of the fish just prior to the first spawning and the wet weight of the fish after the final spawning by the mean fraction dry weight of unfertilized eggs (0.161) and adding the dry weight of the spent ovary. Dry matter was 21.1 ± 3.2 % of the wet weight of the ovary after a single hand-stripping and 12.6 ± 2.6 % of the weight of the fully spent ovary.

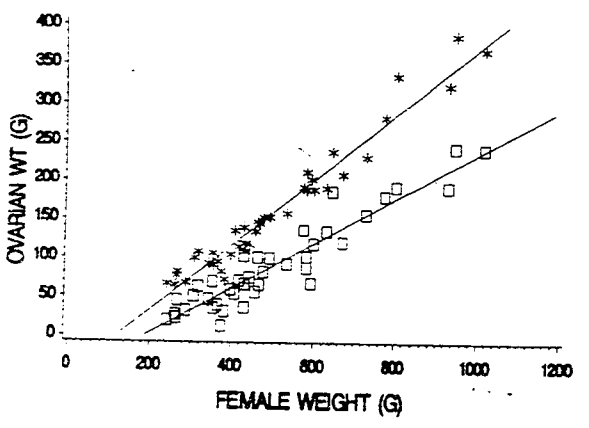

Fig. 1. *Pseudopleuronectes americanus*. Wet weight of ovary before (•) and after (□) hand-stripping

Egg weight and fecundity

The mean dry weight of single eggs produced by individual winter flounder ranged from 35 to 56 µg (mean 45.6 ± 5.9, CV 12.9 %; Table 1). Variability was considerably less among eggs produced by a given individual (average CV 7.2 %). When a fish was hand-stripped more than once, mean egg dry weight decreased an average of 8 % with each subsequent spawning.

Egg weight decreased as the spawning season progressed and increased with female size (Fig. 2). Together, female size and spawning date explained 61 % of the variability in egg weight (Table 2). Analysis of covariance indicated significant differences ($p \leq 0.05$) in mean egg weight between early- and late-spawning fish after adjustment for differences in female size, and among female size groups after adjustment for spawning date (Table 3). Large, early-spawning fish produced the largest eggs while small, late-

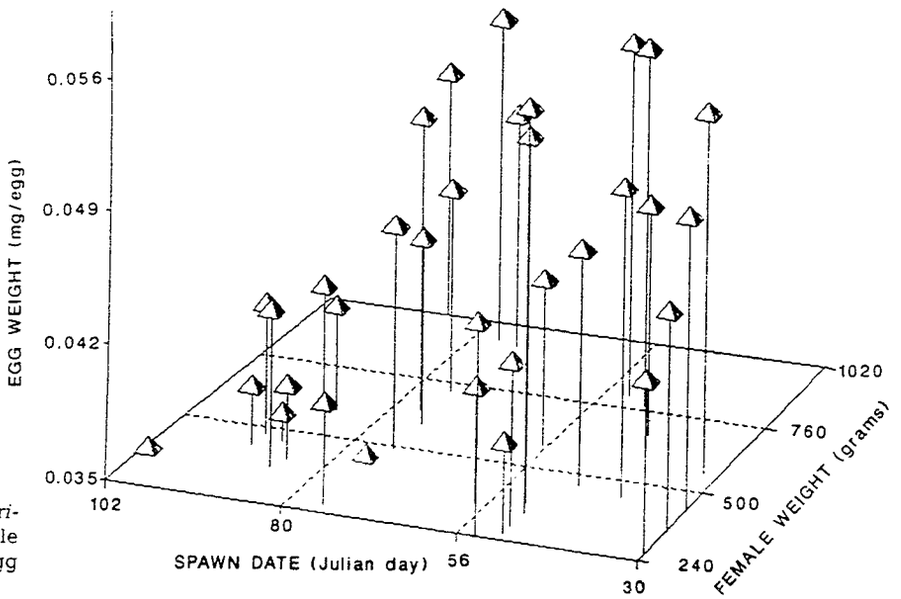

Fig. 2. *Pseudopleuronectes americanus*. Relation among female weight, spawning date, and egg weight of winter flounder

spawning females produced the smallest eggs. On average, dry matter represented 16% of the wet weight of *Pseudopleuronectes americanus* eggs.

Fecundity (b), the sum of the eggs obtained by hand-stripping plus the eggs remaining in the ovary, was estimated using the equation:

$$b = \frac{[(w_e \times C) + (w_o)]}{m} \times 0.894$$

where w_e is the wet weight of eggs obtained by hand-stripping, w_o is the dry weight of the remaining ovary, m is the dry weight of an individual egg and C is the ratio of dry weight to wet weight of unfertilized eggs determined for each spawn. The term 0.894 is an estimate of the fraction of the ovarian dry weight actually made up of eggs just prior to spawning based on completely spent fish from 1990. Connective tissue, membranes, immature oocytes, and blood vessels make up the bulk of the remainder of the ripe ovary.

Fecundity increased with increasing female size (Fig. 3). For a given size of female, fecundity increased as the spawning season progressed due to the decrease in egg size. Female size and spawning date together explained 94% of the variability in fecundity (Table 2). Analysis of covariance indicated a significant difference ($p \leq 0.05$) in mean fecundity between early- and late-spawning fish after adjustment for differences in female size. A significant difference ($p \leq 0.05$) in mean fecundity was also observed among female size groups after adjustment for spawning date (Table 3).

Table 3. *Pseudopleuronectes americanus*. Mean values for egg weight and fecundity of Narragansett Bay fish adjusted for spawning date and female size. [a] Bracketed means are not significantly different ($p \geq 0.05$). LS mean: least-squares mean; SE: standard error

	Egg weight (µg)		Fecundity (no. eggs)	
	LS mean	SE	LS mean	SE
Adjusted for spawning date				
Female size group: small	[42.2	1.5	[313×10^3	148×10^3
medium	[44.8	1.4	[491×10^3	87×10^3
large	48.3	1.2	[892×10^3	68×10^3
Adjusted for female weight				
Spawning date group: early	[48.8	1.1	[599×10^3	321×10^3
late	[42.1	1.1	[742×10^3	227×10^3

[a] The female size groups were created by dividing all females spawned from Narragansett Bay into 3 groups based on standard length. Fish were assigned to a spawning date group depending upon when they spawned in relation to peak spawning (see 'Methods' for details)

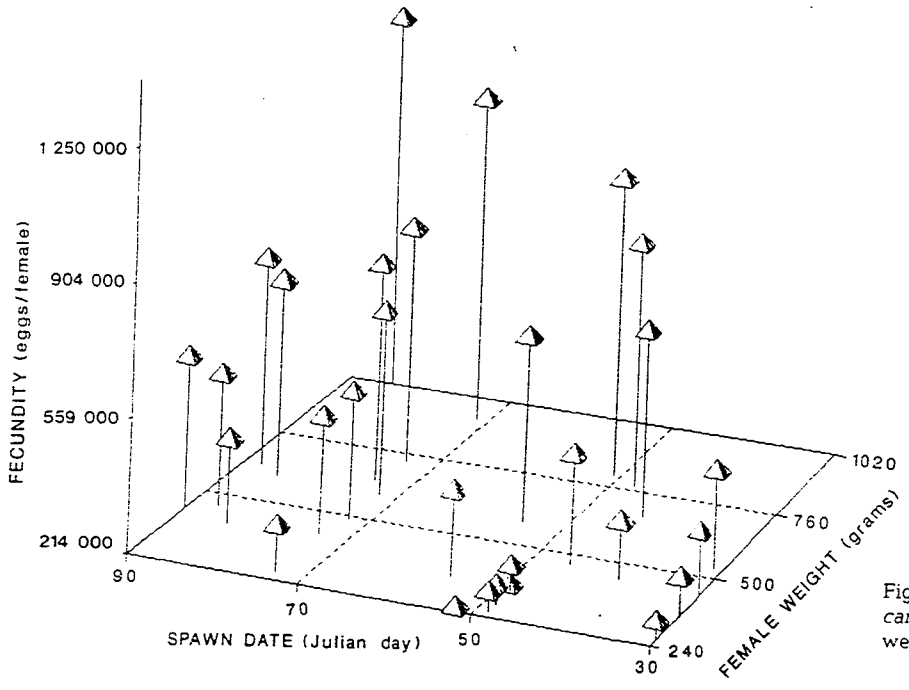

Fig. 3. *Pseudopleuronectes americanus*. Relation among female weight, spawning date, and fecundity of winter flounder

Biochemical composition of eggs

The protein, RNA and lipid composition of unfertilized *Pseudopleuronectes americanus* eggs are given as total content (μg egg^{-1}) and concentration (% dry weight) in Table 4. The content of all 3 components was highly correlated with egg dry weight. Protein and lipid concentration were independent of egg size and spawning date. RNA concentration increased with date of spawning and decreased with egg size. Protein accounted for one-half of the dry weight of the egg.

Fertility, hatch, and survival (upper Narragansett Bay fish)

To eliminate any possibility of the origin of fish affecting the viability of eggs and larvae, these analyses were limited to fish collected in upper Narragansett Bay in 1988. Analysis of variance of transformed data indicated a significant interaction ($p \leq 0.05$) between female size and spawning time for fertility and viable hatch rates of winter flounder from upper Narragansett Bay (Table 5). No interaction was observed for larval survival rates. Fertility of eggs from different female winter flounder ranged from 31 to 99% with a mean of 91%. Fertilization rates were lowest (mean 46%) for small, late-spawning fish (Table 6). Viable hatch ranged from 0 to 96% with a mean of 74% and was again lowest (mean 22.5%) for small, late-spawning fish (Table 6). Survival for the first month of life under standard conditions ranged from 0 to 9% with a mean of 2%. Survival was higher for early-spawning fish (2.6%) than for late spawners (0.7%) (Table 6). Because of the low viable hatch of eggs from small, late-spawning fish no survival data were obtained for this group.

Table 4. *Pseudopleuronectes americanus*. Biochemical composition of winter flounder eggs from Narragansett Bay

Component	Content (μg egg^{-1})	Concentration (% dry wt)
Protein	22.40 ± 3.60	50.0 ± 4.6
RNA	0.79 ± 0.09	1.8 ± 0.1
Lipid	5.58 ± 0.87	12.5 ± 0.9

Correlation among variables (upper Narragansett Bay fish)

Most female winter flounder spawned within a few days of collection. This was anticipated since females showing signs of hydration were selected from the catch. An inverse correlation ($p \leq 0.05$) was observed between spawning date (Julian day) and the length of the embryonic period, although all eggs were incubated at the same temperature (5°C). The length of time females were held in the laboratory prior to spawning was unrelated to any of the primary performance variables (fertility, viable hatch, and survival). Egg weight, larval length and larval weight were all correlated ($p \leq 0.01$; Table 7). Fertility, hatch and viable hatch were highly correlated ($p \leq 0.0001$) but unrelated

Table 5. *Pseudopleuronectes americanus*. Analysis of variance for factors affecting fertility, viable hatch and survival of winter flounder eggs and larvae from upper Narragansett Bay. Where the interaction factor is significant no *F* values are given for the other factors. Prior to ANOVA, fertilization and hatch rates were arcsine transformed, and survival was square-root transformed

Variable		Female size	Spawning date	Size × Date	Total
Fertilization rate (%)	df	2	1	2	30
	F value	–	–	17.48***	
Viable hatch (%)	df	2	1	2	30
	F value	–	–	9.14***	
Survival (%)	df	2	1	1	21
	F value	0.1	4.8*	1.1	

* $p \leq 0.05$, *** $p \leq 0.001$

Table 6. *Pseudopleuronectes americanus*. Fertilization, viable hatch, and survival rates (means ± SD) of winter flounder eggs and larvae from upper Narragansett Bay. Where the interaction between female size group and spawning time group was not significant, main effects means were calculated and Tukey's studentized range effects applied to these values. Where the interaction was significant, simple main effects were tested. Values in a row with a letter in common or bracketed values in a column are not significantly different ($p \geq 0.05$). Prior to analysis of variance, fertilization and viable hatch rates were arcsine transformed. Survival rates were square-root transformed [$(x + 0.5)^{1/2}$]. Numbers in parentheses represent number of observations per cell

Spawning group	Female size group: Small	Medium	Large	$\bar{x}$
		Fertilization rate (%)		
Early	95.6 ± 2.1 (8)[a]	89.2 ± 15.6 (7)[a]	95.7 ± 4.4 (6)[a]	91.4 ± 14.9
Late	46.0 ± 21.2 (2)[b]	97.5 ± 1.3 (4)[a]	96.5 ± 1.3 (4)[a]	
		Viable hatch rate (%)		
Early	72.1 ± 3.9 (8)[a]	63.1 ± 29.5 (7)[a]	83.5 ± 6.2 (6)[a]	74.0 ± 22.3
Late	22.5 ± 19.1 (2)[b]	90.5 ± 2.5 (4)[a]	91.5 ± 2.1 (4)[a]	
		Survival rate (%)		
Early	2.1 ± 1.4 (5)	2.2 ± 2.2 (5)	3.8 ± 4.1 (4)	2.6 ± 2.6
Late	–	1.2 ± 2.2 (4)	0.1 ± 0.2 (4)	0.7 ± 1.6
$\bar{x}$	2.1 ± 1.4[a]	1.8 ± 2.1[a]	1.9 ± 3.3[a]	1.9 ± 2.4

to larval survival (Table 7). The GSI was correlated with fertility, viable hatch and fecundity. The fraction of dry matter in the stripped ovary was correlated with fertility and viable hatch.

Two estimators of the condition of the spawning females were calculated: a standard condition factor (Tyler & Dunn 1976, Burton & Idler 1984) and the residual GSI. Condition was defined as (female wet weight − ovarian weight)/length^b, where b is the exponent of the length-weight regression (Table 2). The residual GSI was defined as (actual GSI)−(GSI predicted from a regression between GSI and standard length).

No correlation was observed between these condition factors and performance variables (fertility, viable hatch, and larval). No correlation was observed between egg size or the concentration (% dry wt) of any single class of biomolecule and egg viability as measured by fertilization, viable hatch or survival rates.

DISCUSSION

Our data on *Pseudopleuronectes americanus* from Narragansett Bay show that female size affected most of the reproductive parameters examined, including absolute and relative measures of total reproductive output (reproductive rate and GSI), egg size, fecundity, and viability. In concert with female size, spawning time was found to have an effect on egg size, fecundity and viability. Spawning time did not affect total reproductive output as measured by reproductive rate or GSI. The interaction of spawning time and female size was significant for fertilization rate and viable hatch but not for egg weight, fecundity or larval survival.

Female size was related closely to the absolute amount of mass directed toward reproduction. Of our 3 measures of the size and age of spawning females (length, weight and age), wet weight explained the largest part (95%) of the variability observed in repro-

Table 7. *Pseudopleuronectes americanus*. Correlation (r) among variables associated with reproduction of winter flounder from upper Narragansett Bay. Numbers in parentheses represent number of observations (GSI: gonadosomatic index)

Variable	Fecundity	Egg weight	Fertility	Viable hatch	Survival
Spawning date	0.47* (11)	−0.65*** (25)	−0.26 (31)	0.14 (31)	−0.44* (22)
Female weight	0.96*** (11)	0.31 (25)	0.16 (31)	0.37* (31)	−0.04 (22)
Female length	0.87*** (11)	0.32 (25)	0.19 (31)	0.36* (31)	0.03 (22)
Female age	0.68* (11)	0.07 (24)	0.11 (30)	0.29 (30)	0.14 (22)
Female condition	0.52 (11)	−0.11 (25)	−0.05 (31)	0.09 (31)	−0.26 (22)
GSI	0.80** (11)	0.29 (25)	0.36* (31)	0.48** (31)	−0.05 (22)
Residual GSI	0.38 (11)	0.13 (25)	0.30 (31)	0.33 (31)	−0.10 (22)
Fraction of eggs spawned	−0.57 (11)	0.03 (25)	−0.37 (31)	−0.34 (31)	−0.07 (22)
Fraction dry weight of ovary	0.51 (11)	−0.10 (14)	0.81*** (15)	0.74** (15)	0.00 (13)
Fraction dry weight of egg	0.49 (11)	−0.02 (22)	0.17 (28)	0.16 (28)	0.25 (20)
Larval length	0.04 (11)	0.54** (25)	0.15 (25)	0.00 (25)	0.37 (22)
Larval weight	−0.96** (5)	0.69** (15)	0.08 (15)	−0.40 (15)	0.18 (13)
Fecundity	–	−0.01 (11)	0.10 (11)	0.58 (11)	−0.43 (11)
Egg weight	0.01 (11)	–	0.21 (25)	−0.06 (25)	0.22 (22)
Fertility	0.10 (11)	0.21 (25)	–	0.80*** (31)	0.10 (22)
Viable hatch	0.58 (11)	−0.06 (25)	0.80*** (31)	–	−0.38 (22)
Survival	0.43 (11)	0.22 (22)	0.10 (22)	−0.38 (22)	–

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

ductive rate (Table 2). Little variability in size-specific ovarian weight was observed over the spawning season of winter flounder. Similarly, Tanasichuk & Ware (1987) found that size-specific ovarian weight of Pacific herring remained remarkably constant over years and between areas.

Given a set amount of mass to devote to reproduction, a change in either egg size or fecundity will result in a corresponding change in the other variable, since these variables are related by the equation:

$$\text{Reproductive rate (g yr}^{-1}\text{)} = \text{egg weight} \times \text{fecundity}$$

Female size appeared to affect the partitioning of the reproductive mass into individual eggs as estimated by egg size and fecundity (Table 3). This is in agreement with an earlier observation that 3 yr old *Pseudopleuronectes americanus* produced smaller eggs than did age 4 or 5 fish (Topp 1968). Topp (1968), however, did not observe a significant correlation between female size and ovum size. This may have resulted from asynchronous ripening, as suggested by Topp (1968), or from differences in spawning time, as suggested by our data. Unlike size-specific ovarian weight that showed little variability over the spawning season, egg size and fecundity varied considerably over the spawning season in Narragansett Bay (Tables 2 & 3).

Seasonal shifts in egg size have been reported for a variety of species that spawn at different locations at different times of the year, notably plaice and herring (Simpson 1959, Blaxter & Hempel 1963, Cushing 1967, Bagenal 1971). Several authors (Cushing 1967, Bagenal 1971) have suggested that this shift in egg size is related to areal changes in the timing of the production cycle. Similar changes in egg size and fecundity have been demonstrated on an interannual scale for Pacific herring (Tanasichuk & Ware 1987). These authors linked this change in fecundity and egg size to water temperatures 60 to 90 d prior to spawning. In this paper we demonstrate a seasonal change in fecundity and egg weight among winter flounder spawning at a single location. This decrease in egg size and the associated increase in fecundity during the spawning season of winter flounder were not due to a progressive decrease in the size of spawning females, as suggested for plaice (Simpson 1959, Bagenal 1971) and herring (Hay 1985, Tanasichuk & Ware 1987), or to a reduction in egg size over successive batches of eggs from individual females, as suggested for Atlantic cod *Gadus morhua* (cited in Knutsen & Tilseth 1985). Rather, in winter flounder the progressive decrease in egg size over the spawning season appeared to result from a change in the balance between egg size and fecundity among females of all sizes as the spawning season progressed.

At least 2 mechanisms for adjusting the number of oocytes brought to full maturity are possible. These are (1) regulation of recruitment of oocytes to the vitellogenic phase, and (2) resorption of developing oocytes prior to spawning (atresia). Both appear to be operative in winter flounder.

A 3 yr cycle for oocyte maturation has been demonstrated for *Pseudopleuronectes americanus* (Dunn & Tyler 1969, Burton & Idler 1984). Tyler & Dunn (1976) demonstrated that food ration can affect the number of

oocytes undergoing maturation in winter flounder, and when faced with a shortage of food, females sacrifice egg production but maintain egg size and body weight. They found no increase in the number of oocytes undergoing resorption among females at low rations, although 10.4% of yolk-bearing oocytes were found to be atretic. They deduced that recruitment of oocytes must have been affected.

Several lines of evidence suggest that female fish initiate development of more oocytes than are brought to full maturation. Pre-ovulatory atresia has been observed in several species (Bowers & Holliday 1961, Polder 1961) including *Pseudopleuronectes americanus* (Dunn & Tyler 1969, Tyler & Dunn 1976). Blaxter & Holliday (1963) and Tanasichuk & Ware (1987) have suggested that the maximum number of oocytes is adjusted downward in response to environmental factors. The actual number brought to full maturity and spawned may be determined as late as 20 to 30 d prior to spawning in Pacific herring (Hay 1985). Tanasichuk & Ware (1987) presented data for the same species suggesting that water temperature 60 to 90 d prior to spawning was critical in determining the number of oocytes brought to maturity. They suggested that water temperature during this 'decisive period' acts through its effect on gonadotropin levels, with warm years resulting in higher fecundity and smaller egg size.

Our data on winter flounder *Pseudopleuronectes americanus* are not wholly consistent with Tanasichuk & Ware's hypothesis of a 'decisive period' for vitellogenesis 60 to 90 d prior to spawning, suggesting that the same mechanism may not operate on an interannual basis in winter flounder. In Narragansett Bay the winter flounder spawning season extends from January to April. Examination of 14 yr of sea-surface temperature records from the NOAA National Ocean Service tide station at Newport, Rhode Island, indicated that the temperature minimum usually occurs between the last week of January and the second week of February (range in Julian days = 20 to 50). The 'decisive period' 60 to 90 d prior to spawning of winter flounder would occur during the descending portion of the annual temperature cycle. Early-spawning fish should, on average, experience warmer temperatures 60 to 90 d prior to spawning during the 'critical period' than do later-spawning fish, even though water temperatures are lower at spawning. In apparent conflict with the 'decisive period' hypothesis, early-spawning winter flounder, which would on average experience warmer water temperatures 60 to 90 d prior to spawning, produce larger eggs and have lower relative fecundity than do late-spawning fish. It is possible, however, that the later-spawning fish remain in warmer water offshore during the 'decisive period'. Our data on experimental manipulation of water temperature, beginning 50 d prior to spawning in winter flounder (Buckley et al. 1990), suggest that water temperature closer to the time of spawning may be critical in determining larval size and viability in winter flounder.

Several authors have published estimates of the fecundity of *Pseudopleuronectes americanus* as a function of female size (Saila 1963, Topp 1968, Kennedy & Steele 1971); however, none considered an effect of spawning time. While female weight explained most (90%) of the variability in fecundity observed in our data (Table 2), the residuals increased with spawning date. Addition of spawning time as a second independent variable significantly improved the regression, explaining an additional 4% of the variability. Our estimates of egg production of winter flounder from Narragansett Bay are in close agreement with those of Saila (1963) for winter flounder collected 30 yr earlier in Narragansett Bay and slightly lower than those of Topp (1968) for fish from Massachusetts Bay (Table 8). The weight-fecundity curves published by Saila (1963) for Narragansett Bay and by Kennedy & Steele (1971) for Conception Bay, Newfoundland, were not statistically different. Our fecundity estimates for early and late spawners encompass estimates based on Saila's female weight-fecundity relation.

Assessment of the spawning condition and reproductive capacity of *Pseudopleuronectes americanus* is confounded by the prolonged period of gametogenesis (Burton & Idler 1984). The possible downward adjustment in the number of recruited oocytes suggests that care should be exercised in using fecundity estimates based on counts of maturing oocytes far in advance of spawning in winter flounder.

The increasing portion of body weight devoted to reproduction in larger individuals (increase in GSI with

Table 8 *Pseudopleuronecies americanus*. Fecundity estimates (no. eggs female^{-1}) for winter flounder from Narragansett Bay in 1958 (Saila 1963) and 1988 (present study), and Massachusetts Bay in 1966 (Topp 1968). JD: Julian Day

Female weight	Massachusetts Bay	Narragansett Bay		
	1966	1963	1988	
			Early (JD 31)	Late (JD 90)
250 g	422 000	252 000	116 000	383 000
1000 g	1 851 000	1 219 000	1 109 000	1 377 000

female size; Table 2) has been observed in several species (Ware & Tanasichuk 1989). An interesting question relates to how this surplus is partitioned between production of larger eggs and production of more eggs. The GSI of a typical large winter flounder (1000 g wet weight) is 39%, compared to 27% in a typical small one (260 g) (Table 2). The extra 12% of the body weight of the large fish directed toward reproduction represents 120 g of surplus production and is independent of spawning time. Among early spawners (Julian Day 32), 83 g or roughly two-thirds of the surplus goes to production of larger eggs, and 40 g, or roughly one-third, is directed toward producing more eggs. Among late spawners an even larger portion of the surplus in large females goes to production of larger eggs.

Several notable observations on batch spawning and ovarian dry weight were made during the course of this study. Individual winter flounder appeared to produce and spawn several batches of ripe eggs over several days before becoming completely spent. Dry matter, as a percentage of wet weight, made up 16% of the unfertilized egg, 21% of the ovary after 1 hand-stripping and 12.6% of the fully spent ovary. These changes in the concentration of dry matter in the ovary point to limitations in the use of vital statistics based solely on wet weight measurements. An inverse correlation was observed between the fraction of the ovary spawned after 1 hand-stripping and both the concentration of dry matter in the partially spent ovary and the size of the female. Apparently the concentration of dry matter is highest just prior to the initial hydration. A portion of the mature oocytes hydrate over several days and are spawned, resulting in a reduction in the density of the partially spent ovary compared to the initial prehydration condition. This cycle of hydration and spawning is repeated over several days until the fish is spent. At this point the concentration of dry matter in the ovary is at a minimum. Dry matter remaining in the fully spent ovary represents 10.6% of the dry weight of the ovary just prior to spawning. No difference in the rates of fertilization or viable hatch was observed among batches of eggs produced by the same female.

Our data on fertilization, viable hatch, and survival rates suggest that female size and spawning time can have important effects on these variables. For both fertility and hatch rate, the interaction of female size and spawning time was significant. Embryos produced early in the season appeared to have a survival advantage over those produced later in the season (Table 6). In most cases embryos produced by small, late-spawning fish appeared to be at a pronounced disadvantage. Trends in size and viability of eggs produced over the spawning season could contribute to differential survival at later life-history stages. Differences in the distribution of birth dates estimated from egg production and from otolith analysis of larvae and juveniles have been observed for several species (Methot 1983, Rice et al. 1987). The role of egg quality in these patterns should be considered along with the role of environmental variability.

Fertilization rate, hatch rate and viable hatch rate were highly correlated but unrelated to larval survival rate (Table 7). For fish spawned from Narragansett Bay in 1988, we were unable to identify a single property of the unfertilized egg or newly hatched larva that was related significantly to larval survival. The parameters examined included egg dry weight and chemical composition (protein, RNA, and lipid content), and initial length and weight of the newly hatched larva. This is in contrast to larvae from *Pseudopleuronectes americanus* collected at several locations in Long Island and Narragansett Bay during the 1987 spawning season, where we observed a strong correlation between initial larval size (and chemical content) and survival for the first month of life (Buckley et al. 1991). There are at least 2 possible explanations for this difference in results: (1) in the earlier work a much broader range of initial sizes was observed, and (2) the rearing conditions used in the current work may have minimized the survival advantage of larger larvae over smaller larvae. In this effort, due to very low fertility and hatch rates, we were unable to obtain sufficient larvae from small, late-spawning winter flounder to determine larval survival rates. These eggs and yolk-sac larvae were among the smallest produced in 1988. This effectively resulted in an even smaller range in initial larval size for survival trials.

We have described an approach to obtaining vital statistics (reproductive rate, GSI, fecundity) on individual fish while obtaining viable eggs from the same individuals. These eggs can be used for studies of viability and survival potential. Using this approach we have demonstrated that female size and spawning time of winter flounder have important effects on egg size, fecundity and survival potential. Small, late-spawning females produced small eggs and larvae that appeared to have a reduced survival potential.

LITERATURE CITED

Bagenal, T. L. (1971). The interrelation of the size of fish eggs, the date of spawning and the production cycle. J. Fish Biol. 3: 207–219

Blaxter, J. H. S., Hempel, G. (1963). The influence of egg size on herring larvae (*Clupea harengus* L.). J. Cons. perm. int. Explor. Mer 28: 211–240

Blaxter, J. H. S., Holliday, F. G. T. (1963). The behavior and physiology of herring and other clupeids. Adv. mar. Biol. 1: 261–393

Bowers, A. B. Holliday, F. G. T. (1961). Histological changes in

the gonad associated with the reproductive cycle of the herring (*Clupea harengus* L.). Mar. Res. Scot. No. 5: 16 p.

Buckley, L. J. (1980). Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and the effects of starvation. Fish. Bull. U.S. 77: 703–708

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

Buckley, L. J., Smigielski, A. S., Halavik, T. A., Laurence, G. C. (1990). Effects of water temperature on size and biochemical composition of winter flounder *Pseudopleuronectes americanus* at hatching and feeding initiation. Fish. Bull. U.S. 88: 419–428

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

Cushing, D. H. (1967). The grouping of herring populations. J. mar. biol. Ass. U.K. 47: 193–208

Dunn, R. S., Tyler, A. V. (1969). Aspects of the anatomy of the winter flounder ovary with hypotheses on oocyte maturation time. J. Fish. Res. Bd Can. 26: 1943–1947

Gall, G. A. E. (1974). Influence of size of eggs and age of female on hatchability and growth in rainbow trout. Calif. Fish Game 60: 26–36

Hay, D.E. (1985). Reproductive biology of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 42: 111–126

Hempel, G., Blaxter, J. H. S. (1967). Egg weight in Atlantic herring (*Clupea harengus* L.). J. Cons. perm. int. Explor. Mer 31: 170–195

Kazakov, R. V. (1981). The effect of the size of Atlantic salmon, *Salmo salar* L., eggs on embryos and alevins. J. Fish Biol. 353–360

Kennedy, V. S., Steele, D. H. (1971). The winter flounder (*Pseudopleuronectes americanus*) in Long Pond, Conception Bay, Newfoundland. Can. J. Fish. Aquat. Sci. 28: 1153–1165

Knutsen, G. M., Tilseth, S. (1985). Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. Trans. Am. Fish. Soc. 114: 507–511

Methot, R. D., Jr. (1983). Seasonal variation in survival of larval northern anchovy, *Engraulis mordax*, estimated from age distribution of juveniles. Fish. Bull. U.S. 81: 741–750

This article was presented by K. Sherman, Narragansett, Rhode Island, USA

Miller, T. J., Crowder, L. B., Rice, J. A., Marschall, E. A. (1988). Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. 45: 1657–1670

Polder, J. J. W. (1961). Cyclic changes in testes and ovary related to maturity stages in the North Sea herring (*Clupea harengus* L.). Archs néerl. Zool. 14: 45–60

Rice, J. A., Crowder, L. B., Holey, M. E. (1987). Exploration of mechanisms regulating larval survival in Lake Michigan bloater: a recruitment analysis based on characteristics of individual larvae. Trans. Am. Fish. Soc. 116: 703–718

Rogers, B. A., Westin, D. T. (1981). Laboratory studies on effects of temperature and delayed initial feeding on development of striped bass larvae. Trans. Am. Fish. Soc. 110: 100–110

Saila, S. B. (1963). The contribution of estuaries to the offshore winter flounder fishery in Rhode Island. Proc. Gulf Carrib. Fish. Inst. 14: 95–109

SAS Institute Inc. (1985). SAS/STAT guide for personal computers, Version 6 edn. SAS Institute, Inc., Cary, North Carolina

Simpson, A. C. (1959). The spawning of plaice in the North Sea. Fishery Invest., Lond. (Series 2) 22: 1–111

Tanasichuk, R. W., Ware, D. M. (1987). Influence of interannual variations in water temperature on fecundity and egg size in Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 44: 1485–1495

Topp, R. W. (1968). An estimate of the fecundity of winter flounder, *Pseudopleuronectes americanus*. J. Fish. Res. Bd Can. 25: 1299–1302

Tyler, A.V., Dunn, R. S. (1976). Ration, growth, and measures of somatic and organ condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypotheses regarding population homeostasis. J. Fish. Res. Bd Can. 33: 63–75

Ware, D. M. (1985). Life history characteristics, reproductive value, and resilience of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 42 (Suppl. 1): 127–137

Ware, D. M., Tanasichuk, R. W. (1989). Biological basis for maturation and spawning waves in Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 46: 1776–1784

Zastrow, C. E., Houde, E. D., Saunders, E. S. (1988). Quality of striped bass (*Morone saxatili*) eggs in relation to river source and female size. ICES 1988 ELH/No. 60

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