AN INVESTIGATION OF BATCH-SPAWNING REPRODUCTION IN CAPTIVE YELLOWTAIL FLOUNDER, Pleuronectes ferrugineus.

ANTHONY J. MANNING

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AN INVESTIGATION OF BATCH-SPAWNING REPRODUCTION IN CAPTIVE YELLOWTAIL FLOUNDER, *Pleuronectes ferrugineus*.

BY

ANTHONY J. MANNING, B.Sc.

A thesis submitted to the School of Graduate Studies
in partial fulfilment of the requirements
for the degree of Master of Science

Department of Biology & Ocean Sciences Centre
Memorial University of Newfoundland

Sept 1996

St. John's Newfoundland

ABSTRACT.

The batch-spawning reproduction of female yellowtail flounder, *Pleuronectes ferrugineus*, was examined in a captive setting over the spawning periods of 1994 and 1995. Individual female yellowtail were examined daily with the objectives of determining the inter-ovulatory period of this species, as well as, the annual egg production, mean batch fecundity and number of batches per female. Egg quality measures (fertilization and hatching rates) were also made on batches from females to quantify maternal differences and inter-batch variation within females in egg survival rates.

A one-day inter-ovulatory period was predominant in this species and the frequency of this period increased in 1995 due to reductions in irregular ovulatory activity detected in certain females in 1994. The mean duration of spawning for individual females ranged between 31 to 48 days, over which time egg diameter and dry weight were observed to decrease within individual females.

Female yellowtail flounder demonstrated considerable individual variation in seasonal egg production. In 1994, the mean female production was 550 000 eggs (7.9×10^5 eggs $\cdot$ kg^{-1} spawning female), in 1995 this increased to 1 187 000 eggs (1.5×10^6 eggs $\cdot$ kg^{-1} spawning female). The number of ovulations per female was high, a mean batch number of 14 was seen in 1994, rising to 22 in 1995. Small batch sizes (< 100 000 eggs) dominated batch fecundity distributions, and the data suggested that females increase the number of ovulations rather than increase batch fecundity when egg production increases between years.

Egg quality in yellowtail flounder was found to be subject to maternal differences. In addition , females demonstrated significant inter-batch variation in fertilization and hatching rates. An inter-annual increase in egg quality was observed, where mean fertilization rates rose from 38 % to 60 %. This increase was not due to slower over-ripening rates since spawning temperatures were not significantly different between years. The egg quality data also did not demonstrate any period within an individual female's spawning season in which the batches with the highest quality eggs were produced. A relationship was seen between gamete potential rates, determined by egg morphological characteristics, and fertilization rates. However, since individuals had their own separate relationships, gamete potential rates can only serve as a rough estimate of egg quality, where fertilization rate were generally 25 % lower than gamete potential rates. Hatching rates were, overall, higher than fertilization rates and showed no relationship with fertilization rates.

Investigations on over-ripening indicated that a daily examination protocol is required to avoid reductions in fertilization rate which were greatest during the first 24 hours after collection. Hatching rates declined less dramatically than fertilization rates with over-ripening.

The inter-annual increase in the reproductive performance for captive yellowtail flounder may have been based upon reductions in stress from an additional year of acclimation to captive conditions or the introduction of a commercial feed in 1995. Either factor may have been responsible for the reduction of females with poor egg quality and the observed increases in egg production for spawning females.

ACKNOWLEDGEMENTS.

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Part 1.

INTRODUCTION

The yellowtail flounder is a batch-spawning flatfish of the family Pleuronectidae. Described as an offshore species inhabiting the continental shelf of the Western North Atlantic, its geographic range extends from Labrador to Chesapeake Bay (Walsh, 1992). This species is eurythermic, tolerating temperatures of -1.4 °C to 16 °C across its geographic range (Colton, 1972; Walsh, 1992). The yellowtail flounder has a protracted spawning season which is characteristic of batch-spawners. The spawning season may begin in mid-March and last into August depending on latitude (Royce *et al.*, 1959; Colton *et al.*, 1979). Information from the yellowtail flounder stocks of the Grand Banks of Newfoundland indicated that spawning can occur in late May with peak activity during the second half of June (Pitt, 1970).

Batch-spawning (serial or multiple spawning) is a reproductive strategy where portions of the late-vitellogenic oocyte population of the ovary are ovulated periodically over a female's spawning season. Histological evidence for batch-spawning in yellowtail flounder has been detected by Howell (1983) and Zamarro (1991) where low percentages of hyaline oocytes were observed in the ovaries of spawning females. Ovulatory activity within a batch-spawner follows a temporal rhythm, which is defined by an inter-ovulatory period. Zamarro (1991) proposed that yellowtail flounder had an inter-ovulatory period of one day. This proposal was partly based on histological evidence which indicated that maturation of oocytes was continuous; as the ovulation of one batch occurs, the oocytes of the next batch in the series have already completed nuclear migration. Howell (1983) revealed that yellowtail flounder also show the group synchronous pattern to oocyte development, where at least two populations of oocytes at different stages of development

can be distinguished in the ovary at any one time (Wallace & Selman, 1981).

Smigielski (1979) injected female yellowtail flounder with pituitary extract preparations which induced maturation and ovulation. A dosage of 2 mg of pituitary extract per kg of body weight was successful only when the gonadosomatic index measurements reached 20 %, where GSI was determined from a subsample of control females. Induced females ovulated one to five times with a periodicity of one or two days. The induced eggs demonstrated fertilization rates ranging from 50 % to 90 % and hatching rates of 40 % to 80 % .

This project is the first to examine the female reproduction of yellowtail flounder without any artificial stimulation of maturation and ovulation. Yellowtail flounder has been commercially important in the Newfoundland fishery and is a potential candidate for aquaculture development. Information on the reproductive biology of this species is therefore required for proper brood stock management in aquaculture. The aim of this two year study was to examine the batch-spawning reproduction of yellowtail flounder in a captive setting.

The thesis is divided into two sections, the first is concerned with egg production. Of particular interest is the frequency of ovulatory events and the pattern of egg production associated with the batch-spawning strategy which can be described by the number and fecundity of a female's batches. The second section concentrates on egg quality in yellowtail flounder. Egg quality as generally defined by Kjørsvik *et al.* (1990) is an " egg's potential to produce viable fry". Egg quality can be characterized by various parameters of a physical, chemical, genetic or biological nature. Useful egg quality

descriptors may include egg size, egg mechanical strength, biochemical composition, egg survival rates or the rate of symmetrical cleavage of the early blastomeres (Kjørsvik *et al.*, 1990).

Studying egg quality is important as it indicates the efficiency by which the diversion of maternal energy resources to the gonad results in the successful production of progeny. The objective of the examination of egg quality in this study was to assess the success with which the eggs of yellowtail flounder are able to complete the fertilization and hatching processes. Often neglected topics in the study of batch-spawners are the maternal differences which exist in egg quality, in addition to the inter-batch variation which may be present within a female. These issues were one of the primary concerns in the investigation of egg quality in yellowtail flounder.

The specific objectives of this study of the batch-spawning reproduction of the yellowtail flounder are listed below.

A: Egg production.

(1) report the timing of reproduction and the environmental conditions coinciding with ovulatory activity in captivity.

(2) determine the inter-ovulatory period for female yellowtail flounder.

(3) estimate the annual egg production and the batch fecundity variation for individual females.

(4) examine the changes in egg diameter and dry weight over a female's spawning season.

B: Egg quality.

(1) determine the overall performance of the group in egg quality parameters.

(2) test the use of egg morphological characteristics as predictors of egg quality.

(3) determine if significant maternal differences in egg quality occur.

(4) determine if individuals show consistency in their egg quality between their batches.

(5) evaluate whether individual females produced their highest quality eggs during a particular time period in their spawning season.

(6) assess the decline in egg quality with over-ripening in the eggs of yellowtail flounder.

Part 2.

METHODOLOGY

Brood stock management.

A group of seventeen females (labelled A-Q) were held in captivity for two years prior to the start of this experiment which was conducted at the Ocean Sciences Centre (OSC) of Memorial University of Newfoundland. The fish were held in an exterior 5000 l tank where they experienced natural light conditions. The tank was aerated and received a water supply which was heated during the winter months to ensure a minimum water temperature of approximately + 1 °C. Temperature data for the tank were recorded daily. From Sept 1993 to mid-May 1994, the experimental fish were fed a shrimp diet three times a week, where over the nine month feeding period females received a mean ration of 1.47 kg $fish^{-1}$. The ration level for the group was based on the report by Collie (1987) which estimated an annual consumption rate of 1.4 -1.6 kg yr^{-1} $fish^{-1}$ for wild yellowtail.

When the first ovulating female was detected in May 1994, the brood stock group was transferred to three interior 400 l tanks. The tanks were aerated and had an approximate water flow rate of 10 l min^{-1}. Light conditions included indirect natural daylight and artificial light based upon the natural photoperiod. During the summer of 1994, chilled and ambient water supplies were mixed and provided a water supply with mean daily tank temperatures which ranged from 4.1 °C to 12.8 °C.

Seven males were kept among the seventeen females to provide possible pheromonal cues for reproductive development and ovulation. Six to eight fish were allocated to each tank, which corresponded to a stocking density of approximately 4.5 to 5.5 kg m^{-2}. Feeding ceased when ovulatory activity began in females to ensure that stripped eggs were not contaminated with faecal material.

The tanks used in 1994 had outflow standpipes which drained water from the surface. Thus, a cylindrical tube, taller than the standpipe and with a perforated bottom, was placed over the standpipe to ensure that spontaneously spawned, floating eggs could be detected (Figure 1). The surface of the water and the bottom of the tank were skimmed daily with a fine meshed net for evidence of natural spawning. The tanks used in 1995 had a bottom drain design, however, since no spontaneously spawned eggs were found in 1994, a bottom drain design was considered to be acceptable.

Following the 1994 spawning season, fifteen surviving females and six surviving males were transferred to an interior 2000 l tank. The brood stock experienced artificial lighting which reflected the natural photoperiod cycle. Feeding of the shrimp diet, three times a week, was resumed in September, until the middle of December when a formulated moist pellet, manufactured by Connors Bros., was introduced with the aim of giving the brood stock a more nutritionally balanced diet. Food consumption by the experimental group between Sept 1994 and the start of the 1995 spawning season in June averaged 1.42 kg fish^{-1}.

When ovulation was detected in 1995, feeding ceased and thirteen of the original females available for study in 1995 were transferred to three 400 l tanks. As in 1994, males were kept with females to provide pheromonal cues. An ambient water supply was available over the spawning season where mean daily temperatures ranged from 2.6 °C to 11.5 °C.

Examination protocols.

Females which had not yet ovulated and had swollen, firm ovaries were checked

once or twice a week. Checking females involved a brief handling at the water surface, if the ovary region felt soft or spongy, the female could be stripped. Stripping of eggs required the removal of females from the water, whereupon the repeated application of gentle pressure along the ovary towards the gonopore produced freely flowing eggs. Egg batches were collected in prechilled 100 ml beakers, labelled and then set on ice. Ovulating females were checked (and/or stripped) daily, usually between 10 am and 11 am, until their ovaries were thin and flat, and eggs had not been obtained for one week. Initially in 1994, nets were used to examine females but their use was discontinued after tail infections appeared in three females. Fish were then captured and handled with gloved hands since nets could not be used.

Milt from two or three males was collected along with the eggs. Males were removed from the water and the area around the urogenital pore was dried of sea water. Milt, which was exuded from each male by gentle pressure above and posterior to the urogenital pore, was immediately aspirated from the pore surface into separate prechilled one cc syringes. Care was taken to avoid contamination of the milt sample with seawater or urine. The milt containing syringes were carefully capped and placed in ice.

After collection of the gametes, egg and milt collections were immediately processed, with egg quality procedures having priority in the process schedule.

Estimation of egg production.

For this study a batch was defined as a 5 ml or greater egg volume which was measured in a cold, clean, graduated cylinder. Triplicate batch fecundity estimates were done by pipetting, from different areas of the batch volume, aliquots of 125 µl (100 µl in

1995). After counting eggs under a dissecting microscope, a mean of the triplicates was calculated and was converted to an egg concentration value (eggs per ml). Batch fecundity was determined by multiplying egg volume by the mean egg concentration . Total egg production was determined from the sum of the batch fecundities.

Egg quality assessment.

Every egg batch in 1994 underwent an assessment of egg quality which was quantified according to the following parameters: gamete potential rate, fertilization rate, and hatching rate. In 1995, although gamete potential rate was again determined for every batch ovulated from a female , the egg quality assessment work was reduced, in order to conserve incubator space, by only determining the fertilization and irregular cleavage rates of a subsample of egg batches produced by any female. The two criteria for determining which 1995 egg batches would undergo fertilization trials were:

1) only batches with a volume ≥ 10 ml were fertilized;

2) providing the volume exceeded 10 ml, every third batch ovulated by a female under went evaluation, otherwise the next batch ≥ 10 ml was used.

Some additional batches underwent fertilization trials which did not meet these criteria, either by chance or because certain extra batches were very large and represented a significant proportion of a female's production. Thirteen extra batches were distributed among six of the 1995 females as follows, where a letter identifies an individual female : A (2), D (2), H (2), I (1), M (2), Q (4).

Gamete Potential Rate - Three samples of 100 unfertilized eggs were visually assessed under a dissecting microscope and the mean percentage of "good quality" eggs was

recorded. Good quality eggs had a transparent cytoplasm, appeared spherical with no damage or dimples, and had no premature perivitelline space. There appears to be agreement on these characteristics of "good quality" eggs among authors of studies of several other species, including, goldfish, *Carassius auratus* (Formacion *et al.*, 1993); turbot, *Scophthalmus maximus* (McEvoy, 1984 ; Fauvel *et al.*, 1992; Omnes *et al.*, 1991); and Japanese flounder, *Pleuronectes yokohamae* (Hirose *et al.*, 1979).

Fertilization Rate- The fertilization technique was modelled after the protocol of Harmin & Crim (1992). Fresh milt collected from 2-3 males was combined only if motility was verified under a compound microscope. Three aliquots of 200-300 eggs (volume of 125 µl) were randomly sampled from each egg batch using an Eppendorf pipette. The aliquots were placed in petri dishes set on ice, and five microlitres of undiluted milt were then added to each aliquot of eggs. Sperm activation was facilitated by stirring in 125 µl of chilled seawater solution followed by an additional two millilitres after two minutes. The seawater solution consisting of 1 µm filtered, UV sterilized seawater with concentrations of 0.1 $g \cdot l^{-1}$ streptomycin and 0.06 $g \cdot l^{-1}$ penicillin. After ten minutes, 20-25 ml of seawater solution was added to the dishes which were then placed in an incubator set at 5 °C. The fertilization rate was determined following incubation of the eggs to the 4, 8 or 16 cell stage (approximately 6 to 8 hours) under an Olympus dissecting microscope at 10- 15 X magnification.

In 1995, the following revisions were made to this protocol: 1- because egg size seemed to be smaller, the egg aliquot volume was reduced from 125 to 100 µl to maintain the 200-300 egg sample size which was established in 1994 with the 125 µl sample

volume. 2- Given that the duration of yellowtail sperm motility is less than two minutes the 10 minute postactivation period was shortened to 5 minutes. This reduction in time was not considered to have an effect on the results of fertilization trials.

Hatching Rate- After the fertilization rate had been determined the eggs were then returned to the incubator until hatching occurred where the incubator temperature was monitored daily. Renewal of the seawater solution in the petri dishes occurred every two days as was done in Chambers & Leggett (1987). The number of larvae that hatched was determined and expressed as a percentage of fertilized eggs.

Larval Production Rate- This term was calculated by multiplying the average fertilization rate and the average hatching rate of a batch (LP %= FR % x HR %/100). This rate indicates the percentage of the eggs in a batch that survived to hatch.

Irregular Cleavage Rate- The percentage of fertilized eggs in the early cell division stages (i.e. 4 to 16) which showed asymmetrical division of cells (or irregular cleavage).

Over-ripening Study.

In 1995, the effects of ageing on egg quality were determined on batches with gamete potential rates exceeding 70 %. Immediately after collection of batches, eggs were sampled for the determination of egg quality parameters including gamete potential, fertilization, irregular cleavage and hatching rates. Afterwards, a 100 ml beaker, covered with perforated Parafilm, was used to hold a one centimetre deep sample of eggs from a batch, which was then stored in an incubator where mean daily temperatures ranged from 6.9 °C to 8.8 °C. The eggs in the beaker underwent sampling for egg quality testing after having been stored in the incubator for 24 hours, 48 hours, 72 hours, etc, until the storage

period had exceeded 96 hours. If the egg fertilization rate fell to < 10 % before the 96 hour limit, sampling was terminated.

Egg diameter and egg dry weight measurements.

A sample of fresh unfertilized eggs were preserved in a solution of 5 % formalin in Borax (sodium tetraborate) buffered sea water (Pépin, pers. comm.). The mean diameter was obtained from 20 eggs using Optimus Image Analysis software with a Hitachi camera at 250X magnification. Additional image analysis software included Frame Grabber , Imaging Technology OFG.

The use of formalin as a fixative and preservative can cause changes in the egg size. Changes in diameters of fresh eggs after preservation was determined from samples of twenty eggs from four different females either freshly measured or measured after 30 days of storage in the formalin solution . Measurements of egg diameters in this investigation were made using an ocular micrometer on a dissecting microscope at 40X magnification.

Egg dry weight was determined for some batches from females. Generally very small batches or batches with very poor gamete potential rates were not used. Small pieces of aluminium foil were placed overnight in a muffle furnace at 450 °C and the average weight obtained from two measurements on a Mettler microbalance to 0.1µg. Eggs were first water-hardened no longer than a day after collection. A sample of eggs from the floating layer of water-hardened eggs was poured into a vacuum filter apparatus under low suction. Separate glass fibre filters were rinsed with distilled water and placed on the apparatus. The seawater in the sample was removed leaving the dry, intact eggs on the

surface of the filter. Next, the eggs were washed of surface salts with a 0.9 % ammonium formate solution (Whyte *et al.*, 1987), followed by distilled water. Three replicates of five eggs represented a batch. After placing five eggs on a weighed foil using small forceps, the foil samples were left overnight in a drying oven at 100°C. Foil egg samples were stored in a desicooler until they returned to room temperature and were ready for weighing. Again, each foil-egg sample was weighed twice on the microbalance and the mean egg dry weight was calculated from the weight data for the three replicate foils.

Statistical Analysis.

Statistical analyses were performed using the Statistical Analyses System (SAS). Residuals from all analyses were tested for normality and transformations of the data were done when deviations from normality were indicated. Linear relationships underwent regression analysis, if normality problems were seen only a logarithmic transformation or squaring the data was attempted.

Statistical analyses were undertaken using the fertilization rate and hatching rate data in order to examine maternal differences in egg quality (maternal effects) and the degree of inter-batch variation which occurred within females (batch effects). Maternal effects were determined by nested anovas, where batches within females served as the nested variable in the model. Nested anovas were done with the General Linear Models (GLM) procedure which is robust to the effects of unbalanced data sets. Unplanned paired comparison tests including the Duncan's Multiple Range Test, the Tukey's Studentized Range Test (HSD), the Bonferroni T-test and the Least Square Means test were done in conjunction with nested anovas to determine which females could be responsible for

significant maternal effects. Batch effects within females were analysed by one-way anovas as were comparisons examining inter-annual effects. For all anova situations, randomization tests were also performed as a non-parametric alternative, where each test was represented by 1000 randomizations of the data.

In 1995, duplicate analyses were done for the egg quality data. The first analysis examined the data set which strictly adhered to the sampling criteria for batches to be tested for egg quality. The second analysis tested data sets which included the extra batches collected outside the criteria. If differences between the duplicate analyses occurred, the results of both analyses were reported.

Since the selection of batches for egg quality testing was modified in 1995, differences between sampling of batches between years had to be removed prior to testing for inter-annual effects in egg quality. Therefore, data sets for 1994 females were reconstructed, where a sample of the data was selected using the criteria used in 1995. Balanced data sets for inter-annual comparisons were ensured by randomly selecting additional batches of ≥ 10 ml from the 1994 data set when needed.

Figure 1. Photograph of one of three tanks containing 1994 spawners showing the standpipe guard which was placed over the elevated standpipe to prevent the loss of any spontaneously spawned eggs. If spontaneous spawning had occurred floating eggs could be collected with a fine-meshed net hours after the spawning event.

Part 3.

BATCH-SPAWNING AND EGG PRODUCTION

3. 1. RESULTS.

Spawning record.

Prior to each spawning season, the spawning condition of the females was assessed by examining the degree of swelling of the gonadal region of each individual (Table 1). In 1994, three females (N, P, Q) were non-reproductive, a state characterized by thin, flat ovaries . The 1994 group spawning season began on May 13 and ended Aug 29, a duration of 109 days. Eleven of the fourteen potential spawners ovulated, seven of which initiated ovulation within two weeks of the summer solstice. The remaining three potential spawners included females J and K, which died due to tail infections before ovulating, and female G, which remained swollen the entire season but did not ovulate.

In 1995, thirteen females of the original group remained in the experiment. As in 1994, three non-reproductive females were observed in the group (E, L, P). The remaining ten females all ovulated in 1995, seven of these females were repeat spawners from 1994. The three non-repeat spawners included female G and two of the non-reproductive females from 1994 (N & Q). The first ovulation was detected on June 14, and the last occurred on Aug 30 (n=78 days). Most 1995 spawners initiated ovulation in mid-July (July 10-23), which was later than in 1994. In addition, five of the seven repeat spawners initiated ovulation ten to eighteen days later in 1995 than in 1994.

Table 1. Female spawning records for the 1994 and 1995 spawning seasons. Spawning condition prior to the season is indicated for each female, as well as the dates of the first and last ovulations for spawning females (Y - capable of reproduction; NR - non-reproductive; N/A - not available ; M - mortality; shaded areas indicate an absence of spawning data).

♀	Spawning condition 1994	First batch 1994	Last batch 1994	Spawning condition 1995	First batch 1995	Last batch 1995
A	Y	July 5	Aug 5	Y	July 11	Aug 9
B	Y	July 15	Aug 16	N/A		
C	Y	June 22	Aug 29	Y	July 10	Aug 30
D	Y	July 1	Aug 8	Y	July 15	Aug 16
E	Y	May 13	May 25	NR	M	
F	Y	July 6	July 31	Y	July 2	Aug 1
G	Y			Y	July 23	Aug 24
H	Y	July 3	Aug 11	Y	July 15	Aug 14
I	Y	June 20	Aug 10	Y	June 23	July 17
J	Y	M				
K	Y	M				
L	Y	May 25	Aug 28	NR		
M	Y	July 3	Aug 16	Y	July 17	Aug 19
N	NR			Y	July 30	Aug 19
O	Y	May 20	Aug 6	N/A		
P	NR			NR	M	
Q	NR			Y	June 14	June 28

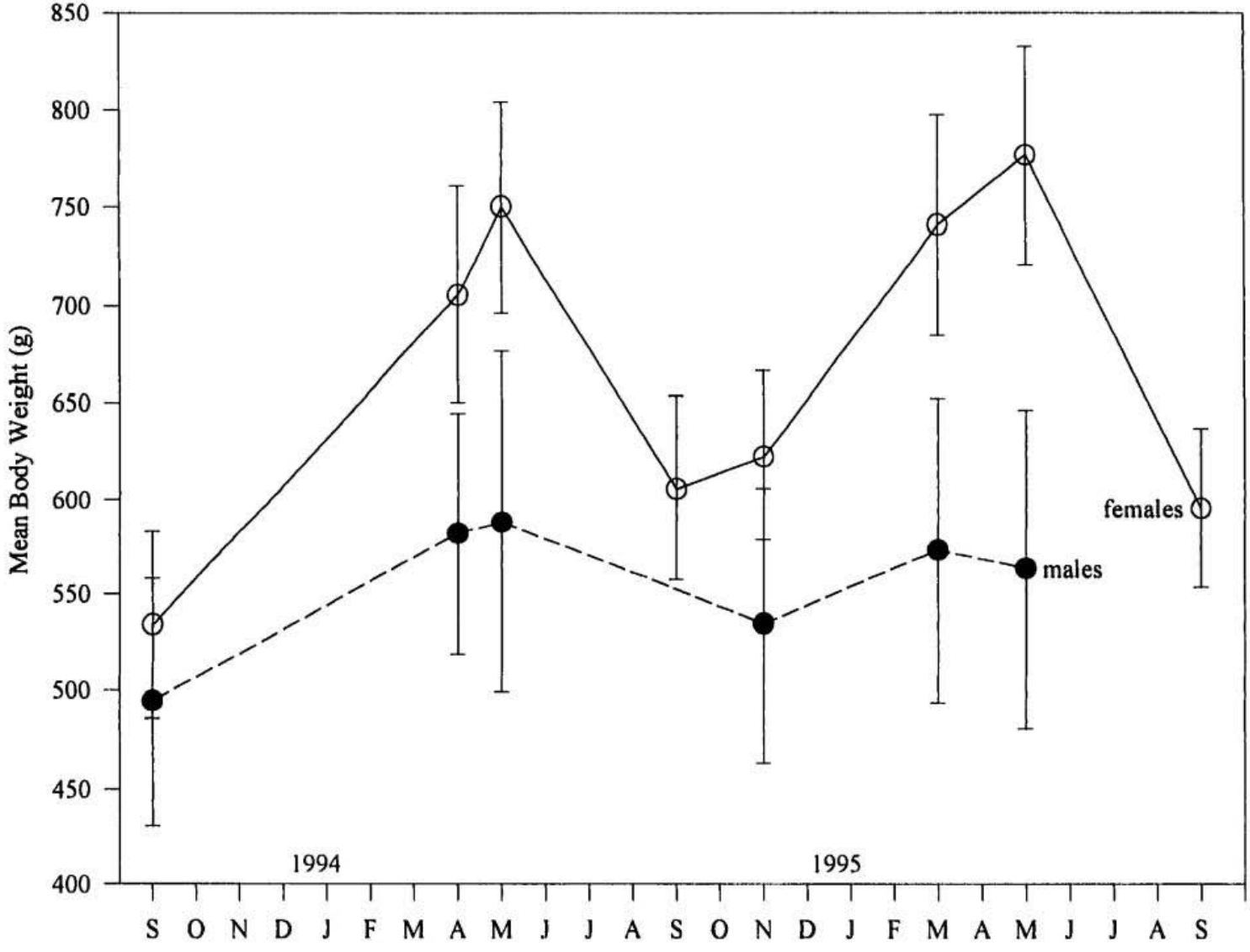
Growth.

Increases in mean body weight for females occurred over the winter months and peaked near spawning (Figure 1). Large decreases in weight were noted for females over the spawning season. Males also showed notable decreases in average body weight over the 1994 spawning season. The average female weight at postspawning in Sept 1994 exceeded that of Sept 1993. However, the average postspawning weight of the same females in Sept 1995 was relatively unchanged from that of Sept 1994 (Figure 1).

Examination of the length data for individual females revealed that some female did not grow, while others grew at rates of 0.5 to 3.5 cm per year. In both years mean growth rates remained at approximately one cm per year. In 1995, length increases were not always accompanied by increases in body weight.

Regarding non-reproductive females, in the first season these fish demonstrated increases in body weight. However, in 1995 non-reproductive females demonstrated decreases in weight.

Figure 1. Mean body weight (g) changes for the six males and ten females which survived from Sept 1993 to Sept 1995. Error bars represent standard errors.


Mean Body Weight (g)
850
800
750
700
650
600
550
500
450
400
females
males
1994
1995
S O N D J F M A M J J A S O N D J F M A M J J A S

Daily Egg Production and Temperature.

Peak egg production occurred during mid July to early August in both 1994 and 1995 (Figures 2 & 3). Prior to the detection of ovulation in either season, temperature fluctuations rising to 6 °C were observed and may have had a positive effect on the initiation of ovulation in the brood stock. However, temperatures decreasing below 4 °C, in 1994, or 3 °C, in 1995, had no negative effect on ovulation in early spawners (Figures 2 & 3).

The mean temperatures over each season were similar, in the first year a mean of 8.10 ± 2.65 °C was recorded, while the second season had a mean of 8.14 ± 2.36 °C. No significant inter-annual difference was detected by statistical analysis in temperature variation between 1994 and 1995 (Figure 4; P=0.8863).

Figure 2. Daily egg yield and temperature records for the 1994 spawning season. The bar plot shows day to day changes in pooled egg volumes among the spawning females. Linear plots are mean daily temperatures for each of the three holding tanks the 1994 spawning season.

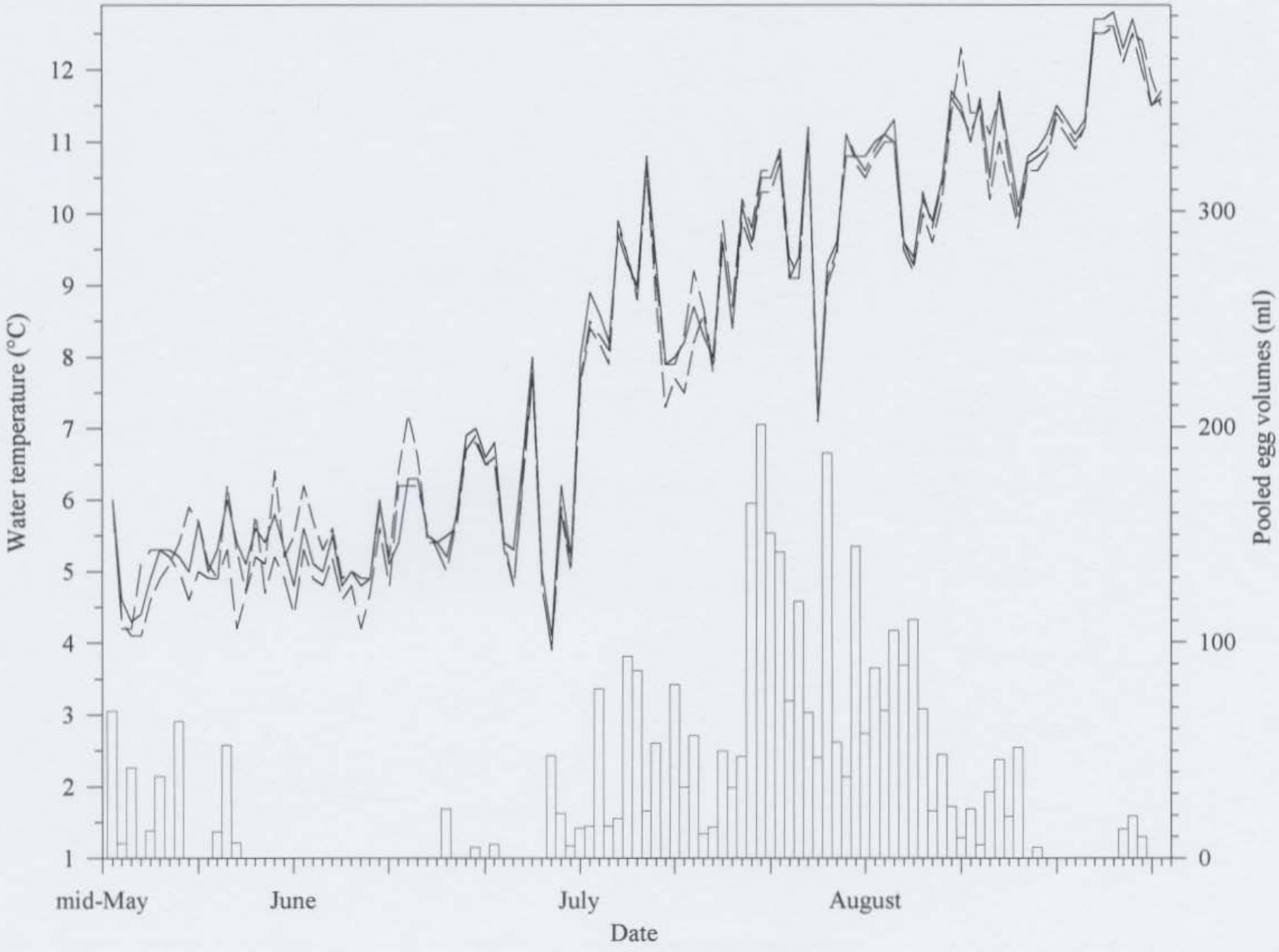

Water temperature (°C)
Pooled egg volumes (ml)
Date
mid-May
June
July
August
1
2
3
4
5
6
7
8
9
10
11
12
0
100
200
300

Figure 3. Daily egg yield and temperature records for the 1995 spawning season. The bar plot shows day to day changes in pooled egg volumes among the spawning females. Linear plots are mean daily temperatures for each of the holding tanks over the 1995 spawning season.

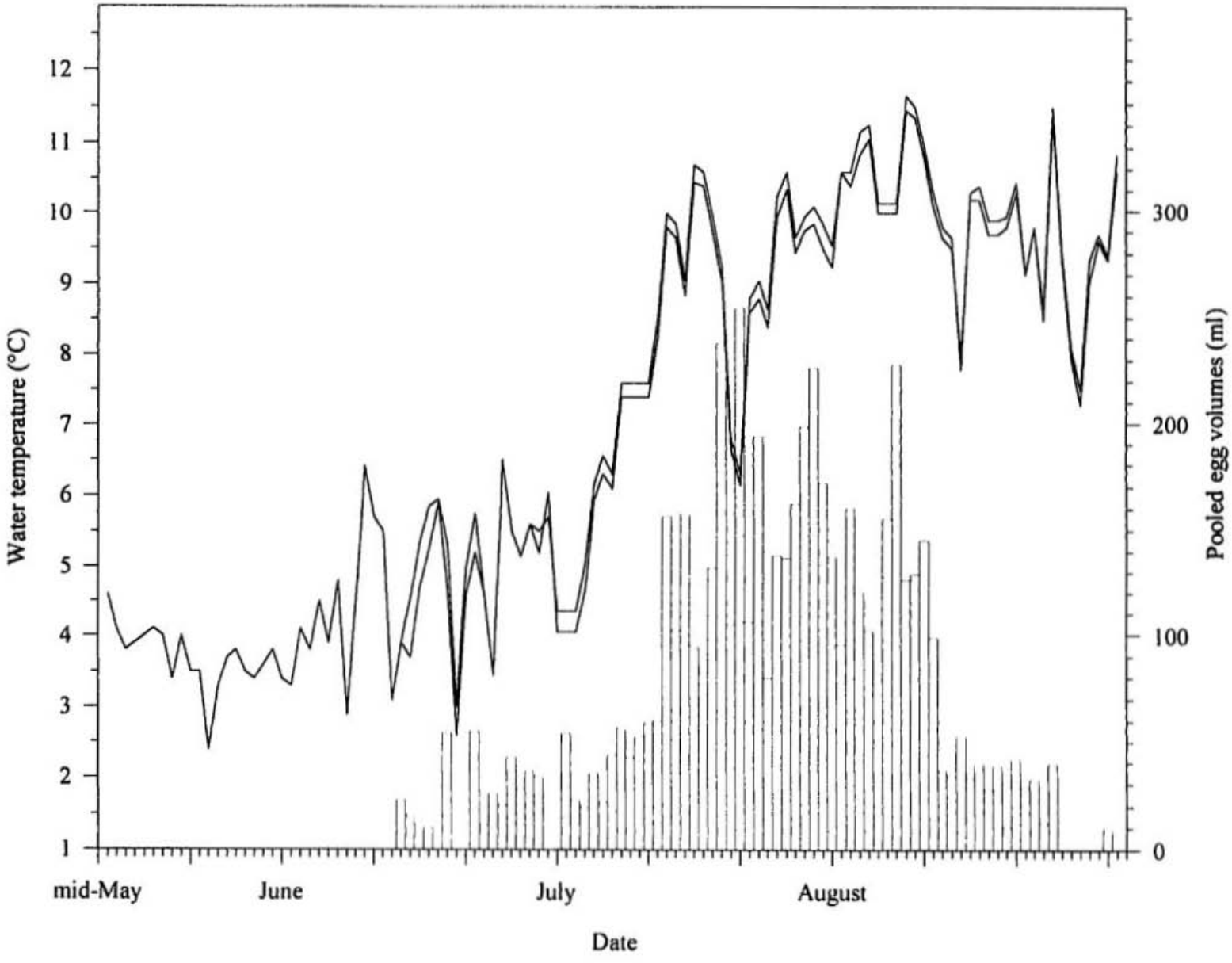
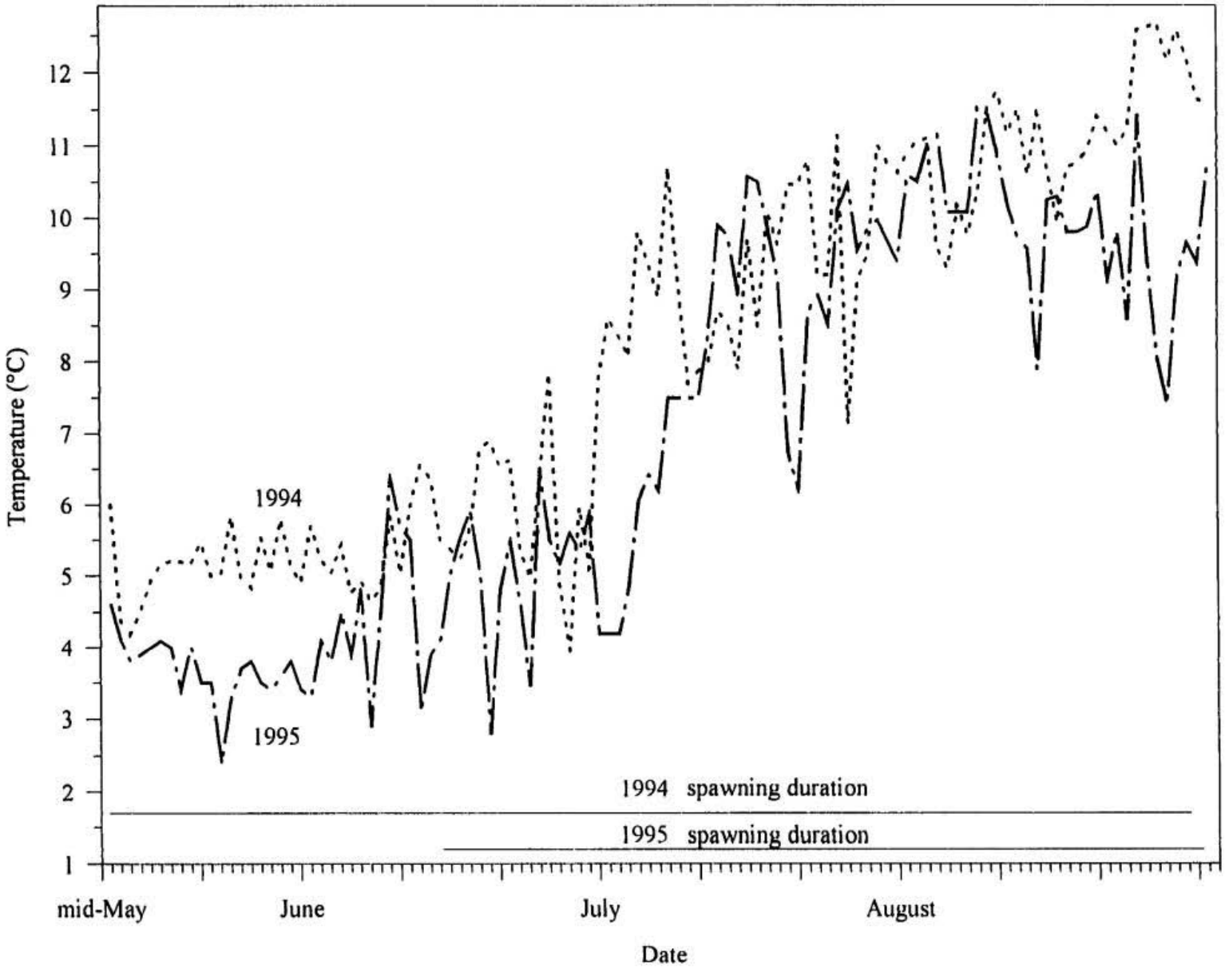

Water temperature (°C)
12
11
10
9
8
7
6
5
4
3
2
1
Pooled egg volumes (ml)
300
200
100
0
mid-May
June
July
August
Date

Figure 4. Comparison of the seasonal temperature profiles for the 1994 and 1995 spawning periods. Daily mean temperatures are plotted for 1994 (dotted line) and 1995 (dashed line) from mid-May to the end of August.


Temperature (°C)
12
11
10
9
8
7
6
5
4
3
2
1
1994
1995
1994 spawning duration
1995 spawning duration
mid-May
June
July
August
Date

Inter-ovulatory period.

In 1994, a one day inter-ovulatory period was the most frequent, accounting for 43 % of the pooled cases (n=146) (Figure 5). A two day interval followed, accounting for another 22 % of the cases. All other intervals had relative frequencies below 10 %. Evidence of irregular ovulatory activity was observed by large inter-ovulatory periods (maximum 39 days). Females demonstrating irregular ovulatory activity had high mean inter-ovulatory periods with large standard deviations (Table 2). Of the 1994 females, subjects L and O were responsible for the largest intervals observed in the group (14 to 39 days ; Figure 5). Six of the eleven females had a one day interval as their most frequent period (Table 2).

In 1995, the frequency of a one-day inter-ovulatory period increased to 74.5 % while the two day interval decreased to 17.5 % (Figure 5). Irregular ovulatory activity was greatly reduced among the females in the second season. For nine of the ten females, a one day interval was the most frequent (Table 2). Female N was the only female to demonstrate irregular ovulatory activity with intervals of 6 or 7 days. All repeat spawners showed decreases in mean inter-ovulatory periods in 1995 (Table 2).

Figure 5. Frequency histograms representing the inter-ovulatory period data observed from the brood stock during the 1994 (n=146) and 1995 (n=209) spawning seasons.

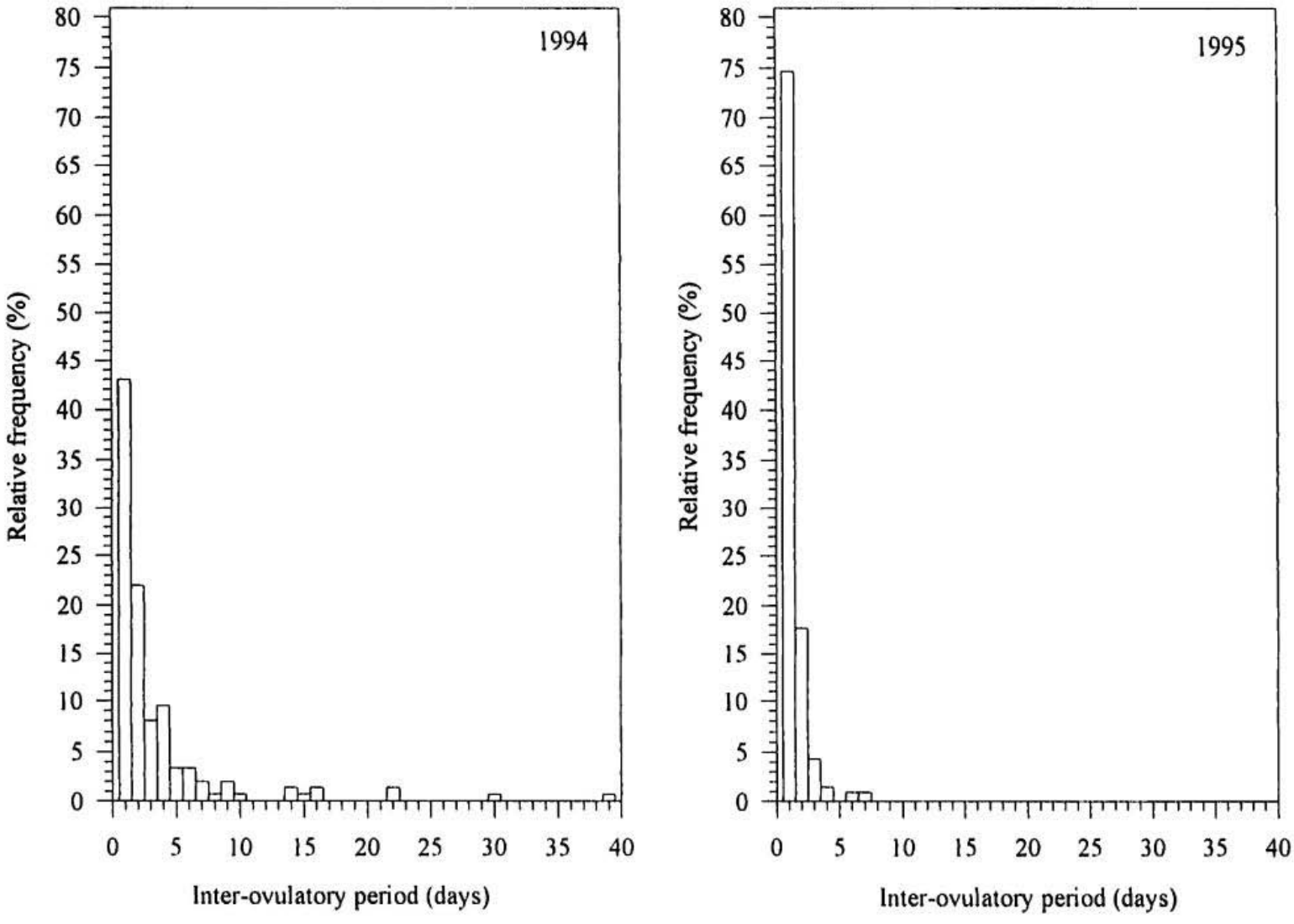

1994
1995
Relative frequency (%)
Inter-ovulatory period (days)
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80

Table 2: Mean inter-ovulatory periods and most frequent intervals for individual females for both seasons (shaded areas indicate an absence of spawning data).

	1994 Spawning season			1995 Spawning season		
♀	mean	s.d.	Most frequent interval	mean	s.d.	Most frequent interval
A	1.7	±1.0	1	1.2	±0.5	1
B	4.6	±2.2	5 & 6			
C	3.6	±3.5	1	1.8	±1.1	1
D	5.4	±7.4	4	1.3	±1.1	1
E	1.7	±1.1	1			
F	2.1	±2.5	1	1.2	±0.4	1
G				1.2	±0.4	1
H	2.2	±1.7	1	1.6	±1.0	1
I	2.0	±1.7	2	1.1	±0.5	1
L	7.9	±7.3	2			
M	2.8	±2.1	1	1.2	±0.5	1
N				5.0	±2.5	7
O	19.5	±17.7	All different			
Q				1.6	±0.7	1

Egg Production.

The range in total length for the spawning females was 33 to 44.5 cm in 1994. The average spawning female measured 37 cm and weighed 694 g prior to spawning (Table 3). In 1994, the group produced an estimated 6 047 316 eggs, the average contribution per female was 549 756 eggs (7.9×10^5 eggs $\cdot$ kg^{-1} spawning female). This mean production was portioned out into 14 batches. Individual spawning seasons lasted an average of 48 days, the influence of females with irregular ovulatory activity increasing the mean. Females lost an average of 22 % of their prespawning body weight over the spawning season.

Table 3. Mean values for prespawning length and weight, number of batches ovulated, spawning duration, egg production and weight loss for spawning females in 1994.

Season 1994 (n= 11♀)	Prespawning Length (cm)	Prespawning Weight (g)	Batches Ovulated	Spawning Duration (days)	Egg Production	% of weight lost
Mean	37	694	14	48	549 756	22.0
s.d.	± 4.1	± 178	± 7	± 25	± 353 864	± 3.7

Table 4. Mean values for prespawning length and weight, number of batches ovulated, spawning duration, egg production and weight loss for spawning females in 1995.

Season 1995 (n= 10♀)	Prespawning Length (cm)	Prespawning Weight (g)	Batches Ovulated	Spawning duration (days)	Egg Production	% of weight lost
Mean	39.9	777	22	31	1 186 881	23.2
s.d.	± 3.3	± 175	± 8	± 10	± 596 461	± 4.9

In 1995, the spawning females were larger, ranging from 34 to 45 cm, with a mean total length of 39.9 cm and mean pre-spawning weight of 777 g (Table 4). There was an increased representation of medium to large sized females in 1995. Females G, N and Q replaced females B, O and E which were small females in 1994. The group egg production increased nearly two fold in 1995 to an estimated 11 868 811 eggs, the average individual contributing 1 186 881 eggs (1.5×10^6 eggs $\cdot$ kg^{-1} spawning female). The variation among the different females in egg production still remained high. Females, on average, ovulated 22 batches in 31 days . The average female lost 23.2 % of her prespawning body weight.

Female A, a medium sized female of 37 cm, had the highest egg production of 1.3 million eggs (Table 5). Notably, the production values of females 37 cm or less, (C, H, I, and A), frequently were greater than the production of larger females. Furthermore, the production of female F exceeded that of female E by 3 787 eggs, yet female F was 11.5 cm larger than E. Females B, D and O had the lowest egg production estimates. These females interrupted ovulatory activity after five or eight batches and resorbed the remaining advanced stage oocytes in their ovaries. The number of batches ovulated by those females which completed their spawning seasons ranged from 8 to 27 (Table 5). The highest number of batches was observed from female I of 33.5 cm. All large females produced less than 20 batches.

The spawning duration for females was highly variable in 1994 (Table 5). The first ovulating female (E) completed her season in 13 days, the shortest duration for 1994. Extremely long spawning durations of 79 and 96 days were seen for females O and L, respectively. Both of these females had demonstrated irregular ovulatory activity in their

inter-ovulatory period data.

Table 5. Prespawning length and weight, number of batches ovulated, spawning duration, and egg production information for individual females in 1994.

♀ 1994	Prespawning Length (cm)	Prespawning Weight (g)	Batches Ovulated	Spawning Duration (days)	Egg Production
A	37	739	19	32	1 324 762
B	34	528	8	33	200 454
C	34.5	579	20	69	769 990
D	40.5	882	8	39	206 592
E	33	444	8	13	489 792
F	44.5	920	13	26	493 579
H	36.5	736	19	40	750 921
I	33.5	468	27	52	723 217
L	41.5	864	13	96	345 135
M	42	880	17	45	671 868
O	34	595	5	79	71 011

In 1995, the egg production of both females M and G exceeded two million eggs, female M had the highest of the group at 2 051 304 eggs (Table 6). As in 1994, the production estimates of small females, notably C and I, were greater than females larger than themselves. The smallest number of eggs ovulated by an individual in 1995 was from female N, which ovulated five times before resorbing her advanced stage oocytes.

In contrast with 1994, six of the ten females had egg production estimates greater than one million eggs (Table 6). When comparing the results of the seven repeat spawners between seasons, females A and H were seen to produce fewer eggs than in 1994. The

five other repeat spawners exhibited large increases in production. Female F, the largest female in both years, while demonstrating an increase in egg production, continued to be exceeded in production by smaller females in 1995.

The number of ovulations per individual was generally higher for 1995 females (Table 6). Batch number ranged from 10 to 30 for the nine females which completed their spawning seasons. Eight of the females had 20 or more batches. Female C had the most batches despite being the second smallest female of the group. This female also had the longest spawning duration of 52 days. Extremely long seasons were not present in the 1995 group. The female with the shortest spawning duration of 15 days was female Q, the first to ovulate in 1995. Six females had spawning durations between 30 and 34 days.

Table 6. Prespawning length and weight, number of batches ovulated, spawning duration, and egg production information for individual females in 1995.

♀ 1995	Prespawning Length (cm)	Prespawning Weight (g)	Batches Ovulated	Duration of Spawning (days)	Egg Production
A	39	821	25	30	1 088 972
C	36	545	30	52	1 622 822
D	42	920	25	33	918 936
F	45	988	26	31	1 367 185
G	43	971	28	33	2 001 472
H	38	712	20	31	738 216
I	34	500	22	25	1 255 915
M	42	925	28	34	2 051 304
N	40.5	719	5	21	211 736
Q	39	666	10	15	612 250

Portioning of production.

The mean batch volume for the 1994 group was 20.9 ± 8.3 ml, which would have an average fecundity of 36 807 ± 16 353 eggs. In 1995, the mean female batch was 24.7 ± 4.9 ml which contained 52 783 ± 13 469 eggs. The batch fecundity means of females ranged from 14 000 to 70 000 in 1994 and from 36 500 to 71 500 in 1995 (Table 7).

Batch fecundity is primarily determined by batch volume changes, however, egg concentration (eggs/ml) was not constant from batch to batch within females. Egg concentration was seen to change erratically over the spawning seasons of individuals. Factors affecting egg concentration within a female include the amount of ovarian fluid and the changes in egg size which occur over a female's spawning season. Six of the repeat spawners had increases in mean egg concentration in 1995. Female C had the largest increase in mean egg concentration, from 2090 eggs/ml in 1994 to 2899 eggs/ml in 1995.

Data collected on batch fecundity indicated that generally there was little change between years in batch fecundity (Figure 6). The top plots show that batches of 10 000 to 20 000 eggs were the most frequent in both years, although a decrease occurred in the relative frequency of this class in 1995 (Figure 6). The second most frequent batch size was 20 000 to 30 000 eggs in 1994, however, this changed to 40 000 to 50 000 eggs in 1995. Examining the data for repeat spawners (the bottom plots Figure 6) shows that, again, the 10 000 to 20 000 egg class was the most prominent in either season, decreasing in 1995. However, the second most frequent batch size was the 40 000 to 50 000 egg

class in both years. The introduction of large batch sizes in 1995 was the most significant inter-annual difference in batch fecundity distributions. Batches exceeding 200 000 eggs appeared in 1995, where none were seen in 1994 (Figure 6). The total relative frequency of batches of this size was small, less than 3 %.

Since most repeat spawners increased their egg production in 1995, the batch fecundity data for repeat spawners was examined to determine if inter-annual differences occurred in these females, and whether increases in batch size accompanied increases in egg production when they occurred. The results of statistical analyses comparing the batch fecundity data for repeat spawners shows that five of the seven repeat spawners did not have a significant inter-annual difference in batch fecundity (C, D, F, H, M; Table 8). Female A had a significant decrease in batch size in 1995, although the decrease in total egg production in 1995 was not large. Female I had a significant increase in batch fecundity which accompanied the increase in her production. Notably, only female I had fewer batches in 1995 than in 1994. All other repeat spawners had an increase in the number of batches between years.

Regarding the changes which occur in batch fecundity within a female's ovulation sequence, an irregular pattern was observed to be typical of female yellowtail in both seasons. Examining the batch fecundity records of females C and I as examples, the irregular pattern is clear, where batch size can increase or decrease quickly between batches (Figure 7). In addition, no reduction in the irregular pattern occurred between years within these repeat spawners.

Table 7. Batch number and mean batch fecundity information for individual females in 1994 and 1995.

♀	1994 Batch Number	1994 Mean Batch Fecundity (eggs)	1995 Batch Number	1995 Mean Batch Fecundity (eggs)
A	19	69 724	25	43 559
B	8	25 056		
C	20	38 500	30	54 094
D	8	25 824	25	36 757
E	8	61 224		
F	13	37 968	26	52 584
G			28	71 481
H	19	39 522	20	36 911
I	27	26 785	22	57 087
L	13	26 549		
M	17	39 522	28	73 261
N			5	42 347
O	5	14 200		
Q			10	61 225

Figure 6. Frequency histograms of batch fecundity data observed among the brood stock during the 1994 and 1995 spawning seasons. The top two plots compare the data from all spawners in each year. The bottom two plots compare the data from repeat spawners only.

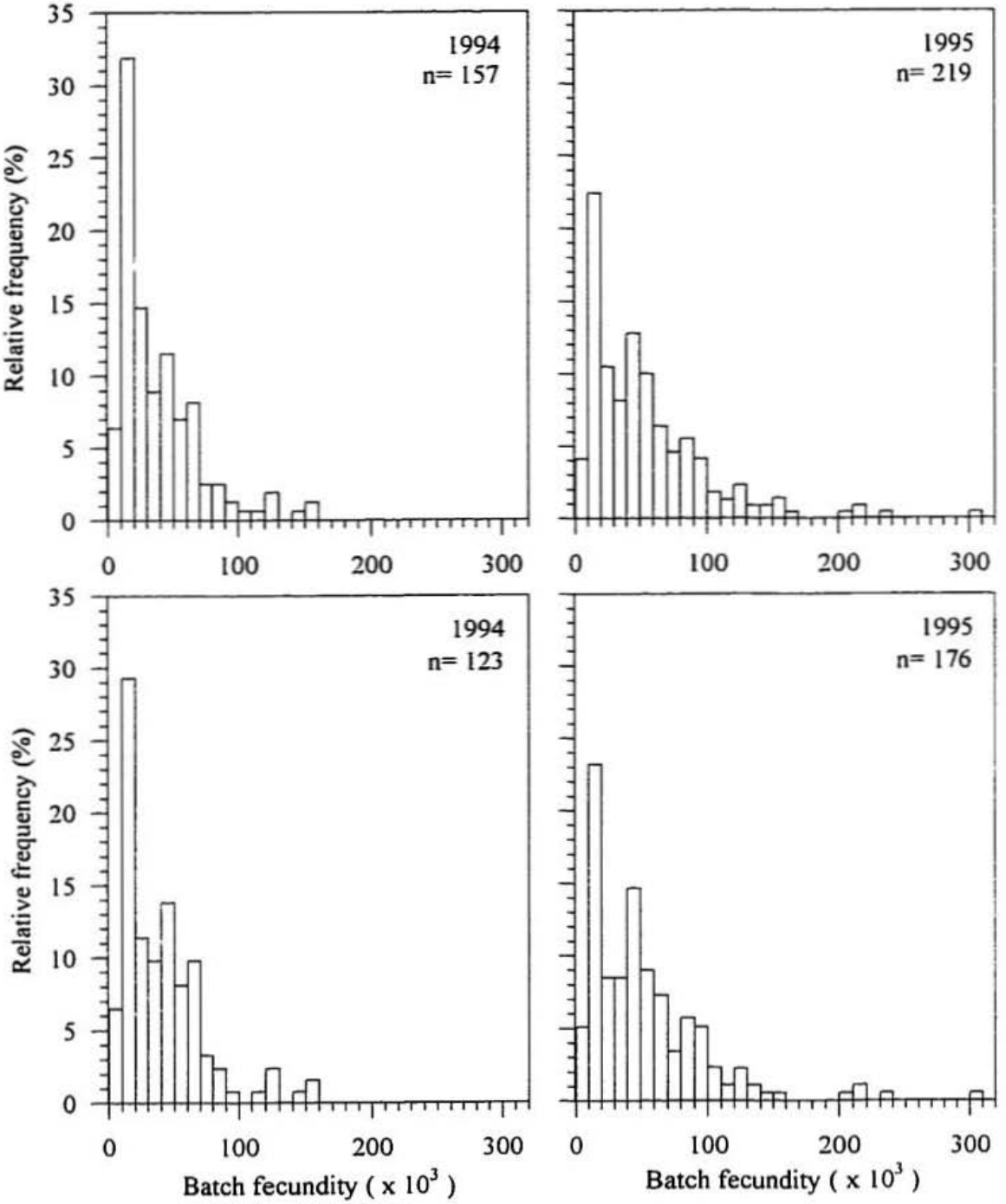
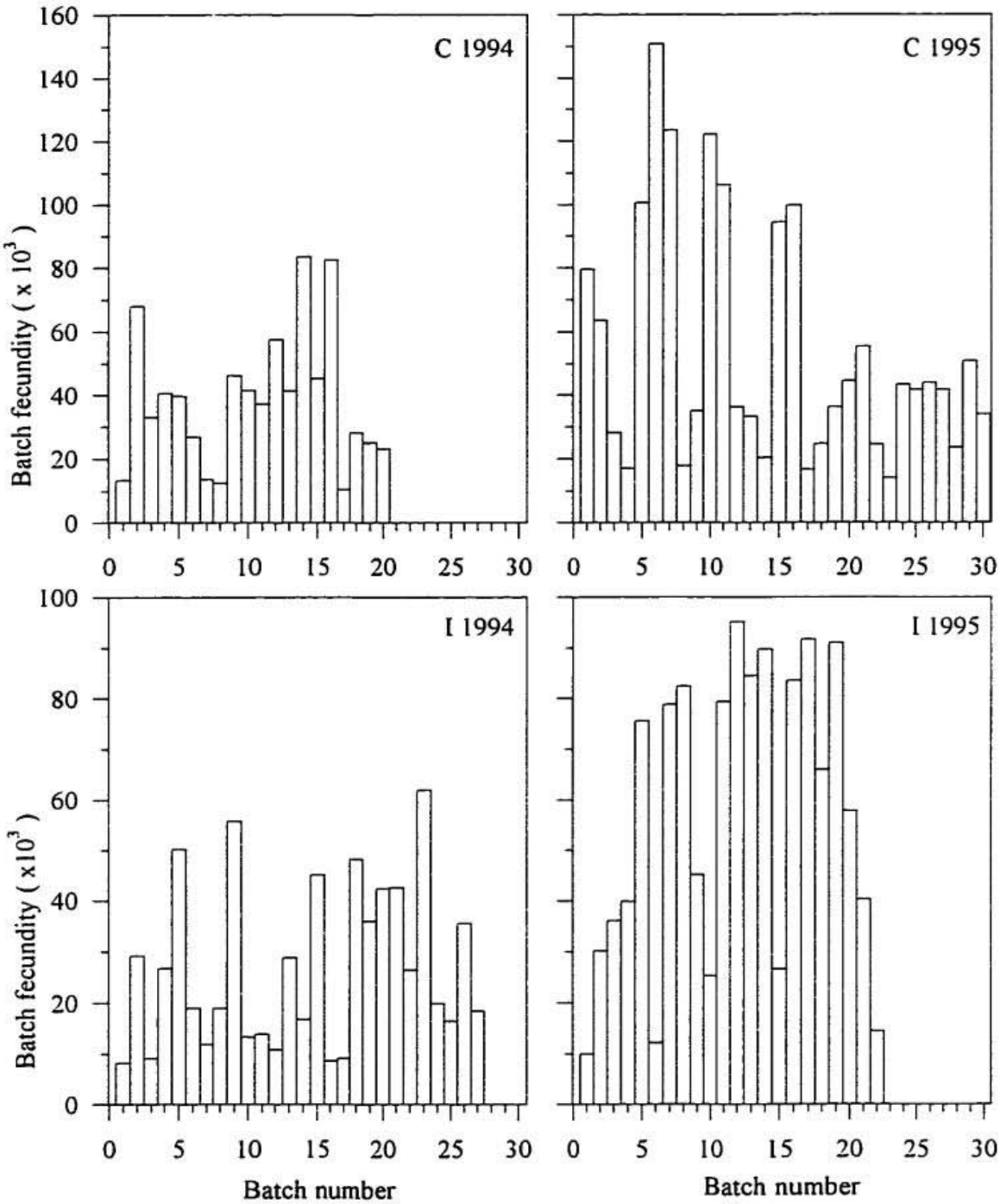

1994
n= 157
1995
n= 219
1994
n= 123
1995
n= 176
Relative frequency (%)
Relative frequency (%)
0
5
10
15
20
25
30
35
0
100
200
300
Batch fecundity (x 10^3)
Batch fecundity (x 10^3)

Table 8 : Anova and randomization test results examining for an inter-annual effect on the batch fecundity data of repeat spawners.

Repeat Spawner	n=	F-value	P<	Randomization P=	Significant Seasonal increase / decrease in batch fecundity
A	44	4.47	0.041	0.045	Yes, decrease
C	50	2.83	0.099	0.078	No
D	33	1.97	0.171	0.178	n.s. prob increase
F	39	1.86	0.181	0.188	No,
H	39	0.11	0.741	0.742	No
I	49	21.03	0.0001	0.001	Yes, increase
M	45	2.95	0.093	0.091	No

Figure 7. Inter-annual comparison of batch fecundity distributions within two repeat spawners. The top plots show the batch fecundity records of female C for 1994 and 1995. The bottom plots show the batch fecundity records of female I for 1994 and 1995.


C 1994
C 1995
I 1994
I 1995
Batch fecundity (x 10³)
Batch fecundity (x10³)
Batch number
Batch number
0
5
10
15
20
25
30
0
20
40
60
80
100
120
140
160

Egg diameter and egg dry weight.

Egg size among the 1994 captive females ranged from 0.983 mm in diameter to 0.829 mm. The mean fresh egg was 0.901 ± 0.033 mm in diameter. Egg dry weight ranged from 34.6 µg of dry matter to 21.9 µg , the mean egg weighing 26.9 ± 2.9 µg. Both egg diameter and egg dry weight decreased as successive batches were ovulated by a given female (Table 9). Data were not obtained for female E and few data were available for females L and O. Decreases in egg dry weight over the spawning seasons of females which completed spawning ranged from 3.2 µg to 10.8 µg. The largest drop was seen in female A which also had the highest egg production of the 1994 females. Resorbing females B and D had changes in egg dry weight of less than 1 µg.

The effect of the formalin preservative on fresh egg diameter was determined. The results from the four different batches indicated that fresh eggs when preserved, experienced a mean decrease in diameter of 1.4 % (± 0.4 % s.e.). The range in the mean was from 0.53 % to 2.29 %. The effect of the formalin preservative on diameters of fresh eggs was small and was not considered to have had a deleterious effect on the results.

Regression analyses revealed a significant negative relationship between the pooled female egg diameter data and day in the spawning season of the group (P=0.0001, r^2 = 0.584; Figure 8). When regressions were done on the data for individual females, seven significant negative relationships were found among the nine females which could be tested ($0.0001 < P < 0.05$). The percentage of variation explained by the regressions for the individual females ranged from 39 % to 95 %. Five of the females had more than 80 % of the variance explained by the relationship. Females D and F did not have significant

relationships.

The next set of regressions analysed the relationship between egg dry weight and corresponding egg diameter. A significant positive relationship was found for the pooled data set ($P = 0.0001$, $r^2 = 0.582$; Figure 9). Examining the data for individuals separately, only four of the six females had significant relationships ($0.0001 < P < 0.02$). The percentage of explained variation ranged between 53 % and 86 %. Females F and H did not have significant relationships.

A significant negative relationship was found between the egg dry weight data of females and the corresponding day in the spawning seasons of individuals ($P = 0.0001$, $r^2 = 0.309$; Figure 10). The pooled data was regressed against corresponding day in the spawning season of the group, however, non-normality of the residuals could not be corrected despite a significant regression. Separate analyses of the data for individuals revealed that all six females tested had significant negative relationships between egg dry weight and time. The amount of explained variance by an individual's regression ranged from 33 % to 90 %.

The total matter involved in reproduction was estimated by using the egg dry weight data in conjunction with the production data (Table 10). Missing data within a female were filled by using the previous regression relationships or by averaging the data. For females E and O, where no data was available, the mean egg weight calculated from all the females was used to estimate their total ovulated egg dry weight. Females which underwent resorption had spawned less than six grams of matter, while females which completed their spawning seasons ovulated between 10.0 g to 37.2 g of total dry matter.

Table 9. Decreases in egg size and dry weight over the spawning seasons of individuals.

1994 ♀	Early Egg Diameter (mm)	Early Egg Dry Weight (µg)	Late Egg Diameter (mm)	Late Egg Dry Weight (µg)
A	0.963	34.6	0.883	23.9
B	0.914	26.6	0.878	25.8
C	0.905	26.9	0.838	21.9
D	0.921	27.8	0.898	27.5
F	0.898	28.7	0.876	25.5
H	0.965	32.0	0.891	28.0
I	0.949	29.2	0.864	23.2
L	0.983	n/a	0.868	24.9
M	0.888	26.6	0.829	22.3
O	0.967 (n=1)			

Table 10. Total estimated dry weight ovulated by individual females in 1994.

Fish	Total Dry Weight (g)
A	37.2
B	5.0
C	18.4
D	5.9
E	13.1
F	13.2
H	22.6
I	19.1
L	10.0
M	16.3
O	1.9

Figure 8. Relationship between egg diameter data and corresponding day in the spawning season of the group in 1994. The regression line for the relationship is shown (n= 109).

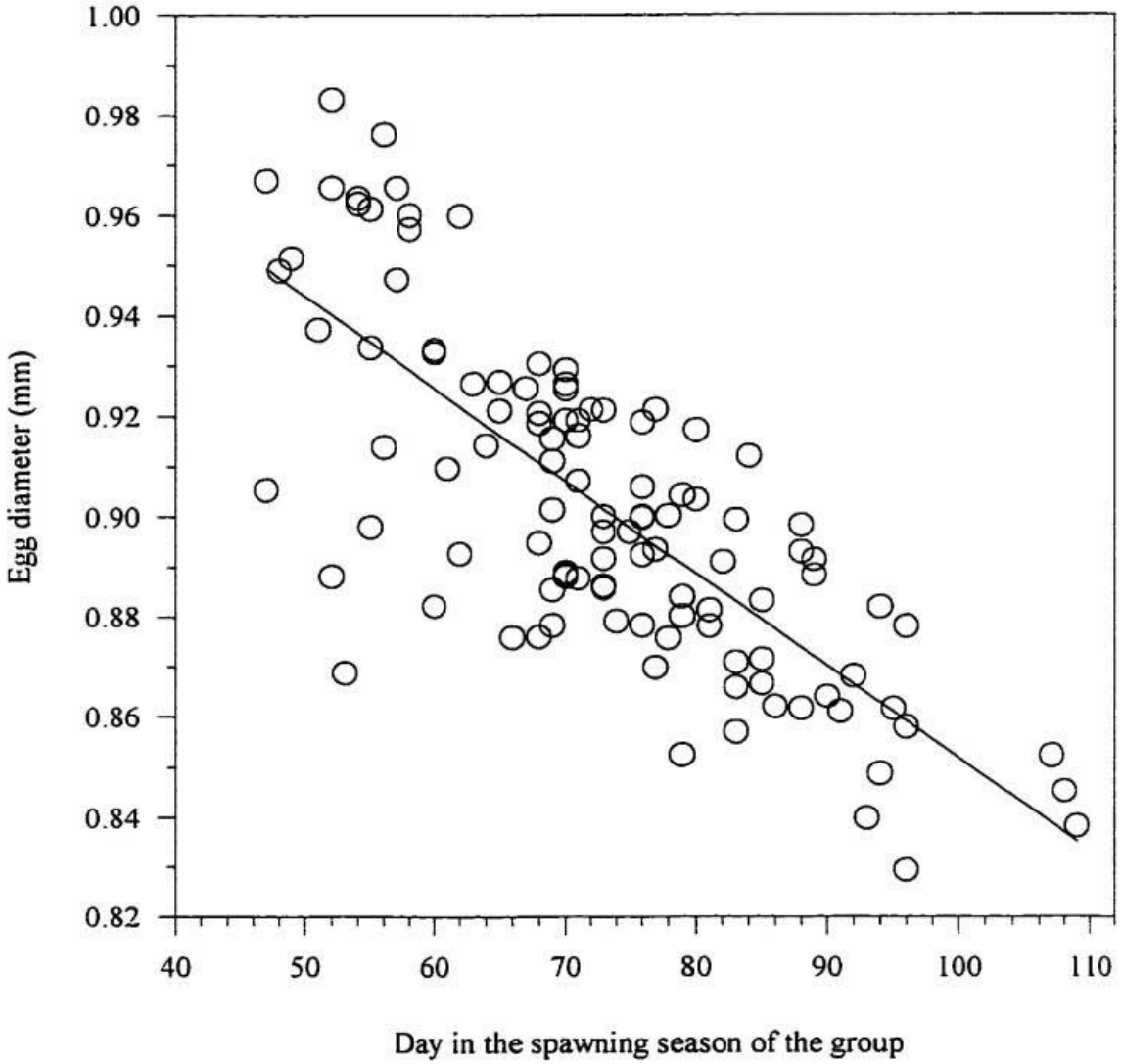

1.00
0.98
0.96
0.94
0.92
0.90
0.88
0.86
0.84
0.82
Egg diameter (mm)
40
50
60
70
80
90
100
110
Day in the spawning season of the group

Figure 9. Relationship between egg dry weight and corresponding egg diameter during the 1994 spawning season. The regression line for the relationship is shown (n= 72).

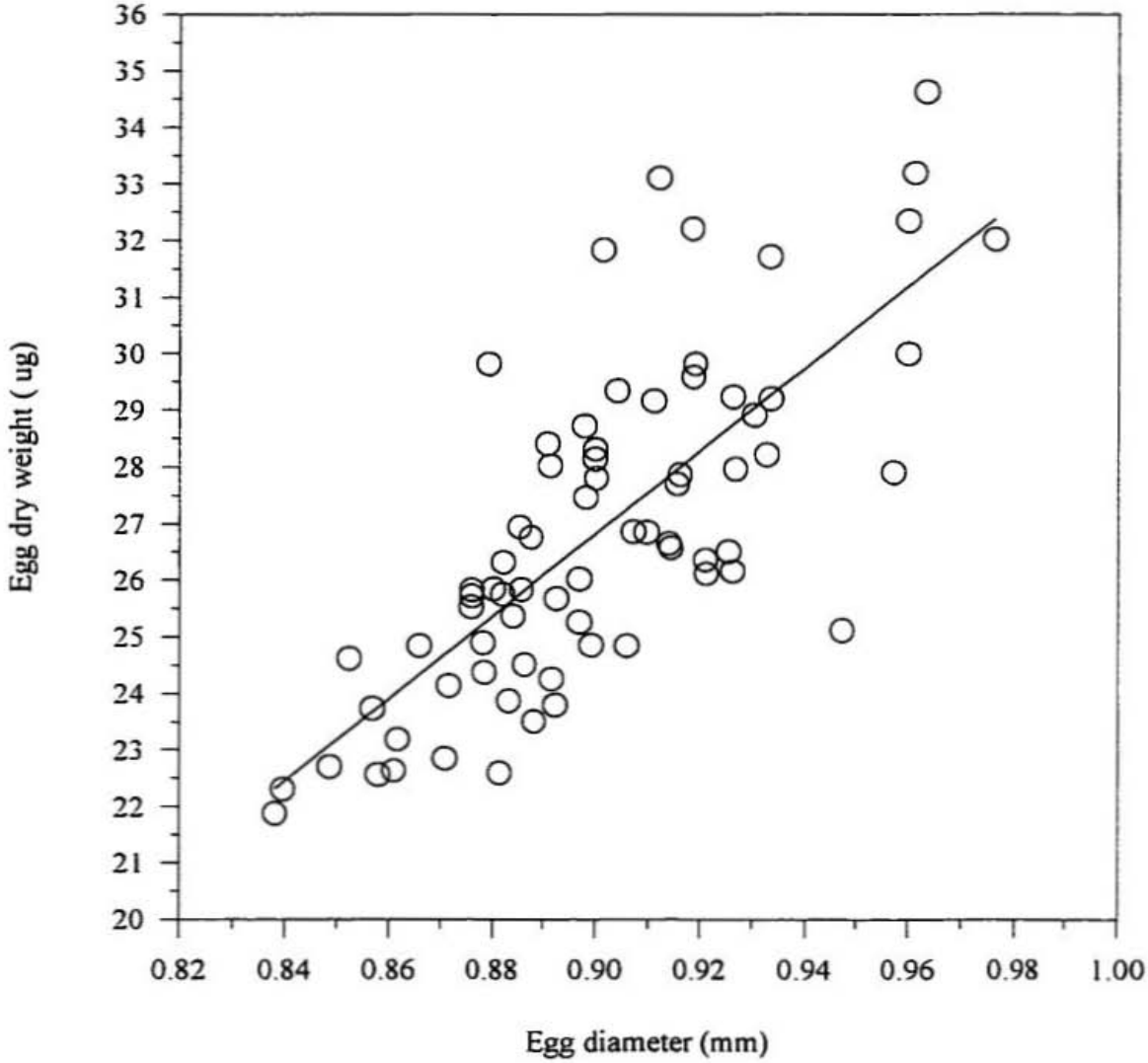
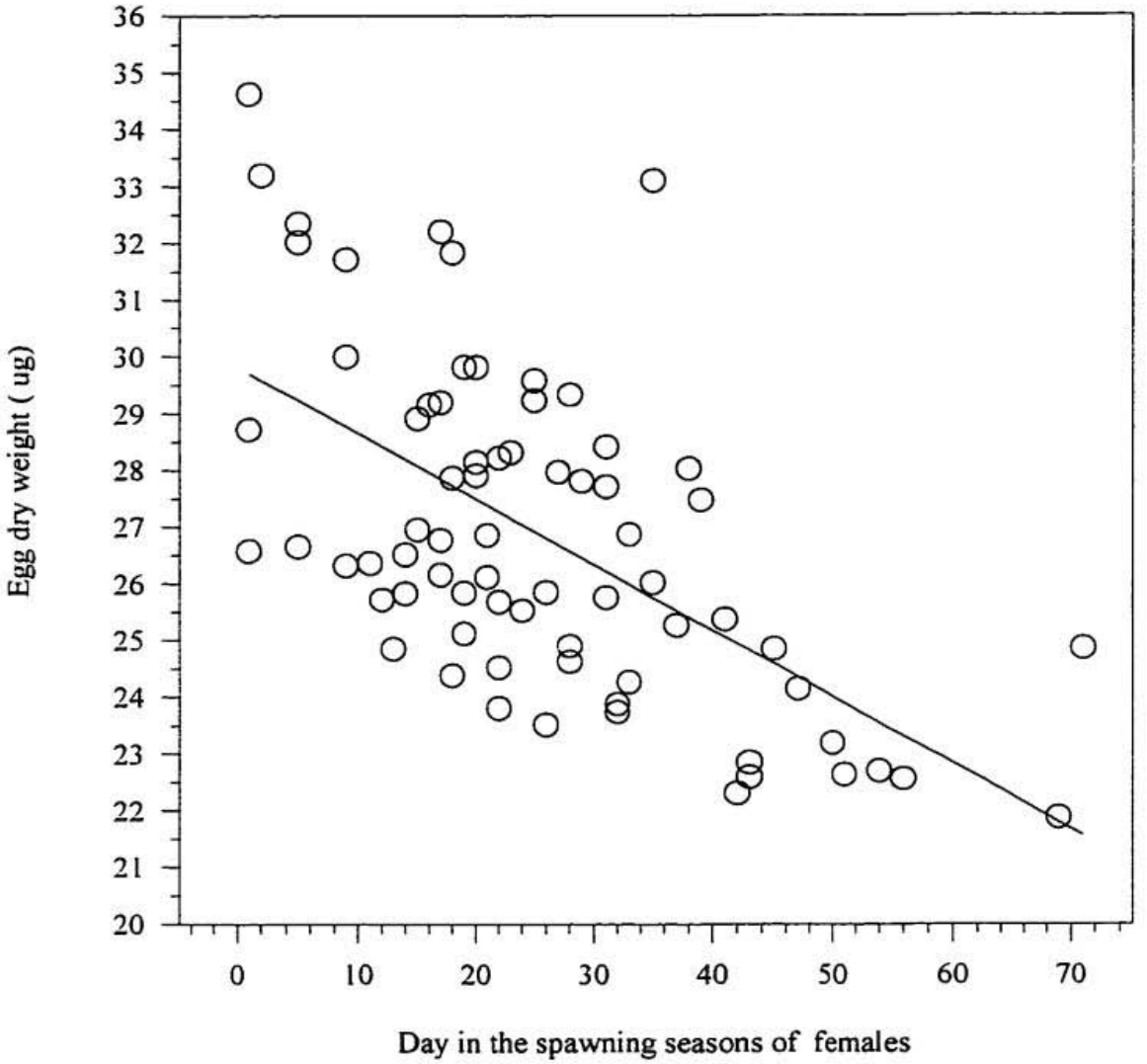

Egg dry weight (ug)
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
0.82
0.84
0.86
0.88
0.90
0.92
0.94
0.96
0.98
1.00
Egg diameter (mm)

Figure 10. Relationship between egg dry weight and corresponding day in the spawning seasons of females in 1994. The regression line for the relationship is shown (n= 72).


Egg dry weight (ug)
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
0
10
20
30
40
50
60
70
Day in the spawning seasons of females

3.2. DISCUSSION.

Photoperiod and temperature are two important environmental cues for the timing of reproduction in fish (Lam, 1983). Since most yellowtail females in the current study initiated spawning near the summer solstice, a long photoperiod appeared to stimulate reproduction in this group. While Pitt (1970) indicated that peak spawning of yellowtail on the Grand Banks occurs in the second half of June, the peak period of egg production for the captive group in my study was in mid-July to early August. This delay may have been due to the effects of captivity.

Regarding temperature, a temporary increase to six degrees may stimulate ovulation in captivity, but is not necessary to maintain the process. Evidence for wild spawners on the Grand Banks showed that spawning temperatures remained between one and five degrees (Pitt, 1970), although a maximum of 8.6 °C has been observed (Walsh, 1992). A possible temperature cue of six degrees may only be associated with captive situations. Increases in temperature may overcome stress associated with captivity which may delay the initiation of ovulation. Future research should examine the role of photoperiod and temperature cues in captivity.

The occurrence of non-reproductive females noted in this study is a problem for the management of captive brood stock. Turbot studies have recorded that 40 % to 50 % of their females were non-reproductive (Howell & Scott, 1989; Omnes *et al.*, 1991). Non-reproductive female winter flounder, *Pleuronectes americanus*, have been encountered in the wild, where the non-reproductive state was observed to be dependent on somatic condition, rather than age factors or senescence (Burton & Idler, 1984).

This state was induced in captive winter flounder by limiting food and reversed by increasing food availability the following season (Burton, 1991). A similar link to nutrition was seen in turbot, where 43 % of females on a low ration (0.5 % wet body weight· day^{-1}) were non-reproductive (Horwood *et al.*, 1989).

The cessation of feeding over the spawning season may have been responsible for the following observations on growth. First, non-reproductive females in 1994 grew over the preceding year, while those of 1995 lost weight. Secondly, there was no increase in the average female's post-spawning weight between 1995 and 1994, compared with 1994 and 1993. The fact that a non-feeding policy was employed for a second consecutive season in combination with high rates of egg production in 1995 may explain why, often, females had post-spawning weights lower than the previous year. Other studies adopting a non-feeding policy included Kjesbu (1989) on spawning cod, *Gadus morhua*, and Smigielski (1979) on the hormonal induction of spawning in yellowtail flounder. According to the ecological literature, wild yellowtail flounder feed during the spawning season, however, spawning fish show reduced consumption rates compared with other times of the year. On the basis of percentage body weight the amount of prey consumed by wild spawning females was 0.06 % compared with post-spawning or recrudescing females (Langton, 1983). Reduced appetites were noted among the prespawning captive females in this study. It appears, however, that feeding during the spawning season may be required to maintain somatic condition, maximize growth, and minimize the number of non-reproductive females.

A one day inter-ovulatory period was the predominant interval seen in the captive group, which supports the proposal of Zamarro (1991) for a daily spawning strategy for yellowtail. In addition, since early spawners have a one day inter-ovulatory period as their most frequent interval, this indicates that high temperature conditions seen later in both spawning seasons were not responsible for the prevalence of the one day interval. Whether a female spawns daily in the wild is uncertain since behavioural factors are involved. Nevertheless, the finding of a one day inter-ovulatory period indicates that females in the wild have the potential of spawning daily.

Other studies of batch-spawning flatfish reported inter-ovulatory periods of two to four days, three days and 3.5 days for the turbot, *Scophthalmus maximus*, Atlantic halibut, *Hippoglossus hippoglossus*, and the barfin flounder, *Verasper moseri*, respectively (McEvoy, 1984; Howell & Scott, 1989; Norberg *et al.*, 1991; Methven *et al.*, 1992; Koya *et al.*, 1994). Scott (pers. comm.) revealed that female plaice, *Pleuronectes platessa*, had a three day interval. The plaice is comparable in size to the yellowtail flounder, unlike the other listed species which range in size from 2 kg to 60 kg. Examination of the data of Fauvel *et al.* (1992) on turbot indicated that a one day inter-ovulatory period was as frequent as three or four day intervals. In contrast, daily ovulation cycles were seldom observed in plaice (Scott, pers. comm.).

Inter-individual and intra-individual variability in inter-ovulatory period was greater in 1994 than in 1995 for the captive group of yellowtail flounder. Inter-individual variation has also been noted in the turbot studies of McEvoy (1984) and Howell & Scott (1989). Regarding intra-individual variation, McEvoy suggested that

female turbot were predictable in their ovulatory rhythms, although oscillation between short and long periods were seen in some females. Conversely, a lack of consistency within a female's ovulatory periods was observed in the turbot and the plaice (Howell & Scott, 1989; Fauvel *et al.*, 1992 ; Scott, pers. comm.).

Irregular ovulatory activity, seen in some 1994 yellowtail females, may be indicative of major disruptions in reproduction. Why certain females displayed extreme irregular ovulatory activity is unclear, but may relate to individual variability in stress tolerance or somatic condition. Stress factors may have caused irregular ovulatory activity since the effect of long term stress has been shown to inhibit ovulation in the batch-spawning red sea bream, *Pagrus auratus* (Carragher & Pankhurst, 1991). Female O, one of the two individuals displaying the largest inter-ovulatory periods in 1994, had a tail infection which may have affected her ovulation patterns, as well as, caused the resorption of her vitellogenic oocytes. Unfortunately, the 1994 females displaying the highest irregularities were not repeat spawners in the 1995 group and no inter-annual comparison could be made.

Inter-annual variability in ovulatory periods was clearly shown by the reduction in irregular ovulatory activity and the increase in the frequency of one day intervals among the 1995 females. As a result, reductions in spawning durations of individuals occurred, even in cases where annual egg production had greatly increased. This led to a reduction in the spawning duration for an individual, even in cases where total production had greatly increased. Since temperature conditions were not significantly different between years these inter-annual differences were not based on changes in environmental

temperature conditions. However, an additional year of acclimation to captive conditions may have been responsible for the inter-annual differences via a reduction in stress levels.

Two studies relating potential fecundity with female length for yellowtail flounder offer an opportunity to compare the performance of captive groups with estimates from the wild. Howell & Kesler (1977) reported a relationship for New England stocks, while Pitt (1971) examined the stocks of the Grand Banks of Newfoundland. The regressions found by these two studies are listed below :

New England : Howell & Kesler (1977) : $F= 0.986\ L\ ^{3.858}$ ($r^2 = 0.784$, $P<0.001$)

Grand Banks of NFLD: Pitt (1971) : $F= 0.0355\ L^{4.69}$ ($r^2 =0.558$, $P< 0.01$)

The 1994 and 1995 egg production data for females in my study was compared with the corresponding potential fecundity estimates of both papers (Tables 11 & 12). Pitt (1971) may offer a better comparison since the captive females used in this project were also collected from the Grand Banks. It is evident from the tables that Howell & Kesler's estimates consistently exceed those of Pitt's. Since females from the New England area mature at a younger age than those of the Grand Banks, due to higher growth rates, they demonstrate higher fecundities than Grand Banks females of the same size (Royce *et al.*,1959; Howell & Kesler, 1977).

The comparison indicates that only half of the captive females were capable of matching or exceeding the egg production estimated for females of the same size in the wild. The females unable to realize their potential fecundity estimates included not only resorbing females but females which had completed their spawning seasons. In 1994, five females realized their potential production estimated by Pitt (1971). These were all small

to medium sized females of 37 cm in length or less. Only female A exceeded both estimates. In 1995, the five females exceeding Pitt's estimates included large females as well as the smallest females. Four females, across the size range, had egg production values greater than both potential fecundity estimates. The ability of smaller females to realize and exceed the potential fecundity estimates of even large females showed that the size- fecundity relationship seen in wild populations was not present among the captive females of this study. Hence, other factors outside of size or age variables were involved in determining egg production of yellowtail flounder in captivity.

These factors include dietary ration, nutritional composition, spawning history and stress. Regarding dietary ration, although group ration was controlled it can not be assumed that individuals received an equal proportion of the ration. Individual variability in feeding rates or aggressiveness play a role in determining the ration a female receives. Consequently, only a proportion of the females may have been able to consume enough food to realize their potential fecundities. According to Horwood *et al.* (1989), female plaice, *Pleuronectes platessa*, fed a higher ration (2-2.3 % wet body wt day^{-1}) had 59 % more vitellogenic oocytes than those on a low ration (0.5 % wet body wt day^{-1} increased later to 1.8 %). The difficulty of larger females in reaching their potential fecundities, compared with smaller females, may have been due to individual ration requirements which were influenced by size. However, any effect relating to size may be interrelated with spawning history. Kjesbu & Holm (1994) revealed that the fecundity of repeat spawning cod, which is also a batch-spawner, was more sensitive to feeding conditions during vitellogenesis than first time recruit spawners. Repeat spawners undergo a severe

depletion of somatic energy during spawning, compared with recruit spawners which have more than one year of feeding to contribute to the fecundity of their first season (Kjesbu & Holm, 1994).

Increases in egg production in 1995 may have been aided by a change in nutritional composition with the introduction of a commercial feed between seasons. The commercial feed may have had a greater energy content than the shrimp diet previously used. It may also have had a greater concentration of limiting nutrients for egg production. The literature suggests that certain dietary nutrients limit egg production in fish. Phosphorous deficient diets negatively affected egg production in the red sea bream, *Pagrus auratus*, (Watanabe *et al.*, 1984) and in the ayu, *Plecoglossus altivelis* (Watanabe, 1985). Diets deficient in α-tocopherol, a major form of vitamin E, produced females lacking vitellogenic oocytes in carp *Cyprinus carpio* (Watanabe, 1985).

Increases in egg production may also be explained by reduced stress levels due to an additional year of acclimation to captive conditions. Stress has deleterious effects on reproduction which are mediated by cortisol, a corticosteroid hormone. *In vivo* and *in vitro* studies have indicated that cortisol causes a depression of reproductive hormones, such as, 17ß- estradiol and testosterone. This depression leads to decreased vitellogenin levels, reductions in oocyte growth and low gonadosomatic index values, as seen in female tilapia, *Oreochromis mossambicus*, brown trout, *Salmo trutta*, and rainbow trout, *Oncorhynchus mykiss* (Carragher *et al.*, 1989; Carragher & Sumpter, 1990; Pottinger & Pickering, 1990; Foo & Lam, 1993).

Resorption in females is another problem regarding reproductive performance and has been encountered in captive turbot, *Scophthalmus maximus*, and sole, *Solea solea* (Bromley *et al.*, 1986; Howell & Scott, 1989). The impact of atretic processes on the potential fecundity of cod, *Gadus morhua*, was examined in Kjesbu *et al.* (1991). Erosion of potential fecundity during the spawning season was linked to maternal nutritional status. Females spawned between 20 % to 80 % of their potential fecundity depending on their somatic condition. Atresia was not observed in prespawning ovaries except for fish in very poor condition, but increased in spawning fish as the season progressed. The energy made available by atresia may fuel reproduction when somatic condition is poor (Kjesbu *et al.*, 1991).

Whether female yellowtail which resorbed were in poorer condition than females which completed their spawning season could not be determined. Weight and length data did not indicate any distinction for these females. A connection may exist between irregular ovulatory activity and resorption. All females which resorbed displayed irregular ovulatory activity, yet not all females with irregular intervals exhibited resorption. Irregular ovulatory activity extends the spawning duration of an individual which may be deleterious for somatic condition, particularly in the current experiment where yellowtail were not fed during spawning. Resorption may be more likely under such circumstances if a nutritional link with resorption exists. Regarding the decrease between years in resorbing females, this may have been due to improvements in nutritional composition or reductions in stress levels over the period of recrudescence.

Zamarro (1991) estimated that a 42 cm female yellowtail producing 1 456 000 eggs would ovulate 7 ± 1 batches of a batch fecundity of 200 000 ± 20 000 eggs. Female yellowtail in captivity ovulated up to four times the number of batches estimated by Zamarro. In addition, the batch fecundity of captive females was shown to be small, remaining in the tens of thousands and rarely exceeding 100 000 eggs. Zamarro's batch fecundity estimate, although greater than the batch fecundities observed in captive females, may accurately reflect batch size in wild yellowtail flounder.

The batch fecundity data for repeat spawners indicated that most females did not demonstrate inter-annual differences in batch fecundity. An increase in the number of ovulations was observed rather than increasing mean batch size, even in cases where total egg production had increased. A reproductive strategy which favours a high number of ovulations may be advantageous by allowing many matings with different males, as well as, permitting the increased distribution of a female's egg batches across a prolonged spawning season. Houde (1989) hypothesized that batch-spawning ensures that some egg batches from a female will produce larvae which will coincide with environmental conditions favourable for growth and survival.

Batch fecundity was observed to change erratically between ovulations in female yellowtail. Kjesbu (1989) indicated that erratic changes in batch fecundity distributions were seen for irregular spawners, while regular spawners had dome shaped distributions of batch fecundity. This was not the case in captive female yellowtail, where all females demonstrated erratic changes in batch fecundity. Furthermore, this erratic pattern was seen in repeat spawners in both years.

Decreases in egg diameter and dry weight with time, and the relationship between egg diameter and dry weight have been previously reported in batch-spawning species such as northern anchovy, *Engraulis mordax*, cod, *Gadus morhua*, turbot, *Scophthalmus maximus* and sole, *Solea solea* (Hunter & Leong, 1981; Devauchelle *et al.*, 1987,1988; Kjesbu, 1989; McEvoy & McEvoy, 1991). Stronger relationships were generally found within individual yellowtail than when the pooled female data was used, which indicates that the changes occurring in diameter and dry weight were specific to the individual. While some females did not have significant relationships between egg diameter and time or between egg dry weight and diameter, all females tested for egg dry weight and time had significant relationships. This seems to support the idea that egg dry weight changes with time are basically responsible for egg size variation over time. Links between female size and the maximum egg size of a female were reported in cod and turbot (Kjesbu, 1989; McEvoy & McEvoy, 1991). However no such relationship was evident in captive yellowtail flounder.

In cod, the egg size of a batch is determined by a final growth phase, which comprises 20 % of a normal inter-ovulatory period prior to hydration or ovulation (Kjesbu *et al.*, 1990). The material fuelling this phase appears to be supplied by somatic reserves (Kjesbu *et al.*, 1991). As spawning progresses and somatic reserves deplete, less material enters oocytes during final growth phases, and the resulting egg size of batches decreases (Kjesbu *et al.*, 1991). Further investigation revealed that yolk uptake during the final growth phase is dependent on the oocyte size (>ca. 100 μg) and that an oocyte may experience a 33 % increase during this phase (Kjesbu & Kryvi, 1993).

Decreasing egg size during the spawning season of batch-spawners is considered to be adaptive (Ware, 1975). Since larval size is correlated to egg size (Blaxter, 1988), batch spawners produce smaller larvae later in the spawning season. The production of smaller larvae coincides with increases in the abundance of small zooplankton prey which permits higher larval growth rates and survivorship (Ware, 1975).

A decline in egg size in the northern anchovy is considered advantageous for this species of indeterminate fecundity. The decrease in egg size and content was estimated to permit the spawning of five additional batches which would not have been possible if a constant egg size were maintained (Hunter & Leong, 1981).

Table 11. Comparison of the 1994 egg production data with fecundity estimates for wild yellowtail flounder females.

♀	Prespawning Total length (cm)	Total Egg Production Collected	Howell & Kesler (1977) Estimate	Pitt (1971) Estimate
A	37	1 324 762	1 106 622	803 701
B	34	200 454	798 588	540 586
C	34.5	769 990	844 857	578 895
D	40.5	206 592	1 568 333	1 227 983
E	33	489 792	711 711	469 958
F	44.5	493 579	2 255 536	1 910 015
H	36.5	750 921	1 050 033	754 018
I	33.5	723 217	754 223	504 299
L	41.5	345 135	1 723 084	1 376 810
M	42	671 868	1 804 566	1 456 356
O	34	71 001	798 588	540 586

Table 12. Comparison of the 1995 egg production data with fecundity estimates for wild yellowtail flounder females.

♀	Prespawning Total length (cm)	Total Egg Production Collected	Howell & Kesler Estimate	Pitt Estimate
A	39	1 088 972	1 355 827	1 028 777
C	36	1 622 822	995 616	706 785
D	42	918 936	1 804 566	1 456 356
F	45	1 367 185	2 354 891	2 012 774
G	43	2 001 472	1 976 051	1 626 280
H	38	738 216	1 226 541	910 780
I	34	1 255 915	798 588	540 586
M	42	2 051 304	1 804 566	1 456 356
N	40.5	211 736	1 568 333	1 227 983
Q	39	612 250	1 355 827	1 028 777

Part 4.

VARIABILITY IN EGG QUALITY

4.1. RESULTS.

The egg quality results for spawning yellowtail of 1994 showed that females had a mean gamete potential rate of 59 % (Table 1). Hence, approximately 41% of the eggs produced by a female were visibly abnormal at collection. Females also had a mean fertilization rate of 38 %, a mean hatching rate of 63 % and a mean larval production rate of 28 % (Table 1). The mean difference between gamete potential and fertilization rates for any batch was 25 %. The mean difference between fertilization and larval production rates was 10 %.

Table 1. Mean values for gamete potential rate, fertilization rate, hatching rate, larval production rate, the difference between gamete potential and fertilization rates (GP-FR) and the difference between fertilization and larval production rates (FR-LP) for spawning females in 1994.

1994 (n= 11♀)	Gamete Potential (%)	Fertilization Rate (%)	Hatching Rate (%)	Larval Production (%)	GP-FR (%)	FR-LP (%)
Mean	59	38	63	28	25	10
s.d.	± 22	± 22	± 17	± 20	± 11	± 4.5

Examining the mean egg quality results for individuals, gamete potential rate means ranged from 17 % (L) to 91 % (E) (Table 2). The highest mean fertilization rate was 82 % from female E, which was the earliest spawner. The females with the lowest mean fertilization rates were C and L, with the respective values of 9 % and 5 % (Table 2). Female E also had the highest hatching rate of 84 %, followed by female D which ovulated during the main part of the spawning season. Female L had the poorest hatching rate of 38 %. The resulting larval production rates ranged from 3 % and 4 %, from females L and C, to 72 % from female E (Table 2). All other females had larval production

rates less than 50 %. The results of nested anova analysis revealed that a significant maternal effect was present in the 1994 fertilization rate data ($P < 0.0001$). Paired comparison tests indicated that all females were significantly different from one another. Regarding the hatching rate data, statistical analyses showed that a significant maternal effect was present ($P < 0.003$). While paired comparison tests displayed some grouping of females, female L was found to be significantly different from all other females.

Table 2. Mean values for gamete potential, fertilization, hatching and larval production rates for individual females in 1994 (N= number of batches which were fertilized).

♀ 1994	N	Gamete Potential Rate (%)	Fertilization Rate (%)	Hatching Rate (%)	Larval Production Rate (%)
A	17	77	46	78	37
B	6	49	27	60	19
C	16	55	9	47	4
D	6	53	42	81	33
E	5	91	82	84	72
F	11	71	57	76	43
H	17	69	37	66	29
I	24	86	53	68	39
L	11	17	5	38	3
M	13	38	22	48	12
O	3	43	42	42	18

Most 1994 females demonstrated considerable inter-batch variability in fertilization rates over the course of their spawning seasons, exceptions were females E, C and L (Figure 1). The inter-batch variability within females in hatching rates was generally larger

than for fertilization rates (Figure 2). Females D and F had the least variation of the group, while females C and L had the most variability. One-way anovas and randomization tests on the fertilization rate data for individuals showed that each female had a significant batch effect ($P<0.001$). For the hatching rate data a significant batch effect was found within all females ($P<0.005$), with the exception of female D ($df=5$, $F= 1.61$, $P=0.2311$).

An increase in gamete potential and fertilization rates was observed for the females of 1995 (Table 3). Females had a mean gamete potential rate of 81 % and mean fertilization rate of 57 %. The mean difference between gamete potential and fertilization rates for any batch was consistent with 1994 results at 25 % (Table 3). Females also had a mean irregular cleavage rate of 14 %. A sample of 21 batches used in the over-ripening study had a mean hatching rate of 88 %. Using this figure, the mean larval production rate for 1995 females was estimated to have been 50 %.

Table 3. Mean values for gamete potential rate, fertilization rate, irregular cleavage rate, and the difference between gamete potential and fertilization rates(GP-FR) for spawning females in 1995.

1995 (n= 10♀)	Gamete Potential (%)	Fertilization Rate (%)	Irregular Cleavage Rate (%)	GP-FR (%)
Mean	81	57	14	25
s.d.	± 7	± 17	± 7	± 14

Figure 1. Variability in fertilization rate (%) data between and within females during 1994. The fertilization rate data for each spawning individual is represented by a single box plot. The top and bottom edges of the box correspond to the 75^{th} and 25^{th} percentiles, the error bar edges correspond to the 10^{th} and 90^{th} percentile points. Dots outside the error bars, when present, represent the 5^{th} and 95^{th} percentile points. The median of a female's data is indicated by a solid line while the mean is shown as follows ****. The numbers on the box plot indicates the female's mean fertilization rate. An additional box plot indicated by ALL represents the fertilization rate data for all the females and show the group mean (n=129).

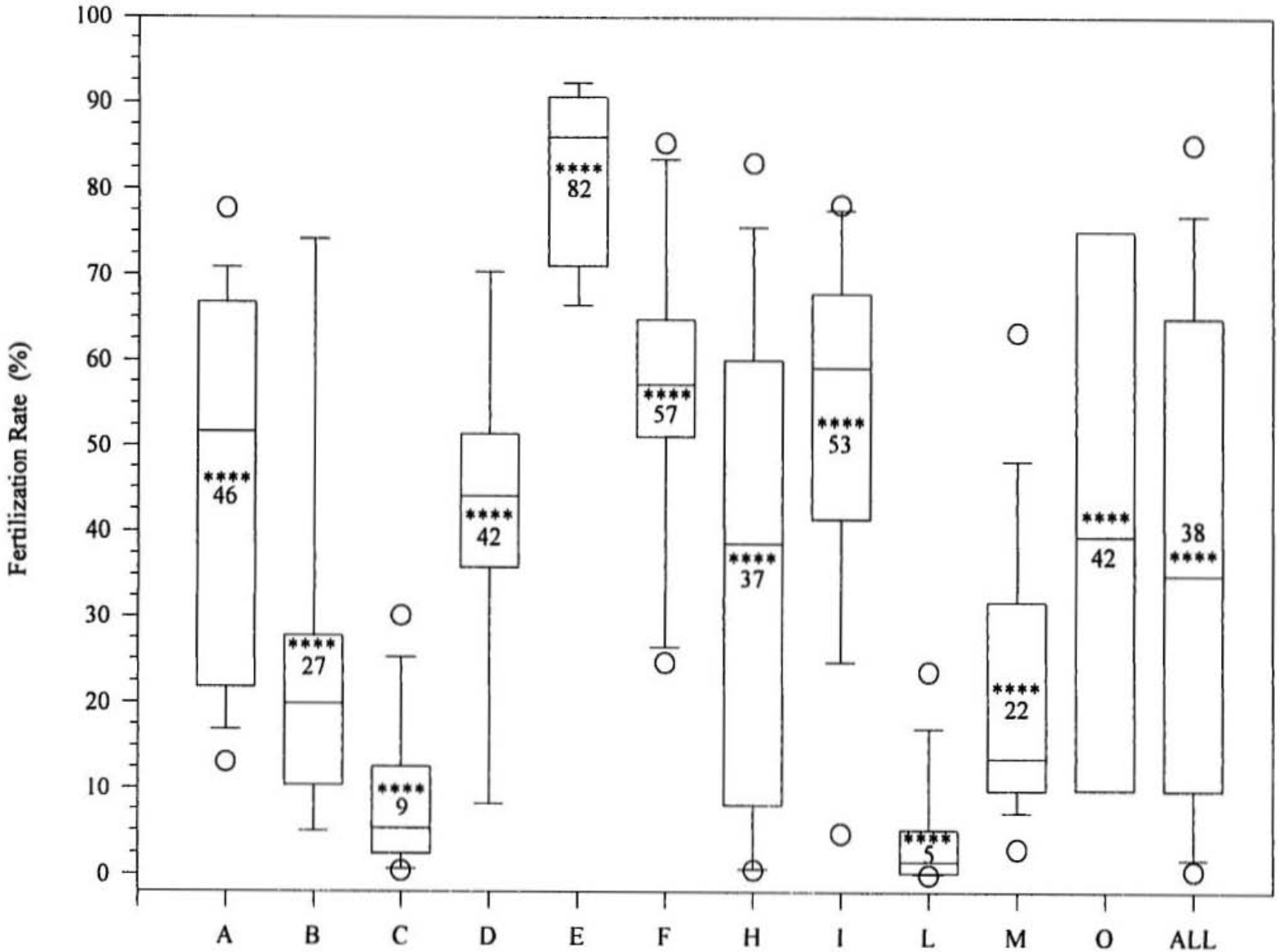

Fertilization Rate (%)
100
90
80
70
60
50
40
30
20
10
0
****
46
****
27
****
9
****
42
****
82
****
57
****
37
****
53
****
5
****
22
****
42
****
38
A
B
C
D
E
F
H
I
L
M
O
ALL

Figure 2. Variability in hatching rate (%) data between and within females during 1994. The hatching rate data for each spawning individual is represented by a single box plot. The top and bottom edges of the box correspond to the 75^{th} and 25^{th} percentiles, the error bar edges correspond to the 10^{th} and 90^{th} percentile points. Dots outside the error bars, when present, represent the 5^{th} and 95^{th} percentile points. The median of a female's data is indicated by a solid line while the mean is shown as follows ****. The number on the box plot indicates the female's mean fertilization rate. An additional box plot indicated by ALL represents the fertilization rate data for all the females and shows the group mean (n=126).

Hatching Rate (%)
100
90
80
70
60
50
40
30
20
10
0
A
B
C
D
E
F
H
I
L
M
O
ALL
78
60
47
81
84
76
66
68
38
48
42
63

Table 4. Mean values for gamete potential, fertilization, and irregular cleavage rates for individual females in 1995 (N= number of batches which were fertilized).

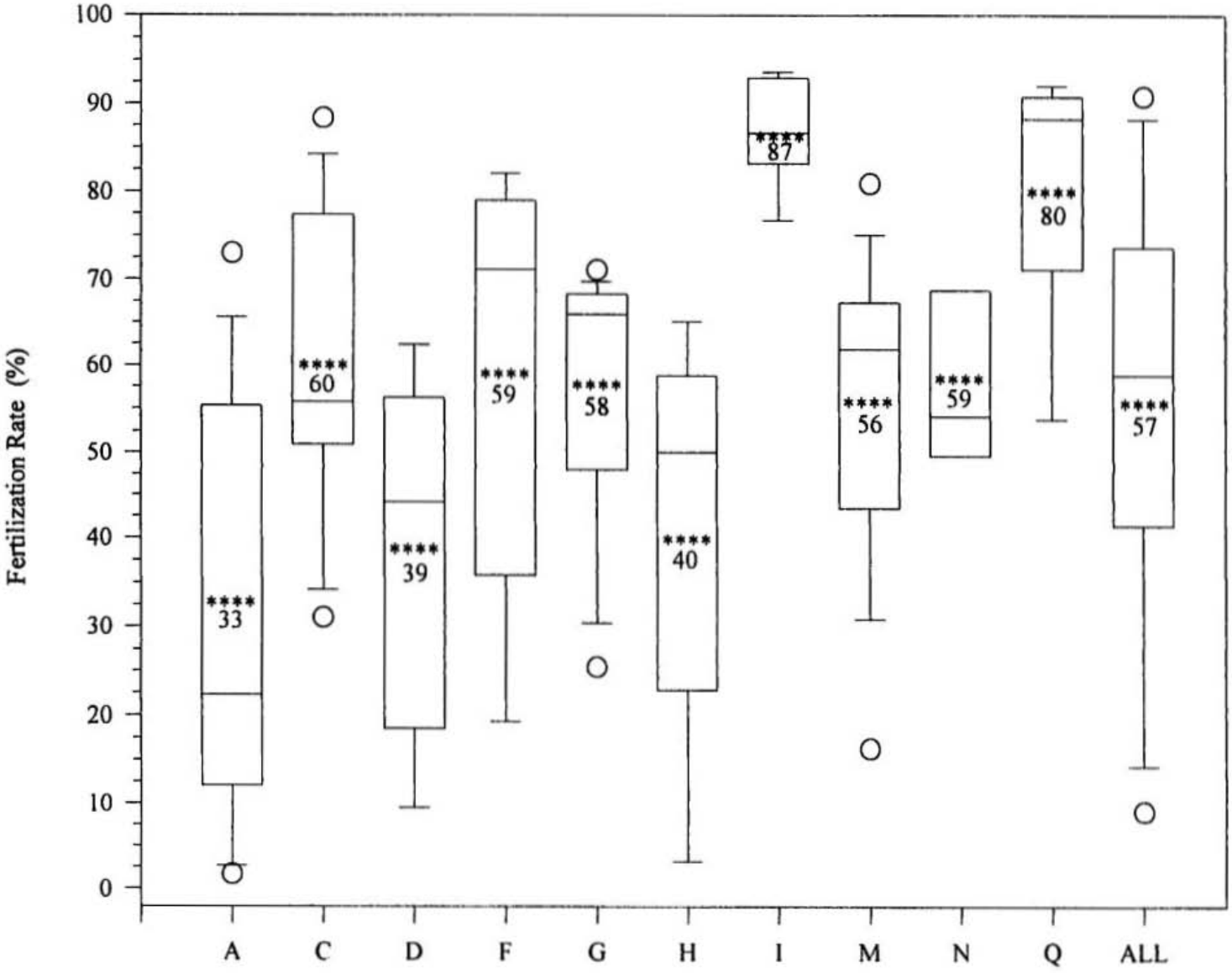
♀ 1995	N	Gamete Potential Rate (%)	Fertilization Rate (%)	Irregular Cleavage Rate (%)
A	11	75	33	13
C	10	86	60	12
D	11	69	39	16
F	8	84	59	10
G	10	78	58	14
H	9	86	40	25
I	9	92	87	8
M	11	80	56	27
N	3	80	59	10
Q	8	76	80	2

There was less variability in egg quality between females in 1995 than in 1994 (Table 4). Gamete potential rates ranged from 69 % (D) to 92 % (I). Underestimation of gamete potential was noted in female Q. The earliest spawners, females I and Q, had the highest mean fertilization rates in 1995 of 87 % and 80 %, in addition, they exhibited the lowest irregular cleavage rates of 10 % and 2 % respectively . The only females with mean fertilization rates less than 50% in 1995 were females A, D and H, with the respective rates of 33 %, 39 % and 40 %. Irregular cleavage was highest in female A at 27 %. Statistical analysis on the fertilization rate data for females of 1995 revealed a significant maternal effect ($P<0.0001$). Paired comparison tests revealed that females I and Q were significantly different from each other and all other females. Female A, with the lowest

fertilization rate mean, was also significantly different from all other females.

Regarding the inter-batch variation within females in fertilization rates, female I had the least amount of variation among her batches, while females A, F and H had the highest amounts (Figure 3). The incidence of poor egg quality females was reduced in 1995, in addition, there were fewer batches of extremely poor fertilization rates in 1995 compared to 1994 (Figures 1 & 3). Statistical analysis on the fertilization rate data of individuals demonstrated that 1995 females had significant batch effects ($P<0.001$), with the exception of female N which ovulated a few times prior to resorbing ($df=2$, $F=3.58$, $P=0.095$).

Figure 3. Variability in fertilization rate (%) data between and within females during 1995. The fertilization rate data for each spawning individual is represented by a single box plot. The top and bottom edges of the box corresponds to the 75th and 25th percentiles, the error bar edges correspond to the 10th and 90th percentile points. Dots outside the error bars, when present, represent the 5th and 95th percentile points. The median of a females data is indicated by a solid line while the mean is shown as follows ****. The number on the box plot indicates the female's mean fertilization rate. An additional box plot indicated by ALL represents the fertilization rate data for all the females and show the group mean (n= 90).


Fertilization Rate (%)
100
90
80
70
60
50
40
30
20
10
0
A
C
D
F
G
H
I
M
N
Q
ALL
****
33
****
60
****
39
****
59
****
58
****
40
****
87
****
56
****
59
****
80
****
57

In each year the earliest spawners exhibited high egg quality means which coincided with low and stable temperature conditions, (i.e. female E in 1994 and females Q and I in 1995; Figures 4 & 5). Since temperature conditions were not constant over the length of the spawning season in either year, significant maternal effects found in statistical analyses may have been due to the early spawners encountering lower temperature conditions. In order to assess the impact of early spawners, their data was removed and the egg quality data set reanalysed for maternal effects by nested anovas. For the 1994 fertilization rate and hatching rate data, significant maternal effects remained with the removal of the data of female E (FR $P< 0.0001$; HR $P<0.002$).

Two analyses were performed on the 1995 fertilization rate data set. The first analysis was performed on the data set which strictly adhered to the 1995 sampling regime criteria (see p. 9). The second analysis included the additional batches collected outside the sampling regime criteria (two batches each for females A, D, H and M). The results of the first analysis revealed that without the data from females I and Q, the significant maternal effect on fertilization rate was lost ($P< 0.1139$). However, the second analysis showed that a significant maternal effect on fertilization rate remained after early spawners were removed ($P<0.012$). Paired comparison tests on the latter result showed that female A, with the additional batches, was significantly different from all other females. Similar tests in the first analysis did not show this result for female A, although groups of females were significantly different.

Figure 4. Temperature data and fertilization rate (%) data of individual females as they were recorded over the 1994 spawning season. The linear plot represents mean daily temperatures (°C). The fertilization rate data (n=129) recorded from the batches of a single female is represented by a character symbol. The characters used correspond to those labelling different females in Table 2 and Figure 1.

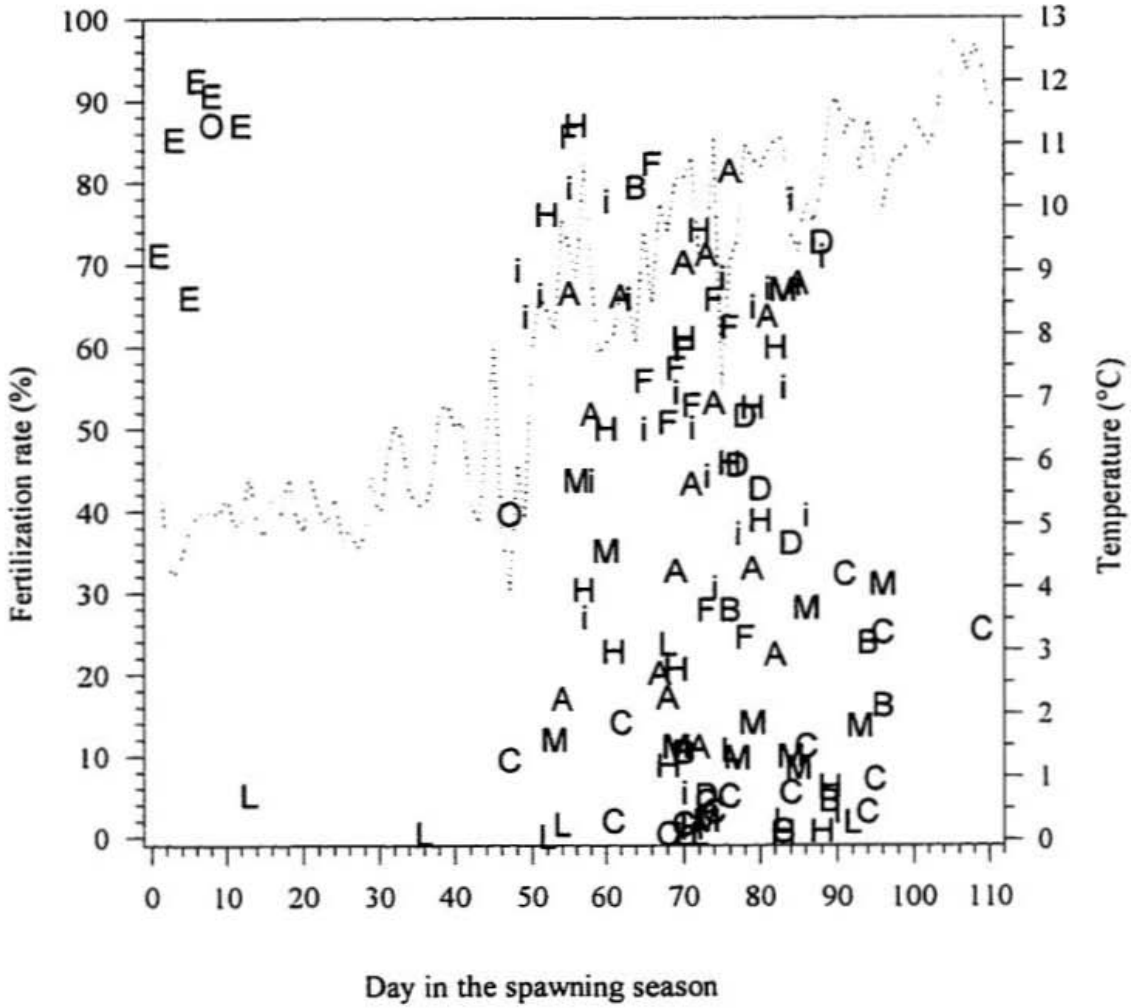
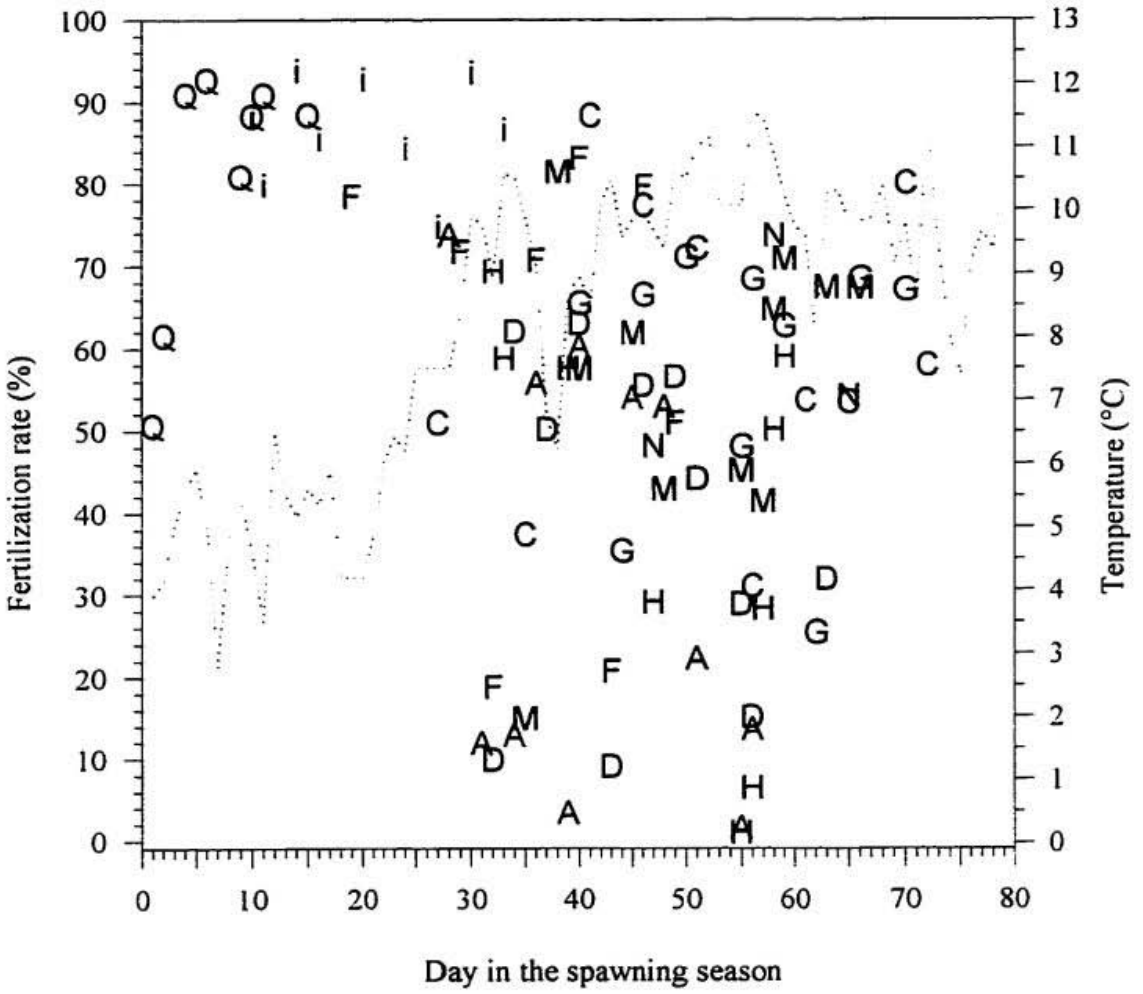

Fertilization rate (%)
Temperature (°C)
Day in the spawning season
0
10
20
30
40
50
60
70
80
90
100
110
0
1
2
3
4
5
6
7
8
9
10
11
12
13

Figure 5. Temperature data and fertilization rate (%) data of individual females as they were recorded over the 1995 spawning season. The linear plot represents mean daily temperatures (°C). The fertilization rate data (n=90) recorded from the batches of a single female is represented by a character symbol. The characters used correspond to those labelling different females in Table 4 and Figure 3.


Fertilization rate (%)
Temperature (°C)
Day in the spawning season
0
10
20
30
40
50
60
70
80
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13

Statistical analysis detected a significant inter-annual increase in fertilization rates for the group of captive females ($P < 0.001$). Inter-annual differences were also examined for the seven repeat spawners. Results of analyses showed that only females C, I and M exhibited significant increases in their fertilization rate data between seasons (Table 5). Additional analyses were performed to determine if inter-annual differences in temperature variation, which may have affected egg quality, occurred between the spawning durations of each individual repeat spawner. Significant differences were seen for females D, F and I (Table 6). While no connection with egg quality was clear for females D and F, female I had a significant inter-annual decrease in temperature conditions which may have been related to the significant increase in fertilization rates. All other females had non-significant inter-annual effects in temperature conditions, including females C and M which demonstrated significant increases in fertilization rates.

Table 5: Anova and randomization test results examining for an inter-annual effect on the fertilization rate data of individual repeat spawners (N= sample size per season).

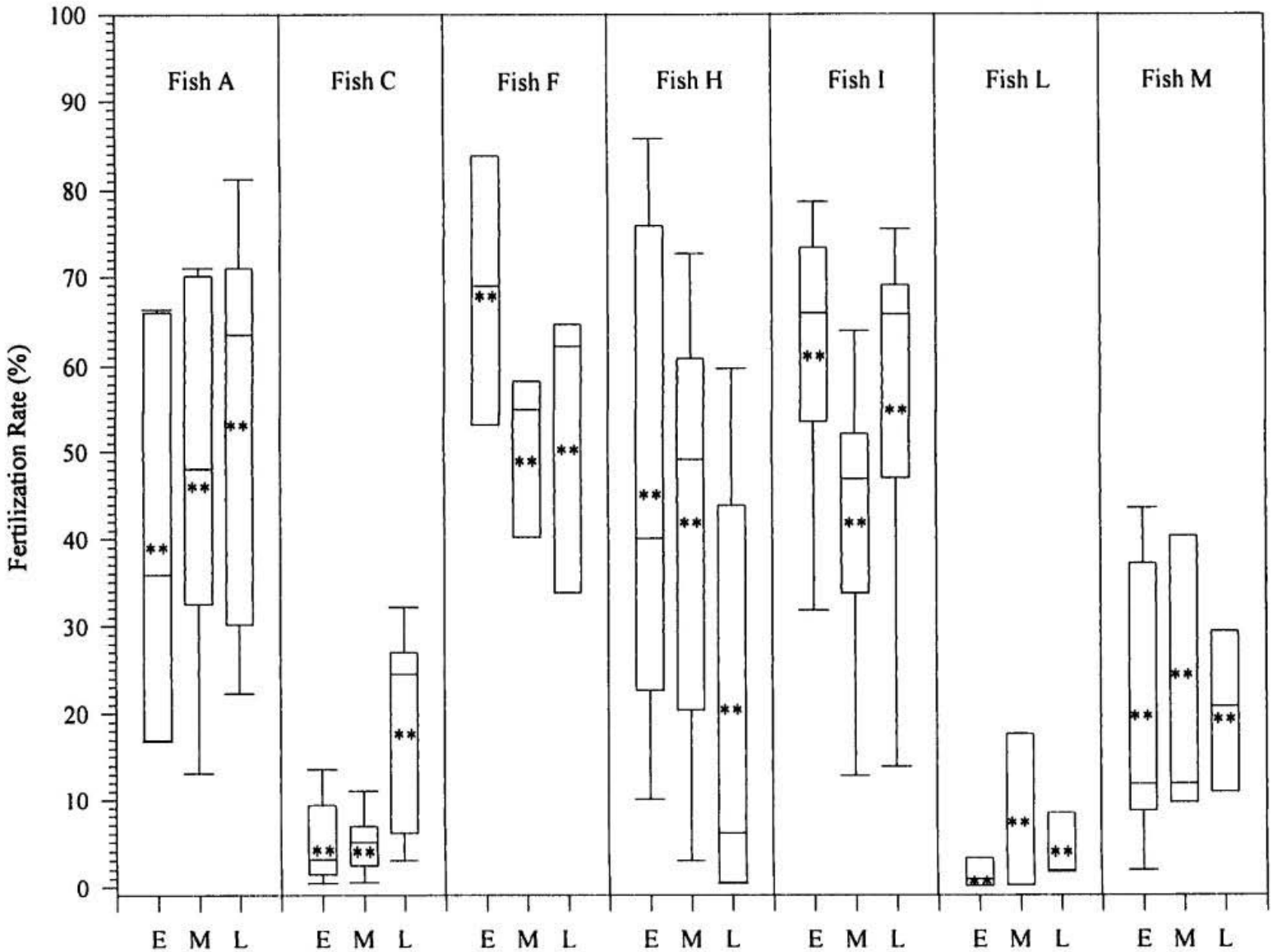
Repeat Spawner	N	F-value	P<	Randomization P=	Significant Seasonal increase / decrease
A	9	0.60	0.4509	0.436	No, decrease
C	10	52.63	0.0001	0.001	Yes, increase
D	6	0.25	0.6265	0.637	No, decrease
F	8	0.05	0.8264	0.826	No, increase
H	7	0.13	0.7231	0.716	No, increase
I	8	24.14	0.0002	0.003	Yes, increase
M	9	12.33	0.003	0.009	Yes, increase

Table 6: Anova results examining for an inter-annual effect on temperature conditions experienced by individual repeat spawners.

Repeat Spawner	1994 mean temperature (°C)	1995 mean temperature (° C)	F-value	P<	Significant Seasonal increase / decrease
A	9.56	9.64	0.07	0.7853	No
C	9.57	9.56	0.00	0.9760	No
D	9.46	9.77	10.45	0.0016	Yes, increase
F	9.38	8.31	6.28	0.0152	Yes, decrease
H	9.68	9.74	0.05	0.8229	No
I	8.78	6.65	19.68	0.0001	Yes, decrease
M	9.80	9.78	0.01	0.9238	No

The 1994 fertilization rate data was used to examine whether individual females produced their highest quality eggs during a particular period in their spawning season. The fertilization rate data of females with the longest spawning records were divided into early, middle and late periods, where the variation in each period was represented by an individual box plot (Figure 6). For most females, the variation in fertilization rate within each period was large. In addition, there was a lack of any consistent temporal pattern for the production of good quality batches among the females (Figure 6). Females L and M had their egg quality peak in the middle period of their seasons, females A and C in the late period, and females F, H and I in the early part of their seasons. Statistical analysis was not attempted due to the small number of batches per period observed in some females.

Figure 6. Fertilization rate (%) variability in the early, middle and late periods of egg production of seven 1994 spawners. The fertilization data within each female was subdivided into early, middle and late periods for the female's spawning season. The top and bottom edges of the box corresponds to the 75^{th} and 25^{th} percentiles, the error bar edges correspond to the 10^{th} and 90^{th} percentile points. The median of a females data is indicated by a solid line. The mean line is indicated by the following **.


Fertilization Rate (%)
100
90
80
70
60
50
40
30
20
10
0
Fish A
Fish C
Fish F
Fish H
Fish I
Fish L
Fish M
E M L
**

Significant relationships between fertilization and gamete potential rates were found in 1994 ($P=0.0001$, $r^2 = 0.611$) and 1995 ($P=0.0001$, $r^2 = 0.519$) when the pooled data was used (Figures 7 & 8). Regression analysis for these parameters in 1994 were performed on all females, except female O due to insufficient data. All 1994 females had significant relationships where the amount of explained variance ranged from 30 % to 87 %. Females D, E, F and H had better relationships than the pooled data relationship. Individual regressions for the 1995 females were significant for all females, except female I possibly because gamete potential rates were occasionally lower than fertilization rates. Female N had too few data points for regression analysis. The amount of explained variance ranged from 50 % to 90 % among the females with significant regressions, of which seven had better relationships than the pooled data had shown. Repeat spawning females A, C and M demonstrated better relationships in 1995 than in 1994.

Regarding relationships between hatching and fertilization rates in 1994, only female I had a significant result ($P<0.01$, $r^2 = 0.234$) all other females had non-significant results ($P> 0.1$). The poor relationship between hatching rate and fertilization rate remained when the data of females was pooled (Figure 9). Hatching rates, however, did have a significant negative relationship with irregular cleavage rate ($P=0.0001$, $r^2 = 0.323$) although the relationship was not strong.

Figure 7. Relationship between fertilization rate (%) and gamete potential rate (%) for the 1994 season. Each point represents an individual batch. The regression line for the relationship is shown (n= 128).

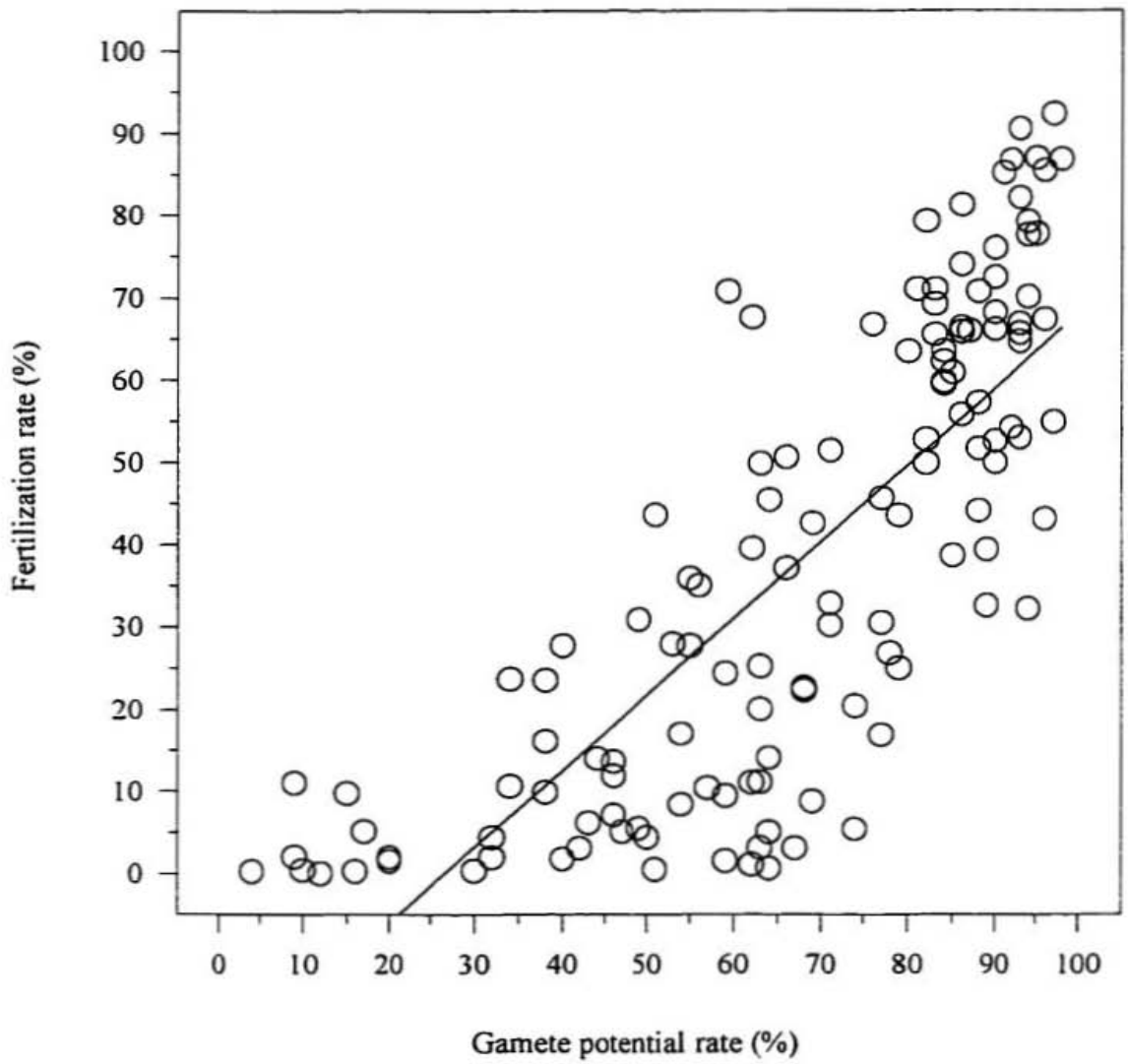

Fertilization rate (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Gamete potential rate (%)

Figure 8. Relationship between fertilization rate (%) and gamete potential rate (%) for the 1995 season. Each point represents an individual batch. The regression line for the relationship is shown (n= 90).

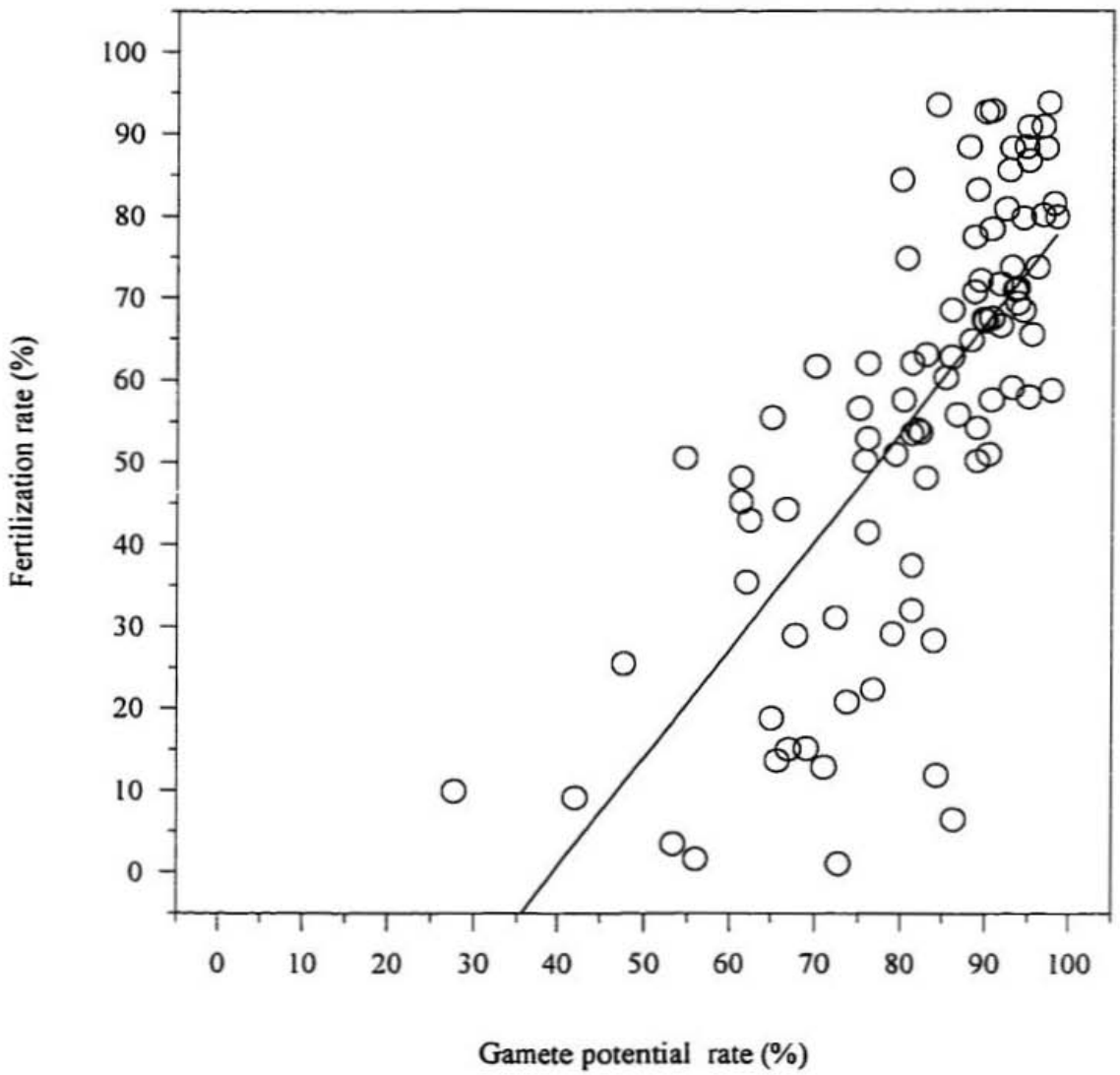
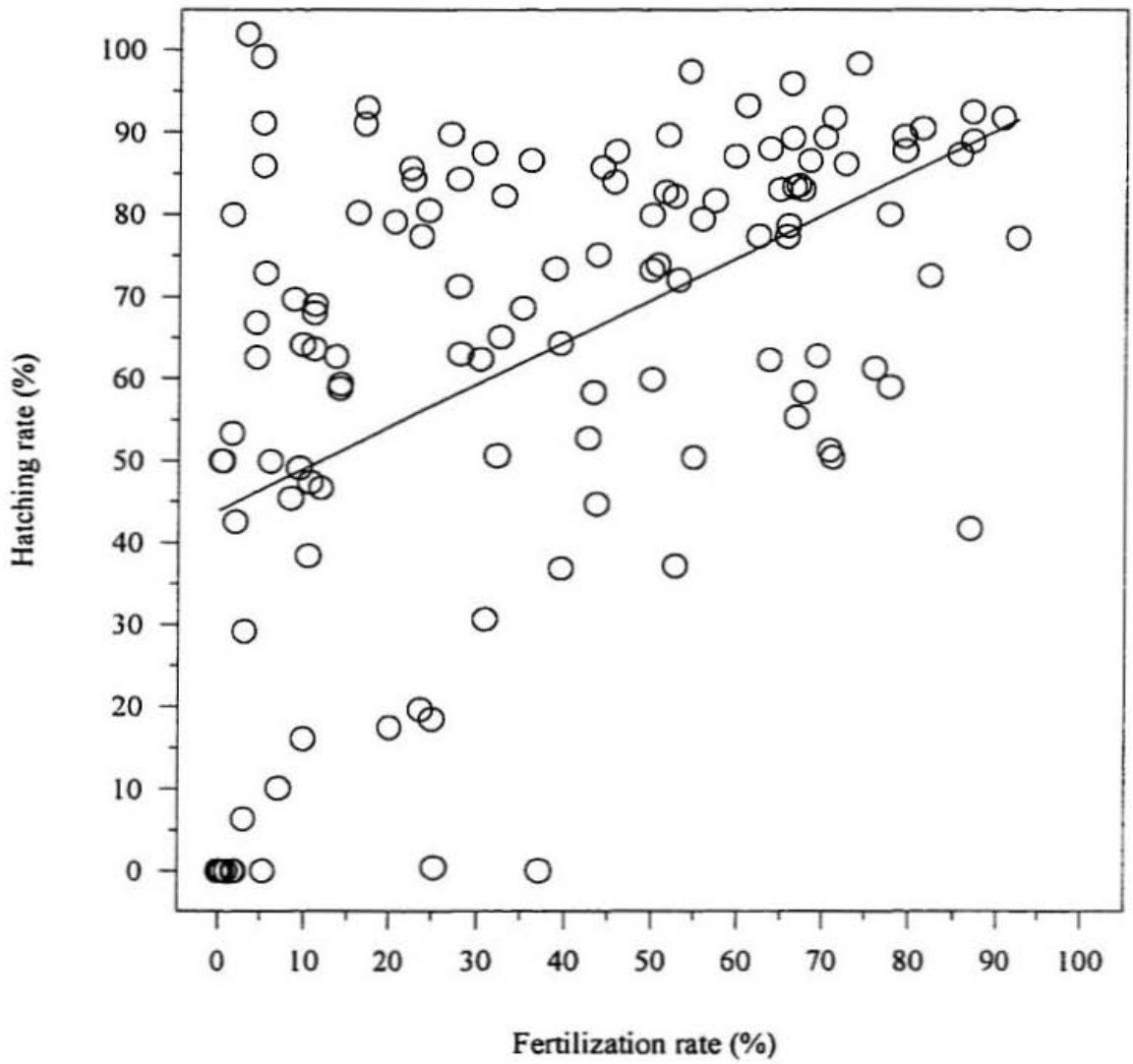

Fertilization rate (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Gamete potential rate (%)

Figure 9. Relationship between hatching rate(%) and fertilization rate (%) for the 1994 season. Each point represents an individual batch. The regression line for the relationship is shown (n= 125).


Hatching rate (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Fertilization rate (%)

A study of egg storage and over-ripening showed that the greatest drop in fertilization rates usually occurred during the first 24 hours post-collection (Figure 10.A). Fertilization rates remained >10 % for a mean of 2.1 days of storage (Table 7), where fertilization rates prior to storage ranged from 35 % to 94 %. Batches with initial fertilization rates over 90 % had the slowest rates of over-ripening where fertilization rates remained >20 % after the maximum of 4 days of incubation. The duration of storage of batches decreased as initial fertilization rates approached the 10 % level (Table 7). High variability in over-ripening rates could be seen in batches belonging within the 50+ to 80+ classes. Initial hatching rates were high even when initial fertilization rates were low. The decrease in hatching rate with ageing was generally less dramatic in comparison with the fertilization rate data (Figure 10B). The steady decrease in hatching rate with time is also reflected in the larval production curve (Figure 10A) .

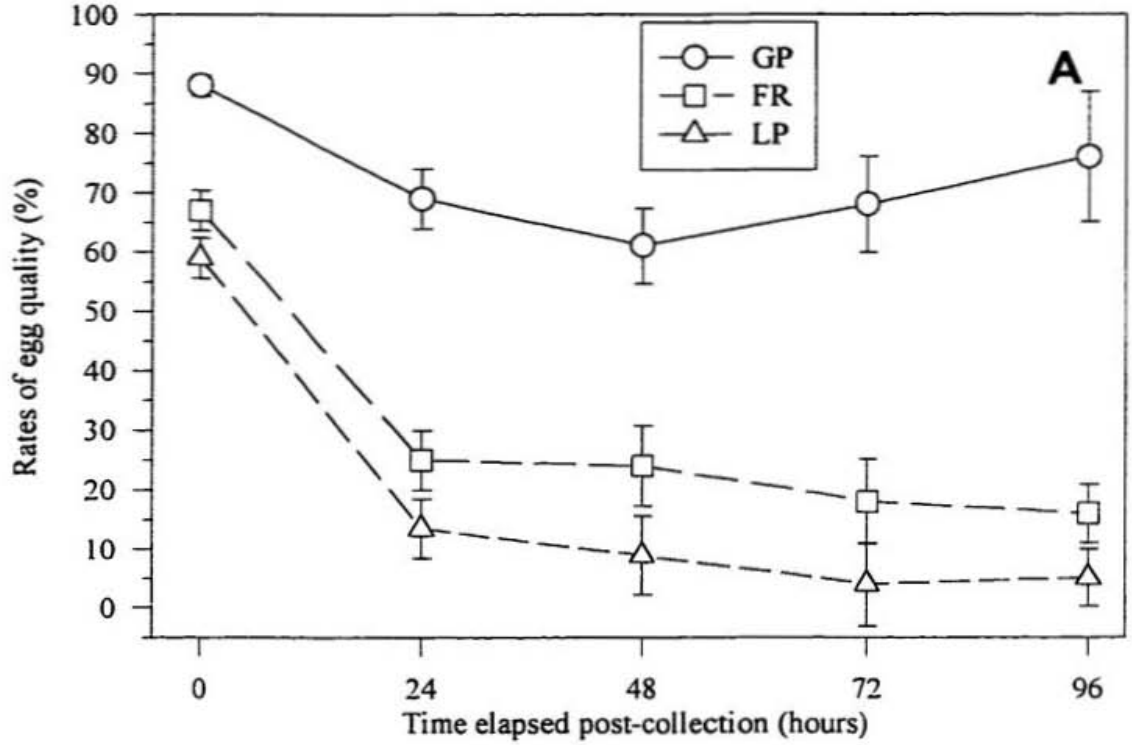
Irregular cleavage rates were low initially at collection (0 hrs) and increased with time. Possibly associated with reductions in hatching rates, irregular cleavage rates were clearly not the only factors contributing to decreasing hatching rates as ageing progressed between 24 and 72 hours post-collection (Figure 10B).

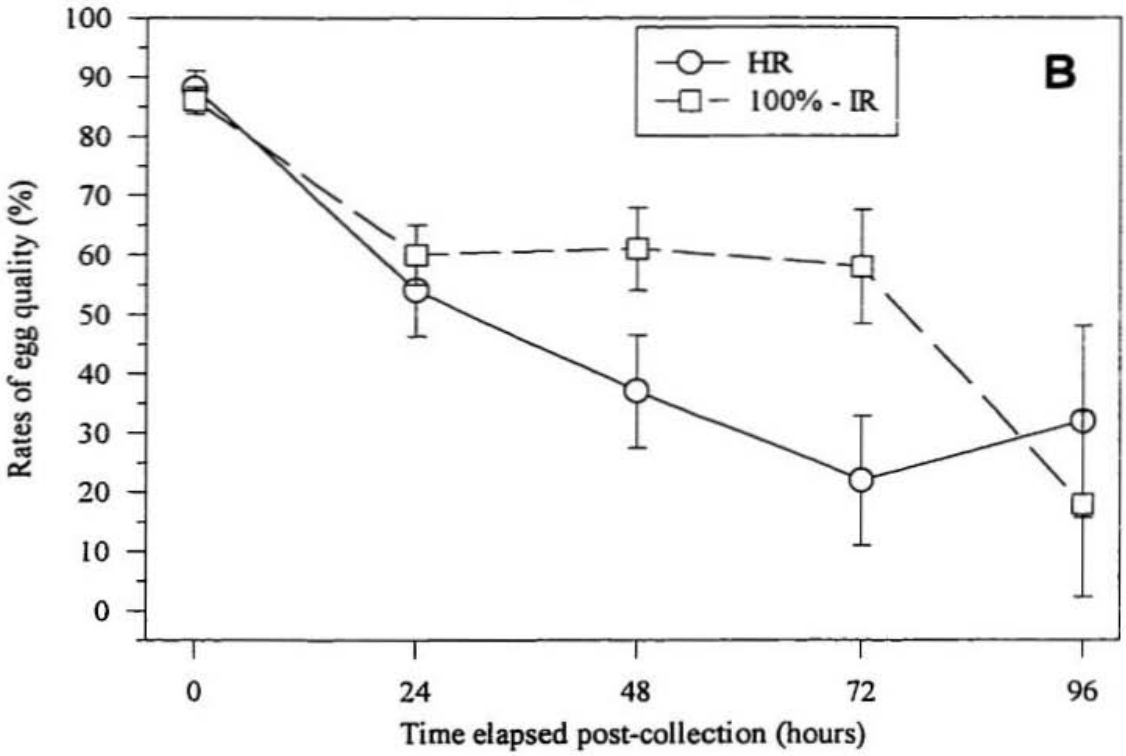
Gamete potential rates, on average, had a decrease after 24 hours of storage, but generally did not decrease linearly with time and remained relatively high (Figure 10A). The large differences seen between gamete potential rate and fertilization rate curves with over-ripening were due to greater rates of decline in fertilization rates (Figure 10A). Therefore, declines in egg viability via over-ripening were not detected by egg morphology characteristics.

Table 7. Mean number of days of egg storage, prior to fertilization rates decreasing to < 10%, listed for each initial fertilization rate class.

Initial Egg Fertilization Rate Class	n=	Mean Duration ± s.d. (days)
90+	2	> 4
80+	3	2.7 ± 1.5
70+	3	2.7 ± 0.6
60+	6	1.7 ± 0.8
50+	5	1.6 ± 0.9
30+	2	1 ± 0
Mean	21	2.1 ± 1.1

Figure 10. Mean changes in egg quality (%) occurring with over-ripening for batches stored *in vitro* in an incubator (n=21). Plot A shows the mean changes in gamete potential rate (GP), fertilization rate (FR) and larval production rate (LP) for the batches tested. Plot B shows the mean changes, for the same batches, in hatching rate (HR) and the potential reductions in hatching rate which may be due to the rate of irregular cleavage (100% - IR). The number of batches representing each mean decreased from 21 (0 - 24 hrs) to: 12 at 48 hrs, 8 at 72 hrs and 3 at 96 hrs. Error bars represent standard errors.


A
GP
FR
LP
Rates of egg quality (%)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
Time elapsed post-collection (hours)


B
HR
100% - IR
Rates of egg quality (%)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
Time elapsed post-collection (hours)

4.2. DISCUSSION.

The egg quality results for yellowtail flounder compare favourably to the results reported in other studies of batch-spawners in captivity. Howell & Scott (1989), Omnes *et al.* (1991) and Fauvel *et al.* (1992) also employed stripping protocols for captive female turbot, *Scophthalmus maximus*, similar to the one used in this study. Omnes *et al.* (1991) reported that females had mean gamete potential, fertilization, hatching and larval production rates of 77 %, 57 %, 50 % and 30 %, respectively. In Howell & Scott (1989), however, the mean proportion of buoyant eggs was only 40 % and the mean fertilization rate was 24 %. In addition, the mean larval production rate seen in Fauvel *et al.*'s study was 25 %.

According to McEvoy (1984), high fertilization rates (>90 %) were obtained if eggs were collected immediately after ovulation, prior to the effects of over-ripening. In contrast, fertilization rates ranged from 30 % to over 95 % within a female Atlantic halibut *Hippoglossus hippoglossus*, despite the eggs having been stripped near ovulation as in McEvoy's study (Norberg *et al.*, 1991).

Regression results indicated that hatching rates were independent of fertilization rates for yellowtail flounder. Optimal survival of embryos has been shown to depend on incubation conditions. Laurence & Howell (1981) indicated that yellowtail flounder embryos were tolerant of a wide range of temperature and salinity conditions, even those exceeding natural conditions. Maximal hatching rates were found to lie between 8 °C and 14 °C with a coinciding salinity range of 31 to 38 ppt. Sources of developmental mortality included irregular cell division and events during the synchronization of blastodermal and

periplast overgrowth of the yolk (Laurence & Howell, 1981). Only a weak relationship was found between irregular cleavage rates and hatching rates for captive females of this study.

The relationship found between gamete potential and fertilization rates revealed that the prediction of fertilization rate was possible. However, individual females demonstrated distinct relationships which were possibly dependent on maternal and environmental factors combined. Since there were such individual influences on the regressions, gamete potential rate can only be used as a rough predictor of fertilization rates, where, on average, fertilization rates can be estimated to be 25 % lower than gamete potential rates.

The large proportion of visibly abnormal eggs at collection was a problem which was observed in both seasons. The appearance of abnormal eggs is commonly attributed to the effects of over-ripening which occurred between the time of ovulation and collection. The over-ripening results for yellowtail eggs aged *in vitro* revealed that, on average, the sharpest decline in fertilization rates occurred within the first 24 hours after collection. In the barfin flounder, *Verasper moseri*, high fertilization rates (>90 %) were initially obtained and remained above 70 % for two days when left to over-ripen *in vivo* (Koya *et al.*, 1994). For the turbot, McEvoy (1984) indicated that fertilization rates of eggs left inside the female could remain above 80 %, even after 30 or 35 hours post-ovulation, yet hatching rates could decrease to 0 % after 18 to 20 hours. In contrast, the results for captive yellowtail showed hatching rates which were higher and fell less sharply than fertilization rates over time, despite *in vitro* incubation. Reductions in egg quality by

over-ripening were not reflected by egg morphology changes in yellowtail flounder. The viability of halibut eggs was also observed to decline prior to any visible structural changes in the eggs (Bromage *et al.*,1994). The results of McEvoy (1984) for turbot are in agreement, where spotting and a premature perivitelline space appeared in eggs prior to 67 hours (12-14 °C) post-ovulation; prior to 85 hours yolk precipitation was under way.

Inter-annual increases in egg quality seen in the mean group results of the current study, as well as some repeat spawners, could not be attributed to any change in environmental temperature, with the exception of one female. Increases in gamete potential rates, without a significant decrease in temperature conditions, between seasons is inconsistent with the idea that visibly abnormal eggs are only due to the effects of over-ripening. Furthermore, sudden peaks in their numbers for egg batches which were less than 24 hours old does not agree with the previously mentioned reports of over-ripening effects on egg morphology. The fact that batches with low gamete potential rates occurred more frequently in some females, rather than others which were experiencing the same conditions, indicates that some other factors may be involved. One such factor could be chorion integrity, where defective chorions of freshly ovulated eggs increase susceptibility to contamination of the ooplasm by ovarian fluid which may lead to osmoregulatory problems and yolk precipitation. Kjesbu *et al.* (1992) indicated that a large proportion of the chorionic membrane is deposited between ovulations in cod. The strength of the chorion may be determined in a short period of time, if this process in cod is the same in yellowtail flounder.

The increases seen in fertilization rates between years may have been due to changes in the egg quality testing protocols in 1995. The effect of the selection of batches over 10 ml and the reduction in the number of batches undergoing fertilization trials was estimated using the 1994 data set. When the batch selection criteria were applied to the 1994 data set, sample size for fertilization rates decreased from 129 to 51 batches. The mean calculated for the reduced data set was 41 %, a 3 % increase from the original 1994 mean. When the means of repeat spawning females were recalculated with the reduced data set, comparison with the original means of these females showed that differences were small compared to the inter-annual changes seen in some repeat spawners. It is therefore concluded that the protocol changes which occurred between years were not the cause of the large inter-annual increases in fertilization rates seen in 1995.

It is proposed that increases in fertilization rates and gamete potential rates in 1995 were not due to slower over-ripening rates or changes in protocol but factors such as the introduction of a commercial diet or an additional year of acclimation to captivity. It is possible that the commercial feed had a greater proportion of nutrients required for producing good quality eggs than the shrimp diet preceding it. A greater level of dietary components needed for chorion strength and integrity may have been responsible for the inter-annual reduction in inviable eggs at collection. Cerdá *et al.* (1994) revealed that low dietary protein negatively influenced egg quality in the sea bass, *Dicentrarchus labrax*, another batch-spawner. Experimental diets deficient of phosphorous or essential fatty acids led to poor egg quality in the red sea bream, *Pagrus auratus* (Watanabe et al., 1984; Watanabe, 1985). Certain fatty acids were noted to affect hatching and fertilization rates

in carp, *Cyprinus carpio*, and rainbow trout, *Oncorhynchus mykiss* (Watanabe, 1985). Investigations into the role of vitamins on fish reproduction indicated that vitamin E increased fertilization and hatching rates in both the ayu, *Plecoglossus altivelis* and the carp (Watanabe, 1985). Lower hatching rates were seen in female rainbow trout fed a diet devoid of ascorbic acid (Dabrowski & Blom, 1994).

An additional year of acclimation to captive conditions may have reduced stress levels in females and led to an increase in egg quality in 1995. Campbell *et al.* (1994) found that female brown trout, *Salmo trutta*, and rainbow trout, *Oncorhynchus mykiss*, subject to confinement stress had a significant decrease in survival rates of embryos. Brooks *et al.* (1995) revealed that stress effects on egg quality in the rainbow trout were not mediated directly by cortisol as no decrease in viability occurred when females or eggs were treated with cortisol. Contreras-Sanchez *et al.* (1995) found that stress did not affect fertilization success in rainbow trout. If stress was affecting egg quality in yellowtail flounder, its effect was probably on rates of vitellogenesis or on the somatic condition of the females. Carragher *et al.* (1989) indicated that cortisol affected the reproductive endocrinology of brown and rainbow trout, and reduced rates of vitellogenesis.

Female yellowtail flounder showed significant individual differences in egg quality. In 1994, maternal effects were present, even when early spawners who experienced cooler conditions were removed, mainly due to the influence of poor egg quality in females C and L. The two analyses determining whether 1995 maternal effects were due to the data from early spawners were contradictory, and indicated that a maternal effect was more likely due to different environmental conditions experienced by the individual females.

The non-significant result of the first analysis reflects the loss of discernibly poor egg quality females. The significant result of the second analysis, after the input of only eight batches, indicates that the result of the previous analysis may have been an effect of the sampling protocol. In any case, it is clear that there was a reduction in the magnitude of a maternal effect on egg quality in 1995.

Various factors may be responsible for maternal effects. Individual variation in feeding rates and ration will affect nutritional condition and reserves of material required for vitellogenesis, oocyte assembly and chorion integrity. Individual variation in stress levels will cause differences in the hormonal environments within each female, which will affect reproductive performance. If feeding ration and nutritional composition are not optimized, individual variation in egg quality may be magnified. A reduction in the significance of a maternal effect in 1995 may have been due to the introduction of the commercial diet which may have improved the nutritional condition of the females.

The environment of the ovarian lumen may also vary among individuals, namely the amount, osmolarity and pH of the ovarian fluid. It was observed that egg batches of low fluidity tended to over-ripen more quickly in yellowtail flounder. Female A, in 1995, had the lowest mean fertilization rate and was noticed to have batches with low amounts of ovarian fluid. Ovarian fluid may serve as a protective buffer for eggs from mechanical damage by muscular contractions in swimming, natural spawning or from pressure in stripping the eggs. Ovarian fluid was noted to vary in both amount and colour among female cod, *Gadus morhua* (Kjørsvik & Lønning, 1983). Furthermore, cod females producing normal eggs had ovarian fluid which was more transparent and less viscous than

those females producing poorer eggs.

Egg quality in female yellowtail flounder was generally not associated with ovulatory activity, with the exception of female L in 1994 which had the poorest egg quality and had very irregular ovulatory activity. Howell & Scott (1989) observed peaks in egg quality which corresponded to the two to four day inter-ovulatory periods found in female turbot. Conversely, egg quality in yellowtail flounder showed no such pattern in a female, where even small batches were capable of high egg quality. According to Kjesbu (1989), only cod females with regular rhythms had consistent egg quality. In contrast, the results for yellowtail flounder demonstrated that egg quality fluctuated widely in both females with regular and irregular ovulatory rhythms.

In both years significant batch effects were found within females, which indicates that females were not consistent in their egg quality. Significant batch effects did not appear to arise solely from temperature variability over a female's spawning season, since even early spawners, which experienced the most stable temperature conditions, had highly significant effects. Therefore the initial conclusion that females are not consistent in egg quality remains tenable, although temperature variability magnifies inter-batch variation by over-ripening.

If it is assumed that all batches have 100 % viability at ovulation, then the batch differences observed would have to be due to variability in degrees of over-ripeness; where either environmental temperature variation altered over-ripening rates between batches or females ovulated egg batches which, when collected, ranged anywhere from 0 to 24 hours old. However, the inter-batch variation in the proportion of visibly

abnormal eggs, with characteristics of over-ripe eggs much older than 24 hours, leads to the hypothesis that not all batches begin with 100 % viability when they are ovulated. The incidence of chorion defects, which may occur randomly among the batches of a female, may contribute to lack of consistency in egg quality within a female. Changes in the amount of ovarian fluid in the lumen may also affect inter-batch variation in egg quality.

Since batch spawning by definition involves the production of a series of ovulations, it is advantageous to determine whether there is a period within a female when eggs of the highest viability are produced. Daniel *et al.* (1993) mentioned that in halibut, *Hippoglossus hippoglossus*, " it is generally known that the middle batches produce better quality eggs". Studies on cod (Solemdal *et al.*, 1992; Kjørsvik, 1994) and barfin flounder (Koya *et al.*, 1994) suggested that there was a periodicity to the appearance of high egg quality batches. However, these studies related the timing of these batches to the group spawning season, which did not indicate whether individual females demonstrated the same periodicity. In some captive, female halibut, which were followed through out their spawning seasons, fertilization rates became more variable towards the end of the spawning season, despite a fairly constant temperature regime (Norberg *et al.*, 1991). McEvoy *et al.* (1993) indicated that turbot showed no differences in survival between early or late batches of a female. The data available for yellowtail flounder revealed no indication of a period within the spawning season of a female where egg quality was highest. In fact each of the three temporal subdivisions of the data for a female showed large amounts of variation.

CONCLUSIONS

This investigation has ascertained that yellowtail flounder is a batch-spawner with a one-day inter-ovulatory period and is capable of efficient egg production in captivity. Yellowtail flounder appear to ovulate a large number of batches of a small size, smaller than has been previously suggested by the literature. Regarding egg quality, despite improvement in 1995, a large proportion of eggs ovulated by the average female do not survive to hatching. While over-ripening may be a major factor affecting egg quality, there is evidence that other factors are involved which are related to nutrition and stress. Furthermore, information on egg quality in yellowtail flounder has lead to the conclusion that individual females can show large differences in their ability to produce viable eggs. In addition, individual females demonstrated inter-batch inconsistency in egg quality and did not show any regular timing in the production of their highest quality batches.

The findings of this investigation has indicated a need to determine the optimal nutritional requirements of this species. Investigations should focus upon the roles of dietary ration and composition on a) minimizing the occurrence of non-reproductive and resorbing females; b) maximizing growth and egg production in females; c) increasing fertilization success and the viability of eggs at collection. Measures should also be taken to lessen the amount of stress experienced by brood stock in captive situations. This may be done by simulating light intensities most frequently experienced by wild fish and decreasing the amount of human disturbances audible or visible to the brood stock. Regarding environmental conditions, spawning temperatures peaking at 6 °C are suggested for yellowtail flounder in captivity, since early spawners had higher egg quality than later spawning females in both years of the study.

The present work has shown some insight into the female reproduction of yellowtail flounder, another batch-spawning species for which little information was originally available. It has also been shown that yellowtail flounder, from a brood stock management point of view, have the potential for aquaculture development. An encouraging aspect with respect to the suitability of yellowtail for aquaculture or for research includes its ability to withstand handling during the stressful spawning period.

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IMAGE EVALUATION
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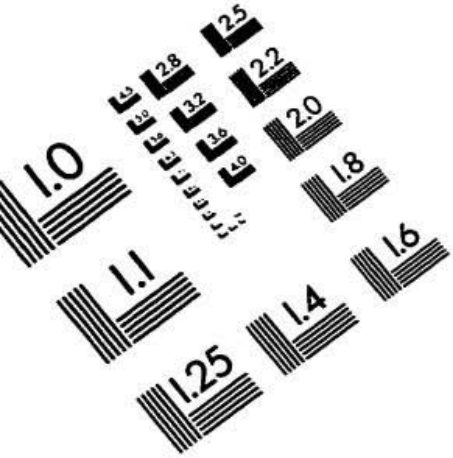

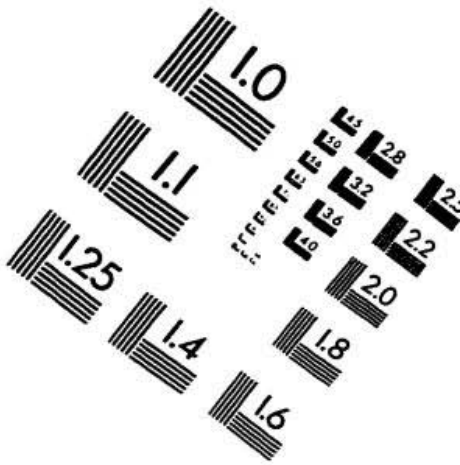

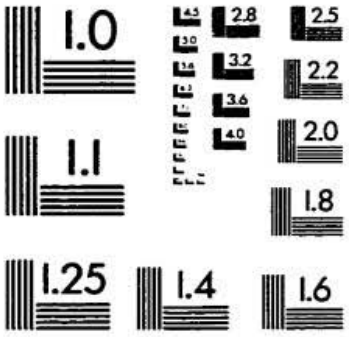

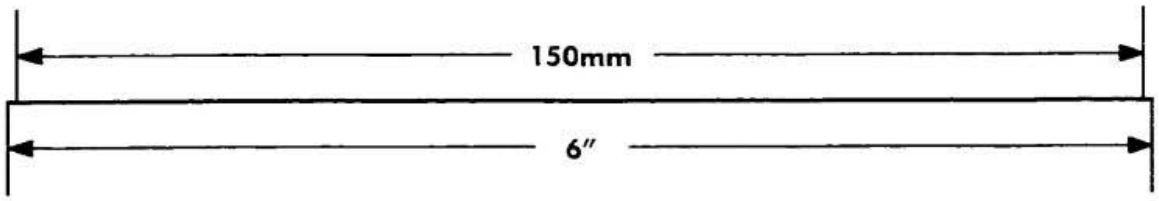

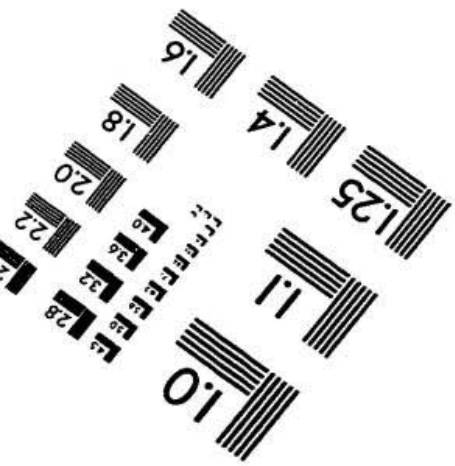

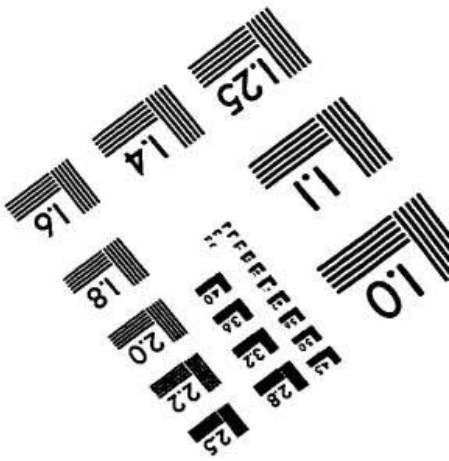

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