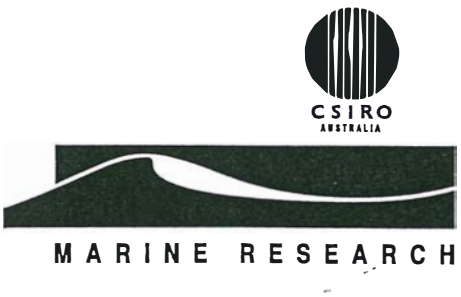
Development of the egg production method to assess the blue grenadier stock in the southeast fishery

Dr. Tony Koslow and Ms Cathy Bulman



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CONTENTS

NON-TECHNICAL SUMMARY

95/035 Development of the egg production method to assess the blue grenadier stock in the South East Fishery

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Objectives:

1. To develop the use of the egg production method to assess the biomass of blue grenadier on the west Tasmania spawning ground. To achieve this overall objective completion of the following sub-objectives was required.

   a. Completion of surveys of the blue grenadier egg distribution during the 1995 and 1996 spawning seasons, covering the full period and area of blue grenadier spawning. The 1994 survey provided the initial biomass estimate.

   b. Determination of the mean fecundity of the blue grenadier.

   c. Determination of the development rate of the blue grenadier eggs as a function of temperature.

Non-technical summary:

The blue grenadier stock is potentially the largest fish stock in the SEF, and following the decline in quota of orange roughy, the need to assess its potential became urgent. The stock has been surveyed using traditional trawl methods and acoustics, but both methods provide only 'snapshot' estimates and are unable to provide absolute estimates. There are two reasons for this: first, the stock is not currently being fished down so relative biomass estimates cannot be calibrated, and second, the proportion of fish spawning on the ground at any time —a factor by which the "snapshot" estimates must be multiplied to obtain an absolute estimate—is unknown.

Annual egg production estimates of stock size based upon egg surveys have been widely and successfully used since 1895 and this method has recently been used in the SEF with orange roughy. The method can provide an absolute measure of spawning stock biomass. Egg surveys tend to be less precise statistically than acoustic surveys, but there is less non-statistical error associated with the supplementary data required for egg production biomass assessment compared with those for an acoustic assessment.

Blue grenadier met the criteria for use of the annual egg production method of stock assessment. The blue grenadier spawning stock off western Tasmania was first surveyed from June through September 1994. The survey design was refined and the area resurveyed the following year.

Fecundity—the number of eggs spawned by a female in a spawning season—was determined from samples of ovaries taken from fish caught by commercial fishermen. It was significantly different in each year, therefore fecundity relationships specific to each year were used in the biomass calculations.

Development rate could not be determined so we used developmental data obtained from earlier experiments. Because the depth distribution of the eggs and their temperature history was unknown, two values for development time were used to bracket the expected development time dpending on whether the eggs rose either slowly or quickly through the water column .

Based on 5% daily mortality and a 1:1 sex ratio, spawning biomass was estimated at 85 478–102 261 tonnes in 1994 and 64 985–77 662 tonnes in 1995, depending on development rate. We examined the sensitivity of the biomass estimates to uncertainty in three parameters: incubation time, egg mortality and sex ratio of the spawning stock. The estimates were approximately inversely proportional to egg incubation time. A 21% increase in development time led to a 16% decrease in estimated biomass. Increasing or decreasing egg mortality by 0.05 per day, had less effect on the estimates. If we assumed no mortality, the estimates decreased by 6% but if daily mortality was increased to 10%, the estimates increased by 6-7%. Uncertainty of the sex ratio of the fish on the spawning ground had the largest effect on the biomass estimate. We assumed monthly sex ratios of 1:1, typical of the non-spawning period because the ratios obtained from during the spawning season (1.68-2.07) could have been biased if the sexes differed in their vulnerability to capture or if they had different residence times on the grounds. Use of the ratios observed during the spawning period led to increases in the biomass estimates of 31-32%.

Keywords: Egg production method, blue grenadier, stock assessment

1 BACKGROUND SUMMARY

The blue grenadier stock is potentially the largest fish stock in the SEF, and following the decline in quota of orange roughy, the need to assess its potential became urgent. The stock has been surveyed using traditional trawl methods and acoustics, but both methods provide only 'snapshot' estimates, and are unable to provide absolute estimates. There are two reasons for this: first, the stock is not currently being fished down so relative biomass estimates cannot be calibrated; and second, the proportion of fish spawning on the ground at any time —a factor by which the "snapshot" estimates must be multiplied to obtain an absolute estimate—is unknown.

Egg production estimates of stock size based upon egg surveys have been widely and successfully used since 1895, and in fact, this method has most recently been used in the SEF with orange roughy. It provides an absolute measure of spawning stock biomass. Egg surveys tend to be less precise statistically than acoustic surveys, but there is less non-statistical error associated with the supplementary data required for egg production biomass assessment compared with those for an acoustic assessment.

Fish suited to this method must have determinate annual fecundity, i.e. all oocytes to be spawned during a spawning season are clearly differentiated from the reserve oocytes—always present in ovaries—at the beginning of the season (Hunter *et al.* 1985). This condition has been demonstrated for blue grenadier *Macruronus novaezelandiae* (Gunn *et al.* 1987, Zeldis 1993). It has also been demonstrated that this species is a multiple spawner, i.e., all potentially mature eggs are spawned in several smaller "batches" over the spawning season. To determine the maximum potential fecundity, it is also necessary to examine the occurrence and extent of atresia, i.e. eggs resorbed at the end of the season.

Another requirement for this method is knowledge of the species' spawning area and the period over which it spawns. The major spawning ground of blue grenadier in Australia is off the west coast of Tasmania. Spawning occurs in winter, generally from June to September but can begin as early as May (Smith 1994). The fish do not stay on the ground for the whole season. The length frequency distribution and sex ratio change throughout the season indicating movement of fish to and from the spawning area, however the rate of these movements is unknown for either sex (Smith 1994).

Therefore, the blue grenadier stock that spawns off western Tasmania was reasonably well suited to assessment based upon the egg production method. Spawning stock biomass (B) may be estimated simply from estimates of egg production summed over the spawning season (E), mean stock fecundity (F), the male:female ratio (m/f), and the mean weights of male and female grenadier in the spawning run (W_m and W_f, respectively)

$$B = (E/F)\cdot(W_f + W_m\cdot m/f) \quad (1).$$

In 1994, the egg production method was trialed on the west coast blue grenadier stock. Two-week egg surveys were carried out during each month of the spawning season (June-August). The surveys covered the expected overall area of distribution of grenadier eggs, and more intensive sampling was carried out in areas of grenadier aggregation. It is critical that the survey area encompass the entire area of distribution of grenadier eggs, and a primary objective of the first years sampling was to ascertain the pattern of distribution of the eggs. This was achieved.

Although the 1994 egg survey was intended primarily to serve as a pilot study, the sampling proved adequate to provide a preliminary biomass estimate, albeit with wide confidence limits due to statistical and non-statistical sources of uncertainty. The initial estimate of spawning stock biomass varied between 365,000 and 558,000 tonnes, however this estimate was found to be excessively high because of patchiness in the egg distribution and use of a very high egg mortality rate. The 1994 estimate was recalculated after post-stratifying the survey area and adopting a more realistic egg mortality rate. Fecundity was assessed based on analysis of 40 blue grenadier collected in 1994. The success of the 1994 survey only became apparent after the proposal to FRDC to continue the survey in 1995 and 1996 had been submitted, i.e. following analysis of the samples and data.

The sampling design in 1995 was modified so that fewer stations were assigned to areas around the periphery of the 1994 egg distribution and more stations assigned to the core area of the egg distribution. It was anticipated that the CV of the 1995 survey would be reduced both because more stations could be sampled and the stations were stratified into areas of high, medium and low density. Fresh running ripe fish for incubation experiments were not caught. The observer on board a commercial grenadier vessel provided improved data on the sex composition of fish in the catches, although this did not resolve the issue of a possible bias due to either variable vulnerability of the sexes to capture or a different residence time on the spawning ground.

It appeared that valid biomass estimates would be forthcoming from the 1994 and 1995 field seasons, so the 1996 egg survey was cancelled. However, in 1996, a technician was onboard a commercial vessel to initiate egg development experiments and obtain fecundity samples. Running-ripe fish were not caught so incubation experiments were not run. However more samples for fecundity were collected.

2 NEED

In the SEF, the blue grenadier fishery has possibly the greatest potential for further expansion, as well as some of the greatest uncertainty concerning present stock size. Due to the contraction of quotas in the orange roughy fishery, there is an urgent need to define the sustainable yield for the blue grenadier fishery, which may have potential to take up excess effort from the roughy fishery.

A research project based upon development of the egg production method for use with blue grenadier was proposed for 1994-96 to assess the biomass of the western Tasmania stock. The egg survey method was selected as most likely to provide a reliable biomass estimate in the near term. Because the turnover of grenadier over the protracted spawning period is presumably large and difficult to ascertain, 'snapshot' survey methods (e.g. acoustic or trawl surveys) can only assess a fraction which may vary from year to year. No significant technical problems were anticipated in carrying out an egg survey on this stock. However, it was unlikely that a single year's egg survey could provide a reliable biomass estimate. Because the 1994 survey was originally envisaged as a pilot survey, two further surveys were first proposed for the 1995 and 1996 spawning seasons. Based on the success of the 1994 survey, only one further egg survey was carried out (in 1995) and the effort in 1996 concentrated on remedying remaining gaps in the method, namely improving estimates of fecundity and egg development (and hence egg mortality) rate.

3 OBJECTIVES

Overall objective: To develop the use of the egg production method to assess the biomass of blue grenadier on the west Tasmania spawning ground. To achieve this overall objective completion of the following sub-objectives was required.

1) Completeion of surveys of the of the blue grenadier egg distribution during the 1995 spawning seasons, covering the full period and area of blue grenadier spawning. The initial (1994) survey provided the first biomass estimate.

2) Determination of the mean fecundity of the blue grenadier.

3) Determination of the development rate of the blue grenadier eggs as a function of temperature.

4 METHODS

4.1 Sampling Design

In 1994, the west coast of Tasmania was surveyed from 40° 30'S to 43° 15'S (Fig. 1a). Transects were spaced at 15 nautical m intervals and stations on the transects were spaced at 10' longitude (= 7.5 nautical m or 13.9 km) from as close inshore as practical (min 50 m depth) to 1000m depth. In addition, four canyons containing aggregations of blue grenadier suspected of spawning, were surveyed more intensively, in a 3 x 3 station pattern with 2 nautical m between each station, giving a total of 9 stations in 16 sq. nautical m (=123.48 sq. km).

The coast was surveyed three times over the span of the spawning season: at the beginning (7–12 June), midway (22 July–5 August), and at the end (25 August–8 September). Only one of the canyons was sampled during the first survey because of bad weather but all areas were sampled during the second and third surveys.

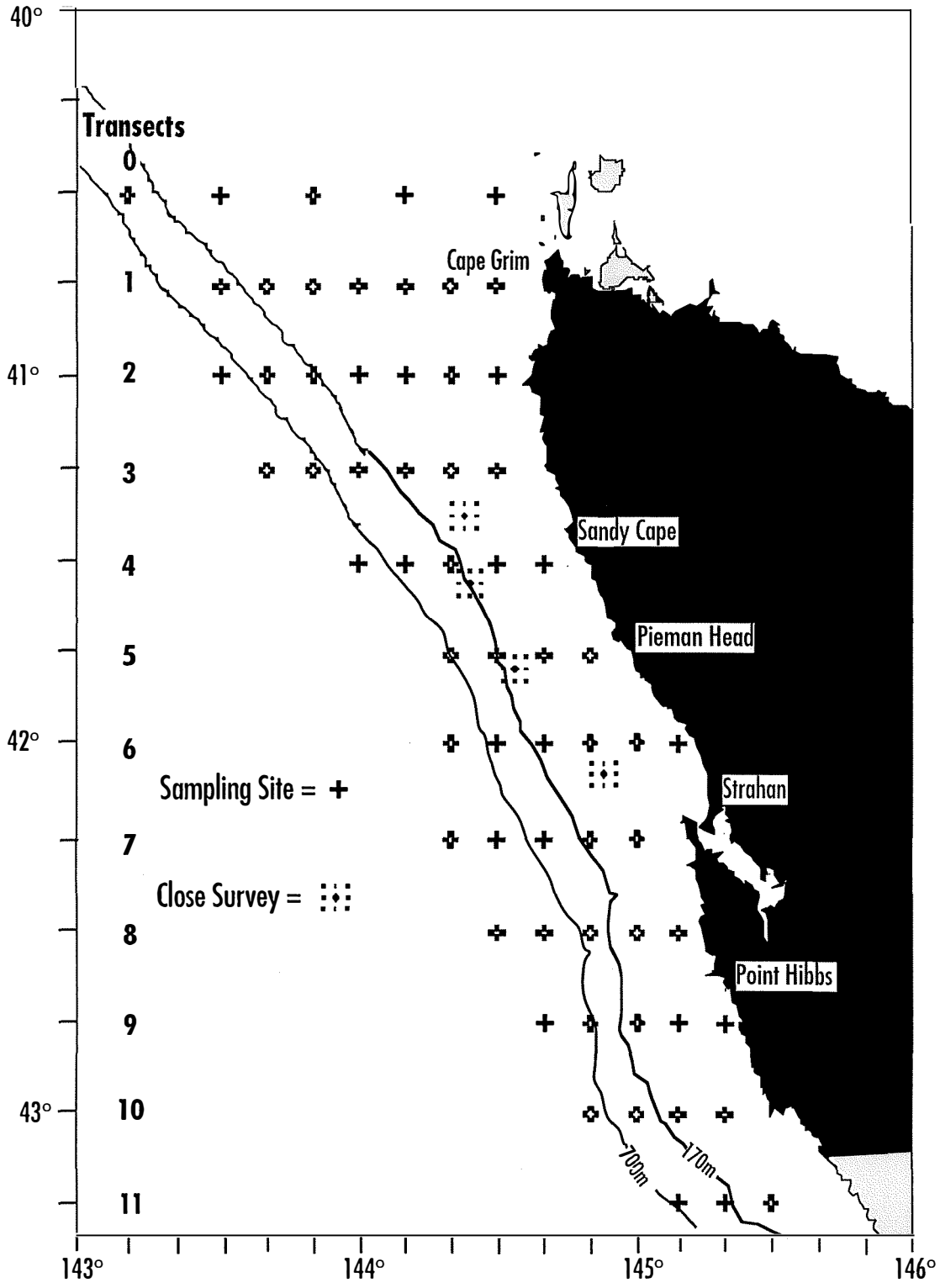


Fig 1 a. Survey area off the west coast of Tasmania showing survey design used in 1994.

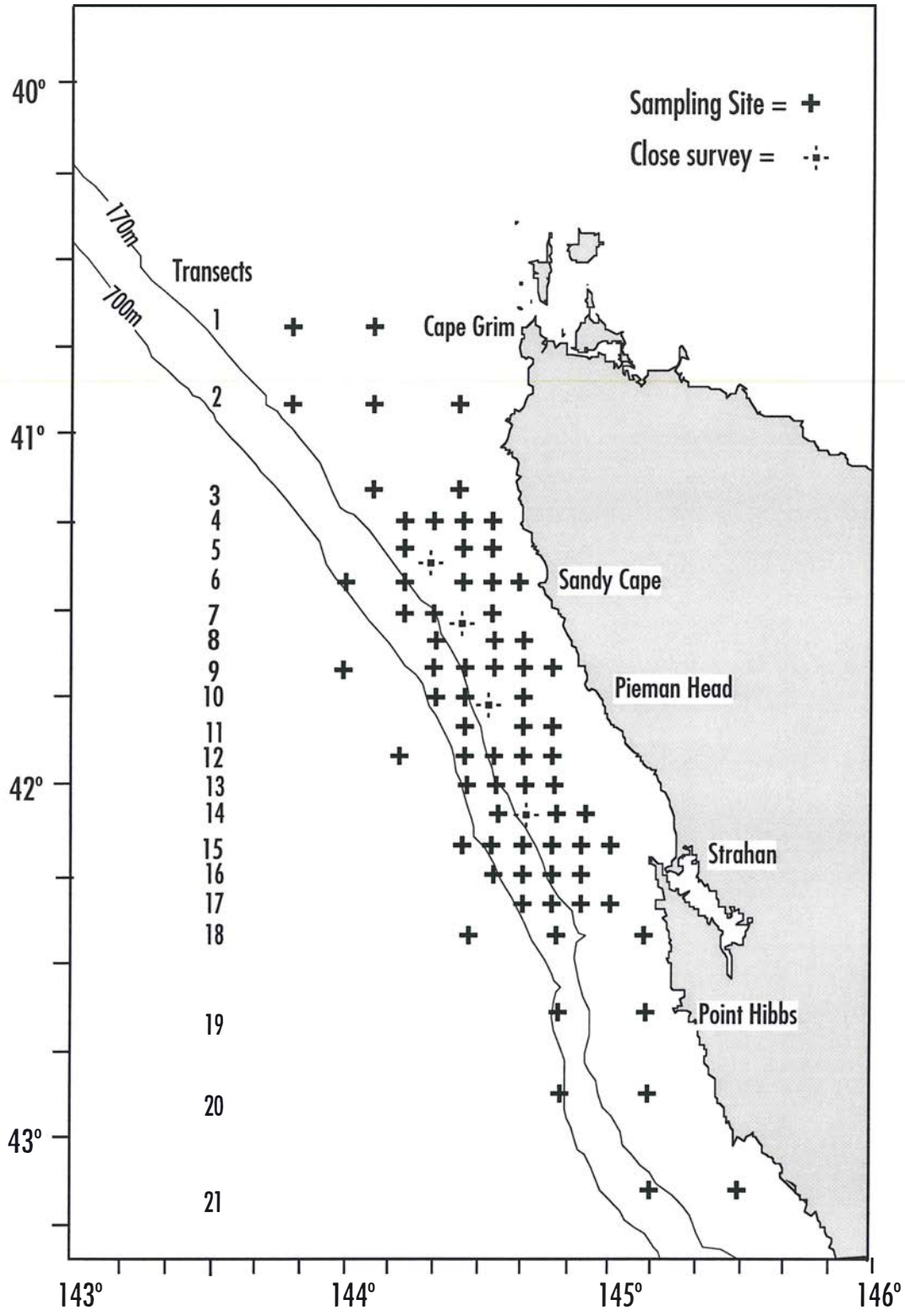


Fig 1 b. Survey area off the west coast of Tasmania showing survey design used in 1995.

In 1995, the survey design was modified based on information from the previous survey. We surveyed from 40° 40'S to 43° 10'S (Fig. 1b). The area was sampled at three densities: low on the periphery of the survey area, medium in the centre and high at the 4 localities of high spawning activity. Around the periphery of the survey area, i.e. in the low density area, transects were spaced at 15 nautical m intervals and stations on the transects were spaced at 10' longitude (c. 7.5 nautical m). In the medium density area, transects were spaced at 5 nautical m apart and stations were about 5 nautical m apart along the transects, except where they overlapped a high-density station. The four high-density areas were sampled by 5 stations, with each of 4 stations placed 2 nautical m north, south, east and west of a central station.

As in 1994, the coast was surveyed three times over the span of the spawning season: at the beginning (15-27 June), midway (12-25 July), and at the end (8-14 August).

At each station a 2 m^2 plankton net with 500 µm mesh was deployed vertically to within 10 m of the bottom. A sounder attached above the net enabled us to monitor the depth of the net in depths less than 900 m. In depths greater than 900 m (up to 1000 m), a length of wire the same as the bottom depth was deployed. The net was hauled at about 60 $m.min^{-1}$. A calibrated Rigosha flowmeter measured the water flow through the net. In 1995, an external Rigosha flowmeter was also fitted to determine whether there was significant clogging or other problems with the internal flowmeter. In 1994, at about one-third of the stations, a Sea Cat water profiler was deployed to record temperature and salinity of the water column. In 1995 a submersible data logger was used.

The plankton samples were preserved in 5% formalin buffered with sodium ß-glycerophosphate. In the laboratory, blue grenadier eggs were identified and removed from the plankton.

4.2 Staging of blue grenadier eggs

Table 1. Development times of *Macruronus novaezelandiae* reared artificially.

Reference	Temperature range (°C)	Mean temperature (°C)	Time to hatch (h)
Patchell *et al* (1987)	10-11		96
"	14-14.5	14	52-60
"	11.2-13.2	11.9	80-84
"	11.2-12.4	12	81-82
Bruce (1987)	14-19	14	55-60
Bulman (unpub.)	10-14	12	66

Our staging criteria were based on observations from incubation experiments in 1992 (Bulman unpub. data) and those by Patchell *et al.* (1989) and Bruce *et al.* (1989) (Table 1):

Stage 1 (Fertilisation to first division, 0-2 h). One hour after fertilization, a clear "disc" of cytoplasm accumulated at one pole. Small vesicles were observed around the edge of the blastodisc. Oil droplets accumulated into a single droplet at the other pole.

Stage 2 (First division to morula, 2-10 h). At 2 h, 2 to 4 cells; 8 cells at 4 h; 16-32 cells at 5 h: 128 cells at 8 h. Periplast formation was evident.

Stage 3 (Morula to early gastrulation, 10-20 h). The small-cell morula became very concentrated. The morula then spread over the periplast to cover it entirely. The blastula formed revealing periplast or germ ring, which thickened at the beginning of gastrulation.

Stage 4 (Gastrulation to organogenesis, 20-30 h). The embryo formed along the embryonic shield. The neural tube was just visible.

Stage 5 (Germ ring from 2/3 down yolk to blastopore closure, 30-40 h). Embryos had optic vesicles and developed notochords.

Stage 6 (Blastopore closure to separation of tailbud, 40-48 h). Pigment was evident on body of embryo. More than 20 somites were visible towards the end of this stage.

Stage 7 (Tail lifted up to 1/4 of a body length, 48-60 h). The tails were quite pointed. Pigment increased; two long stripes extended from the head down the back.

Stage 8 (Tail lifted more than 1/4 of a body length to hatching, 60-66 h). The tail twists to one side when more than half the length of the embryo is separated from the yolk.

Development rate depends on the depth distribution (and, consequently, temperature) of the eggs but we were unable to define their depth distribution. However we have temperature profiles of the spawning areas from both surveys. In 1994, the temperature of the water column in area B, an area of high spawning activity, at 457 m was 9.7°C and was evenly mixed in the upper 200m at 14°C (Fig 2). At temperatures from 10-11°C, egg incubation

took 96 h; between 11.2 and 13.2°C it took 82 h (Patchell *et al.* 1987). At higher temperatures incubation time is reduced: 52-60 h at 14°C (Patchell *et al.* 1989) and 55-60 h at 14-19°C (Bruce 1987). The eggs are positively buoyant and are expected to ascend through the water column but their rate of ascent is unknown. We used two estimates of incubation time in our biomass estimates: 66 h based on an experiment where incubation temperature rose from 10°C at fertilization to about 14°C at hatching, and 80 h assuming the eggs remained largely at 11-13°C.

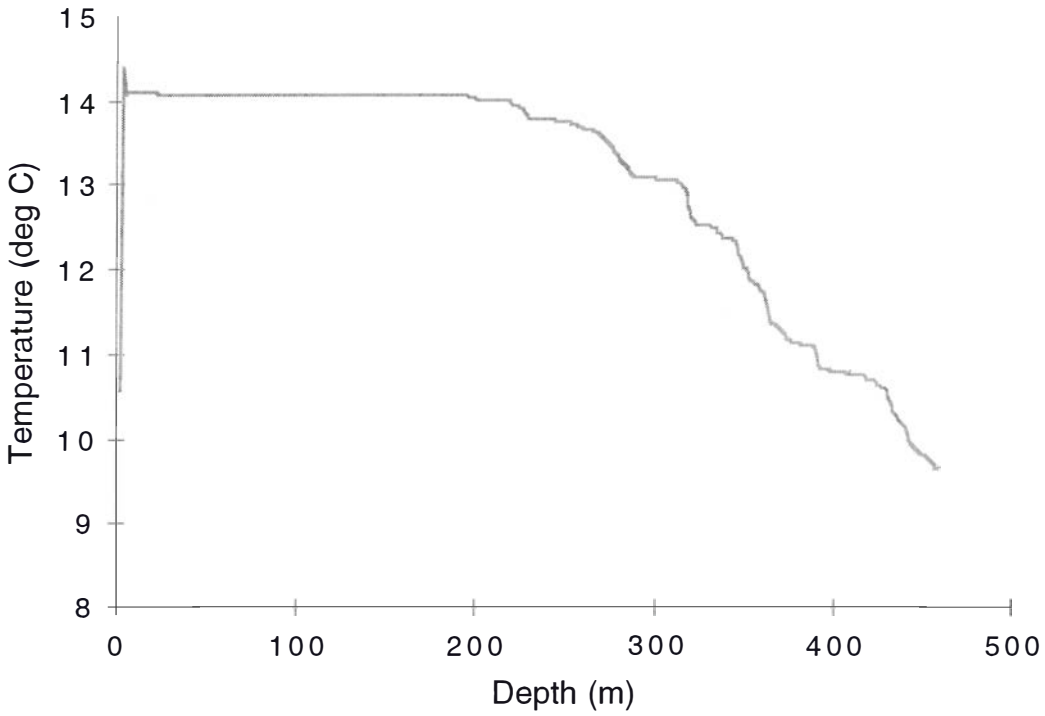


Fig 2. Temperature profile of water column at area B, during survey 2 in 1994.

4.3 Adjustment of egg counts

Unstageable eggs in a sample (station) were pro-rated according to the age structure of the stageable eggs in that sample. Then the pro-rated counts per stage in each sample were adjusted to eggs per m^2 so that

$$N = \frac{N_{obs}}{a}\frac{d}{b} \qquad (2)$$

where N is the adjusted number of eggs per m^2 of sea surface area, N_{obs} is the number of observed and pro-rated eggs, a is area of the ring net (= $2m^2$), d is the tow depth determined by the net sounder on the ring net, and b is tow length determined from flowmeter or wire-out readings.

4.4 Mortality

Stratum egg abundance per stage, N_{sj}, was calculated by normalising the mean egg abundances per stage for stage duration, h_s, total incubation time, and the area of the stratum, A_j, where s is egg stage, j is stratum, i is samples (stations), n is the number of samples in stratum j, and N_{sji} is the number of eggs per stage per sample per stratum:

$$N_{sj} = \frac{\sum_{i=1}^{n} N_{sji}}{n} * \frac{\sum_{s=1}^{8} h_s}{h_s} * A_j. \quad (3)$$

The survey egg abundance per stage was obtained by summing the stratum egg abundances per stage,

$$N_s = \sum_{j=1}^{n} N_{sj}. \quad (4)$$

The instantaneous mortality rate per hour, z, was estimated from the slope of the regression of the egg abundances per stage, N_s, (log transformed), against the ages at the midpoint of each respective stage, t_s, using the model,

$$N = N_0 e^{-zt} \quad (5)$$

where N_0 is the initial number of eggs spawned.

4.5 Sex ratio

The sex ratios of blue grenadier caught on the west coast of Tasmania during the spawning season were determined from commercial catches from the Tasmanian Department Primary Industries and Fisheries, Bureau of Resource Sciences (Scientific Monitoring Program) and the Victorian Marine and Freshwater Institute and from our own data collected during factory visits. Sex ratios for the months of June, July and August were calculated from data combined over the years 1992-1995.

Using the length-weight relationship of $W = 0.00375*SL^{3.013}$ (J. Lyle, pers. comm.; SEFSAG 1993), the female and male length frequency distributions were converted to weight frequency distributions and weighted by relative proportions in catches for each month. The monthly values were used in the biomass estimates.

4.6 Fecundity

4.6.1 Sampling

In 1994, ovary samples were taken from a total of 40 females and in 1995, from 51 females. Females with ovaries composed of stage 2 (maturing) or stage 3 (ripe or hyaline) eggs were selected. Total length and weight (with ovary) of the fish were measured and the gonads removed intact, weighed and frozen. Where macroscopic staging of an ovary was uncertain, samples for histology were collected from ovaries for microscopic verification.

4.6.2 Sampling sites in ovary

We first tested for differences in oocyte density (i.e. numbers of oocytes g^{-1}) within the ovaries and for the optimum number of samples from the ovary needed to determine fecundity. In 1994, a subsample of 10 fish was used to test for the effects of lobe (i.e. right or left side of ovary), and position of sample within a lobe on fecundity. Three positions were sampled (posterior, medial, anterior) in each lobe of the ovary of each fish, giving a total of 6 samples per ovary.

Samples of about 1 g were "cored" from the frozen ovary. The cores were weighed and thawed and preserved in Gilsons fluid to digest tissue and loosen the oocytes for counting. All visible, i.e. yolked, oocytes were counted. Reserve oogonia were too small to be visible in this process, and therefore were easily excluded from the counts.

An analysis of variance was performed on the square root of the oocyte counts per gram of ovary weighted by the sample weights:

$$\sqrt{\frac{c_n}{z_n}} . z_n \qquad (6)$$

where for fish m, c_n is the oocyte count for sample n and z_n is the weight of sample n. The square root approximately stabilised the variance of the oocyte counts which followed a Poisson distribution so that larger samples had larger variances. Since the variance of $\sqrt{c_n}$ was approximately constant, the variance of $\sqrt{\frac{c_n}{z_n}}$ then became proportional to the inverse of the sample weights.

4.6.3 Optimum number of samples per ovary

The subsample of 10 fish was also used to estimate the optimum number of samples, q, required to estimate fecundity. We attempted to follow the method of Hunter *et al.* (1985, 1992), however modifications were necessary to allow for unequal variances and a lobe effect found in the previous analysis.

We assumed a linear model to estimate fecundity for fish m of weight W_m and ovary weight Z_m, with error A_m and error in the oocyte count per gram of ovary, f_m,

$$\hat{Y}_m = \alpha + \beta W_m + A_m + Z_m f_m$$

$$= \alpha + \beta W_m + \xi_m \quad (7)$$

where

$$\xi_m = A_m + Z_m f_m \quad (8)$$

The oocyte count error, f_m, is composed of the variance of the random lobe effect , σ_L^2 and the variance of the random error, σ_ε^2 , and

$$\mathrm{var}(f_m) = \frac{1}{2}\sigma_L^2 + \frac{1}{q}\sigma_\varepsilon^2 \quad (9)$$

therefore

$$\sigma_{\xi_m}^2 = \sigma_A^2 + Z_m^2\left(\frac{1}{2}\sigma_L^2 + \frac{1}{q}\sigma_\varepsilon^2\right) \quad (10)$$

where $\sigma_{\xi_m}^2$ is the variance about the line in the fecundity regression and σ_A^2 is the true fecundity.

Hunter *et al.* (1985) estimated $\sigma_{\xi_m}^2$ from residuals from the fitted model (equation 10), assuming $\sigma_{\xi_m}^2$ was constant, and then estimated σ_A^2 by difference. Although their method was not strictly valid in our case because $\sigma_{\xi_m}^2$ was not constant, we followed it so we could estimate σ_A^2. So, the within-ovary variances, $Z_m^2\left(\frac{1}{2}\sigma_L^2 + \frac{1}{q}\sigma_\varepsilon^2\right)$, were estimated for a range of ovary weights, Z_m, between 405.2 g and 1215 g, and for a number of samples, q = 2, 4, 6 and 8 (or q/2 per lobe). We estimated σ_ε^2 and σ_L^2, (or s_ε^2 = error MS and $s_L^2 = \frac{\mathrm{lobeMS} - \mathrm{errorMS}}{3}$ respectively), from the components of an analysis of variance on the oocyte counts per sample. Across the range of weights and sample sizes, the within-ovary variance was subtracted from $\sigma_{\xi_m}^2$ to estimate σ_A^2. Optimum sample size is obtained when the ratio of $\sigma_{\xi_m}^2$ to σ_A^2 is minimised.

4.7 Potential annual fecundity

Potential annual fecundity, i.e. annual fecundity uncorrected for atresia, was determined using a gravimetric method (Hunter *et al.* 1985, Hunter *et al.* 1992), where eggs were counted from a subsample of known weight. The number per ovary was obtained by extrapolating the subsample weight to whole ovary weight. Fecundity per fish was calculated from the average of the two lobe counts. Fecundity was regressed against fish weight to obtain relative fecundity (Fig 3). We tested for significant differences in fecundity between the two years.

4.8 Atresia

Gonad sections were taken from spent fish to determine the existence and extent of atresia in spent ovaries and to verify macroscopic staging. Samples were preserved in 10% formalin until preparation. Sections were prepared and stained with haemotoxylin & eosin.

4.9 Biomass estimation

Biomass for each survey was estimated. The adjusted egg counts per stage, s, per station, i, per stratum, j, N_{sij}, were adjusted to allow for mortality using the mortality model,

$$N_0 = \frac{N_s}{e^{-zt}}. \qquad (11)$$

Therefore, the daily production rate per station per stratum, P_{ij}, summed over all stages is

$$P_{ij} = \sum_{s=1}^{8} N_{0i} * \frac{24}{\sum_{s=1}^{8} h_s} \qquad (12)$$

where h_S is the stage duration of stage, s, and $\sum_{s=1}^{8} h_s$ is the total egg development time, e.g. 66 h or 80 h.

The mean daily production rate of the stratum, j, is given by

$$\bar{P}_j = \sum_{i=1}^{n} \frac{P_{ij}}{n} \qquad (13)$$

and its variance by

$$\operatorname{var} \bar{P}_j \quad \frac{1}{n} \frac{1}{n-1} \sum (\quad - \bar{P}_i)^2 \qquad (14)$$

The egg production of stratum j was calculated by

$$P_j = \bar{P}_j A_j \qquad (15)$$

where A_j is the area of stratum, j. Daily egg production for the cruise, P, was obtained by summing the egg production over all k strata;

$$P = \sum_{j=1}^{k} P_j \qquad (16)$$

and variance was calculated by

$$\operatorname{var} P = \sum_{j=1}^{k} \operatorname{var} P_j A_j^2. \qquad (17)$$

The total annual egg production was modelled on a normal curve. The normal curve is described by the function

$$P_t = \frac{\alpha}{\sqrt{2\pi\sigma^2}} e^{-\frac{(t-\mu)^2}{2\sigma^2}} \quad (18)$$

where P_t is the area under the curve, α is the height of the curve, σ^2 describes the spread of the curve, t is the time in days from the start of the season and μ is the position of the peak of the curve.

For each year, the model was fitted to the three points corresponding to the daily egg productions calculated for each survey and their respective midpoints. The area under the curve was integrated, month-by-month over the 3 months, to give the total egg production for the whole spawning season.

The spawning biomass of blue grenadier in each month, u, of the spawning season was estimated by

$$B_{fu} = \frac{P_{tu}}{F_u} W_{fu} \quad (19)$$

where P_{tu} is the monthly egg production, W_{fu} is the monthly average female weight, and F_u is the average fecundity per month. The monthly biomass, B_{tu}, is calculated from the sex ratio, R_u, and average male weight, W_{mu};

$$B_{tu} = B_{fu} + (R \frac{P_{tu}}{F} W_{mu}). \quad (20)$$

The total biomass of spawning blue grenadier is the sum of the three months' biomass estimates.

Confidence limits for the estimates were determined by "bootstrapping" the estimate. Each of the surveys was resampled and a new biomass calculated using the same parameters and models. This procedure was done 800 times and the upper and lower 5th percentiles of the range of estimates were taken as the 95% confidence limits of the biomass estimate.

5 RESULTS

5.1 Sub-Objective 1: Surveys

Peak abundance of eggs occurred during the middle surveys in both years as expected (Table 2). Fewer eggs were caught in the first and last surveys in 1994 than in 1995 because those surveys were conducted further from the peak of spawning than the corresponding surveys in 1995.

Table 2. Details of 1994 and 1995 blue grenadier surveys.

Year	Survey	# stations occupied	# stations eggs present	# eggs
1994	1	50	19	305
	2	84	61	100 944
	3	90	55	1 640
		224	135	102 889
1995	1	86	46	9 511
	2	93	69	71 792
	3	59	21	6 970
		238	136	88 273

5.2 Sub-objective 2: Fecundity

5.2.1 Sampling sites in ovary

The analysis of variance on the square of the weighted oocyte counts did not show any significant effect of position within a lobe nor any consistent effect of lobe ($p=0.659$; Table 3). However a significant effect of lobe within fish ($p=<0.001$) suggested that although the effect was not consistent between left or right lobes, there was a significant difference between the lobes of the ovary within the same fish. Therefore, we used samples from each lobe to determine fecundity.

Table 3. Analysis of variance on square root of oocyte counts per gram of ovary weighted by the sample weights.

Source	DF	SS	Adj SS	F	P
Fish	9	8253.99	917.11	28.78	0.000
Lobe(Fish)	10	318.64	31.86	4.2	0.001
Position	2	6.40	3.20	0.42	0.659
Error	38	288.23	7.59		
Total	59	8867.26			

5.2.2 Optimum number of samples per ovary

The components of within ovary variance were estimated from the results of the analysis of variance on oocyte counts (Table 4). The variance of the true fecundity, $\sigma^2_{\xi_m}$, was estimated from the analysis of variance of the regression of fecundity on weight, i.e. 52.69 x 10^{10} (Table 5). The within-ovary variances were calculated and subtracted from the variance of the fecundity. The ratios, $\frac{\sigma^2_{\xi}}{\sigma^2_{\Lambda}}$, were generally not improved by increasing sample sizes although in larger ovaries it could be argued that 4 samples would be more appropriate (Table 6). We concluded that two samples per ovary (one from each lobe) were adequate to estimate fecundity of the set of fish sampled .

Table 4. Analysis of variance of oocytes per gram of ovary, F_{mn}.

Source	DF	SS	MS	F	P	Expected MS	Variance component
Fish	9	112598328	12510925	22.16	0.000		
Lobe	10	5646556	564656	4.07	0.001	$=\sigma^2_{\varepsilon}+3\sigma^2_{L}$	141961 (σ^2_{L})
Error	40	5550907	138773			$=\sigma^2_{\varepsilon}$	138773 (σ^2_{ε})
Total	59	123795792					

Table 5. Regression statistics of fecundity on weight for the subsample of 10 fish.

Source	DF	SS ($x10^{10}$)	MS ($x10^{10}$)	F	P
Regression	1	105.826	105.826	2.01	0.194
Error	8	421.545	52.6931		
Total	9	527.371			

Table 6. Ratio of variance of the fecundity regression, $\sigma^2_{\xi_m}$, to the estimated variance of the true fecundity, σ^2_A, for a range of ovary sizes and samples per ovary, q.

		Ratio = $\frac{\sigma^2_\xi}{\sigma^2_A}$			
q	Ovary weight	405.2 g	700 g	1000 g	1215 g
2		1.04	1.15	1.36	1.65
4		1.03	1.11	1.25	1.42
6		1.03	1.10	1.22	1.36
8		1.03	1.08	1.20	1.33

5.3 Potential annual fecundity

The fecundity regressions for each year (Fig 3) were significantly different (ANCOVA year: F=4.44, P=0.038) although their slopes were similar (ANCOVA wt*year effect: F=1.16, P=0.285). Therefore fecundity relationships specific to the year were used.

5.4 Atresia

Of the 37 histological sections examined in 1994 and the 67 in 1995, only 1 and 6 respectively were from spent fish. Only one section examined in 1995 showed any atretic oocytes. This is consistent with results from New Zealand blue grenadier (Schofield and Livingston in press), which indicated atresia was not significant.

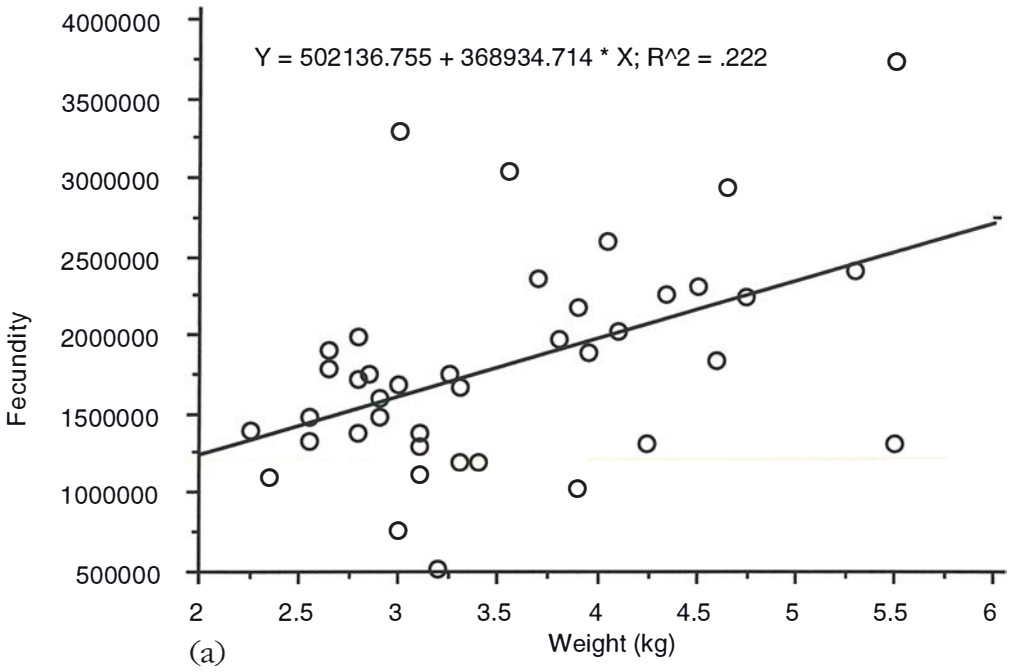


Fig 3a. Regression of fecundity on weight for blue grenadier in 1994 (a: n=40).

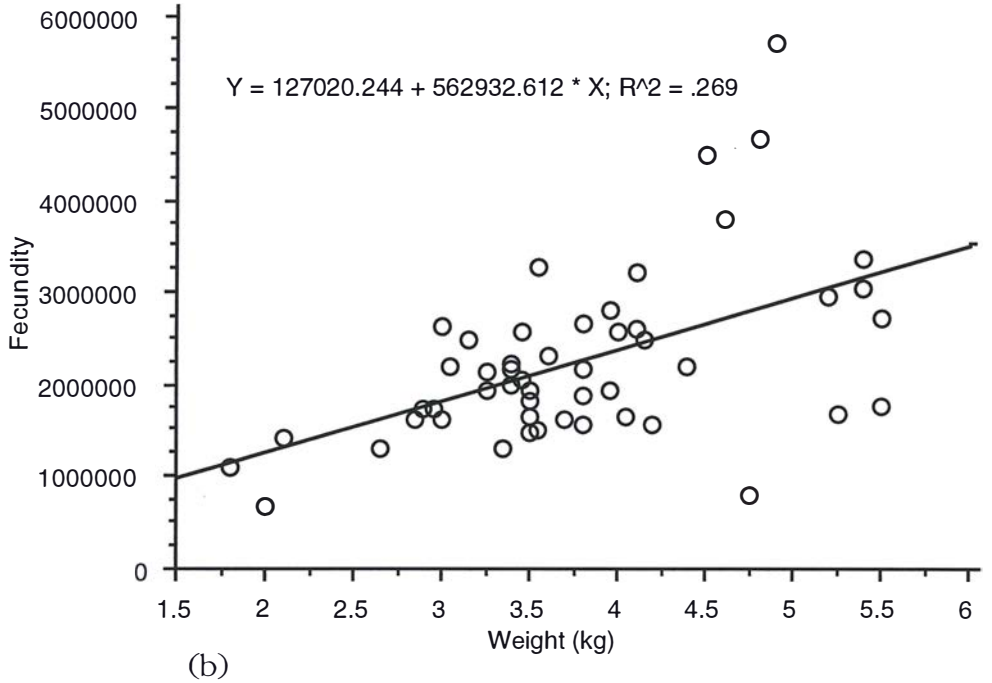


Fig 3b. Regression of fecundity on weight for blue grenadier in 1995 (b: n=51).

5.5 Sub-objective 3: Development rate

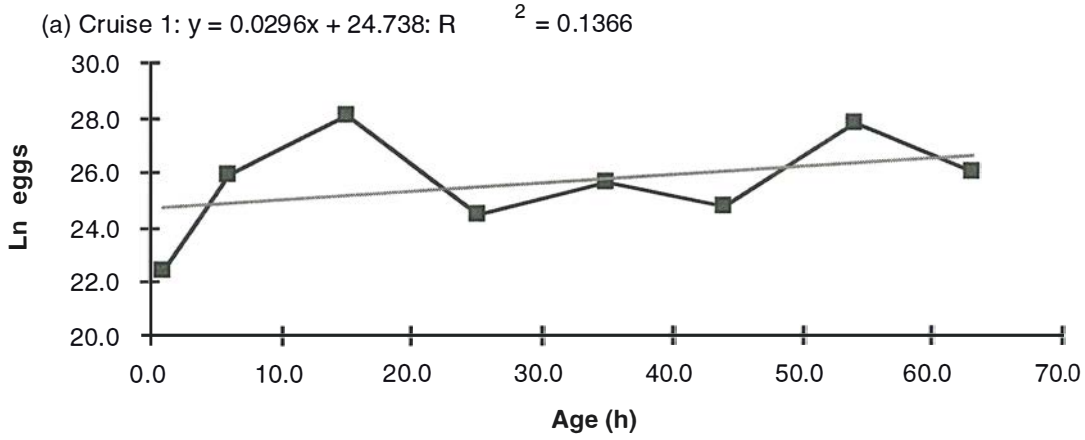
During the two spawning seasons, we were unable to use running-ripe fish in fresh enough condition for successful incubation and so development rate under different temperature regimes could not be determined (refer to Background paragraphs 3 & 4, p. 4). However in 1994, an experiment was conducted on FRV *Southern Surveyor* using onboard incubators that were being used for orange roughy egg incubations. Although only one experiment was run on blue grenadier it provided what we believed was a reasonable estimate of incubation time given the temperature profile of the water column in an area where fish were aggregating.

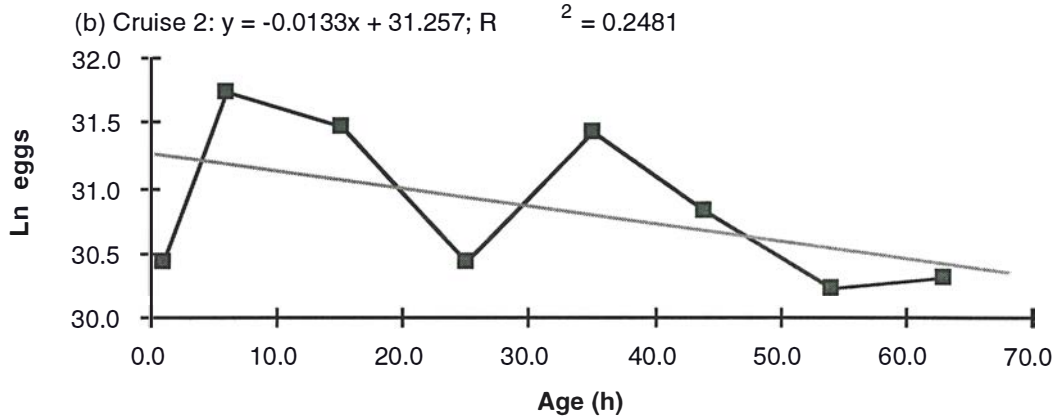
5.6 Mortality

Mortality was not significantly different from 0 during any cruise in either year (Figs 4a-f). In our estimates of egg production, we assumed a mortality rate of 5% per day, since zero mortality was unlikely. Because spawning is largely localised to the canyon areas, amalgamating data from the whole survey area could lead to a positive bias in the monthly estimates. Mortality was also calculated only from the areas where aggregations were found spawning. An opposite bias might be expected from this procedure but significant mortality was still not observed.

5.7 Sex Ratio

The ratio of males to females was between 1.68 and 2.09 to 1, based on the combined data collected during the spawning seasons of 1992 through 1995 (Table 7). The sex ratio outside the spawning season is about 1:1 (Smith pers. com.). The skewed sex distribution seemed more likely to be biased, reflecting a difference in residence time on the spawning ground or in availability to the gear. We used the 1:1 sex ratio in the biomass estimates but we also provide estimates using the ratios observed on the spawning grounds.





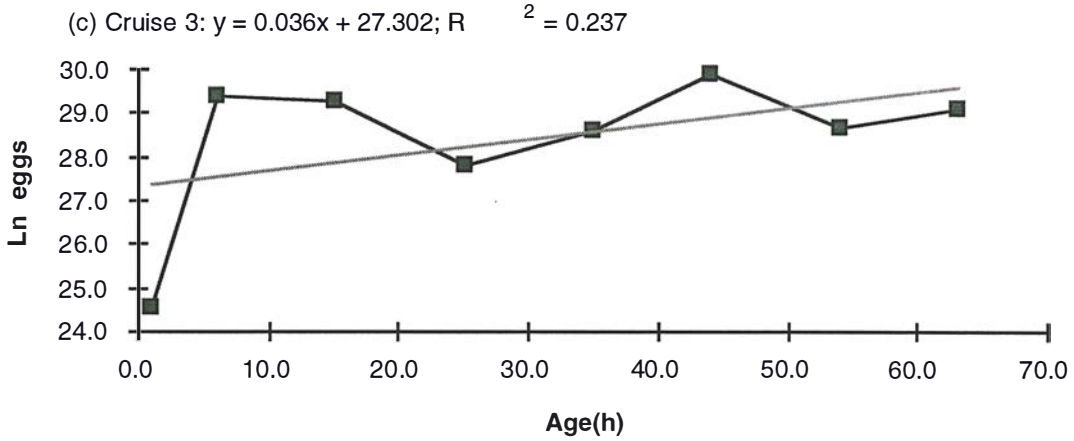
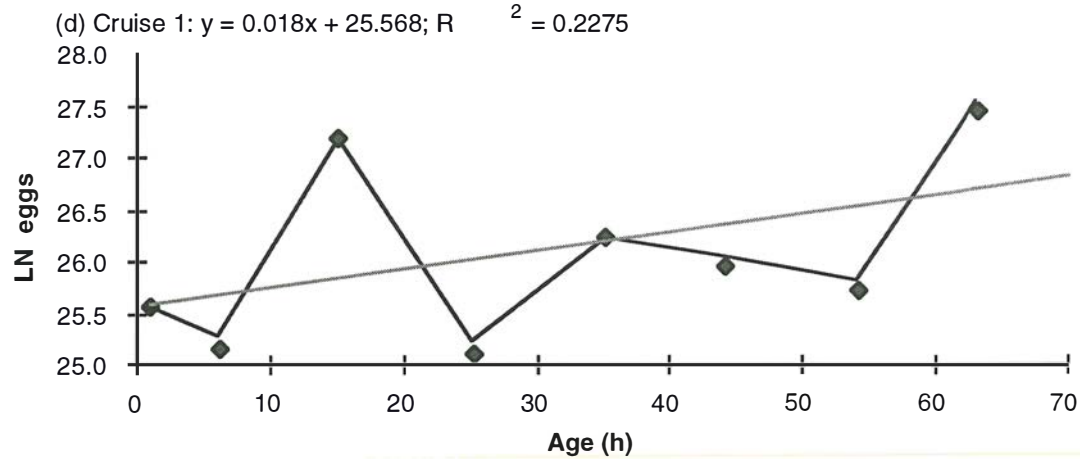
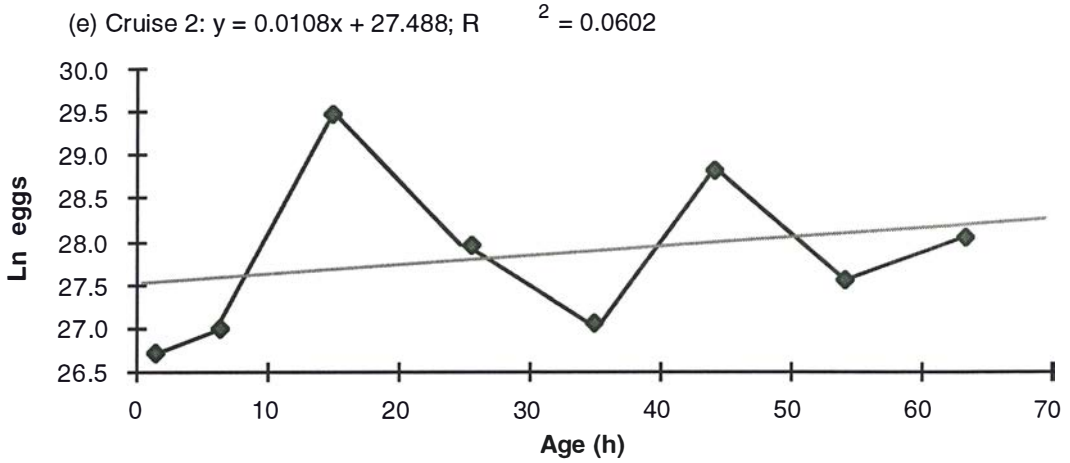


Fig 4 (a-c). Mortality rates for cruises in 1994.





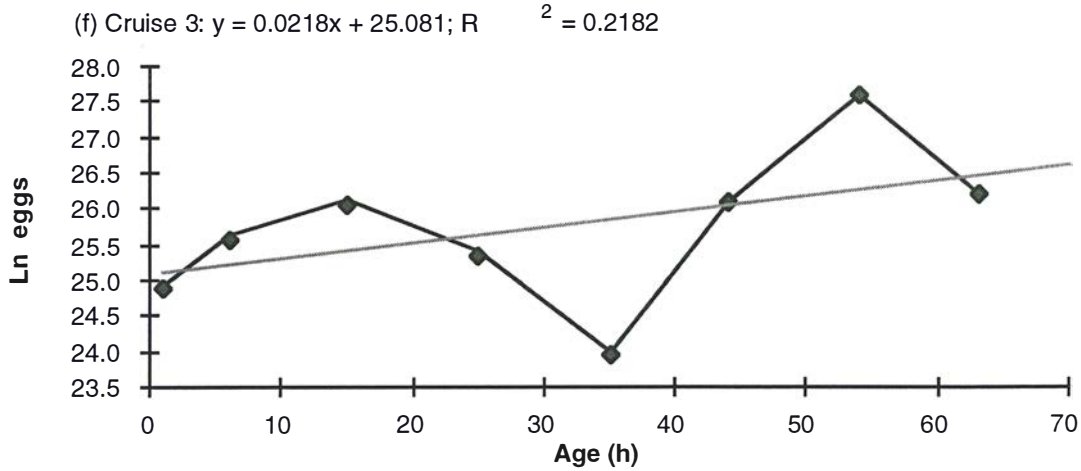


Fig 4 (d-f). Mortality rates for cruises in 1995.

Table 7. Summary of length frequency data (source: BRS, TDPIF, CSIRO) used in estimating sex ratios and average weights of male and female blue grenadier.

		1992	1993	1994	1995	Total	Av Wt (kg)	Sex ratio
June	Males	15473	11800	21218	26604	75095	2.38	1.68
	Females	7332	5800	11204	20392	44728	3.54	
	Wt. measured (kg)	86000	46070	93200	4894	225270		
	No. shots measured	3	3	1	7	14		
	Total wt caught (kg)	91230	48182	93200	131527	364139		
	Total shots	6	7	1		14		
July	Males	638		21117	23721	25814	2.26	1.70
	Females	1478		17175	7872	13317	3.14	
	Wt. measured (kg)	7350		91078		98428		
	No. shots measured	3		3	9	15		
	Total wt caught (kg)	8166		91678	78000	177844		
	Total shots	3		5		8		
August	Males		6900			6900	2.01	2.09
	Females		3300			3300	2.40	
	Wt. measured (kg)		16600			16600		
	No. shots measured		3			3		
	Total wt caught (kg)		19950			19950		
	Total shots		3			3		

5.8 Overall objective: Biomass Estimates

Daily egg production (DEP), its variance and coefficient of variation (CV) were estimated for each survey over the two years (Table 8). The CV based on the egg sampling for egg production during the period of peak spawning was 14-26%. Based on 5% daily mortality, a 1:1 sex ratio and incubation times of 80—66 h, spawning biomass was estimated at 85 478—102 261 tonnes in 1994 and 64 985—77 662 tonnes in 1995 (Fig 5, Tables 9 & 10).

Table 8. Daily egg production estimates for 1994.

Year	Cruise	Daily Egg Production (millions)	Variance (millions)	CV (%)
1994	1	5534	8289192	52
	2	818872	41062260065	26
	3	29318	189999225	47
1995	1	118787	1462061714	32
	2	811000	13323546929	14
	3	120736	3414570702	48

Table 9. Spawning biomass of blue grenadier for 1994 and 1995, assuming incubation period of 66 h.

Year	Month	Av. wt female (kg)	Av. wt male (kg)	Monthly Egg Production (eggs $x10^8$)	No. females	Female Biomass (tonnes)	Spawning Biomass , B (tonnes)
1994	June	3.54	2.38	14622	809291	2862	4784
	July	3.14	2.26	266383	16055698	50351	86709
	August	2.40	2.01	33899	2440488	5867	10769
	Total						102261
						Lower CL	63039
						Upper CL	140417
1995	June	3.54	2.38	30726	1450998	5131	8577
	July	3.14	2.26	209530	11072339	34723	59796
	August	2.40	2.01	31161	2105071	5061	9289
	Total						77662
						Lower CL	43736
						Upper CL	132983

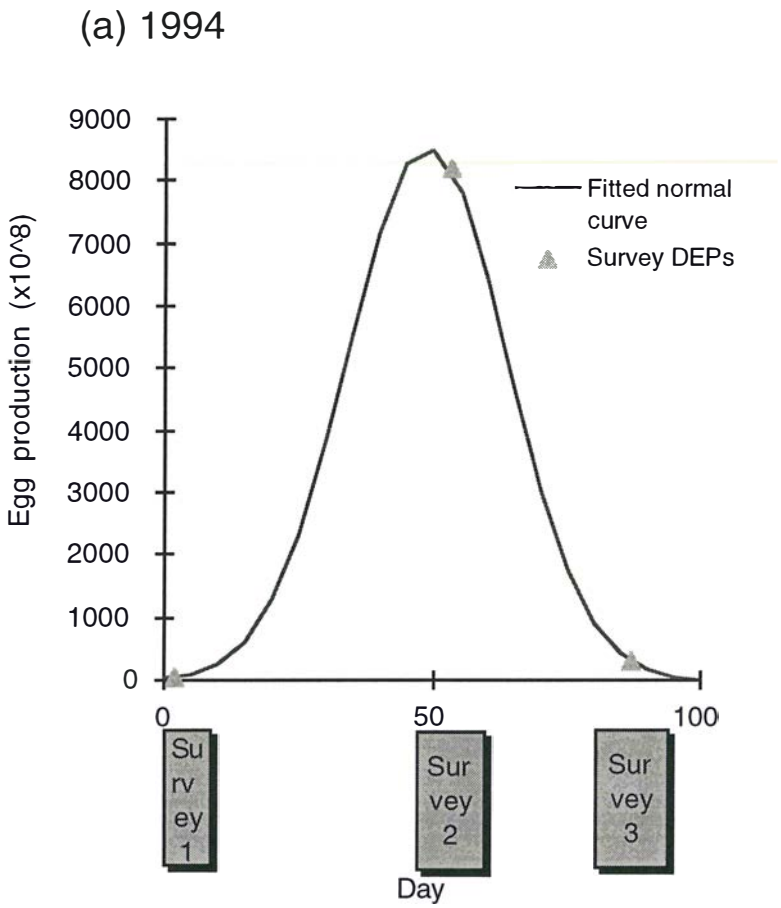


Fig 5a. Annual egg production curves for blue grenadier based on daily mortality rate of 0.05 for 1994. Width of title boxes indicates length of surveys.

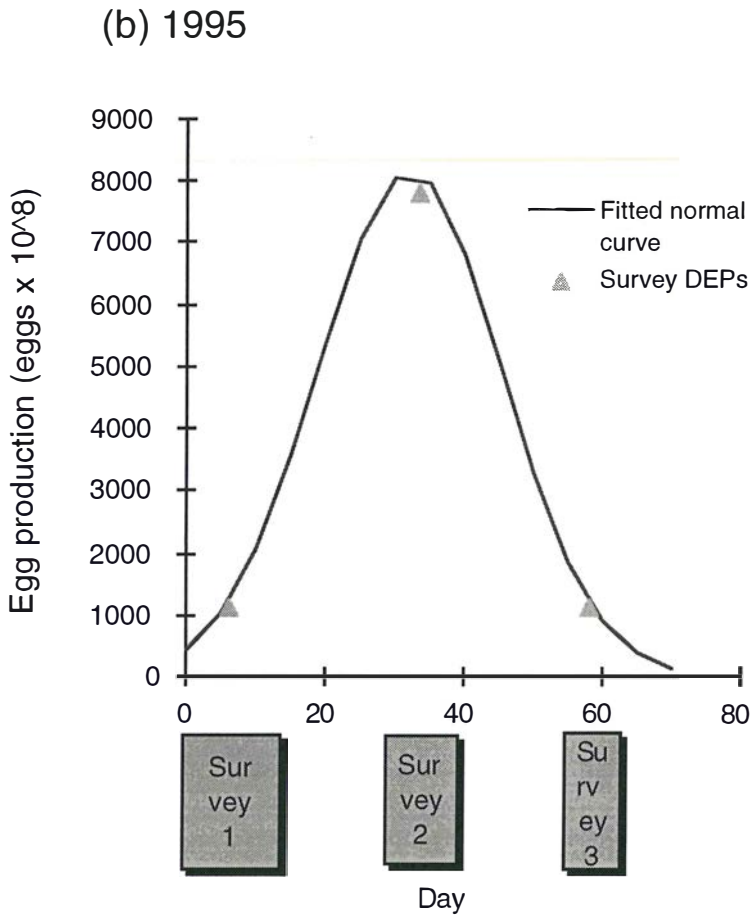


Fig 5b. Annual egg production curves for blue grenadier based on daily mortality rate of 0.05 for 1995. Width of title boxes indicates length of surveys.

We examined the sensitivity of the biomass estimate to uncertainty in several parameters (Table 10). The biomass estimate was approximately inversely proportional to egg incubation time; biomass decreased by 16 % for a 21% increase in incubation time. Due to uncertainty in the mortality rate, we examined the implications of increasing and decreasing the egg mortality by 0.05 per day. If we assumed zero mortality, the estimates decreased by 6% and if mortality was increased to 10%, estimates increased by 6-7%. If we used the monthly sex ratios based on commercial data taken during the spawning season, the estimates increased by 31-32%.

Table 10. Estimates of blue grenadier biomass using different values: incubation time (66h based on our data and 80h based on New Zealand data); mortality (none, 5% and 10%); and sex ratio (monthly ratios from commercial catch data and non-spawning period). Percentages represent the differences from the best estimate.

Incubation Time (h)	Mortality	Monthly Sex Ratio	1994		1995	
66	0	1.0	96 210	-6%	72 708	-6%
	0.05	1.68-2.09	134 327	+31%	102 140	+32%
	0.05	1.0	102 261		77 662	
	0.1	1.0	108 833	+6%	83 076	+7%
80	0.05	1.0	85 478	-16%	64 985	-16%

6 DISCUSSION

The statistical variance of the biomass estimate is affected primarily by the patchiness of the egg distribution. However the high variance in the 1994 surveys was successfully lowered in 1995 by modifying the survey design to include a third stratum of intermediate sampling density. The coefficient of variation for the survey at the peak of spawning in 1995 was reduced to 14%.

A normal curve was fitted to the daily egg production data but the real distribution of spawning effort may be somewhat different. This could prove a significant source of variation in egg surveys that we could not quantify.

Uncertainty of the sex ratio of the fish on the spawning ground had the largest influence on the biomass estimate of any single factor over the range of uncertainties identified. The ratios obtained from commercial market sampling may have been biased because the sexes may differ in their vulnerability to capture or they might have different residence times on the grounds. To avoid such specific biases, we chose to use the sex ratio typical of the non-spawning period.

To estimate the biomass of the whole stock, it is necessary to know the proportion of the population that spawns annually. This proportion is estimated to be from 66 to 84% in some New Zealand stocks (Livingston *et al.* 1997). Because the largest fish in the Tasmanian stock are not observed outside the spawning season, a survey conducted at this time to assess the proportion of the population maturing would have limited reliability. Our estimate is relevant only to the spawning stock.

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8 BENEFITS

Knowledge of the biomass of the blue grenadier standing stock is the single most critical piece of information required to establish a TAC on a scientifically sound, sustainable basis. This is therefore the major direct benefit of this research, and the major beneficiary is the deepwater component of the SEF fishing industry. The previous estimates of sustainable yield are based upon a trawl and an acoustic survey, which may severely underestimate the true extent of the resource because there is no proper estimate of turnover rate. However, to expand the fishery prematurely, i.e. beyond its sustainable limits due to lack of adequate information, risks losing much of the present value of the fishery as well as rendering proper management extremely difficult. Based on 1996 landings in the SEF of over 3000 tonnes - about 24% of the TAC - the present value of the fishery is about $4.5 million. Based on the current stock assessment the sustainable value of the fishery could expand several-fold.

The proposed research may considerably benefit other sectors of the SEF, which are at considerable risk of overexploitation if there is a massive transfer of effort out of the orange roughy fishery. Continued development of biomass assessment methodology at CSIRO is a long-term benefit to Australian fisheries insofar as there are a number of fisheries to which the egg survey method may be applied potentially, e.g. the warehou, mackerel, gemfish, and others.

Estimated percentages of total benefit:

Deepwater fisheries, SEF: 75%

Other SEF: 15%

Other Australian fisheries: 10%

9 INTELLECTUAL PROPERTY

No intellectual property however the biomass estimates are valuable information for management of the fishery.

10 FURTHER DEVELOPMENT

No further development of this method is envisaged. The results from the 1995 & 1996 surveys will be published in Marine and Freshwater Research. The results have been disseminated at various stock assessment group and scientific-industry meetings during the past two years.

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APPENDIX 1
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