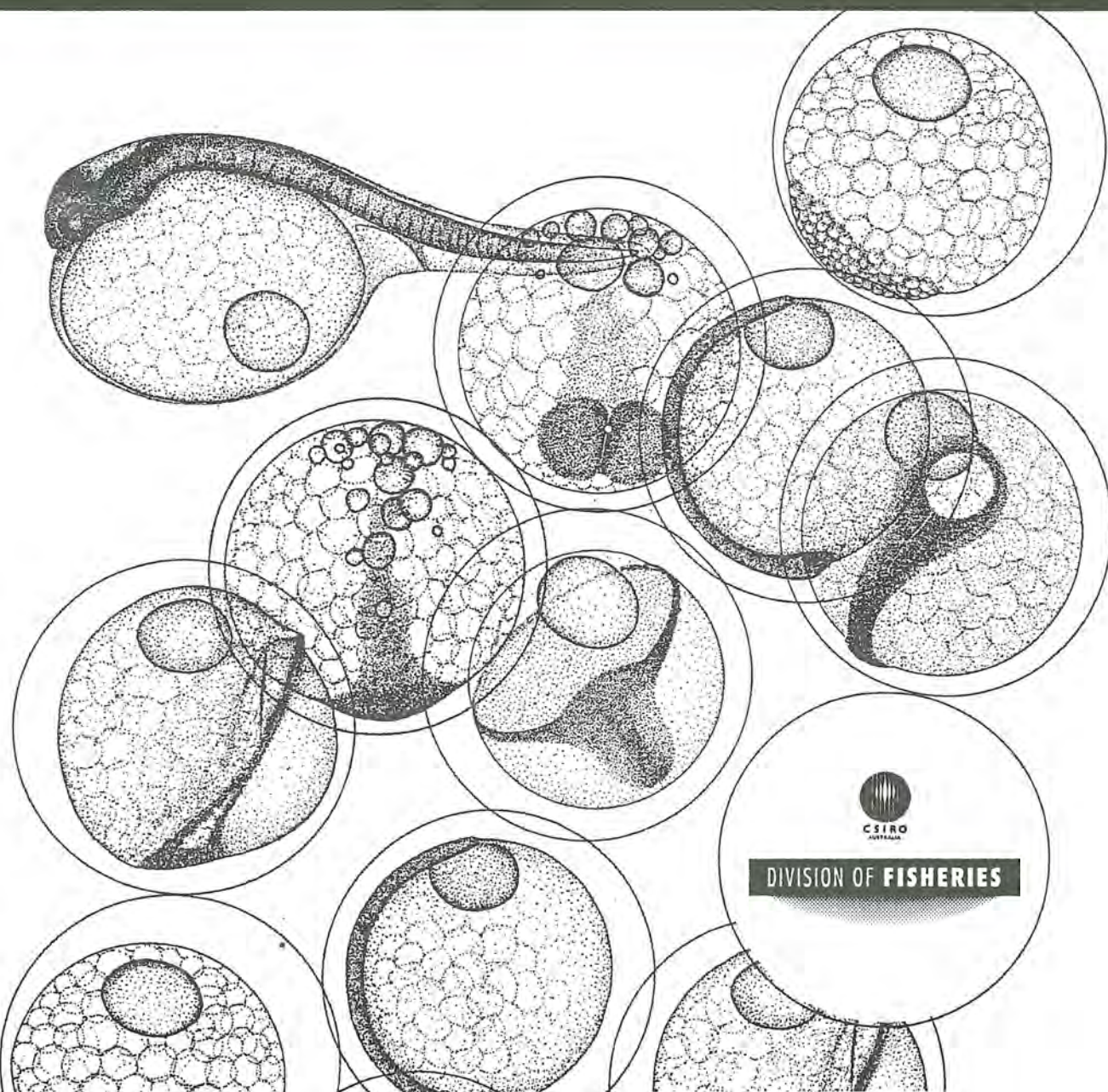


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FINAL REPORT TO FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

GRANT # 90/9

DEVELOPMENT AND USE OF THE EGG PRODUCTION METHOD TO ASSESS THE BIOMASS OF ORANGE ROUGHY OFF EASTERN TASMANIA



CSIRO. Division of Fisheries.

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PART ONE

SUMMARY



INTRODUCTION

Orange roughy is one of Australia's most valuable finfish. However, its aggregating behaviour during spawning, slow growth, and longevity render it vulnerable to over-exploitation. Some stocks in New Zealand are at extremely low levels after only ten years of exploitation.

Good management is vital to maintaining orange roughy as a sustainable fishery. The model currently used for management estimates maximum sustainable yield (MSY) from estimates of natural mortality, M , and virgin biomass, B_0 . Virgin biomass is estimated from the total catch to date and current stock size. Current stock size was the parameter about which there was greatest uncertainty.

Several methods of stock assessment appeared inadequate for orange roughy. Catch per unit effort indices are not very useful for highly aggregating species. Trawl surveys were also deemed to be of limited value because the fish occur largely over rough, untrawlable ground. Acoustic and egg production methods appeared feasible and were proposed to assess the orange roughy stock that spawns off eastern Tasmania. Egg surveys have been used world-wide to assess stocks of species such as anchovy, sardine, and mackerel (Lasker 1985; Lockwood *et al.* 1981; Lo *et al.* 1992). However, until now, this method had only been used on one deepwater species, Dover sole (Lo *et al.* 1992).

Using the conventional egg production method (Saville 1964), biomass is estimated from data on:

- annual egg production: egg production is estimated from plankton surveys conducted during the spawning period. The eggs must be staged and ages assigned to the stages. Age, or rate of development, can be determined from incubation of fertilized eggs under controlled conditions.
- mean fecundity, i.e. the mean number of eggs per kg body wt that a female produces annually;
- the proportion of males and females in the spawning aggregation; and
- the proportion of males and females that actually spawns in a particular year.

Orange roughy have several attributes particularly suited to the egg production method of assessment:

- the major known spawning stock spawns in a very small area over about a month and is therefore relatively easy to survey;
- orange roughy are group-synchronous spawners, i.e., they spawn one batch of eggs annually, and therefore have determinate fecundity; and
- their eggs are distinctive and easy to identify in plankton samples.

We proposed to develop the method for orange roughy and compare our results with the results of acoustic surveys (FRDC Grant # 90/25).

OBJECTIVES

1. To assess the standing stock of orange roughy off east Tasmania based upon a survey of the egg production of the spawning aggregation off St Helens.
2. To carry out studies of orange roughy reproductive biology and early life history to determine:
 - a) the temperature-dependent development rate of orange roughy eggs;
 - b) the sex ratio of orange roughy in the spawning stock;
 - c) the relation between fecundity and body weight;
 - d) the proportion of non-reproductive fish in the population.

RESEARCH SUMMARY

SURVEYS

The spawning aggregation off St Helens was surveyed each winter from 1990-92. In 1990, egg and acoustic pilot surveys were carried out aboard the chartered vessel, *Tasmanian Enterprise*. In 1991, *Tasmanian Enterprise* was chartered solely to conduct the egg survey. As in the first year, stations were occupied along transects at distances ranging to 30 nm north and south of the spawning site. Due to the extensive survey area and poor weather conditions, the full survey area was often not fully sampled during survey periods. For the final survey in 1992, which was carried out aboard the *Dell Ritchie*, the survey was changed to a random stratified design with stations in 2 strata, which were defined by the area 0-5 nm and 5-10 nm from the spawning site. The 24-h old eggs under survey were most abundant within the first stratum and fully contained within the two strata. This sampling strategy reduced the survey area and led to an approximate 2-fold increase in the number of samples obtained, which substantially improved the precision of the survey estimate of egg production.

EGG DEVELOPMENT

Egg fertilization and incubation experiments were carried out during each field season. However, successful *in vitro* fertilization and incubation through to hatch was only achieved in the final year of the project. The experiment was carried out in three controlled-temperature baths, and egg development was described as a function of temperature. The vertical distribution of the eggs through the water column was also assessed during the 1992 field season. The eggs were sampled with the EZ opening-closing plankton net system, which was deployed from the surface to 1000 m along a track around the spawning site. The eggs ascended through the water column at $18.4 \text{ m}\cdot\text{h}^{-1}$ until they reached the upper mixed layer at 200 m. Based upon CTD sampling of the temperature structure of the water column, the temperature history of the egg development was described. The time to hatch was estimated to be 7.3 d *in situ* (PartTwo, Chapter One).

FECUNDITY

Between 120 and 200 females of mature stage 4 were collected for fecundity analysis during early July in 1990-92. Fecundity was highly variable with only 10-17% of the variance explained by standard length. Egg size, a condition factor based upon liver weight, and age also contributed significantly to explaining the variance in fecundity, such that together, these factors explained 41% of the total variance in fecundity. Fecundity was reduced in fish over 50 years old, in fish in poor condition, and in fish bearing relatively large eggs. Fecundity was also significantly correlated to lipid levels in the ovary.

Fecundity increased by 20% between 1987 and 1992, apparently a density-dependent response to the 50% reduction in stock size due to fishing. Combined with an apparent increase in the proportion of spawning fish from 55% to 76%, the populations' annual egg production may have actually increased over this period (Part Two, Chapter Two).

BIOMASS ESTIMATION

The estimated biomass of spawning fish was 33,700 tonnes with a coefficient of variation (CV) of 35% (Part Two, Chapter Three). Based on the proportion of non-spawning fish observed in the population (24% of females and 10% of males) and estimated catch of spawners in 1992 (16,300 tonnes), total stock biomass was estimated to be 28,800 tonnes at the end. Based upon the catch history of the fishery, virgin biomass of the stock was approximately 96,100 tonnes, so the stock was at 30% of virgin biomass at the end of season. The variance of the estimate was dominated by the spatial variability of the egg distribution: the CV increased < 3% when the variance in fecundity and in the proportion of non-spawners was incorporated. The egg production estimate of stock size was in close agreement with an estimate derived from an acoustic survey conducted at the same time.

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TRANSFER OF RESULTS

Preliminary results of the egg surveys were made available to industry and management committees as soon as possible. Results have also been communicated to the scientific community at scientific meetings and will be reported in *Australian Fisheries* and refereed scientific journals (see Part Two for draft manuscripts).

1990

Smith, T. & T. Koslow. Biomass survey of orange roughy at St Helens. *Australian Fisheries* 40 (10), 29-31.

Demersal and Pelagic Fishery Research Group (DPFRG)
Government, Industry & Technical Liaison Committee (GITLC)
Public seminar GITLC, CSIRO Marine Laboratories, Hobart

1991

GITLC meeting
DPFRG meeting

1992

GITLC meeting
DPFRG meeting
Koslow, J.A. South East Fishery Inaugural Workshop,(October) Bendigo, Vic.

1993

Bulman, C. M. & J.A. Koslow. A method of orange roughy stock assessment using egg production surveys. Australian Marine Sciences Association Conference 6 July, Melbourne, Vic.

Koslow, J. A. Australian Society for Fish Biology Annual Conference, August, Perth, WA.

Bulman, C. M. & J. A. Koslow. A method of orange roughy stock assessment using egg production surveys. CSIRO Divisional Seminar (September), Hobart.

Koslow, J. A. South East Fisheries Workshop, (October) Geelong, Vic.

CONCLUSIONS AND RECOMMENDATIONS

The primary objective of the project was to estimate the biomass of orange roughy off northeastern Tasmania based upon an egg survey; the secondary objective was to study the reproductive biology of the population to assess its mean fecundity, sex composition on the spawning ground and proportion spawning, and the rate of egg development. Both objectives were successfully achieved and the details of the studies are documented in Part 2.

Of particular interest, we found fecundity increased 20% over the period 1987-92, a period when the stock declined by about 50% (Chapter 2.). The proportion of females spawning each year increased from 55% to 76%, another example of changes in reproductive effort to compensate for changes in population density.

Based on our results and the survey in 1992, total stock biomass was estimated to be 45,100 tonnes at the beginning of the 1992 fishing season and 28,800 tonnes at the end. Including the total catch removed from the fishery, virgin biomass of the stock was estimated to be 96,100 tonnes, so the stock was at 30% of virgin biomass at the end of the 1992 season. The coefficient of variation (CV) of the estimate was 35%, which is comparable to the variance generally obtained in egg production estimates of fish stock biomass. The variance is almost entirely due to the variability of the egg density estimates, resulting from the highly patchy distribution of eggs. The variability of the estimates of fecundity, sex ratio, and proportion of non-spawners increased the CV by less than 3%.

Results of the egg survey were similar to those obtained from the acoustic surveys of the same spawning aggregation, adding to confidence in the overall biomass assessment. This assessment provided the basic data for the management strategy developed for the stock. Confidence in the assessment allowed substantial quota reductions on the orange roughy to be made.

Although the egg production estimates agreed well with those of the acoustic survey, the statistical precision of the acoustic estimate was higher than that of the egg survey (CV = 10% cf. 35%). However there are fewer non-statistical sources of error with the egg production estimate, whereas the acoustic target strength of orange roughy and the species composition of acoustic marks in the vicinity of the spawning area still present major sources of uncertainty in the acoustic biomass assessment. The use of two independent survey methods therefore appears justified.

In conclusion, egg surveys have proven to be an effective method of stock assessment for Australian deepwater fisheries. We recommend that its use be considered in assessing other high-priority species with suitable reproductive

biology. This is consistent with CSIRO's recent proposal, which AFMA funded, to use egg surveys to assess the blue grenadier stock that spawns off western Tasmania. We also recommend that egg surveys be considered for continued monitoring of high-value fisheries. For example, egg surveys are a reasonable option for the continued monitoring of the east Tasmanian orange roughy. The costs of egg and acoustic surveys are comparable.

PART TWO

DETAILS OF THE STUDY



CHAPTER ONE

DEVELOPMENT AND DEPTH DISTRIBUTION OF

THE EGGS OF ORANGE ROUGHY *Hoplostethus atlanticus* (PISCES: TRACHICHTHYIDAE)



**DEVELOPMENT AND DEPTH DISTRIBUTION OF THE EGGS OF ORANGE ROUGHY,
HOPLOSTETHUS ATLANTICUS (PISCES: TRACHICHTHYIDAE)**

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ABSTRACT

The mid-slope benthopelagic fish, orange roughy (*Hoplostethus atlanticus*), is the first beryciform, and only one of a few deep-sea species, whose egg development has now been described. Eggs of the orange roughy were fertilized and incubated at three temperatures. Eggs incubated at 7°C hatched at 13 days; wild eggs were estimated to hatch at 73 days. The development rate of orange roughy is similar to those of fish from other orders, and of shallower-living species. Depth-stratified plankton sampling indicated that the early stage eggs were found at 500-700 m and rose to the upper mixed layer 18.4 m·h⁻¹. Buoyancy of orange roughy eggs was estimated to be 0.005 g cm⁻³, which is at the upper end of the range of measured fish egg buoyancies. Orange roughy eggs ascend faster than those reported for other fishes because of their relative buoyancy and large egg size. However, large egg size is apparently not an adaptation to enhance development rate because the decrease in incubation time due to a more rapid ascent into warmer water does not compensate for the longer time required for large eggs to develop.

INTRODUCTION

The fishery for orange roughy (*Hoplostethus atlanticus* Collett 1889; Order: Beryciformes) at mid-slope depths (700-1200 m) became one of Australia's most valuable fisheries after the discovery of a spawning aggregation off northeastern Tasmania in 1989. Since then, this spawning ground has produced nearly half of the Australia's orange roughy landings. The considerable longevity of the species, a 100 years or more (Fenton *et al.* 1991), its low productivity (Mace *et al.* 1990) and its aggregating behaviour, particularly during spawning, render orange roughy highly vulnerable to over-fishing. There is therefore an urgent need to assess the biomass of the stock so it can be managed as a sustainable resource.

Egg surveys have proved to be an effective method of stock assessment in other species. It has been successfully applied to fishes such as anchovy, sardine, and mackerel (Lasker 1985; Lockwood *et al.* 1981; Lo *et al.* 1992). Until Lo *et al.* (1992) applied this method to Dover sole, it had not been used on deepwater species.

Biomass is estimated from data on annual egg production, mean fecundity, the proportion of females in the spawning aggregation and the proportions of males and females that actually spawn in a particular year (Saville 1964).

Egg production is estimated from plankton surveys conducted during the spawning period. However, the eggs must be staged and ages assigned to the stages. Ages were obtained by incubating fertilized eggs under controlled conditions. In this paper we describe the stages and rate of development of orange roughy eggs. The depth distribution of eggs in the water column at the spawning site is also described, as are the temperatures to which the eggs are exposed in the wild. These data are used to estimate the egg buoyancy.

This is the first published description of the development from fertilization to hatching of orange roughy, or of any beryciform fish. Studies of other species have found that the rate of development of eggs is a function of temperature and egg size (Ware 1975, Kendall *et al.* 1984, Pauly and Pullin 1988). Oxygen and salinity also influence development (Hempel 1979). However, few of the studied species were deepwater fish. Here, we examine whether the development of orange roughy is consistent with these general empirical relationships.

METHODS

EGG DEVELOPMENT

On board FRV *Southern Surveyor*, a ripe female and a ripe male were retained from a catch on the northeastern Tasmania spawning ground, St Helens Hill (41° 14' S, 148° 45.5' E), on 17 July, 1992. Eggs and milt were extruded from the fish, combined in a 1 litre jar of clean surface seawater and incubated in a water bath at 5°C for two hours. About two hundred eggs were transferred into each of nine 500 ml polycarbonate containers (incubators) of seawater. Three containers were incubated at each of the three experimental temperatures, 5° ($\pm 0.1^\circ\text{C}$), 7° and 9°C ($\pm 0.5^\circ\text{C}$), in controlled-temperature water baths.

Initially, samples of up to 10 eggs from each temperature were taken every 2 h and were preserved in 2.5% formalin (in seawater) buffered with 4% sodium acetate, after examination. As the changes in development became slower and stages lasted longer, samples were taken less often, until, after 6 d, eggs were sampled only once a day. When eventually there were too few live eggs to continue sacrificing them, the sampled eggs were returned to their respective incubators after examination.

The developmental stages we describe are based on descriptions of the egg development of northern anchovy *Engraulis mordax* (Moser and Ahlstrom 1985), Alaska pollock *Theragra chalcogramma* (Yusa 1954) and Dover sole *Microstomus pacificus* (G. Moser, Southwest Fisheries Science Centre, La Jolla, pers. comm.) and

on general characteristics of eggs reviewed by Ahlstrom and Moser (1980) and Matarese and Sandknop (1984).

Lengths of larvae are notochord lengths, NL (Leis and Rennis 1983), and all measurements are on fixed material.

VERTICAL DISTRIBUTION

The vertical distribution of orange roughy eggs around the spawning site was determined from two depth-stratified sets of plankton tows. We used an EZ opening-closing plankton net system, similar to the BIONESS net system (Sameoto et al. 1980). It consists of 10 nets, each with a 1 m² mouth opening and 335 μm mesh netting. It was deployed with the first net open from the surface to 900 m (bottom depth ~ 1000 m). The water column was then sampled by 100 m strata to the surface. Each stratum was sampled with a 15 minute oblique tow. Net depth was continuously monitored and the nets were closed electronically from the ship.

Each set of tows followed a semi-circular track at a distance of 3-4 nm from the spawning hill. A constant distance from the spawning site was maintained to minimize bias between strata due to differences in drift time from the source-the hill. The first set was towed from north to south of the spawning area and the second from south to north. To assess the depth distribution of the eggs, the numbers per depth stratum were summed over the two tows.

The samples were preserved in 5% formalin (in seawater) buffered with 4% sodium acetate. Orange roughy eggs, which are distinctly identifiable from any other eggs, were later removed from the samples in the laboratory. They were staged and assigned ages, using criteria from the egg-development experiments.

Temperature and salinity profiles of the water column around the spawning area were obtained from a CTD cast.

RATE OF DEVELOPMENT

The development time of orange roughy eggs in nature was estimated by summing the estimated durations of each stage. The mean temperature experienced by eggs of each stage *in situ* was determined by calculating, from the vertical distribution data, the weighted mean depth of occurrence of each stage and its corresponding temperature from the CTD profile. Stage duration was estimated directly from the incubation experiment when the mean *in situ* temperatures were within the range of our experimental temperatures. Where *in situ* temperatures were beyond our range, stage duration (D_{iT}) was estimated by weighting the duration obtained from the 7°C incubation, (D_{i7}), by the relationship between the total egg development time at the *in situ* temperature

(D_{Tt}) predicted by Pauly and Pullin (2), and the measured development time at 7°C (D_{T7}):

$$D_{it} = D_{i7} \cdot \frac{D_{Tt}}{D_{T7}} \quad (1)$$

The empirical relationship of Pauly and Pullin (1988) between development (D), in days, temperature (t) in °C, and egg diameter (d), in mm is:

$$\log_{10}D = 7.10 + 0.608\log_{10} d - 4.09\log_{10} (t + 26). \quad (2)$$

EGG BUOYANCY

The buoyancy of orange roughy eggs, $\Delta\rho$, defined as the difference in densities of seawater (ρ_w) and the eggs (ρ_e) (i.e. $\Delta\rho = \rho_w - \rho_e$), is dependent upon egg diameter ($d = 2.26$ mm), the molecular viscosity of seawater ($\eta = 0.00139$ kg m⁻¹ s⁻¹ at salinity = 35, $T = 10^\circ\text{C}$ (Sverdrup et al. 1942, p. 69)), and the terminal velocity of eggs, v . We estimated v from a least-squares regression of the mean depth of the eggs as a function of their age, which was determined as the midpoint of the duration of the stage of the eggs.

The relationship used to estimate buoyancy from terminal velocity is dependent upon the Reynolds number, $Re = vd/\eta$. When the Reynolds number is less than 0.5, buoyancy can be estimated by the classical Stokes equation (Sverdrup et al. 1942, p. 956):

$$v = \frac{gd^2\Delta\rho}{18\eta}, \quad (3)$$

where g is the acceleration due to gravity ($= 9.8$ m s⁻²). When $Re > 0.5$, viscous forces become less important. In a region of intermediate Re , where $Re > 0.5$ and before motion becomes turbulent, v may be defined by:

$$v = \frac{Kd_0\Delta\rho^{2/3}}{\eta^{1/3}} \quad (4)$$

(Dallavalle 1948 in Sundby 1983). Sundby (1983) demonstrates that $K \cong 19$ in the region of intermediate Re , and $d_0 = d - \zeta d_m$, where $\zeta = 0.4$ for spheres and d_m is the uppermost diameter for which the Stokes equation applies, i.e. at which $Re = 0.5$.

RESULTS

EGG CHARACTERISTICS

Orange roughy eggs are large and spherical: the diameter of formalin-preserved eggs is 2.17-2.33 mm ($\bar{x} = 2.26 \pm 0.07$ mm, $n = 34$). The chorion is smooth, unpigmented and thin. There appears to be no vitelline membrane. The yolk is clear and coarsely segmented. The egg usually contains one large bright orange oil droplet ($\bar{x} = 0.76 \pm 0.066$ mm, $n = 30$) that fades to a greenish colour when preserved in formaldehyde. When an egg was removed from a fish or a plankton tow, the oil droplet was often shattered into smaller globules distributed throughout the yolk mass. The globules coalesce after several hours if they are not disturbed. The perivitelline space was at its widest at stage 2 development, with a mean maximum width of 0.23 ± 0.06 mm ($n = 33$).

EGG DEVELOPMENT

Although the eggs were incubated at three temperatures, the 5°C incubation was curtailed after 193 h due to a waterbath malfunction and the 9°C incubation ended at 96 h when no live eggs remained. Only the 7°C eggs were successfully hatched, so the following descriptions pertain only to that incubation temperature.

DEVELOPMENT STAGES

Stage 1 (Fig. 1a: fertilization to first division, 0-10 h). Four hours after fertilization, cytoplasm accumulated at the vegetal pole on the yolk surface. Later, "strands" extended from the cell to the centre of the egg.

Stage 2 (Figs 1b-1e: first division to gastrulation, 10-40 h). "Early" stage 2 was defined as 2 to 32 cells (Fig. 1b); "mid" stage 2 as 64 to 128 cells (Figs 1c & 1d); and "late" stage 2 as greater than 128 cells until gastrulation (Fig. 1e). As Moser and Ahlstrom (1985) found difficulty in observing the formation of the segmentation cavity-the beginning of gastrulation-they considered gastrulation to have started when the blastoderm looked like tissue rather than cells.

Stage 3 (Figs 1f, 1g: gastrulation to epiboly halfway down yolk, 40-120 h). The rim of the blastula continued to thicken (Fig. 1f) and the margins developed into the germ ring (epiboly). The embryonic shield developed from emboly of the germ ring (Fig. 1g). At the end of this stage, the germ ring extended halfway down the yolk sac.

Stage 4 (Fig. 1h: development of embryo to blastopore closure, 120-144 h). The embryo formed along the embryonic shield. The blastopore continued to shrink.

Stage 5 (Fig. 1i: blastopore closure to separation of tail bud from yolk, 144-216 h). Embryos had optic vesicles and a developed notochord. The oil droplet is near the notochord tip in the specimen illustrated but in most other specimens, it was opposite the embryo.

Stage 6 (Fig. 1j: separation of tailbud to elongation of tail to 1/4 body length, 216-264 h). The tail became much thicker and sometimes slightly pointed, which suggested the fin folds were developing. The oil droplet was uppermost in the yolk sac. From this stage, normally developing eggs sank to the bottom of the containers which suggests their density increased.

Stage 7 (Fig. 1k: tail lifted up to 1/4 of a body length, 264-288 h). The tails were quite pointed. Myomeres were clearly visible.

Stage 8 (Fig. 1l: tail lifted more than 1/4 of a body length to hatching, 288-312 h). Before hatching, the embryos were wrapped nearly 3/4 around the yolk sac and about 1/3 of the body length was lifted from the sac. The vent was clearly visible and attached to the sac.

Stage 9 (yolk-sac larva, 312 h+). The length of larva on hatching was 3.7 mm. At four days the larva was 4.5 mm NL (Fig. 1m). The length of free tail was about equal to the egg-yolk length, which was 2.0 mm. Pectoral fins and otic capsules (dashed circle on figure) were developing.

The oldest larva lived for 12 days. During those 12 days, its head became broader and more rounded in profile. The heart, which is located ventral to the eye, was visibly beating at 11 d. The yolk sac became smaller relative to the body of the larva. The larva did not develop pigmentation. Mouth parts had not developed enough to allow feeding. It was 4.3 mm NL after preservation, shorter than the 4 d larva, but it was preserved at least several hours after death during which time the larva may have shrunk.

VERTICAL DISTRIBUTION

The water temperature near the spawning area rose from 5.6°C at 1000 m to 12.5° in the surface 100 m (Fig. 2). Our incubation temperatures of 5°, 7° and 9°C corresponded to the water temperatures below 1000 m, at 850 m and at 350 m respectively.

In the first set of tows, 6561 eggs were caught. In the second set, which was from south to north, finishing due east of the hill, 205 eggs were caught. The low catch may have been due to a predominantly northward drift. However, the relative proportions of the eggs by depth and stage were similar from the two tows, so the results were combined.

The earliest-stage eggs were found at 500-700 m depth (Fig. 3). As the eggs developed, their mean depth of occurrence decreased. By the end of stage 2 most eggs were caught in the upper 300 m (Fig. 3, Table 1). Very few older-stage eggs were caught, probably due to advection out of the area.

RATE OF DEVELOPMENT

The rate of development of the eggs at three temperatures during the incubation experiment is shown in Fig. 4. Although the 5° and 9°C incubations ended prematurely, they provided data for the early stages of development.

Regressions were fitted to the time (Y, in hours) for development of the eggs at the three temperatures through the first eight cell divisions, x, (i.e. from 1 to 256 cells) (Fig. 4) of stages 1 and 2. The formulae for the regressions were:

$$5^{\circ}\text{C: } Y = 3.89 (x + 1) - 1.49; R^2 = 0.91$$

$$7^{\circ}\text{C: } Y = 2.47 (x + 1) + 2.11; R^2 = 0.99$$

$$9^{\circ}\text{C: } Y = 1.79 (x + 1) + 2.31; R^2 = 0.99$$

Eggs beyond mid-stage 2 in the wild experienced higher temperatures than the incubation temperatures (Table 2), so development time was extrapolated from the rate at 7°C as modified by the empirical relationship of Pauly and Pullin (1988) (see Methods). The total observed development time at 7°C was 13 d, which is close to the time of 12.7 d predicted by the Pauly and Pullin (1988) relationship. It therefore seemed reasonable to use their relationship. We estimated time to hatch in the wild at 174 h (= 7.3 d), almost half the development time at 7°C (Table 2). Given the temperature distribution of the eggs in the water column, an egg would reach late-stage 2 during the first 24 h.

EGG BUOYANCY

The rate of ascent of orange roughy eggs was estimated from the average depth of capture of each stage (Table 1) and its predicted age (Table 2). Up to late-stage 2, the eggs rose from about 600 m to 200 m at 18.4 m h⁻¹ (Fig. 5). The ascent rate of later-stages decreased to 1.6 m h⁻¹; This decrease is, at least in part, due to mixing and turbulence in the upper water column: the water column was mixed to about 250 m (Fig. 2). Buoyancy may also decrease in the later-stage eggs, as their sinking in the incubators suggests. However, the decrease in upward velocity is not due to decreasing seawater density, because salinity in this region increases toward the surface. As a result, the relative buoyancy of the eggs should increase as they rise through the water column, assuming the eggs are spherical packets of seawater whose densities are primarily a function of temperature, salinity and pressure, and that their temperature and pressure are in equilibrium with the surrounding seawater.

Based upon an ascent rate (terminal velocity) of 18.4 m h^{-1} , Reynolds number, Re , for the eggs is approximately 10, so we used the Dallavalle (1948) equation to estimate egg buoyancy. The difference in density with seawater was computed to be 5.0 kg m^{-3} . The density of seawater midway through their ascent to the mixed layer was 1026.5 kg m^{-3} , based upon a salinity at 500 m of 34.6 and $T = 10^\circ\text{C}$ (Fig. 2) (Pond and Pickard 1978, p. 5), so we estimated the density of the orange roughy eggs to be 1021.5 kg m^{-3} .

DISCUSSION

There are few data in the literature on the development of eggs of deepwater fishes to which we could compare our results. The only trachichthyid egg described, that of *Paratrachichthys trailli*, had a segmented yolk similar to orange roughy but no oil droplet (Robertson 1975). However, no developmental data were given. The review by Pauly and Pullin (1988) had no data for any species in the Order Beryciformes.

Compared with the 140 experiments in Pauly and Pullin's data set, our incubation temperature was cooler than all but 9 of them, and they were larger than 82 of the 84 species for which Pauly and Pullin had data. Ahlstrom and Moser (1980) estimated only 14% of marine planktonic eggs are greater than 2 mm in diameter.

Although orange roughy eggs differ from those reviewed in some respects, their development rate at 7°C (13 d) was close to that predicted by Pauly and Pullin (1988) (12.7 d) from an empirical relationship based upon temperature and egg diameter. This supports the robustness of the relationship of the incubation rate of fish eggs with temperature and egg size over a broad range of fish taxa and conditions (Ware 1975; Pauly and Pullin 1988).

The mean diameter of orange roughy eggs in our study (2.26 mm) was similar to that reported for orange roughy from New Zealand waters by Pankhurst and Conroy (1987) (2.0-2.5 mm) but slightly smaller than that reported by Grimes and Zeldis (1993) (2.45 mm). Grimes and Zeldis (1993) may have used live material, whereas our measurements were on formalin-preserved material, which is subject to shrinkage (Theilacker 1980). Based upon the Pauly and Pullin (1988) relationship, the time to hatch if orange roughy eggs were 0.2 mm larger would be only 5 h longer - a minor difference.

Data from experimental determinations of egg buoyancy for North Sea mackerel (*Scomber scombrus*) and plaice (*Pleuronectes platessa*) and Arcto-Norwegian cod (*Gadus morhua*) were reviewed by Sundby (1983). The eggs from all three species were positively buoyant with buoyancies ranging from 0.5 - 3 kg m^{-3} for the plaice

and cod and 4 - 6 for mackerel. These values, particularly that of mackerel, are similar to the relative buoyancy of orange roughy eggs. However, orange roughy eggs are larger, and because ascent rate is proportional to egg diameter (Equation 4), they rose faster than eggs of the North Atlantic species (18.4 m h^{-1} cf. 3.46 - 6.48). Due to their larger size and more rapid ascent, orange roughy eggs have a higher Reynolds number than plaice, cod or mackerel eggs ($Re = 10$ cf. $0.5 < Re < 5$ respectively). However the ascent rates of eggs for all species fell within a range where neither viscous nor frictional forces predominate.

Pelagic eggs of a broad range of fishes living over the continental shelf or at mesopelagic depths generally fall within a narrow size range (0.7 - 1.5 mm diameter) (Ware 1975; Pankhurst and Conroy 1987; Pauly and Pullin 1988). However, the range of egg diameters of deepwater species extends from this size up to 3-4 mm (Marshall 1979; Golovan and Pakhorukov 1984; Pankhurst and Conroy 1987). In teleosts, larger egg size is usually associated with lower fecundity (Elgar 1990). The greater parental bestowal per egg is generally attributed to the poor food conditions in this environment. This is consistent with the large yolk-sac of the newly-hatched orange roughy larva. Grimes and Zeldis (1993), who raised orange roughy larvae from wild-caught fertilized eggs, found that they did not begin feeding until about a month after hatching.

However, larger egg size of orange roughy, and other deepwater fishes, may minimize incubation time and, hence, risk of predation during the vulnerable egg stage, by increasing the rate of rise into warmer near-surface waters. A 1.0 mm diameter egg with the same density as the orange roughy egg would rise at 7.92 m h^{-1} (cf. 18.4 m h^{-1} for an orange roughy egg) and would require 63.6 h to rise from 500 m into the upper mixed layer, compared with 27.2 h for orange roughy eggs (Equation 4).

This could decrease development time by up to a day, based on the difference in temperature regime. However, egg development rate is inversely related to egg size (Ware 1975; Pauly and Pullin 1988). Based upon Pauly and Pullin's empirical relationship (Equation 2), a 1.0 mm egg that experienced the same mean temperature as the orange roughy eggs should hatch in 4.4 d, compared with 73 d for an orange roughy egg. Thus the direct effect of increased egg size on development time outweighs the indirect benefit obtained from a faster ascent into the warmer, upper mixed layer.

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DEVELOPMENT AND DEPTH DISTRIBUTION OF
ORANGE ROUGHY EGGS

TABLE 1. DEPTH OF OCCURRENCE BY STAGE OF DEVELOPMENT

| Stage | n | Depth range (m) | Temp range (°C) | Mean depth (m) | Mean temp (°C) |
|---------|------|-----------------|-----------------|----------------|----------------|
| 1 | 159 | 0-900 | 6.5-12.3 | 588 | 8.7 |
| early 2 | 396 | 0-700 | 8.0-12.3 | 601 | 8.5 |
| mid-2 | 5162 | 0-700 | 8.0-12.3 | 486 | 9.2 |
| late 2 | 879 | 0-400 | 10.1-12.3 | 249 | 11.9 |
| 3 | 187 | 0-400 | 10.1-12.3 | 211 | 12.1 |
| 4 | 2 | 200-300 | 11.2-12.1 | 250 | 11.9 |
| 5 | 15 | 0-300 | 11.4-12.5 | 115 | 12.6 |
| 8 | 1 | 0-100 | 12.4-12.5 | 50 | 12.5 |

TABLE 2. PREDICTED TIME TO HATCHING OF ORANGE ROUGHY EGGS *IN SITU*.

| Stage | Mean ambient temperature at average depth of capture °C | Cumulative development time (h) | |
|---------|---|---------------------------------|--------------------------|
| | | Experimental (7°C) | Predicted <i>in situ</i> |
| 1 | 8.7 | 10 | 8.6 |
| early 2 | 8.5 | 18 | 14.8 |
| mid-2 | 9.2 | 24 | 18.2 |
| late 2 | 11.9 | 40 | 27.2 |
| 3 | 12.1 | 120 | 72.0 |
| 4 | 11.9 | 144 | 85.4 |
| 5 | 12.6 | 216 | 123.6 |
| 6 | - | 264 | 149.0 |
| 7 | - | 288 | 161.7 |
| 8 | 12.5 | 312 | 174.4 |

FIGURE LEGEND

Figure 1 a) stage 1; b) two-cell development (stage 2); c) stage 2 about 64-cell stage (dorsal view); d) stage 2 at 24 hours (128-cell); e) stage 2 (late); f) stage 3; g) stage 3: epiboly, beginning of embryonic shield; h) stage 4: blastopore clearly visible; i) stage 5; j) stage 6; k) stage 7; l) stage 8; m) yolk-sac larva 4 d post-hatch. Actual length 4.5 mm NL. The dent behind the head is probably due to damage from handling. N.B. Stippling on egg diagrams does not represent pigmentation.

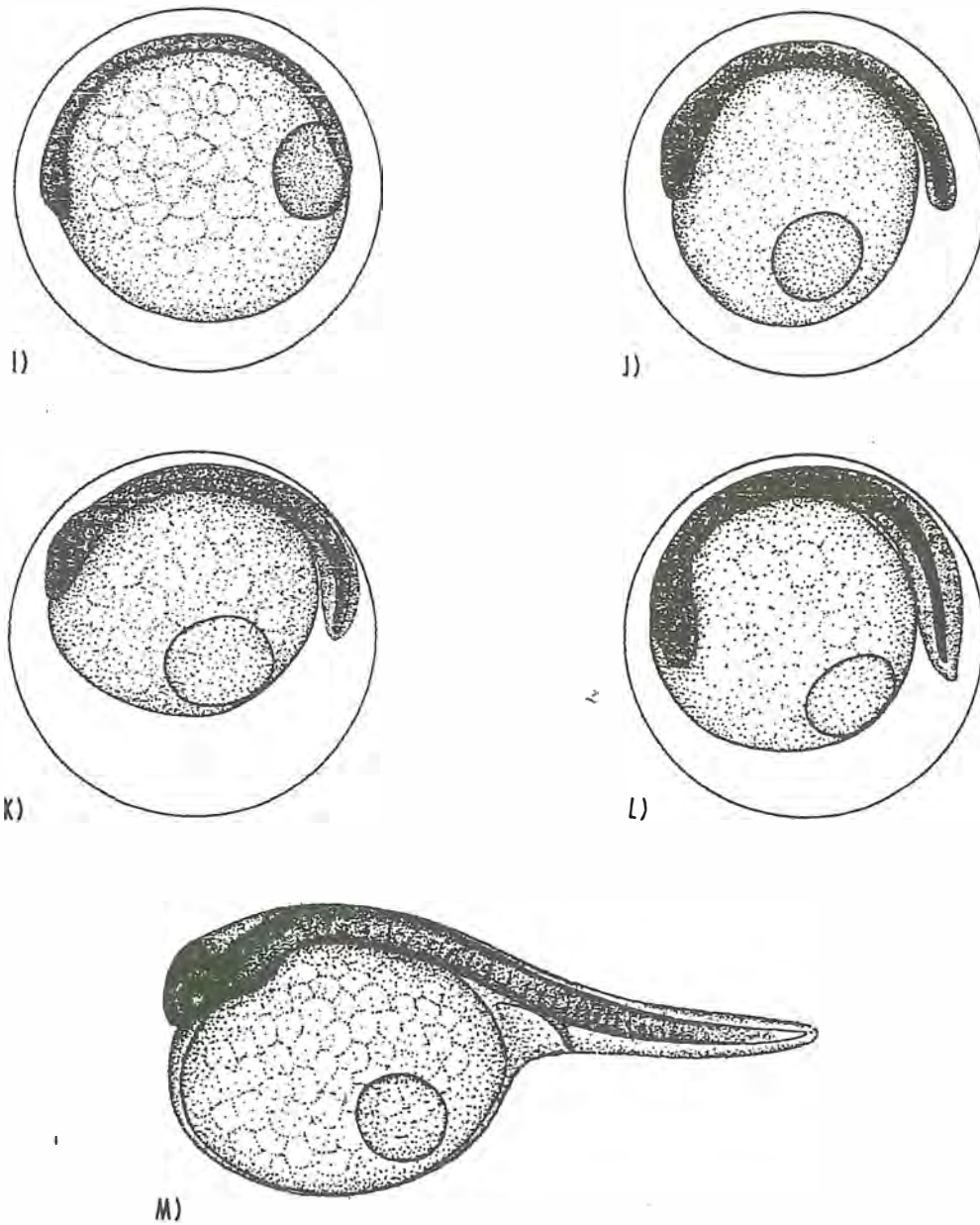
Figure 2 Profile of temperature and salinity distribution of the water column near the St Helens spawning ground during winter 1992.

Figure 3 Proportions (%) of eggs by stage caught per 100 m depth stratum from both EZ tows during SS3/92. 2e=2-32 cells; 2m=64-128 cells; 2l=>128 cells.

Figure 4 Rate of egg development for three incubation temperatures; 5°, 7° & 9°C.

Figure 5 Ascent of eggs from 1) 600 m to 200 m ; 2) 200 m to 50 m based on the average depth of capture of each stage and the predicted age (in h).

DEVELOPMENT AND DEPTH DISTRIBUTION OF
ORANGE ROUGHY EGGS



DEVELOPMENT AND DEPTH DISTRIBUTION OF
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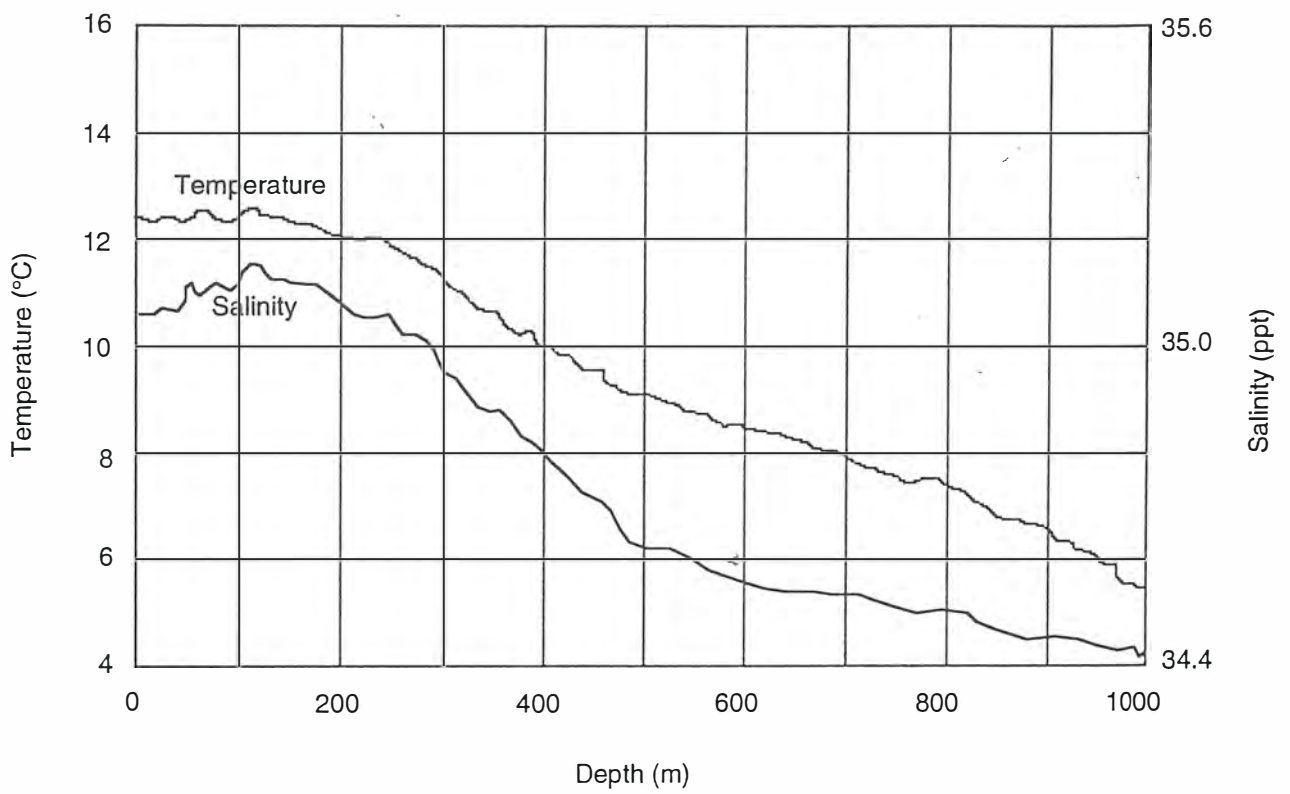


FIGURE 2

DEVELOPMENT AND DEPTH DISTRIBUTION OF
ORANGE ROUGHY EGGS

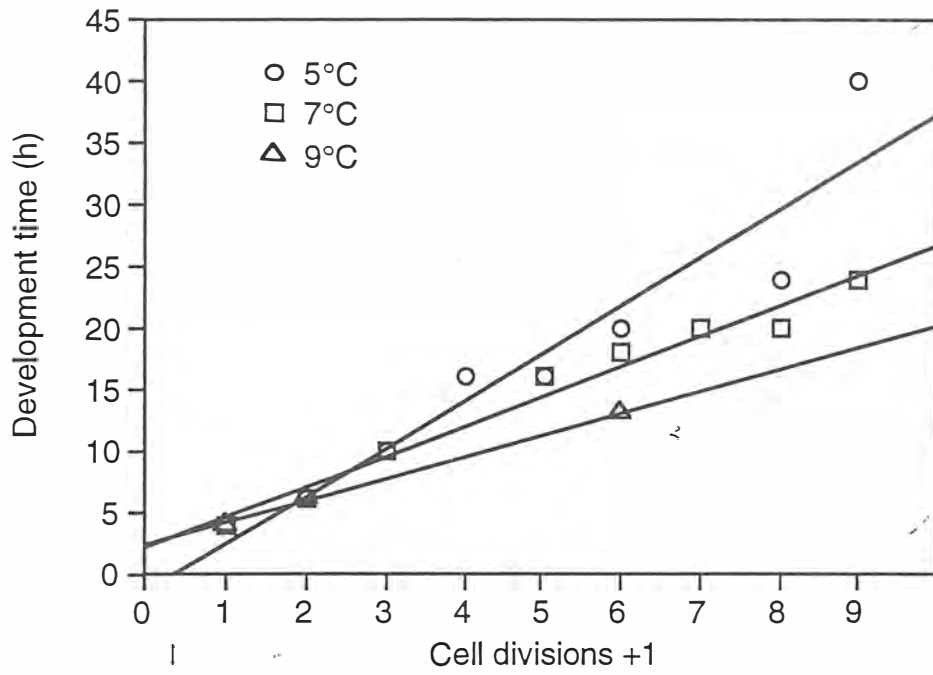


FIGURE 3

DEVELOPMENT AND DEPTH DISTRIBUTION OF
ORANGE ROUGHY EGGS

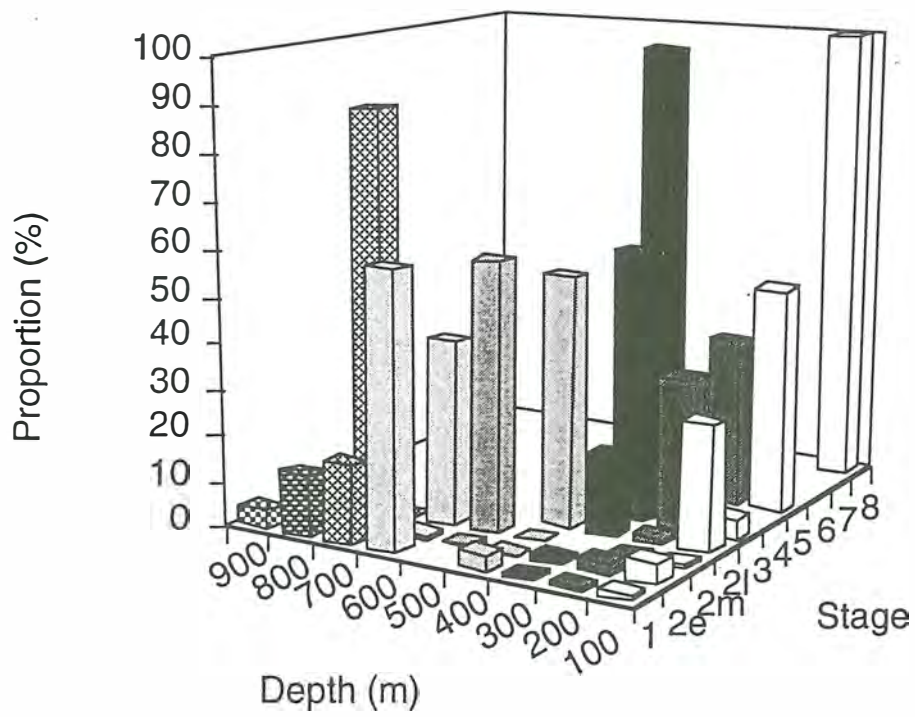


FIGURE 4

DEVELOPMENT AND DEPTH DISTRIBUTION OF
ORANGE ROUGHY EGGS

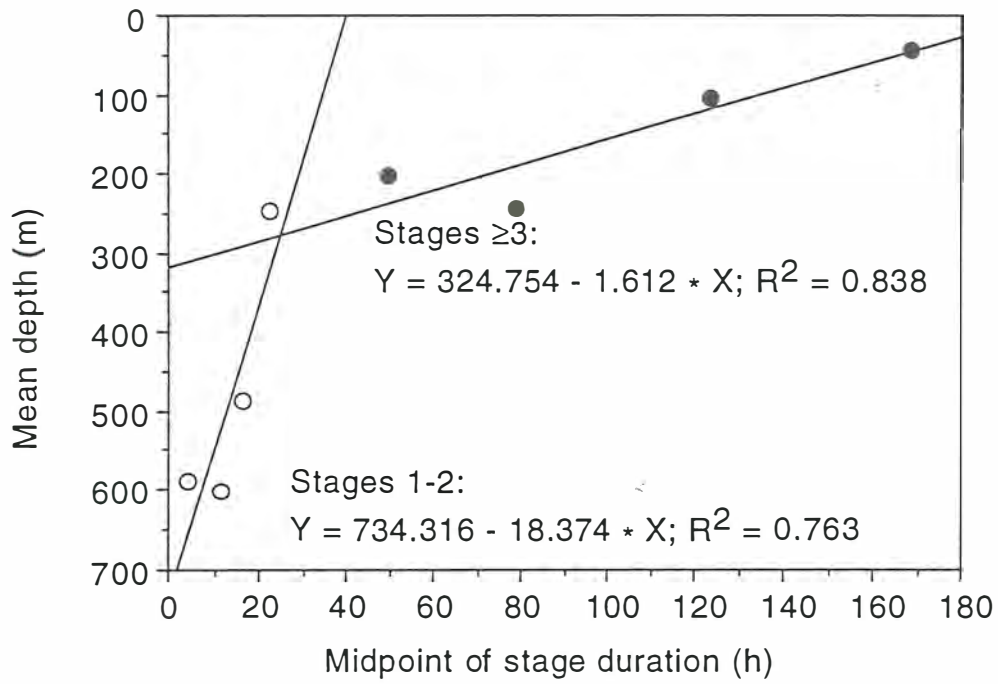


FIGURE 5

CHAPTER TWO

FECUNDITY AND ITS VARIABILITY IN ORANGE ROUGHY (*Hoplostethus atlanticus*)

OFF SOUTHEASTERN AUSTRALIA



FECUNDITY AND ITS VARIABILITY IN ORANGE ROUGHY (*HOPLOSTETHUS ATLANTICUS*) OFF SOUTHEASTERN AUSTRALIA

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ABSTRACT

The fecundity of orange roughy was examined from 1987-90 in three areas off southeastern Australia and from 1987-92 off eastern Tasmania, site of the largest spawning ground for orange roughy in Australian waters. Fecundity in 1987-90 adjusted for standard length (SL) varied significantly in the three areas around southeastern Australia, being highest off New South Wales (42,787 eggs/female) and lowest off east Tasmania (31,085 eggs/female).

Fecundity in orange roughy was highly variable. Only 10-17% of the variability in fecundity for fish off east Tasmania in any year from 1987-92 was explained by SL. However, egg size, liver condition, and age of the fish also contributed significantly to explaining the variance in fecundity, such that in combination with SL, 41% of the variance in fecundity was explained within a particular year. Orange roughy is an exceptionally long-lived fish, and fecundity declined in fish over 60 years old. Chemical analyses indicated that fecundity was significantly correlated with lipid levels in the ovary, and in particular, with triacylglycerol as a proportion of the total lipid fraction.

Significant interannual changes in fecundity appeared related to the impact of fishing. From 1987-92, the orange roughy stock off east Tasmania was reduced by 50% due to the fishery, and mean fecundity increased 20% over that period. This compensatory increase in individual fecundity, combined with an apparent decline in the proportion of non-spawning females in the population from 45% to 24% may have led to an actual increase in the population's egg production over this period. These factors indicate that the unexploited orange roughy population was at or near its carrying capacity and represents one of the few recorded examples of a senescent marine population.

INTRODUCTION

Fecundity has been of interest to fishery scientists as both a critical parameter of stock assessment based on egg production methods (Saville 1964, Lasker 1985b) and a basic aspect of fish biology and population dynamics. This mix of broad and highly specific objectives has characterized studies of the fecundity and reproductive biology of orange roughy (*Hoplostethus atlanticus*), a mid-slope fish inhabiting depths of 700-1400 m that has been fished commercially off New Zealand and Australia since the 1980s.

Initial studies in New Zealand and Australia showed that the orange roughy is a group synchronous spawner that produces a single batch of large eggs (2.0-2.5 mm diameter) that are released during a relatively brief (approximately one month), well-defined spawning season in mid austral winter (Pankhurst et al. 1987; Pankhurst and Conroy 1987, Pankhurst 1988, Bell et al. 1992). The absolute fecundity of orange roughy is relatively low (20,000 - 90,000 eggs per female), which has been attributed to the large size of its eggs and the relatively low biological productivity at mid-slope depths (Pankhurst and Conroy 1987). Fecundity and the timing of spawning varies between localities, which has been used to distinguish among spawning groups (Pankhurst 1988; Bell et al. 1992). Generally relationships of fecundity with either weight or standard length for orange roughy tend to be variable with the proportion of variance (r^2) explained by such regressions being between 0.3 - 0.6 (Pankhurst 1988; Bell et al. 1992). The causes of this high variability have not been apparent.

As Nikolsky (1969) noted, density dependent regulation of fecundity may serve to stabilize fish population dynamics. Nikolsky et al. (1973) pointed out that two hypotheses underlie this possibility: first, that fecundity is density dependent, and second, that variation in egg production regulates recruitment. Within broad limits of egg production, there is only weak empirical evidence that it is significantly linked to recruitment for most fish species, presumably due to the interaction of their very high fecundity and variable mortality during the early life history (Koslow 1992). However, the evidence even for density dependent regulation of fecundity is equivocal, apparently due to the influence of density independent factors. Thus recent studies of northern anchovy (Lasker 1985a) and northeast Atlantic herring (Almatur and Bailey 1989) revealed interannual changes in fecundity unrelated to changes in adult abundance, although other studies have reported variation in fecundity consistent with density dependence (see examples cited in Nikolsky 1969, Nikolsky et al. 1973, Bagenal 1973, Rothschild 1986, chapter 6, and Rothschild and Fogarty 1989). However, there are still relatively few studies that have monitored the fecundity of a marine fish population over a period of major change in spawning stock size. In particular, there have been few opportunities to study the response of reproductive output to the initial 'fishdown' of a fishery, when the population is reduced from its ostensible carrying capacity to some fraction of its virgin biomass.

The only known major spawning ground for orange roughy in Australian waters consists of a single seamount on the continental slope off northeast Tasmania. The region was first fished for orange roughy in 1986 but the catches prior to 1989, when the spawning aggregation was first discovered and exploited, were less than 5000 tonnes. Acoustic and egg surveys of the stock were carried out from 1990-92, and fecundity was sampled for five years off eastern Tasmania in 1987 and from 1989-92. Over this period, the biomass surveys indicated that the adult stock was reduced by almost half from a virgin biomass of approximately 100,000 to 52,000 tonnes (Smith et al. 1994, Kloser et al. in prep., Koslow et al. in prep.).

The major focus of our study is on the factors contributing to the high variability noted in orange roughy fecundity. In general, fecundity may vary among spawning stocks or spawning areas, in relation to fish condition, and inversely with egg size. Orange roughy were collected from smaller spawning groups off New South Wales and South Australia, as well as from the main spawning aggregation off eastern Tasmania (Fig. 1), so variability among spawning areas could be analysed. Studies based on morphometric, parasite and otolith trace-element analyses (Lester et al., 1988; Edmonds et al., 1991; Elliott et al., submitted) indicate that each of these spawning groups represents a distinct stock, although more conservative allozyme and mitochondrial DNA analyses only clearly separate the New South Wales stock from the others (Elliott and Ward, 1992; Smolenski et al., 1993). The influence of fish condition and relationships between egg size and fecundity were also examined, as was the relationship between fecundity and specific lipid levels in somatic and reproductive tissue. Lipids are the major source of stored energy in marine fishes (Sargent, 1976; Sargent et al., 1989), and the importance of lipids to ovarian development is well documented (Kjorsvik et al., 1990).

The possible influence of aging on fecundity was also examined. Age has generally been found to be a poorer predictor of fecundity than size, but investigations hitherto have examined only how fecundity increases with age (Bagenal 1978). However, orange roughy is an exceptionally long-lived fish, with otolith ring counts and radiometric dating both indicating ages well in excess of 100 years (Fenton et al. 1991; Smith et al. submitted). Because the population has not been previously exploited, it still contained very old fish. This enabled us to examine whether reproductive effort declined among older members of the population.

METHODS

The primary area of collection of fish for this study was off eastern Tasmania, the presumed area of distribution of orange roughy that spawn off northeast Tasmania. Orange roughy were collected there in 1987 and 1989-92. Orange roughy were also collected for fecundity analysis off South Australia and New South Wales in 1988-89. The collection dates and numbers of fish collected are tabulated by area in Table 1, and the areas are shown in Figure 1.

Total fecundity was estimated based on the gravimetric method. The mean number of yolky exogenous vitellogenic (EV) oocytes per gram of ovarian tissue was estimated and multiplied by the total ovarian weight, consistent with Pankhurst and Conroy (1987). Oocytes were enumerated when their mean diameter was greater than 11 μ m. These conditions were found prior to spawning from May to early July: spawning in orange roughy off eastern Tasmania begins in the second third of July and peaks during the last 10 days of the month (Bell et al. 1992). Sample collection was concentrated in the period just prior to spawning from 1990-92 following analysis in 1989 of changes in fecundity during the pre-spawning period from April until July. Fish were rejected for fecundity analysis if hydrated oocytes were present.

The ovaries from each fish were removed and frozen prior to analysis in the laboratory, except for the ovaries collected in 1987, which were stored in Gilson's fluid. The gonads were weighed individually both when initially collected on board ship or in the fish processing plants and after thawing prior to sub-sampling and counting the eggs. The weights in the laboratory were more accurate, and these values were used in calculations requiring gonad weight, such as the gonadosomatic index (GSI), which is the total gonad weight divided by the eviscerated weight (EW) of the fish and expressed as a percent, and the estimated egg size, which is the gonad weight divided by the fecundity.

Egg counts per gram of ovary were based on four 1-2 g subsamples prior to 1990 and on three 2 g subsamples subsequently from different sections of the ovary (i.e. center, anterior and posterior). Preliminary sampling indicated there could be significant differences in the number of eggs per gram of ovary between the two ovaries in a fish, so the number of eggs was determined for each ovary separately and the numbers added to determine total fecundity for each fish.

Egg numbers were counted manually. The person employed to count the oocytes varied between years, so samples were retained for initial training and intercalibration. The mean differences between years based on these samples were 25%. When samples were blindly re-enumerated, the differences between counts by the same person were of the same magnitude.

For each fish, data were obtained for standard length (SL), total weight and gonad weight. From 1990-92, the EW and liver weight were also recorded.

Sagittal otoliths from fish obtained in 1991 and 1992 were retained for ageing. Ages were read from sectioned otoliths because whole otoliths are inadequate for ageing mature fish (Smith and Robertson 1992; Smith et al. submitted). Otoliths were embedded in a block of clear polyester resin and sectioned longitudinally along the anterior/posterior axis using a "Gemmast" circular saw with a diamond blade of 0.15 mm thickness. Sections were approximately 0.4 mm thick. No further preparation was required other than to mount them on slides with a clear mountant and cover slip. In most cases, three sections were taken through each otolith to ensure that sections went through the primordium. Increments were counted using a

compound microscope at magnifications of 40 to 100 times. Each otolith was read "blind", that is with no reference to fish size. All were read twice by the primary reader and about 50% were also read by a secondary reader. The index of average precision (Beamish and Fournier 1981) was 3-4%. The first ages from the primary reader were used in analyses except where a considerable disparity occurred between readings or readers. In these cases, if agreement was not reached after re-examination, the otoliths were discarded.

Lipid analyses were carried out using fish of similar length (36 cm). Samples of liver, muscle and gonad tissue were frozen at -10°C prior to extraction. Wet tissue samples were weighed before grinding in CHCl₃. Lipids were extracted quantitatively using the modified one-phase CH₃-MeOH Bligh and Dyer method (1959) (White *et al.* 1979). A portion of each sample was freeze dried to determine the water content of the tissue, which was used to convert wet tissue weights to dry weights. After phase separation, the lipids were recovered in the lower CHCl₃ layer (solvents were removed in vacuo) and stored at -20°C. For selected samples of ovarian tissue, fatty acid methyl esters (FAME) and free alcohols were formed by direct transesterification of an aliquot of the total lipids with methanol/CHCl₃/HCl (10:1:1; 100°C; 120 min). After cooling, 1ml milli-Q-water was added and products were extracted into hexane/CHCl₃ (4:1). Solvents were removed under a stream of nitrogen and the alcohols converted to their corresponding O-trimethylsilylethers (OTMS ethers) by treatment with bis(trimethylsilyl)trifluoroacetamide (BSTFA).

A portion of the total lipid extract was analysed for total lipid composition with an Iatroscan MK III TH10 TLC-FID analyser (Iatron Laboratories, Japan) as described in Volkman & Nichols 1991. Gas chromatographic analysis of fatty acid methyl esters were performed on a Hewlett Packard 5890 GC equipped with a methyl silicone fused-silica capillary column and a flame ionization detector as described in Nichols *et al.* 1991. Fatty acids and alcohols were identified by comparing retention time data with that obtained for authentic and laboratory standards. GC-MS analysis of samples was performed on a HP 5890 GC and 5970 Mass Selective Detector fitted with a direct capillary inlet and a split/splitless injector as described in Nichols *et al.* 1991.

RESULTS

The mean gonadosomatic index (GSI) for orange roughy from eastern Tasmania, where EW was recorded, was 6.5. However, the mean weight of the gonads after thawing was 80% of their initially recorded weight, presumably due to loss of water from the tissue. The GSI corrected for weight loss due to preservation was 8.1.

The relationship of fecundity with SL varied significantly between orange roughy from New South Wales, eastern Tasmania, and South Australia based upon data from 1987-89, when fecundity data were collected from the three areas (Fig. 2).

This period is prior to the onset of intensive fishing for orange roughy off eastern Tasmania. Analysis of covariance indicated that the slope of the relationship of fecundity with SL did not differ significantly between areas (Area by SL interaction: $F = 2.29$, $df = 2$, $p > 0.05$). Assuming a common relationship of fecundity with SL, fecundity varied significantly among areas ($F = 7.12$, $df = 2$, $p < 0.001$). Based upon the samples, the observed mean fecundity (f) was lowest for orange roughy in New South Wales ($f = 31,056$) and highest in South Australia ($f = 42,809$). However, the orange roughy in New South Wales were smaller than in the other areas. When the fecundity values were adjusted for SL, the orange roughy in New South Wales had the highest fecundity ($f_{adj} = 42,787$) compared with east Tasmania ($f_{adj} = 31,085$) and South Australia ($f_{adj} = 35,339$).

Orange roughy fecundity around southeastern Australia was highly variable. Table 2 shows the percent of the variance explained by ordinary least-squares regressions of fecundity as a function of either standard length (SL), total weight (TW) or eviscerated weight EW for fish collected off eastern Tasmania in 1990-92. Although the regressions of fecundity with SL were highly significant, they explained only 10-17% of the variance in any year. The relationships with TW and EW were slightly improved but these relationships may confound the influence of condition factor and, in the case of TW, gonad weight. The mature orange roughy span a relatively small size range (30-45 cm), and there was no evidence of a non-linear relationship of either fecundity or TW with SL (Fig. 3). In order not to introduce the effect of possible confounding variables, the ensuing analyses are based on the relationship of fecundity with SL, as recommended by Bagenal (1978). The effects of population density, age, fish and liver condition, and egg size will be examined to assess their influence on orange roughy fecundity.

Data from 1987-92 indicate that development of the fishery and decreasing stock size led to a significant increase in fecundity (Fig. 4) (ANCOVA, $F = 6.47$, $df = 4$, $p < 0.001$; interaction of SL and year: ns). Fecundity varied little from 1987-90 with a mean adjusted for SL of $35,145 \pm 344$ (1 SE) eggs per female through this period (no data for 1988). Relative to this baseline period, fecundity increased 14% and 20% to 40,098 and then 42,108 eggs/female in 1991 and 1992, respectively. Relative fecundity varied over this period from 24.75 to 29.65 eggs per gram eviscerated body weight based upon a mean eviscerated wet body weight for orange roughy from eastern Tasmanian samples of 1420 g.

SL explained only 14% of the variance in fecundity (regression analysis, $F = 31.70$, $df = 201$, $p < 0.001$) for 1991-92 when data on orange roughy age and liver weight were obtained. Data for these two years was pooled because there was no significant effect of year on fecundity during this period (ANCOVA, $p > 0.05$). However, egg size, liver condition, and age each contributed significantly to the variance (21, 5, and 3%, respectively), such that in combination with SL, 41% of the variance in fecundity was explained (Table 3). Egg size was more closely correlated with fecundity ($r = -0.52$) than was SL ($r = 0.37$). As expected, fecundity and egg size are inversely related, such that there appears to be a trade-off between egg size and egg number for a given gonad weight. Age was inversely related to fecundity in

the regression analysis. A scatterplot indicated that fecundity appears to decline after about age 60, a possible sign of senescence (Fig. 5). The variables, egg size and age, showed significant departures from homogeneity of variance (Cochran's C and Bartlett-Box F tests, $p < 0.05$). This was corrected by log transformation, and the log-transformed variables were used in all analyses.

The significant relationship of a condition factor related to liver weight with fecundity (Table 3) indicated that the liver in orange roughy probably serves as an organ for energy storage. Lipid comprised 41% of the dry weight of the liver with a range of 16-61%, based upon a sample of 9 fish. Triacylglycerol, a short-term energy storage form of lipid (Benson and Lee 1975), was the dominant constituent of the lipid in the liver (range: 38 - 79%). A condition factor based on eviscerated body weight normalized for SL did not enter the regression significantly, but in the absence of a variable based on liver condition, the body weight condition factor entered the regression significantly ($p = 0.01$). The mean lipid content of orange roughy muscle was 40% ($n = 9$; range: 10 - 61%) but the predominant lipid component was wax ester, which comprised up to 99% of the total lipid. Wax ester is considered a long-term energy storage form of lipid (Benson and Lee 1975). The two condition factors were significantly correlated with each other ($r = 0.28$, $p < 0.001$), and the variance in fecundity that they explained presumably overlapped.

Because egg size was most closely linked to fecundity, we examined factors that might contribute to variation in egg size. Stepwise regression analysis of egg size (log-transformed) against age (log-transformed), SL, and body and liver condition factors indicated that only liver condition factor was related to egg size (Table 4). Orange roughy with higher liver condition factor had smaller eggs. The relationship may not be causal but arise from the positive correlation of liver condition to fecundity and the strong inverse relationship of fecundity and egg size. Only 6.5% of the variance was explained by the regression.

Fecundity was significantly correlated with the amount of lipid per gram of ovary ($r = 0.68$, $p = 0.01$) (Fig. 6a). The lipid content of the ovary was examined in 22 specimens and ranged from 15% to 27% dry weight with an average of 23%. There was no significant correlation between lipid levels in either liver or muscle and fecundity, but the sample size was small ($n=9$).

Lipid composition varied between the muscle, liver, and ovary samples. Wax ester was the major lipid class of muscle tissue comprising up to 99% of the total lipid. However, triacylglycerol (TAG) was the major lipid constituent of the liver and ovary with values ranging from 38-79% and 40-58% in the two tissue types, respectively. Fecundity was significantly correlated with the proportion that TAG represented of the total lipid fraction (Fig. 6b). Wax ester was somewhat more important in the liver with the percent of total lipid ranging from 7-20% but averaged only 7% of the ovarian lipid. The percentage of polar lipid as a component of ovarian lipid varied from 27-41% and declined with increasing fecundity. Polar lipid ranged from 9-32% as a fraction of the liver lipid.

DISCUSSION

VARIABILITY IN FECUNDITY AND LIPID CHEMISTRY

Orange roughy liver and muscle lipid content was highly variable in this study with no apparent relationship between these levels and fecundity. This variability may reflect the dramatic changes taking place in the liver during vitellogenesis (Aida et al 1973). Changes induced by estrogen result in an increase in plasma levels of lipid and protein (Plank and Woodhead 1966, Craik 1978). The lipid mobilising effect of estrogen has been well documented (de Vlaming et al 1977, Fostier et al 1983), but little is known about hormone sensitive lipases that hydrolyse storage lipids during egg production. Both the rates of catabolism of dietary wax esters and triacylglycerols and the mobilisation of lipid stored in tissue may fluctuate depending on the individual metabolic need encountered in ovarian growth and spawning. Analysis of fish during previtellogenesis or post spawning stages would probably provide a more reliable index of reproductive condition in terms of somatic lipid levels. Fecundity during these stages, however, would be difficult to assess. An analysis of tissue from intracranial fat, skull and swim bladder, all of which are major lipid stores in orange roughy (Grigor et al 1990), may also be appropriate in understanding the energetics of fecundity in this species.

The major lipid components in the ovary were triacylglycerol and polar lipid. Triacylglycerol levels increased significantly with increasing fecundity. Both triacylglycerol and wax ester serve as sources of metabolic energy, and one can only speculate as to the physiological advantage of triacylglycerol as the preferred lipid component in the ovary. Many species of fish accumulating oil globules in their eggs rather than yolk or yolk platelets have large amounts of wax and sterol esters in their eggs (Kaitaranta and Ackman 1981; Thomas and Walsh 1988). Other species like orange roughy accumulate both polar lipid and triacylglycerol (Kaitaranta and Ackman 1981). Wax esters are the major lipid component in the somatic tissue of orange roughy and constitute a considerable proportion of their dietary lipid (Sargent et al 1983, Grigor 1990). Triacylglycerol, which is high in polyenoic acids, provides most of the fatty acids for energy and the biosynthesis of membrane lipids. Because waxes are usually deficient in long chain polyunsaturated fatty acids, they are used primarily as an energy source rather than for structural purposes, such as in cell membranes (Cowey and Sargent 1972).

Triacylglycerol is considered a short term storage lipid. It is preferentially mobilised over wax esters by copepods during starvation (Benson and Lee 1975). The utilisation of wax esters is carefully regulated and serves primarily as an energy source during long term starvation and hence its provision for reproductive energy may be limited for orange roughy.

COMPARISONS BETWEEN AREAS AND SPECIES

Although orange roughy fecundity varied significantly between spawning areas across southeastern Australia, their fecundity was broadly similar between southeast Australia and New Zealand. Pankhurst and Conroy (1987) reported that fecundity of orange roughy off the Kaikoura coast of South Island of New Zealand ranged from 26,000 to 90,000 eggs per female with an average relative fecundity of 22.00 eggs/g TW or 25.28 egg/g EW, based on the relationship from our data: $EW = 0.87 TW$. This compares with values of 24.75 - 29.65 eggs/g EW from east Tasmania orange roughy from 1987-92.

Pankhurst and Conroy (1987) noted that orange roughy fecundity was substantially lower than the fecundity of most commercial species, often by 1-2 orders of magnitude, based on a comparison with species both around New Zealand and in the northern hemisphere. However, although orange roughy, like most other commercially-important species, is a dominant component of its community, several aspects of its ecology and life history set it apart from other commercial species: its depth of occurrence (700-1200 m), longevity (~150 years), and age at first maturity (~30 years) (Fenton et al. 1991). Two species of oreos (family Oreosomatidae), the smooth oreo (*Pseudocyttus maculatus*) and the black oreo (*Allocyttus niger*) that are also commercially exploited at mid-slope depths and are of comparable size and longevity (Stewart et al. submitted) have comparable fecundities: 12.41 and 20.11 eggs/g EW, respectively. These oreosomatids also, like orange roughy, are single batch spawners and have a relatively brief, well-defined spawning period (Pankhurst et al. 1987). Virtually nothing is known of the larval ecology of either orange roughy or these oreosomatids.

Because there is broadly a trade-off in teleosts between egg size and number, comparisons of fecundity per se may reveal little about relative expenditure on reproductive output, because orange roughy oocytes are considerably larger than those of most teleosts. Comparison of the GSI or other indices of reproductive expenditure relative to body size may be more informative. Orange roughy reproductive output appears to be considerably less than that of most teleosts inhabiting shelf and upper-slope waters, for which commercial fisheries have developed. For example, the mean orange roughy reproductive expenditure off east Tasmania varied between 1987-92 from 10.15 - 12.16 mg dry weight of egg/g EW, which may be compared with values of 16-38, 36, 52, and 88 for a range of gadoids: Norway pout, cod, haddock, and whiting, respectively (Hislop 1984). (The conversion of egg numbers to 'dry weight (DW)' is based upon the regression employed by Hislop (1984): $\log DW, \text{ mg} = 3.35 \text{ egg diameter, mm} - 1.57$, from Simpson (1956)).

However, orange roughy reproductive expenditure appears comparable to or slightly greater than that of other bathypelagic and benthopelagic species. The mean GSI for orange roughy from east Tasmania was 8.1, based on eviscerated body weight and 7.0 based on total weight. The median GSI, apparently based on total weight, for 13 species of benthopelagic fish from the Indian and Atlantic Oceans

was 4.0 (range: 0.2 - 15.7) and for 20 collections of 12 bathyal species was 2.95 (range: 0.9 - 11.9 (Golovan and Pakhorukov 1984). However, at least some of the fish in their study appear to have been multiple batch spawners, so their total annual reproductive expenditure may be greater.

COMPENSATORY CHANGES IN ORANGE ROUGHY FECUNDITY

Changes in fecundity are potentially an important compensatory mechanism contributing to the stability of fish populations undergoing exploitation (Nikolsky 1969; Nikolsky et al. 1973; Rothschild et al. 1989). However, there has been considerable skepticism regarding the role of fecundity as a regulatory mechanism both because of the lack of a clear relationship between egg production and subsequent recruitment (Koslow 1992) and the relatively few studies that have demonstrated the degree to which fecundity may vary in relation to physical and biological conditions.

Under extreme conditions, fecundity may vary dramatically. For example, Johnson (1972) studied the reproductive activity of lake trout in an unfished far northern Canadian lake. The population was dominated by older fish, fecundity was reduced to a mean of 609 eggs per female, and mature females spawned only once every three years.

Such extreme conditions have not been observed in a marine system. In an oft-cited study, Raitt (1968) reported that fecundity in Norway pout, *Trisopterus esmarkii*, a small, short-lived, planktivorous gadoid in the North Atlantic, increased 25 times from 1964 to 1966, apparently in response to intense exploitation. However, from Raitt's description, Norway pout are repeat spawners, and Raitt only measured the number of relatively large eggs, i.e. the number of eggs in the batch next to be spawned. The relationship between fecundity of a single batch and annual fecundity is not straightforward (Hunter et al. 1992). Moreover, because the industrial fishery had reached high levels of exploitation by 1962, it is not clear that the density of Norway pout was significantly higher in 1964 than in 1966 (Figures 3, 6 in Raitt 1968).

We found that fecundity in orange roughy increased 20% from 1987 to 1992 during a period when the spawning stock was reduced by approximately 50% (Fig. 4). Population fecundity may have increased even further. Trawl surveys carried out along the east coast of Tasmania from 1990 through 1992 during the several months prior to the spawning period examined the proportion of orange roughy with developing gonads that would spawn that season. The proportion of non-spawners in the population declined from 45% (+11%) in 1990 to 24% (+17%) in 1992 (Koslow et al. in prep.). Assuming a mean weight for orange roughy of 1.5 kg, these results indicate that population fecundity for orange roughy may have actually increased from 1989 to 1992 from 1.5×10^{12} to 1.8×10^{12} eggs, a factor of 17%. A further compensatory effect on the population's reproductive output may be the replacement with new recruits of old fish whose egg production is significantly reduced (Fig. 5). However, there is no evidence at this time, based upon the

apparently unchanged size structure of the population, of new recruits entering the spawning stock (Koslow et al. in prep.). In general, though, our data, which extends from a previously unexploited population of orange roughy to its reduction to 50% of virgin biomass, supports the hypothesis that population fecundity may serve as a strongly compensatory mechanism. These results also indicate that the orange roughy population prior to exploitation was at or near its carrying capacity, such that its productivity was highly constrained by intra-specific competition. The reduced fecundity of the older fish is a further sign of a senescent population.

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TABLE 1. THE AREAS, MONTHS OF COLLECTION, AND NUMBER (IN PARENTHESES) OF ORANGE ROUGHY COLLECTED FROM SITES AROUND SOUTHEASTERN AUSTRALIA (FIG. 1) FOR FECUNDITY ANALYSIS.

| YEAR | AREA | | |
|------|---------------|-----------------|-----------------|
| | East Tasmania | New South Wales | South Australia |
| 1987 | May (13) | | |
| | June (16) | | |
| 1988 | | May (21) | May (33) |
| 1989 | May (10) | May (21) | June (41) |
| | June (10) | | |
| | July (8) | | |
| 1990 | July (161) | | |
| 1991 | July (121) | | |
| 1992 | July (115) | | |

TABLE 2. THE PERCENT VARIANCE EXPLAINED BY ORDINARY LEAST-SQUARES REGRESSION ANALYSIS OF FECUNDITY WITH STANDARD LENGTH (SL), TOTAL WEIGHT (TW), AND EVISCERATED WEIGHT (EW) FROM ORANGE ROUGHY COLLECTED OFF EASTERN TASMANIA IN 1990-92. ALL REGRESSIONS WERE HIGHLY SIGNIFICANT ($P < 0.001$). THE NUMBERS OF FISH IN THE REGRESSIONS WERE 119 IN 1990, 121 IN 1991, AND 114 IN 1992.

| | 1990 | 1991 | 1992 |
|----|------|------|------|
| SL | 0.17 | 0.17 | 0.10 |
| TW | 0.34 | 0.29 | 0.17 |
| EW | 0.28 | 0.22 | 0.12 |

TABLE 3. RESULTS OF ANALYSIS OF VARIANCE AND MULTIPLE REGRESSION ANALYSIS FOR THE EFFECTS OF STANDARD LENGTH (SL), CONDITION (COND), LIVER CONDITION (LIVER), AND THE LOG₁₀-TRANSFORM OF EGG SIZE (LOGEGG) AND AGE (LOGAGE) ON FECUNDITY OF THE EAST TASMANIA STOCK OF ORANGE ROUGHY. B: COEFFICIENT OF REGRESSION; SE: STANDARD ERROR; B: STANDARDIZED REGRESSION COEFFICIENT; DF: DEGREES OF FREEDOM; T: STUDENT'S T; P: PROBABILITY; R²: CUMULATIVE PROPORTION OF VARIANCE EXPLAINED.

ANALYSIS OF VARIANCE

| | df | Sum of squares | Mean square |
|-------------------|-----|----------------|-------------|
| Regression | 4 | 20237680696 | 5059420174 |
| Residual | 189 | 28624070409 | 151450108 |

F = 33.41, p < 0.001.

REGRESSION ANALYSIS

| Variable | B | SE B | b | t | p < | R ² |
|---------------------------------------|---------|-------|-------|-------|-------|----------------|
| SL | 2710 | 464 | 0.41 | 5.84 | 0.001 | 0.13 |
| Log egg | -55880 | 7638 | -0.43 | -7.32 | 0.001 | 0.36 |
| Liver | 459 | 150 | 0.18 | 3.06 | 0.01 | 0.39 |
| Log age | -19862 | 7923 | -0.17 | -2.51 | 0.05 | 0.41 |
| Constant | -172092 | 23485 | | -7.33 | 0.001 | |
| Cond (not in equation) | | | | 1.62 | 0.11 | |

TABLE 4. RESULTS OF ANALYSIS OF VARIANCE AND MULTIPLE REGRESSION ANALYSIS FOR THE EFFECTS OF STANDARD LENGTH (SL), CONDITION (COND), LIVER CONDITION (LIVER), AND THE LOG₁₀-TRANSFORM OF AGE (LOGAGE) ON THE LOG TRANSFORM OF EGG SIZE IN THE EAST TASMANIA STOCK OF ORANGE ROUGHY. ABBREVIATIONS AS IN TABLE 3.

ANALYSIS OF VARIANCE

| | df | Sum of squares | Mean square |
|-------------------|-----------|-----------------------|--------------------|
| Regression | 1 | 0.18 | 0.18 |
| Residual | 192 | 2.65 | 0.014 |

F = 1338, p < 0.001.

REGRESSION ANALYSIS

| Variable | B | SEB | b | t | p < | R² |
|--|----------|------------|----------|----------|---------------|----------------------|
| Liver | -0.0051 | 0.0014 | -0.26 | -3.66 | 0.001 | 0.065 |
| Constant | -2.18 | 0.15 | | -14.66 | 0.001 | |
| SL (not in equation) | | | | -1.54 | 0.13 | |
| Log age (not in equation) | | | | 0.002 | 0.99 | |
| Cond (not in equation) | | | | -0.37 | 0.71 | |

FIGURE LEGEND

Figure 1. Map showing the areas from which fish were retained for fecundity analysis.

Figure 2. The relationship of fecundity with standard length for orange roughy off a) eastern Tasmania, b) New South Wales, and c) South Australia.

Figure 3. The relationships of a) total weight and b) fecundity with standard length (SL) for fish collected off southeastern Tasmania in 1991.

Figure 4. Changes in mean fecundity adjusted for standard length and estimated spawning stock size of orange roughy from 1987-92. Stock size is estimated from egg and acoustic surveys and catch records (Kloser and Koslow in prep; Koslow et al. in prep; Bax 1993). No fecundity data are available for 1988. Fecundity: squares; stock biomass: diamonds.

Figure 5. Fecundity (egg number/female) in relation to age for orange roughy from the eastern Tasmania spawning stock.

Figure 6. The relationship between fecundity and a) total lipid per gram of ovary and b) percent of triacylglycerol (TAG) in ovarian lipid.

FECUNDITY IN ORANGE ROUGHY

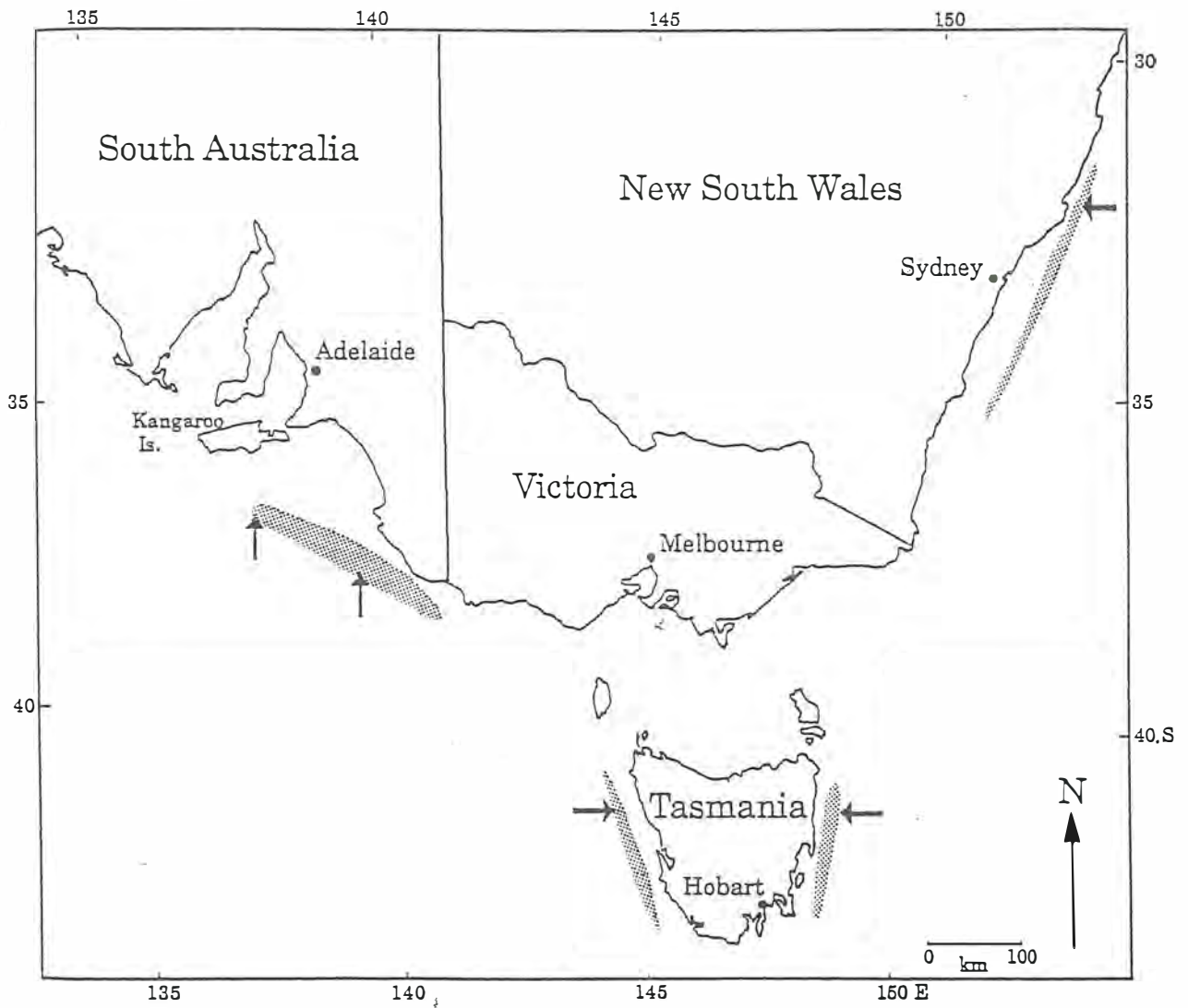
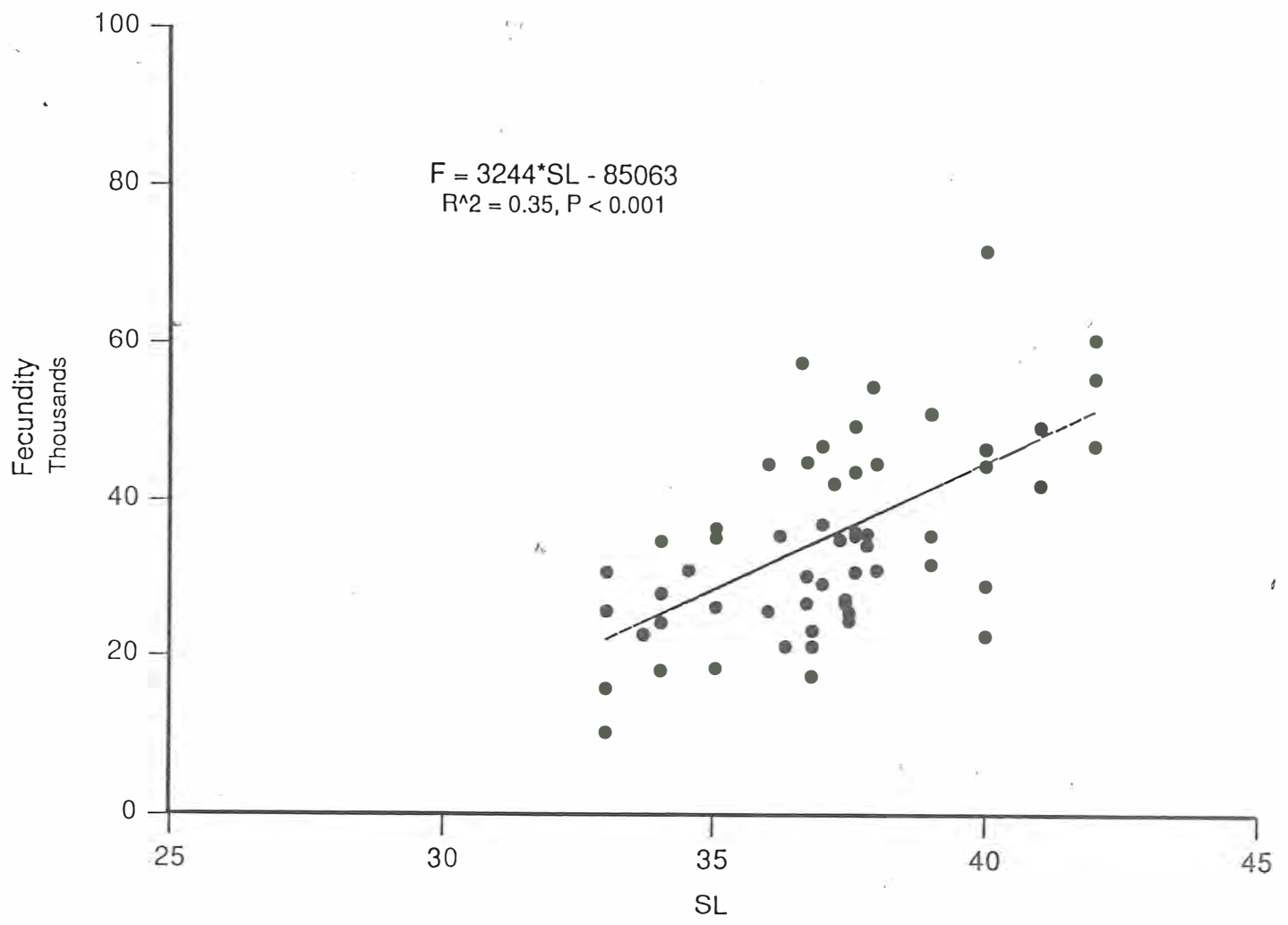
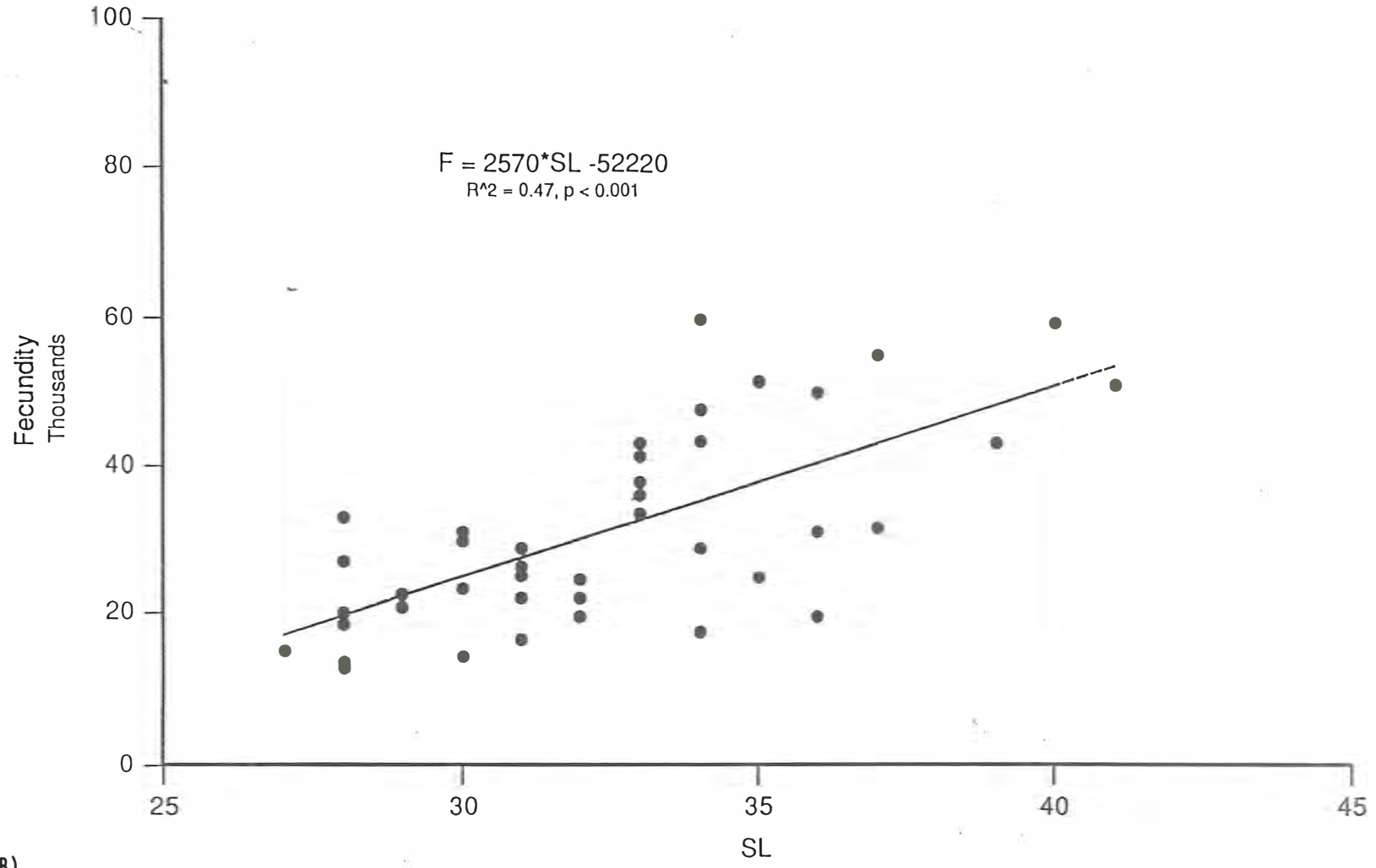


FIGURE 1

FIGURE 2

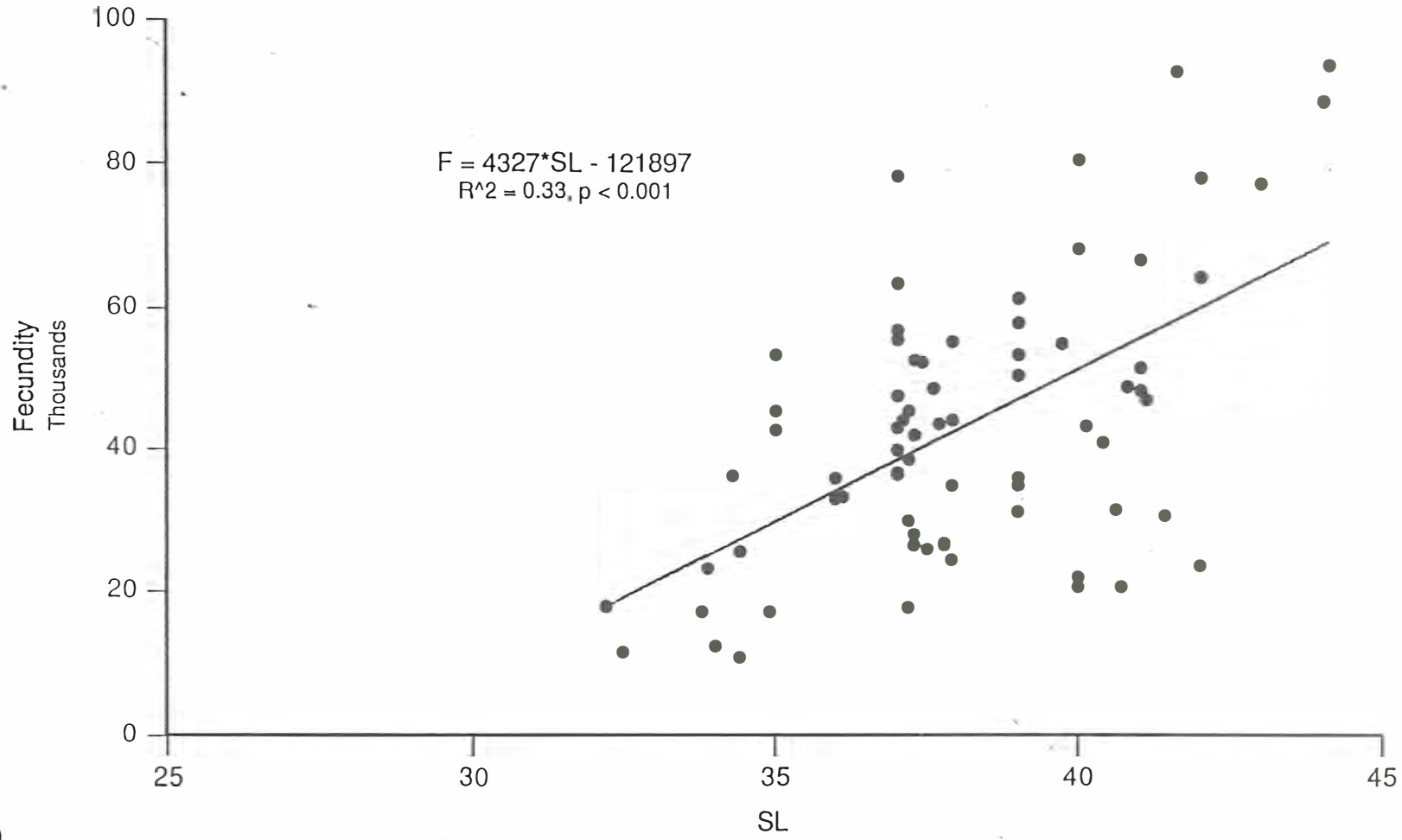
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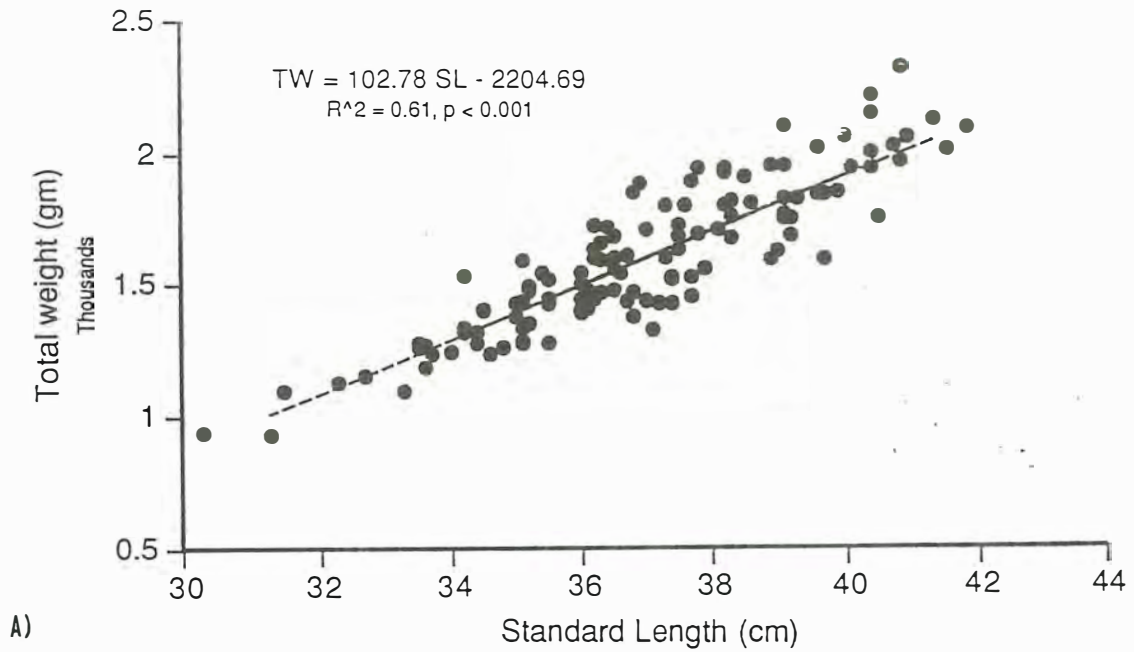


B)

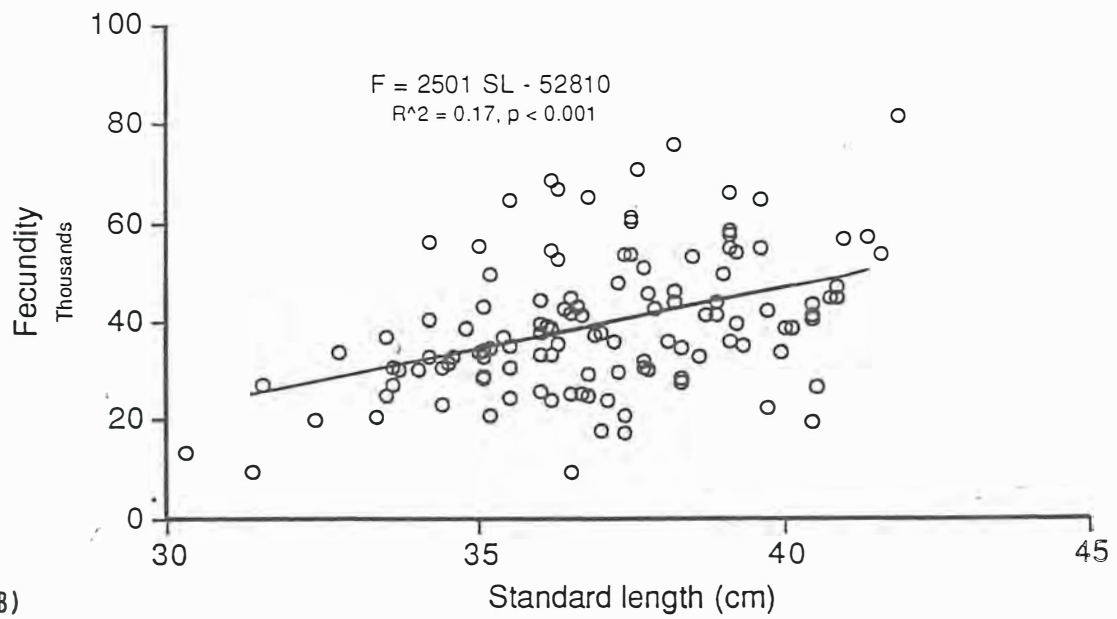
c)



FECUNDITY IN ORANGE ROUGHY



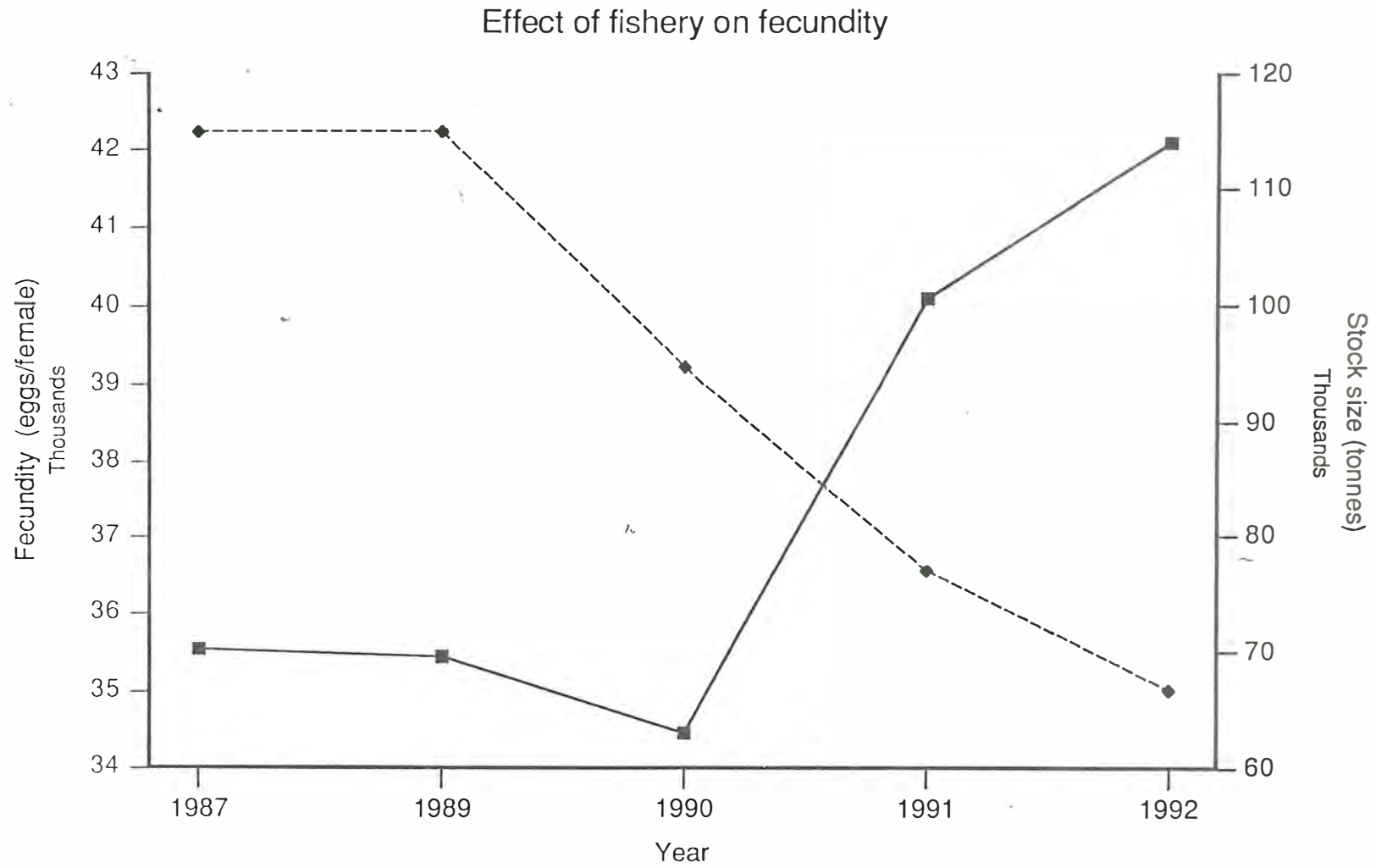
A)



B)

FIGURE 3

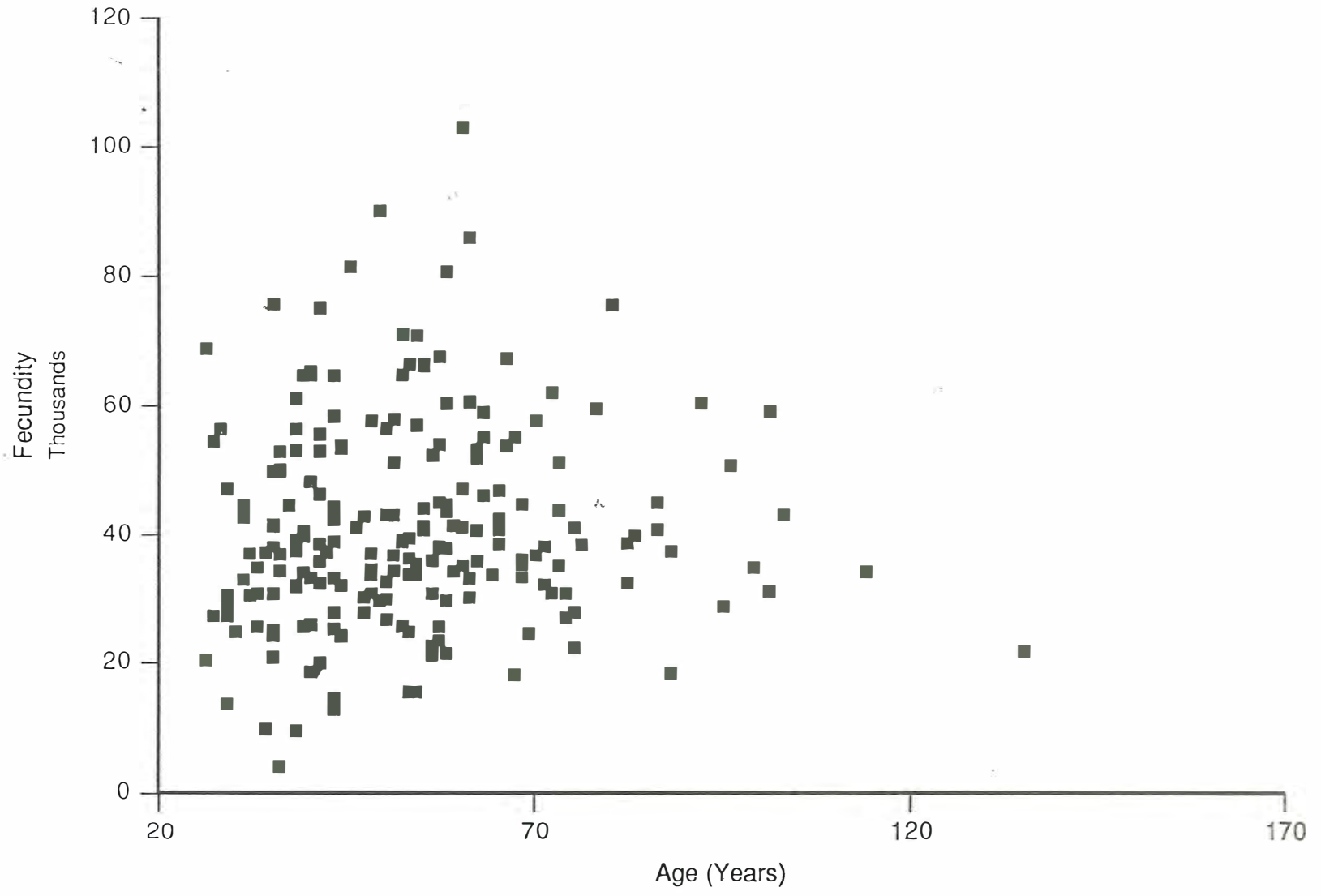
FIGURE 4



Fecundity is adjusted for SL
No data for 1988

FECUNDITY IN ORANGE ROUGHY

FIGURE 5



FECUNDITY IN ORANGE ROUGHY

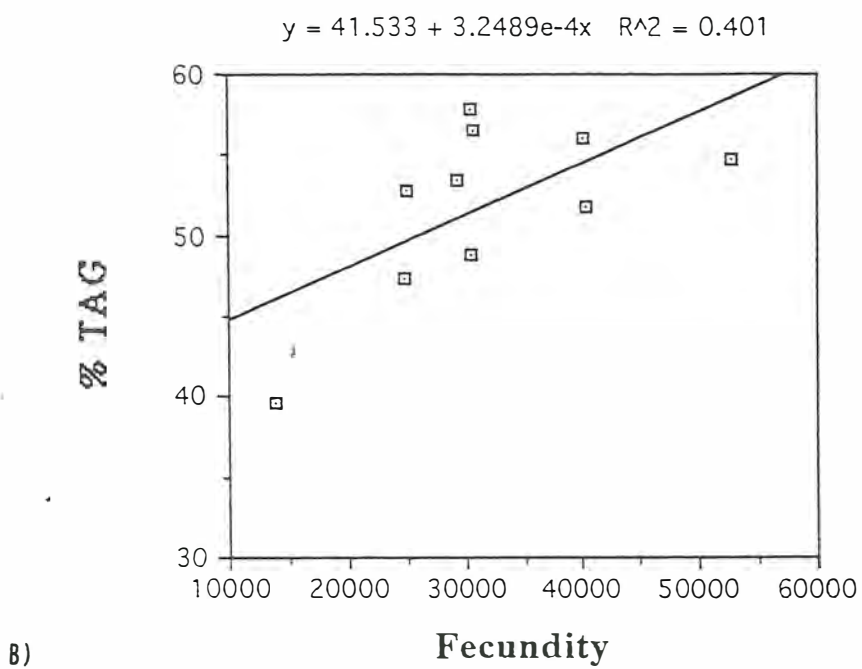
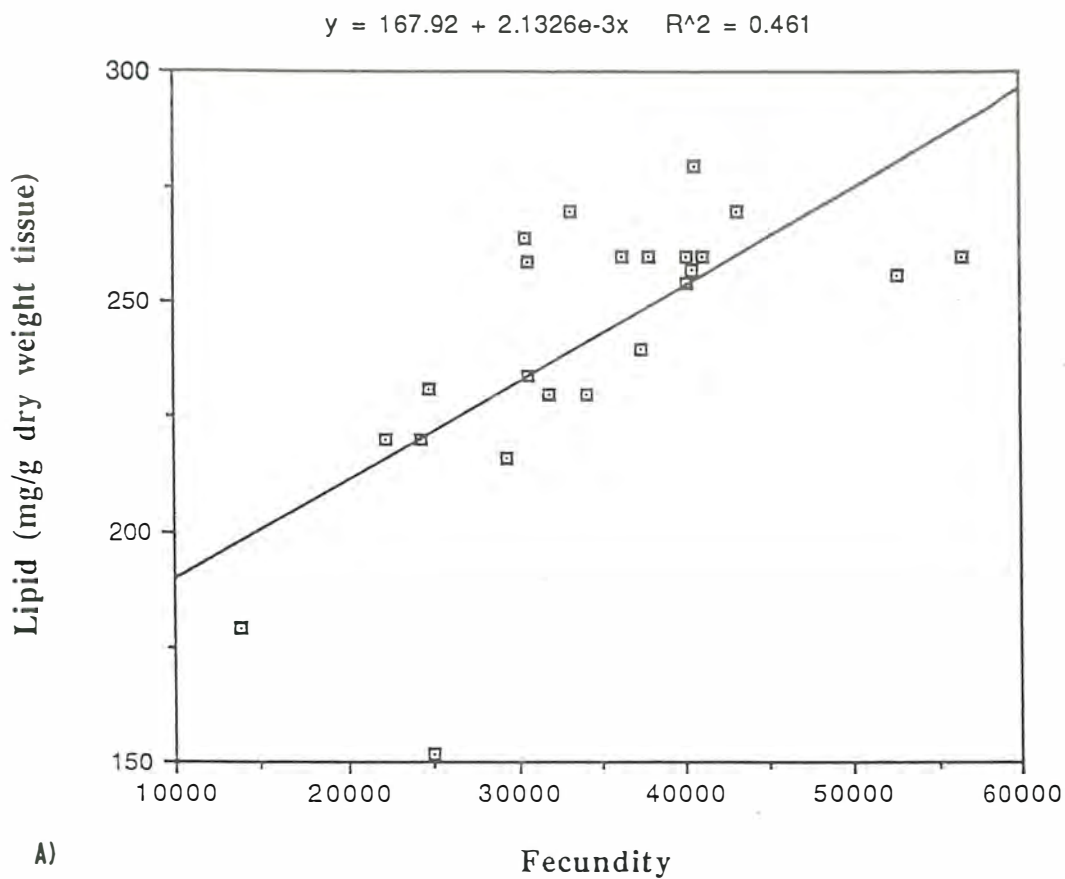



FIGURE 6

CHAPTER THREE

**BIOMASS ASSESSMENT OF A DEEPWATER FISH,
ORANGE ROUGHY *Hoplostethus atlanticus*, OFF EASTERN TASMANIA, AUSTRALIA,
BASED UPON AN EGG SURVEY**



**BIOMASS ASSESSMENT OF A DEEPWATER FISH, THE ORANGE ROUGHY
(*HOPLOSTETHUS ATLANTICUS*), OFF EASTERN TASMANIA, AUSTRALIA BASED
UPON AN EGG SURVEY**

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ABSTRACT

An egg survey was carried out in 1992 to estimate the biomass of the orange roughy stock that spawns off northeastern Tasmania. Spawning occurs at depths of 600-1000 m around a single seamount from early July through early August. The orange roughy are single-batch spawners. The fecundity of the adult females and the depth distribution and development rate of the eggs are described elsewhere. The eggs were sampled with vertical tows from 1000 m to the surface using a plankton net with a 2 m² mouth opening. A random stratified survey was designed to fully sample the eggs during their first day of development. The estimated biomass of spawning fish was 29,100 tonnes with a coefficient of variation (CV) of 35%. Based on the proportion of non-spawning fish observed in the population (24% of females and 10% of males) and estimated catch of spawners in 1992 (16,300 tonnes), total stock biomass was estimated to be 45,100 tonnes at the beginning of the fishing season and 28,800 tonnes at the end. Based upon the catch history of the fishery, virgin biomass of the stock was approximately 96,100 tonnes, so the stock was at 30% of virgin biomass at the end of season. The variance of the estimate was dominated by the spatial variability of the egg distribution: the CV increased < 1% when the variance in fecundity and in the proportion of non-spawners was incorporated. Comparison of lognormal and arithmetic estimates of the mean and variance indicated that the arithmetic estimate was far more sensitive to the influence of extreme values. The egg production estimate of stock size was in close agreement with an estimate derived from an acoustic survey conducted at the same time.

INTRODUCTION

A family of methods has evolved to estimate the biomass of fish populations based upon estimation of their egg production (Saville 1964; Lasker 1985; Lo et al. 1992). The common elements of this methodology are estimates of the number of eggs spawned, female fecundity, the proportion of the population engaged in spawning, and the spawners' male/female ratio. Variations on the basic model outlined by Saville (1964) have arisen largely to overcome logistic problems, such as surveying the ichthyoplankton over protracted spawning periods, or difficulties posed by a particular species' biology, such as multiple batch spawning. As a result, the egg production method has been successfully applied to fishes such as anchovy, sardine, and mackerel that spawn over broad geographical areas for extended periods of time and that have indeterminate fecundity (Lasker 1985; Lockwood *et al.* 1981; Armstrong *et al.* 1988; Lo *et al.* 1992) - indeed such apparently 'difficult' species might arguably be considered those for which the method is most typically applied. Until recently, however, the method was not extended to biomass estimation for deepwater fishes (Lo *et al.* 1992).

Orange roughy (*Hoplostethus atlanticus*) is a dominant member of the fish community at mid-slope depths (700-1200 m) around southeastern Australia and New Zealand (Koslow *et al.* in press). Following the discovery in 1989 of a major orange roughy spawning ground off northeastern Tasmania and a summer fishing ground off southern Tasmania, orange roughy became one of Australia's largest and most valuable fisheries. Orange roughy are highly vulnerable to overfishing because they are both highly accessible, forming large aggregations around seamounts, and very unproductive, attaining maturity at 25-30 years and living well in excess of 100 years (Mace *et al.* 1990; Fenton *et al.* 1991). Orange roughy fisheries in New Zealand were severely depleted following less than ten years of exploitation. It was therefore considered a matter of some urgency to estimate sustainable yield for the newly-developed orange roughy fishery off northeast Tasmania. Maximum sustainable yield (MSY) may be approximated from estimates of virgin biomass (B_0) and natural mortality (M) (Gulland 1971):

$$MSY = 0.5 M B_0 \quad (1)$$

Due to the recent development of the fishery, B_0 could be estimated from data on current stock biomass and catch history, assuming annual recruitment to be negligible. Orange roughy aggregate on ground not amenable to standard trawl operations, so trawl surveys were deemed unsuitable as a means to estimate stock biomass. Catch per unit effort is a poor indicator of orange roughy stock size because the stock is highly aggregated. Acoustic and egg surveys of the spawning stock were therefore proposed.

Although orange roughy spawn at 600-1000 m depth, their reproductive biology otherwise appeared well-suited to the use of the egg production method. Off northeast Tasmania, orange roughy spawn around a single seamount of

approximately 10 km² area that rises from depths of about 1100 m to ~600 m below the sea surface. The orange roughy spawn over a one-month period from mid-July to mid-August that has not varied noticeably in the four years that it has been monitored (1990-1993). Orange roughy produce a single batch of eggs and have determinate fecundity (Pankhurst *et al.* 1987). We report here on the use of an egg survey to estimate the biomass of the orange roughy stock that spawns off northeast Tasmania. The fecundity of this orange roughy stock and its egg development are reported elsewhere (Koslow *et al.* submitted; Bulman and Koslow submitted).

The distribution of fish eggs tends to be highly patchy, and field data on their density are generally highly skewed and contain many zero values. There has been some controversy concerning the best estimators for the mean and variance of egg survey data. Aitchison and Brown (1957) showed that there were more efficient estimators of the mean and variance of a lognormal distribution than the arithmetic sample mean and variance. Pennington (1983) and Pennington and Berrien (1984) applied these results to estimate the mean and variance of egg survey data, assuming that the egg data follow a Δ -distribution, a lognormal distribution modified to include the observation of zeros. Myers and Pepin (1990) criticized this approach, pointing out that many survey distributions do not follow a lognormal distribution and that such violations of model assumptions can lead to bias and reduced efficiency in the estimators. They recommended use of the arithmetic sample mean and variance as more robust. We applied both approaches to our survey data and compare results here to illustrate some possible advantages and disadvantages of the two methods.

METHODS

FIELD SURVEY

The objective of the ichthyoplankton surveys, which were carried out in July and August of 1991 and 1992, was to fully sample the area where 1-day old eggs were found. A systematic survey was carried out in 1991, which will be treated as a pilot study. The transects covered an area 30 nm N and S of the spawning hill, which is located at 41° 14' S lat. and 148° 45.5' E long. Stations extended from the shelf break to between 7.5 and 25 nm offshore, depending upon distance from the spawning ground. There were 5 transects within 5 nm of the spawning ground, with the distance between stations on the transect 2 nm and between transects 2.5 nm. At distances greater than 10 nm from the spawning ground, transects were 10 nm apart and stations along the transect were 5 nm apart. Three cruises were carried out over the course of the spawning season. The pilot study indicated that egg density declined markedly with distance from the spawning site: almost no 1-day old eggs were sampled at distances greater than 10 nm from the spawning site.

In 1992, a random stratified survey was designed based upon results of the 1991 survey. The study area was divided into two strata: a core stratum within 5 nm N and S of the spawning ground extending from 148° 44' E long. to 7.5 nm offshore, and an outer stratum that extended a further 5 nm N, S, and seaward of the core area (Fig. 1). Potential station positions were at intervals of one minute of latitude and longitude in the core stratum (=1.0 nm in a N-S direction and 0.75 nm in an E-W direction) and twice that distance in the outer stratum. Stations were selected randomly from the grids of potential station positions: 50 stations from the core stratum and 20 from the outer stratum. The inshore boundary of the survey area was extended 2 nm seaward of the inshore boundary in 1991, because virtually no 1-day old eggs were found along the shelf break. However, early stage eggs were frequently obtained at the inshore edge of the newly-defined survey area, so calculations of egg abundance in 1992 were based upon a boundary midway between that used in the two years, i.e. 1 nm onshore of the actual edge of the sampling grid in 1992.

The plankton was sampled with a ring net with a 2 m² mouth opening and 500 µm mesh. The water column was sampled vertically, such that the net was raised and lowered with its mouth directed upward. Tows were carried out to a maximum depth of 1000 m or until the mouth of the net was within a few metres of bottom. Height above bottom was monitored with a 50 kHz acoustic transducer mounted above the net. The acoustic signal was transmitted to the surface with a single-core conducting cable, which was used as the tow cable. Plankton samples were preserved in 4% formalin in seawater and buffered with sodium β-glycerophosphate, which was at saturation in the concentrated formalin.

In the laboratory, the orange roughy eggs were removed from the samples and enumerated to 9 developmental stages described by Bulman and Koslow (submitted). The egg development rate and age of the egg stages for this spawning stock were based on temperature-controlled experiments, the vertical distribution of the eggs through the water column, and the temperature structure of the water column (Bulman and Koslow (submitted)). Orange roughy eggs are distinctive and can be identified to species based upon their large size (2.1-2.3 mm) and bright orange oil droplet. Because only early stage eggs were fully sampled, daily egg production was assumed equal to the number of one-day old eggs. This neglects the effects of natural mortality during that day, so we examined the decline in egg numbers with age.

The proportion of reproductive fish in the spawning stock was estimated based upon a trawl survey carried out off eastern Tasmania in early May, 1992. Gonad development is advanced in all but the non-spawning fraction of the population at this time.

The male:female ratio, the size-frequency distribution and length-weight relationship by sex within the spawning stock were estimated based upon routine sampling of the commercial catch.

DATA ANALYSIS

The egg production method to estimate spawning stock biomass is based upon the following relationship between the number of spawning females (N_f), the number of eggs that they produce each day (E_j), which is summed over the n days of the spawning season, and their mean fecundity (F):

$$N_f = \frac{\sum_{i=1}^n E_i}{F} \quad (2)$$

The variance of N_f ($s_{N_f}^2$), the variance of a quotient, was estimated using the delta method, an approximation based on Taylor series expansions using the variance and mean associated with the estimates of total egg production (s_e^2 , E) and of mean population fecundity (s_F^2 , F) (Kotz and Johnson 1988):

$$s_{N_f}^2 = \frac{s_e^2 \cdot F + s_F^2 \cdot E}{F^4} \quad (3)$$

F was estimated to be 42,100 eggs per female in 1992 based upon the ordinary least-squares regression of fecundity to standard length and the size frequency distribution of the orange roughy in commercial landings (Koslow *et al* submitted). The variance of F (s_F^2) was estimated, following Snedecor and Cochran (1961) (p. 137), where s_r^2 is the residual mean square, n is the sample size, l_p is the mean standard length of mature female orange roughy in the population (Table 1), l_s is the mean length of orange roughy in the fecundity sample (37.5 cm), and ss_T is the total sum of squares of the regression:

$$s_F^2 = s_r^2 \left(\frac{1}{n} + \frac{(l_p - l_s)^2}{ss_T} \right) \quad (4)$$

The egg data were grouped into six time periods based upon breaks in the sampling and marked changes in egg abundance. The data were highly skewed and contained many zero counts. Means and variances for each stratum and time period were calculated both as the simple arithmetic mean and variance and following the method of Pennington (1983) and Pennington and Berrien (1984), which assumes that the non-zero egg counts follow a lognormal distribution. During gaps in sampling, egg production was extrapolated from adjacent sampling periods up to the mid-point between them. Daily egg production during each sampling period, j , (E_j) of length in days (d) was computed based upon the mean number of one-day old eggs in each stratum (E_s), where A_s is the area of the inner and outer strata (261.0 km² and 568.8 km², respectively):

$$E_j = \sum_{s=1}^2 A_s E_s \quad (5)$$

The variance in daily egg production during each sampling period (s_j^2) and overall (s_e^2) were calculated as follows:

$$s_j^2 = \sum_{s=1}^2 A_s^2 s_s^2 \quad (6)$$

$$s_e^2 = \sum_{j=1}^6 d_j^2 s_j^2 \quad (7)$$

The number of spawning males (N_m) was estimated based on the number of females and the sex ratio (m:f) in the commercial catch.

$$N_m = N_f(m:f) \quad (8)$$

Total adult stock size is the sum of the spawning and non-spawning components of the mature stock. The non-spawning component of the stock may be estimated from the size of the spawning stock and the proportion of non-spawners. However, the proportion of mature non-spawning males and females (NS_m and NS_f , respectively) was based upon a pre-season survey. Therefore, in calculating the number of non-spawners, the estimates of the number of spawners derived from equations (2) and (8) must be corrected for the fish that migrated to the spawning ground but were captured prior to spawning. We assumed that half the catch was captured prior to spawning. The correction for the number of females in the spawning run that were captured prior to spawning (N_f') is derived from the weight of the catch (C , in kilograms), the male:female ratio (m:f) and mean weights of females (w_f) and males (w_m) in the spawning aggregation (Table 1):

$$N_f' = 0.5 \cdot \frac{C}{w_f + w_m \cdot m:f} \quad (9)$$

The total number of female orange roughy in the stock (N_{ft}) was then estimated:

$$N_{ft} = \frac{(N_f + N_f')}{(1 - NS_f)} \quad (10)$$

The variance of N_{ft} is based upon a ratio of two estimated values and was calculated using an equation analogous to (3) using the means and variances of N_f and NS_f .

The total number of male orange roughy was calculated using an equation analogous to (10). No estimate of the variance in the proportion of spawning males was available, so its coefficient of variation was assumed, conservatively, to be equal to 0.5 (Table 1).

The variances associated with the male:female ratio and the mean weight of males and females were not incorporated in estimates of total variance. These estimates were based on large sample sizes and had relatively low variance, so their contribution to the total variance of the stock biomass estimate was negligible.

Total stock biomass (B_t) was estimated finally based upon the corrected numbers and mean weights of males and females:

$$B_t = w_f N_{ft} + w_m N_{mt} \quad (11)$$

RESULTS

Daily egg production for the orange roughy off northeast Tasmania in July and August, 1991 and 1992 is shown in Figure 2. The timing of spawning and estimates of daily egg production were similar between years, and both surveys reasonably encompassed the period of spawning: daily egg production during the first and last portions of either survey was ~10% of mean egg production during the central survey period. Far fewer stations were sampled in 1991 (109 compared with 240) because of the larger survey area, and a higher proportion of stations contained no early stage eggs. The 1991 data are therefore not considered further to estimate spawning stock size.

Summing the daily egg production shown in Figure 2, egg production in 1992 was estimated to be 4.01×10^{11} eggs with 95% confidence limits of $\pm 2.73 \times 10^{11}$ (CV = 35%), based upon lognormal estimators of the mean and variance of the egg abundance (Pennington 1983; Pennington and Berrien 1984). The number of spawning orange roughy females was estimated to be 9.52×10^6 with a biomass of 14,600 tonnes, given the estimated mean fecundity and weight of orange roughy females (Table 1). The number of males was estimated to be 1.08×10^7 based upon the observed male:female ratio (0.468:0.532, $n = 6243$ fish) (Table 1), and their biomass was 14,500 tonnes. The total biomass of orange roughy spawners was therefore 29,100 tonnes with 95% confidence limits of $\pm 19,800$ tonnes based upon the variance in egg production alone.

Total stock biomass was estimated from the proportion of non-spawners in the population. 23.7% of adult female orange roughy were non-reproductive (standard deviation: 17.6) in the pre-spawning trawl survey ($n=1,134$ fish from 14 trawl samples). Approximately 10% of males were non-reproductive. The estimated catch from the spawning aggregation in 1992 was 16,330 tonnes (Table 2), of which half was assumed to have been caught prior to spawning. Based upon the sex ratio and mean weight of fish in the orange roughy stock (Table 1), the expected sex composition of the catch was 6.09×10^6 males and 532×10^6 females. Based on the proportion of non-spawners in the population (Table 1), total adult stock biomass was 45,100 tonnes $\pm 30,800$ tonnes at the beginning of the 1992 season. The estimated virgin biomass of the stock was estimated to be 96,100 tonnes based upon the catch history of the east Tasmania fishery (Table 2) and assuming that natural mortality was balanced by recruitment and growth and that this region encompasses the extent of a discrete stock. At the end of the 1992 season, 30% of the virgin biomass remained (28,800 tonnes).

When the variances for the proportion of non-spawning females and of the fecundity estimate (Table 1) were incorporated into the estimate of total variance, the CV increased from 34.8 to 35.4%. Thus the variance associated with the egg distribution dominated the variance of the biomass estimate.

The frequency distribution of the non-zero values for egg abundance shows that the egg data still exhibit significant positive skew even after log transformation (skewness = 1.35, Student's $t = 5.64$, $n = 102$, $p < 0.001$) (Fig. 3). The distribution is also significantly kurtotic (kurtosis = 1.439, $t = 3.04$, $p < 0.01$). The distribution thus contains a higher proportion of low values (i.e. values less than 10) than would be expected from a normal distribution. These data span the spawning

season and the two strata based upon distance from the spawning site. There are relatively few data from individual cruises and strata, but the general characteristics of positive skewness and kurtosis remain in smaller subsets of the data. It is not uncommon for sample data for animal abundance to remain positively skewed following log transformation (Myers and Pepin 1990).

In view of the possible bias of lognormal estimators where there is significant departure from a lognormal distribution (Myers and Pepin 1990), we estimated orange roughy stock biomass using the arithmetic means and variance of the untransformed data and based upon the same strata and estimates of fecundity, the male:female ratio, and the proportion of non-spawners. The biomass of spawners was estimated to be 35,700 (\pm 28,300) tonnes and total stock biomass was estimated to be 53,100 (\pm 42,100) tonnes. The CV of the estimate based upon the variance in egg abundance alone was 40%. The estimate of stock size based on the arithmetic mean was thus 18% higher than that based on the lognormal estimator, and the CV was also higher (40% vs 35%).

It was not unexpected that the arithmetic estimates of the mean and variance were higher, since arithmetic estimators are affected more by extreme values than lognormal estimators. The sample with the highest density of eggs (1628/m²) contained almost 5 times more eggs than the next largest sample. When this value was removed and replaced by the resulting mean for that stratum, the estimated total stock biomass based upon the lognormal and arithmetic mean estimators was 36,600 tonnes (CV = 33%) and 37,500 tonnes (CV = 28%), respectively. Thus the biomass estimates from the two methods varied by only 2% when the extreme value was removed. Both the mean and variance of the arithmetic estimator were more sensitive to the influence of the extreme value.

The distribution of 1-day old eggs in relation to distance from the spawning ground indicates that the sampling area was sufficiently large to sample them adequately (Fig. 4). Virtually all stations with egg numbers greater than 5 were within the core stratum, and there were only two non-zero stations among the 21 stations sampled that were greater than 10 nm from the spawning area. The survey design thus seemed adequate to sample the 1-d old eggs.

A plot of the decline in numbers of eggs with age also indicated that 1-d old eggs were adequately sampled within the sampling area (Fig. 5). There was no detectable decline in egg abundance normalized for stage duration over the first 27 h (up to the stage of gastrulation). There was a highly significant ($p < 0.01$) regression through the data from 27 h onward that indicated an instantaneous hourly loss rate of 0.038 (or daily instantaneous loss rate of 0.92). This is presumably due primarily to advection and diffusion from the sampling area, although mortality is a contributing factor.

There was weak evidence of diel spawning periodicity suggesting that spawning occurred predominantly at night in the hours after midnight (Fig. 6). This was examined by testing whether the number of fertilized eggs that had not yet undergone the first cell division differed over the daily cycle. At ambient temperature conditions, eggs at this stage were less than 10 hours old (Stage 1, Bulman and Koslow in prep.). The day was divided into 4 six-hour periods (0000-

0600, 0600-1200, 1200-1800, and 1800-2400 h). As seen in Figure 6, early stage eggs were sampled throughout the day, but the percentage of samples without early stage eggs increased from 50% at 0000 - 0600 h to 76-79% from 1200 - 2400 h. The abundance of Stage 1 eggs differed significantly over these periods (Kruskal-Wallis test corrected for ties, $\chi^2 = 11.48$, $n=239$, $p < 0.01$).

DISCUSSION

Despite the considerable depth of orange roughy spawning (~700-1000 m), the classical egg production method (Saville 1964) proved relatively straightforward as a means to estimate stock size because of the fish's relatively brief spawning period, restricted size of its spawning ground, precise timing of spawning, and development of a single batch of eggs. The depth of spawning did not greatly affect the sampling. By undertaking vertical tows with a large-opening net, we avoided the potential biases arising from unrepresentative sampling of the water column due to concatenation of the tow cable during a deep oblique tow.

Most species for which egg surveys have been employed, such as anchovy, sardine, and mackerel, spawn over relatively large coastal areas (Lockwood et al. 1981; Lasker 1985; Lo et al. 1992), and the egg surveys have been concomitantly large in scale. In encompassing the full area of distribution of eggs within a survey, the full age distribution of the eggs was typically sampled and utilized in the analysis, such that daily egg production may be estimated as the intercept of a regression of the natural logarithm of egg numbers with their age. However, the orange roughy off eastern Tasmania spawn around a single seamount, thereby providing a virtual point source of eggs. Due to advection and diffusion, the abundance of eggs at distances greater than 10 nm from the spawning site was too low to obtain adequate precision. However, limiting our survey to this area and to sampling the early stage eggs alone enabled us to increase the density of sampling for these stages. We thereby achieved greater precision in our estimate of the abundance of early-stage eggs, though at the cost of rendering the egg production estimate more sensitive to error in the estimated rate of initial egg development (Bulman and Koslow in prep.).

Comparing the results of arithmetic and lognormal estimators of the mean and variance, the substantially greater sensitivity of the arithmetic estimates to the influence of an extreme value seems to belie the statement of Myers and Pepin (1990) that "the sample mean and variance are more robust than lognormal-based estimators of mean and variance of population abundance." The primary reason for this discrepancy appears to be that Myers and Pepin used sampling distributions (the Weibull and gamma distributions) in their simulations that are less skewed than the lognormal distribution, whereas the data in our study were considerably more skewed. Their simulations were also based largely upon data with very high variability ($CV = 2$). The CV of our arithmetic estimate of egg production was only 0.35 or 0.40 depending upon whether log-normal or arithmetic estimators were used. Comparable levels of variability are reported from egg surveys of other fish

populations (Table 3). Myers and Pepin showed that the potential bias of the lognormal estimator of the mean was trivial at such levels of variability, although the potential bias of the lognormal estimator of the variance could be significant. However, the considerably greater sensitivity of the arithmetic mean and variance to the influence of a single extreme value leads us to conclude that it is generally a less robust estimator for pelagic egg surveys than the lognormal estimator of Pennington and Berrien (1984).

Results of the egg survey were in close agreement with an estimate of total stock biomass from an acoustic survey: $47,400 \pm 9,200$ tonnes (Kloser and Koslow in prep.). The acoustic survey was carried out during the peak of the 1992 spawning season and is based upon the same catch data and assumptions of the proportion of non-spawners in the stock. The statistical precision of the acoustic estimate was superior: CV = 0.10 compared with 0.35 for the egg survey. However, the acoustic survey provided only a snapshot estimate of spawning biomass, and the acoustic estimate required estimation of several additional factors: species composition in the spawning area, the target strength of orange roughy and other species, and the turnover of spawning fish on the spawning ground. Due to uncertainty surrounding these parameters, the accuracy of the acoustic estimate may be more questionable, although its precision is greater. However, the close agreement of these two independent estimates of stock biomass considerably enhanced confidence in the probable validity of the overall assessment.

ACKNOWLEDGMENTS

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TABLE 1. PARAMETERS OF THE EASTERN TASMANIA ORANGE ROUGHY STOCK USED TO ESTIMATE STOCK BIOMASS. SEE TEXT FOR SOURCES AND DERIVATION. CV: COEFFICIENT OF VARIATION.

| PARAMETER | VALUE | CV |
|----------------------------------|-------------|------|
| Mean fecundity | 42,100 eggs | 0.04 |
| Mean standard length, females | 36.2 cm | |
| Mean weight, females | 1.536 kg | |
| Mean weight, males | 1.339 | |
| Ratio, male:female | 0.532/0.468 | |
| Proportion non-spawners, females | 0.237 | 0.20 |
| Proportion non-spawners, males | 0.90 | 0.50 |

BIOMASS ASSESSMENT OF ORANGE ROUGHY
BASED UPON AN EGG SURVEY

TABLE 2. CATCH HISTORY OF THE FISHERY FOR THE ORANGE ROUGHY OFF EASTERN TASMANIA. (SOURCE: SMITH ET AL. 1993)

| YEAR | CATCH (TONNES) |
|------|----------------|
| 1986 | 36 |
| 1987 | 341 |
| 1988 | 2,114 |
| 1989 | 20,149 |
| 1990 | 17,845 |
| 1991 | 10,496 |
| 1992 | 16,330 |
| 1993 | 3,850 |

TABLE 3. THE COEFFICIENTS OF VARIATION (CV) FOR EGG SURVEYS FOR A RANGE OF SPECIES IN THE NORTH ATLANTIC AND NORTH PACIFIC. THE LIST IS INTENDED TO BE REPRESENTATIVE BUT NOT EXHAUSTIVE.

| SPECIES | SOURCE | CV |
|--|-------------------------------------|-----------|
| Orange roughy (<i>Hoplostethus atlanticus</i>) | This study | 0.35-0.40 |
| Benguela anchovy (<i>Engraulis capensis</i>) | Armstrong <i>et al</i> 1988 | 0.35-0.41 |
| Atlantic mackerel (<i>Scomber scombrus</i>) | Pennington and Berrien (1984) | 0.42 |
| Scad (<i>Trachurus trachurus</i>) | Eaton (1989) | 0.16-0.46 |
| Silver hake (<i>Merluccius bilinearis</i>) | Pennington and Berrien (1984) | 0.14 |
| Yellowtail flounder (<i>Limanda ferruginea</i>) | Pennington and Berrien (1984) | 0.38 |
| Dover sole (<i>Microstomus pacificus</i>) | Lo <i>et al</i> (1992) ² | 0.24-0.51 |

FIGURE LEGEND

Figure 1. Chart showing the sampling grid from which stations were randomly selected for the egg survey in austral winter, 1992. 25 times more stations were occupied in the core stratum. The location of the survey area is shown in the inset.

Figure 2. Daily egg production based upon lognormal estimators of the mean and variance for 6 sampling periods during the orange roughy spawning in a) 1991 and b) 1992. The solid areas represent the periods actually sampled; the hatched areas are interpolated from adjacent periods. The error bars represent 1 standard error.

Figure 3. The frequency distribution of the log-transformed numbers ($\log_e(x + 1)$) of 1-day old eggs in samples from the 1992 survey. A) The frequency distribution including observations of 0 eggs, $n = 240$; B) the frequency distribution excluding observations of 0 eggs, $n = 102$.

Figure 4. A scatterplot of the \log_{10} -transformed numbers of 1-d old eggs per sample in relation to distance (in nautical miles) from the spawning site. At a distance > 10 nm, there were only 2 non-0 samples out of 21.

Figure 5. A plot of the decline with age of the \log_e -transformed numbers of eggs summed by stage over the 1992 survey and corrected for stage duration. Stage durations follow Bulman and Koslow (in prep.). The steep decline after 24 hr is presumably due primarily to advection out of the survey area.

Figure 6. The abundance of Stage 1 (< 10 hr old) eggs (circles) and the proportion of zero observations of Stage 1 eggs (squares) by time of day of sampling. The differences in abundance are significant: Kruskal-Wallis test corrected for ties: $\chi^2 = 11.48$, $n=239$, $p < 0.01$.

BIOMASS ASSESSMENT OF ORANGE ROUGHY
BASED UPON AN EGG SURVEY

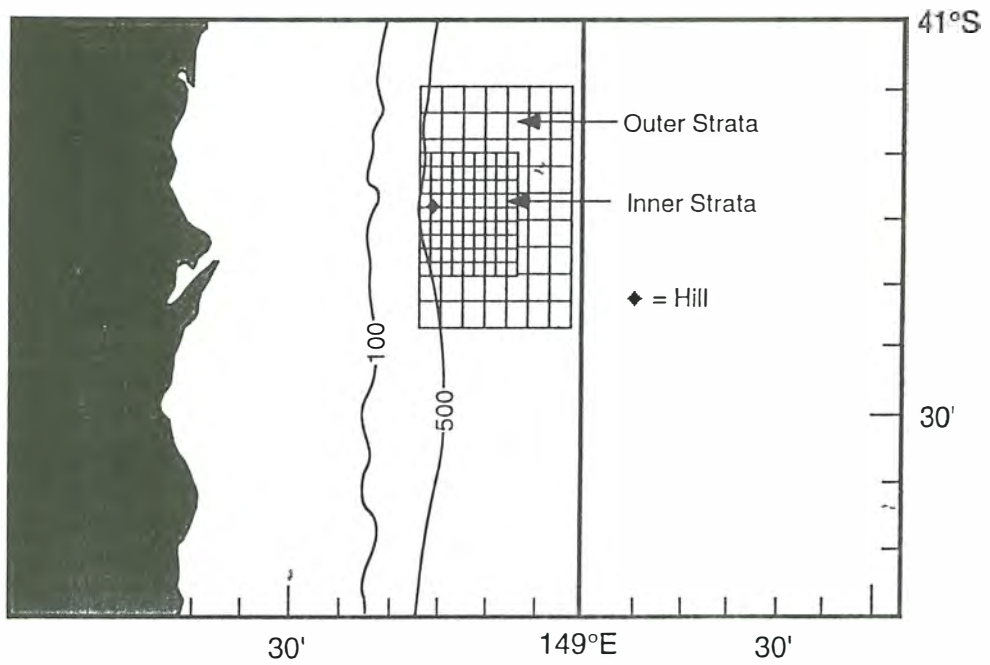


FIGURE 1

BIO MASS ASSESSMENT OF ORANGE ROUGHY
BASED UPON AN EGG SURVEY

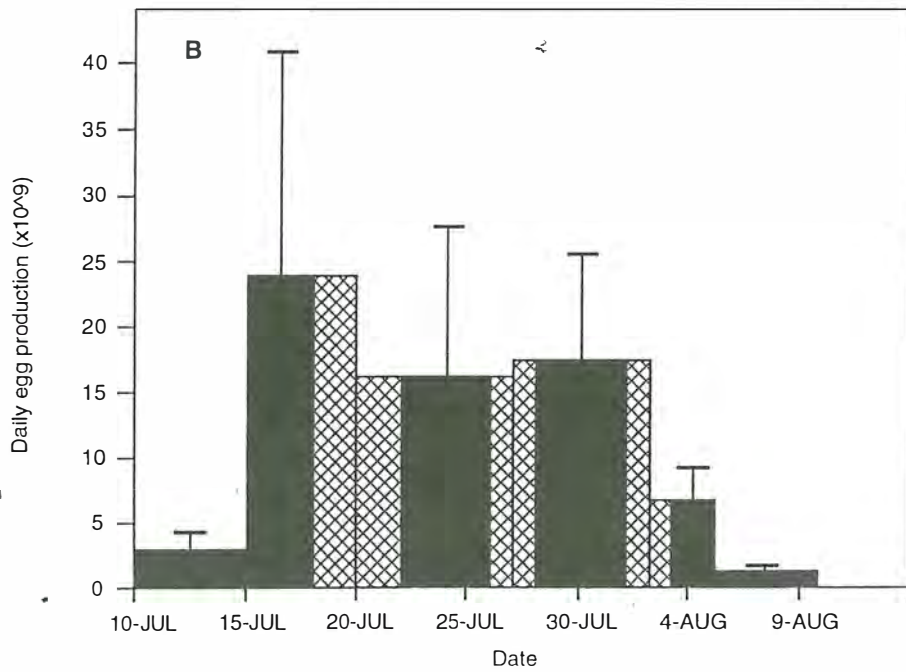
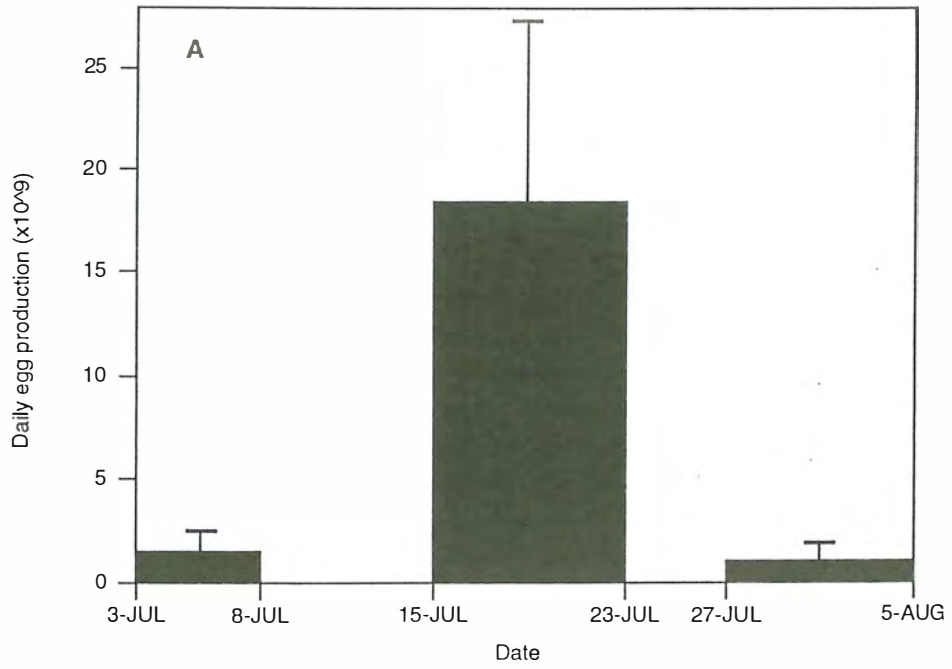


FIGURE 2

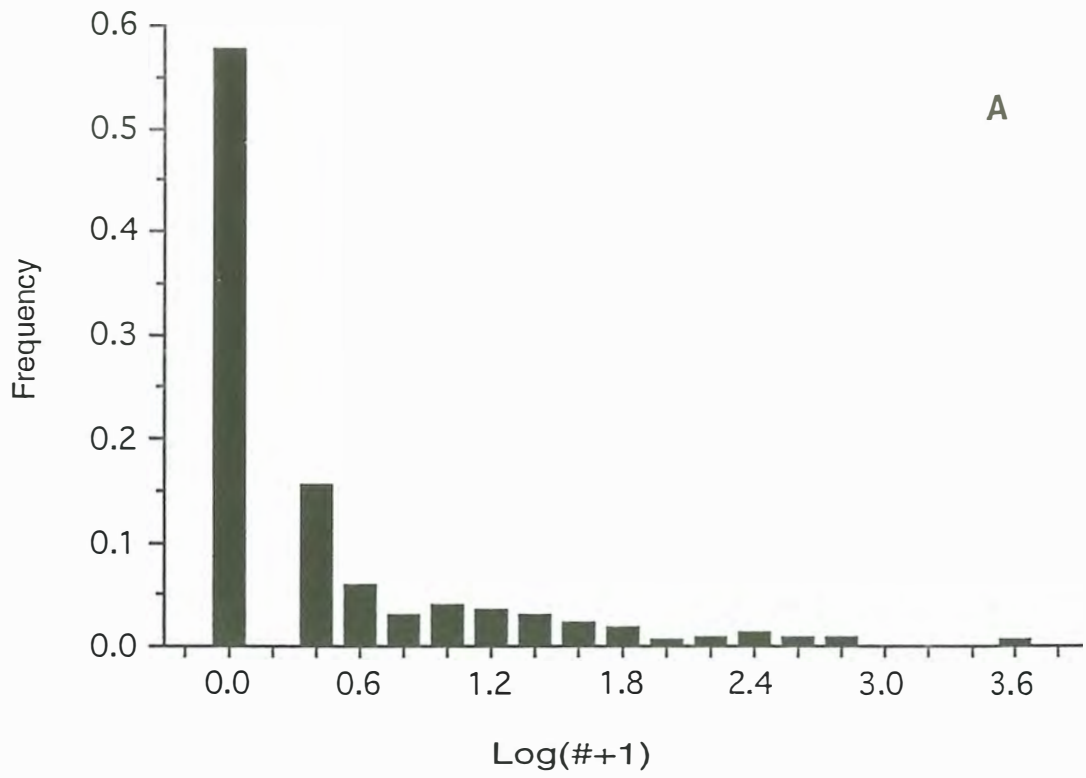
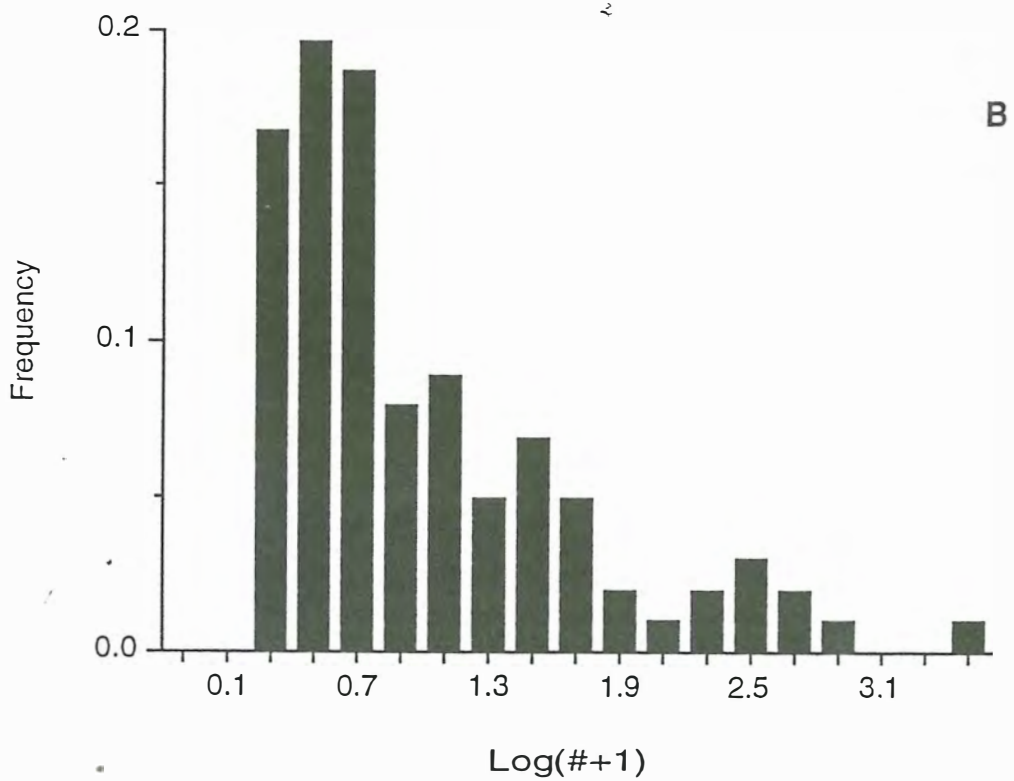


FIGURE 3



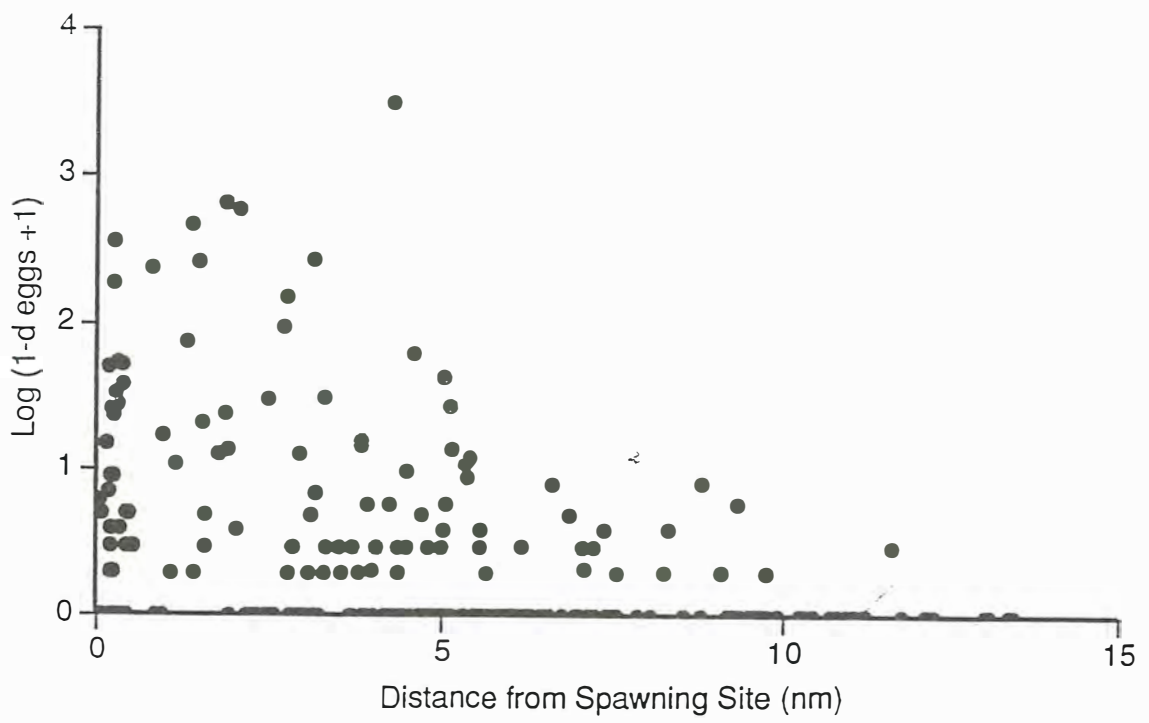


FIGURE 4

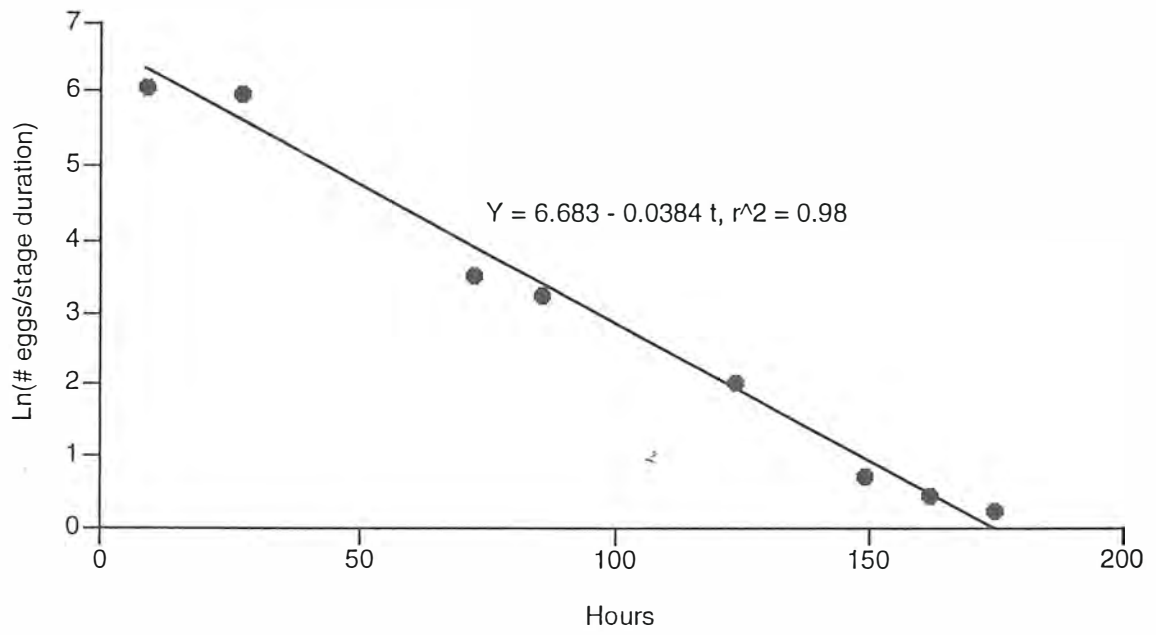


FIGURE 5

APPENDIX

ORIGINAL PROPOSAL & PROGRESS REPORTS



ORIGINAL PROPOSAL

FIRDC 1989 NEW APPLICATION GRANT



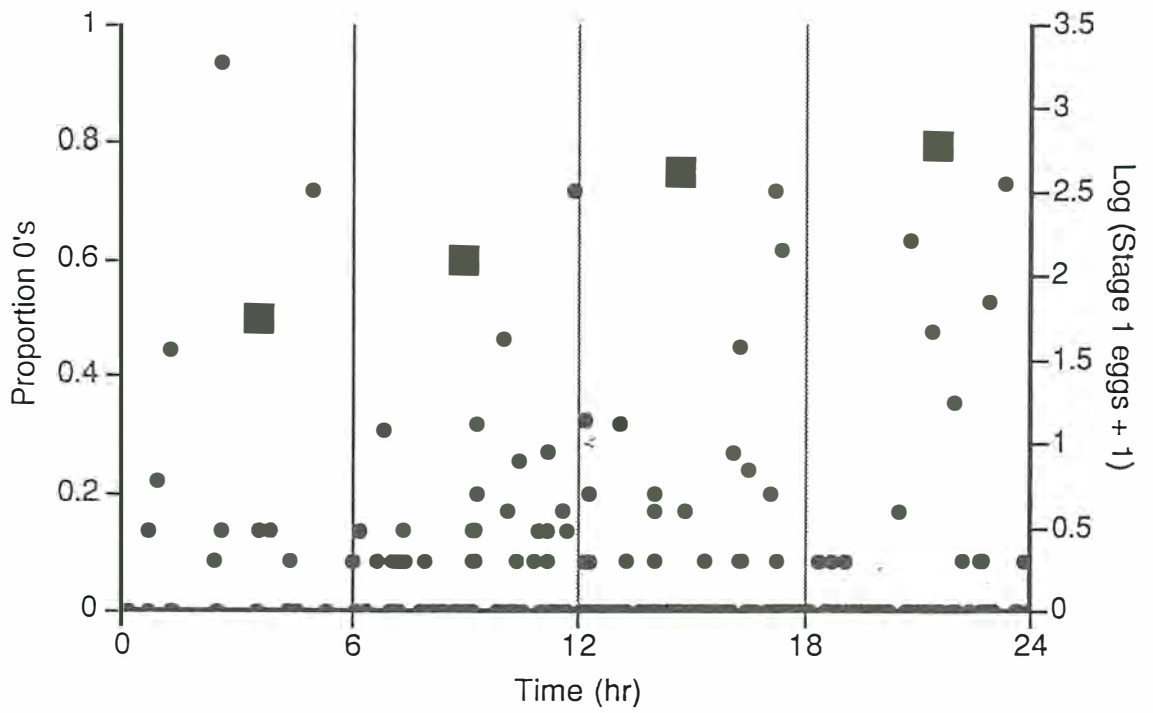


FIGURE 6

**FISHING INDUSTRY RESEARCH AND DEVELOPMENT COUNCIL
1989 NEW APPLICATION GRANT 1989/****1. TITLE OF PROJECT**

Development and use of the egg production method to assess the biomass of orange roughy off eastern Tasmania

2. KEYWORDS

Orange roughy, *Hoplostethus atlanticus*, egg survey, fecundity, stock assessment

3. OBJECTIVES

- To assess the standing stock of orange roughy off east Tasmania, concentrating upon the spawning aggregation off the northeast coast, based upon the egg production survey method.
- To carry out studies of orange roughy reproductive biology and early life history, which are required as ancillary information for the survey method:
 - the temperature-dependent development rate of orange roughy eggs;
 - the sex-ratio of orange roughy in the spawning stock;
 - the relation between fecundity and body weight; and
 - the proportion of non-reproductive fish in the population.

4. JUSTIFICATION

The southeast Australian trawlfish resources occur for the most part in deep water over the continental slope, i.e. orange roughy, gemfish, blue grenadier, and others. Management of these fisheries in Australia and New Zealand has proven exceptionally difficult, largely due to problems of stock assessment. Cohort analysis has not been possible for orange roughy due to inability to age the mature fish, and catch per unit effort (CPUE) indices are generally not valid for highly aggregated species such as roughy or grenadier. The value of trawl surveys for orange roughy and blue grenadier is also questionable due to their occurrence over untrawlable ground, variable distribution in the water column (i.e. only partial accessibility to sampling gear), and saturation of the trawl when aggregated.

There is a critical need to properly manage these resources. The orange roughy seems particularly liable to overfishing due to the combination of its apparent longevity (~75 years) and massive, highly predictable spawning aggregations. As a result, segments of the New Zealand orange roughy fishery are on the verge of collapse after only 10 years of exploitation.

With the discovery last year of the first major spawning aggregation of orange roughy off St. Helens, northeast Tasmania, the catch of orange roughy (~20,800 t) more than doubled and likely exceeded the catch for all other fin fish in the Southeast Australia trawl fishery combined. Estimates of the biomass of the aggregation vary by more than an order of magnitude (50,000 - >1,000,000 t), placing management of the fishery in a state of crisis, since first-order models indicate that only ~3% of virgin biomass may be removed annually on a sustainable basis (Mace et al. in press).

The following proposal and a related proposal ("Development and use of acoustic techniques for the assessment of deepwater commercial fish stocks") are framed within the specific need to assess the biomass of orange roughy off eastern Tasmania. However, they are designed as well to meet the critical long-term need to develop alternative survey methods for major trawl fisheries off southeast Australia. We believe that their use may be extended both to other regions (if orange roughy spawning aggregations are discovered elsewhere) and to other species, such as blue grenadier, which form reasonably localized, predictable spawning aggregations.

We believe that the acoustic/trawl and egg production survey methods, as entirely independent survey methods, should both be used to estimate the biomass of orange roughy due to: 1) the value of the resource, 2) present uncertainties associated with the use of either method, and 3) the present inability to use catch statistics (eg. catch per unit effort or cohort analysis) to evaluate the status of the resource. It is standard practice in fisheries to develop several methods of resource assessment for significant fisheries, particularly in the face of uncertainty. Furthermore, since both methods require ancillary trawl sampling, their ship use and data requirements are somewhat complementary, which has led to their being combined in surveys of other fisheries (eg. Hampton et al. in press).

Egg production methods to estimate stock biomass are well documented (Smith and Richardson 1977; Lasker 1985) and have been applied successfully on a world-wide basis with pelagic and shelf fisheries (eg. Saville 1981; Berrien et al. 1984; Lasker 1985; Bates 1987; Eaton 1989; Hampton et al. in press, among others). Use of the egg production method has recently been extended to deep-water fishes (Dover sole, *Solea vulgaris*, and sablefish, *Anoplopoma fimbria*) off the western USA (Hunter and Smith in prep.). Much of the requisite background information on orange roughy reproductive biology has already been obtained for New Zealand and Australian stocks (Pankhurst 1988, Pankhurst and Conroy 1987, Pankhurst et al. 1987; Bell 1989). The only technical problem encountered in surveying the egg abundance of deepwater spawners is ensuring that plankton samples are quantitative, i.e. that all depths over which the eggs are distributed are equally sampled.

This is readily resolved through use of a computerized net-monitoring system, such as that developed by T. Davis and A. Heron (CSIRO), which monitors depth, time, and filtration in real time so winch speed may be varied to sample all depths equally.

Several aspects of orange roughy biology indicate that this species may be particularly well suited to use of this method. - in fact, better suited than most species to which the method has been successfully applied. Unlike anchovy and mackerel, for example, the orange roughy are single batch spawners, so determination of fecundity is straightforward (Pankhurst and Conroy 1987; Bell 1989). Orange roughy eggs are exceptionally large (~2 mm diam.) and distinctive, containing a large orange oil droplet, so they can be readily identified. Again unlike anchovy and mackerel, which spawn for several months over an area of thousands of square kilometres, orange roughy spawn for only several weeks within a highly restricted area (i.e. the area occupied by the main spawning aggregation of orange roughy off northeast Tasmania in 1989 was ca. 10 km²).

The timing and location of spawning also appears highly predictable (Pankhurst 1988). A survey program can thus readily cover the spawning period. (Our assumption that orange roughy will return to spawn either on the same pinnacle or somewhere in the vicinity appears reasonable given the consistent location of their spawning off New Zealand (Pankhurst 1988), and the catches of orange roughy in spawning or spent condition in previous years in the St. Helen's area (C. Bulman, unpubl. data)).

Due to the localized nature of the east Tasmania spawning aggregation, the eggs should be highly localized when initially spawned, which greatly facilitates design and execution of a survey of egg production at particular times during the spawning season. (Note that the method only requires that the egg 'population' be surveyed through an initial stage of development.)

Finally, the research required to implement the egg production method provides important background information on the early life history of the orange roughy: i.e. development rate, vertical distribution and pattern of dispersal of the eggs. Studies of egg dispersal may enable us to locate orange roughy larvae, whose distribution remains unknown at present. This information could provide the basis for subsequent research on recruitment to the orange roughy fishery.

Funds are requested for three year's field work. The validity of the method needs to be tested by comparing results of the biomass assessments over two field seasons. We anticipate that results of the first year's survey will prove provisional as the method is initially developed. See Table 1 for project work schedule.

In summary, the egg production method, in providing a second, independent estimate of orange roughy spawning stock biomass, is critical to the immediate and long-term management needs for this fishery, considering its value and the extreme uncertainty presently associated with it. Aspects of the proposal were presented to the 1989 Orange Roughy Workshop (Taroona, Tasmania) and discussed with the DPFRG and GITLC at their 1989 meetings. The final performance of the project should be judged primarily on our ability to successfully develop the egg production survey method and apply it to assessment of the orange roughy resource off east Tasmania. The longer term success of the project should be viewed in terms of its use as a routine tool for assessment of orange roughy, and possibly other deepwater fishes, on a regional basis.

5. PROPOSAL IN DETAIL

METHOD OF PROCEDURE

Field work will be carried out on a cruise during the winter spawning period of each year. The egg production method for estimating orange roughy spawning stock biomass will be based upon survey estimates of

- total orange roughy egg production, which is the daily egg production (i.e. the abundance of early stage eggs corrected for their temperature-dependent development rate) measured and summed over the spawning period; and
- the proportion of the stock that is female and reproductive, and their fecundity. (See Appendix I for details of the egg production method.)

The following research program is required to obtain this information:

- sampling with an opening-closing plankton net through the water column to measure the vertical distribution of orange roughy eggs.
- a survey to estimate the abundance of orange roughy eggs by stage based upon quantitative plankton tows through the vertical range of the eggs along a series of onshore-offshore transects (see Figure 1).
- ship-board experiments to describe the stages in development of orange roughy eggs and their temperature-dependent duration, based upon successive staging of eggs incubated at temperatures from 4-10°C that were either a) stripped from ripe females and fertilized in vitro or b) collected from within the spawning aggregation by slowly towing nets with solid cod ends. We will use light microscopy to describe and identify the developmental stages, but samples will be supplied to Dr. C. Crossley (Zoology Department, University of Sydney) for his general study of trachthyid early life history, based upon electron microscopy.
- measurement of the density of orange roughy eggs at several stages, using a density-gradient column, to estimate their expected mean depth in the water column.
- measurement of water column temperature and salinity using a CTD or SDL simultaneous with the egg sampling to determine the temperatures at which the eggs develop, based upon 1) and 4).

- trawl sampling, combined with commercial catch sampling, of the spawning aggregation with ~10 demersal and 20 midwater trawls to determine the proportion of females in the aggregation, using a net with a large 'window' near the cod end to avoid excessive catches of fish.
- determination of fecundity based upon collection of ~200 fish stratified over the size range of mature fish and obtained from the commercial catch of pre-spawning fish (i.e. May/June) (a collaborative study with J. Bell (NSW Agriculture and Fisheries)).
- a survey to determine the proportion of the mature population that is non-reproductive, based upon histological examination of the female gonads, (i.e. lack of oocyte development (Bell 1989)). This will be based upon either a) collections in the pre-spawning period (i.e. March) when oocyte development is underway but the fish have not yet begun their spawning migration (Bell 1989) or b) a stratified acoustic/trawl survey during the spawning period but outside the spawning aggregation (Fig. 1) to estimate the biomass of fish that are non-reproductive as opposed to still ripening or spent. Histological examination will be carried out in collaboration with J. Bell.

The egg survey (Fig. 1) is designed to sample at least one day's egg production over the full range of their distribution. At this time, we assume that the eggs are distributed below 200 m because no orange roughy eggs or larvae were found in samples of ichthyoplankton from the upper 200 m obtained bi-monthly from around Tasmania (the CSIRO Southern Program).

Based upon observed tidal currents at mid-slope depth off eastern Tasmania (15-25 cm/sec (=13.0-21.6 km/d) alongshore and 5-10 cm/sec (=4.3-8.6 km/d) onshore-offshore) (V. Lyne, CSIRO, Hobart, pers. commn), 9 parallel transects are planned to cover the area 55 km north and south of the spawning aggregation. Each transect extends from the 200 m isobath to 33-55 km further offshore, the length of the transects increasing with increasing distance from the spawning aggregation. The transects nearest to the spawning aggregation are spaced more closely (i.e. 5 nm (=9.2 km) versus 10 nm (=18.4 km) for transects more distant from the spawning) in order to increase sampling density and decrease the variance associated with the estimate of egg abundance in the region of greatest abundance. Approximately 55 stations are planned, requiring 3-4 sampling days to complete.

The egg survey will be carried out 4 times during the spawning season: at least twice aboard the CSIRO FRV 'Southern Surveyor' during the expected height of the spawning season in July (in 1990, the Australian Maritime College FRV 'Bluefin' will be chartered), and possibly aboard the Tasmanian Sea Fisheries FRV 'Challenger' at the onset and end of the spawning season, depending upon the duration of the spawning period.

The onset and end of the spawning season will be determined from monitoring of the commercial catch for the incidence of fish in maturing, ripe and running, and spent condition. Commercial catch monitoring will be carried out routinely by Tasmania Sea Fisheries Department (J. Lyle, pers. comm.). Spawning aggregations of orange roughy around New Zealand have consistently returned to either the same topographic feature or the same general area (Pankhurst 1988). We assume that the fishing fleet will locate the spawning aggregation. If the location varies, our survey transects will be shifted accordingly. The adequacy of our overall design to survey orange roughy eggs through an early stage of development will be evaluated after the first year of the program.

The possible effect of condition on incidence of spawning will be examined by comparison of body condition (based upon both the relationship of length and weight and proximate body composition) in mature fish that are or are not developing oocytes in the pre-spawning period (March-May). Samples of the gonads for histological examination must be preserved fresh, requiring that they be collected by a field technician aboard a commercial vessel.

In addition to estimation of spawning stock biomass, the egg survey will also provide a quantitative description of rates of egg dispersal and early mortality, based upon the distribution of orange roughy eggs by stage and experimental determination of egg stage duration (see above) (Okubo 1980; Koslow et al. 1985). If the related CSIRO proposal to FIRDC (Fisheries oceanography of east Tasmanian slope waters) is funded, a physical/biological model of egg dispersal will be developed in collaboration with Dr. V. Lyne (Biological Oceanography Section, CSIRO Fisheries Division). Following the general approach of Page and Smith (in press), the model will be based upon:

- the vertical distribution of eggs in the water column ; and
- the current profile in the water column, based upon current meter measurements.

The model will be tested based upon data from the egg survey and several additional vertical profiles of the distribution of orange roughy eggs (and possibly of larvae) obtained over the range of our survey (see Figure 1).

FACILITIES AVAILABLE

Field work will be carried out aboard the newly outfitted CSIRO research vessel, 'Southern Surveyor.' The vessel will contain tanks with cooled circulating seawater to maintain orange roughy for reproductive work and laboratory space with electronic balances for wet laboratory fish work.

CSIRO has the following equipment required for field and experimental work: a temperature-controlled system for the orange roughy egg-rearing experiment; and an EZ multiple opening-closing net system for series of depth-stratified ichthyoplankton tows. FRV 'Southern Surveyor' will not be available during the first year of the study, and the EZ net cannot be deployed on the anticipated charter vessel, FRV 'Bluefin.' An opening-closing Bongo net, which requires some modifications to work at mid-slope depths, will be used during the first year of the study. To survey and obtain samples of adult fish, CSIRO has Engel High Lift (rough bottom) commercial demersal trawls (19 m long mouth opening; headrope 5 m high maximum) (an additional net is requested for replacement purposes); Engel 152 commercial midwater trawls; a Simrad EK500 scientific sounder system with tow body and faired conducting cable and a Photosea stereo camera system that can be lowered to 2000 m. Computers and software are available for data analysis.

SUPPORT DATA

Staff have been actively involved as part of FIRDC Grant 1987/129 in carrying out trawl surveys and biological investigations of orange roughy (Bulman and Elliot 1988; Bulman et al. 1989). Our group has expertise in carrying out ichthyoplankton survey work on several species of fish (Koslow et al. 1985; Davis 1989). The study of orange roughy reproductive biology off southeast Australia has been carried out primarily by Bell (1989).

6. RESEARCH PRIORITY

The proposed study is directly related to the Council's first priority, fish resource assessment, i.e. to develop and implement use of survey methods to assess the orange roughy resource off east Tasmania. It is expected that the egg production method can be extended to other aggregations of orange roughy as discovered, as well as to other species, such as blue grenadier, which spawn in discreet areas and spawning periods.

7. TRANSFER OF RESULTS TO INDUSTRY

Results of this project are of critical importance to the proper management of the developing orange roughy fishery off southeast Australia. Survey results will be presented in a timely manner to committees and agencies responsible for management of the fishery (i.e. DPFGR, SETMAC, AFS, BRR, GITLC) at annual meetings and as requested. Results will also be presented at national and international scientific meetings and published in the scientific journals in order to receive critical review and contribute to the development of fishery science. Developments will also be published in the fishing industry literature (e.g. Australian Fisheries).

8. COMMENCEMENT AND COMPLETION DATE

Commencement date: July 1, 1990
 Completion date: June 30, 1993.

9. REQUESTED BUDGET

| | 1992/93 | 1990/91 | 1991/92 |
|--------------------------------|---------------|---------------|---------------|
| Salaries and wages (\$) | 80,560 | 80,560 | 88,005 |
| Operating Expenses (\$) | 11,200 | 9,000 | 4,200 |
| Travel Expenses (\$) | — | — | — |
| Capital Items (\$) | — | — | — |
| Total (\$) | 91,760 | 89,560 | 92,205 |

10. FUNDS SOUGHT FROM OTHER SOURCES

1990: Vessel charter (RV Bluefin), 4 weeks: \$108,000.

Funds sought from the Government-Industry Technical Liason Committee (GITLC).

11. FINANCIAL CONTRIBUTION OF APPLICANT

| | 1990/91 | 1991/92 | 1992/93 |
|---|---------------|---------------|---------------|
| Salaries | | | |
| Dr. T. Davis SRS max (30%) | 13,700 | 14,400 | 15,100 |
| Mr. C. Liron STOF 1/max (20%) | 6,500 | 6,800 | 7,100 |
| Hydrographic technician TOF 1/max(20%) | 6,500 | 6,800 | 7,100 |
| Salary sub-total (\$) | 26,700 | 28,000 | 29,300 |
| Salary oncots and overheads x 1.1 | 29,370 | 30,800 | 32,230 |
| Salary Total (\$) | 56,070 | 58,800 | 61,530 |

| Operating costs | | | |
|---|------------------|----------------|----------------|
| Full operating costs for FRV Southern Surveyor for 3 weeks at sea, port and harbour dues, normal crew airfares and fuel costs | 473,000 | 496,000 | 521,000 |
| Laboratory facilities and research support | 20,000 | 20,000 | 20,000 |
| Total funds provided by applicant (\$) | 549,070 | 574,800 | 602,530 |
| Total for 3 years (\$) | 1,726,400 | | |

Contribution of NSW Agriculture and Fisheries

| Salary | 1990/91 | 1991/92 | 1992/93 |
|---------------------|---------|---------|---------|
| Dr J Bell, RS (15%) | | | |

12. BUDGET IN DETAIL

| | 1990/91 | 1991/92 | 1992/93 |
|---|---------------|---------------|---------------|
| Salaries | | | |
| J.A. Koslow SRS 4 (50%) | 22,850 | 22,850 | 22,850 |
| Exp Scientist, ES 2 max (25%) | 8,700 | 8,700 | 8,700 |
| Tech asst, TA 2 max (50%) | 12,057 | 12,057 | 12,057 |
| Tech asst (25%) (supervised by J. Bell, NSW FRI) | 6,029 | 6,029 | 6,029 |
| Salary Sub Total (\$) | 49,636 | 49,636 | 49,636 |
| Superannuation 18.4% | 9,133 | 9,133 | 9,133 |
| Comcare 25% | 1,241 | 1,241 | 1,241 |
| Leave loading | 750 | 750 | 750 |
| Accrued leave | — | — | 7,445 |
| Marine Survey allowance & O/T 3 sea wks @\$1100/wk x 6 staff | 19,800 | 19,800 | 19,800 |
| Total salary (\$) | 80,560 | 80,560 | 88,005 |
| Operating expenses | | | |
| Plankton nets (+ Yr 1:2 Bongo & 2 ring nets; Yr 2: 10 EZ nets; Yr 3: 4 replacement nets@ \$800/net) | 3,200 | 8,000 | 3,200 |
| SDL & Bongo net modifications for use at depthsolenoids, pressure, conductivity sensors & pressure case | 7,000 | | |
| Sample jars, preservatives | 1,000 | 1,000 | 1,000 |
| Total operating expenses (\$) | 11,200 | 9,000 | 4,200 |
| Budget total (\$) | 91,760 | 89,560 | 92,205 |

13. ORGANIZATION

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14. PROJECT SUPERVISOR

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15. STAFF QUALIFICATIONS AND ROLE

J.A. Koslow, PhD Project supervisor, (50%) SRS 4
Overall responsibility for project planning and execution; data analysis, interpretation, write-up, and reporting.

Experimental Scientist/Biologist, (30%) (to be named)
Carry out biological experiments (e.g. egg development) and data collection and assist in their analysis, interpretation, and write-up.

Technical assistant/Field and laboratory technician, (50%) (to be named)
Assist in field sampling; sort plankton samples for orange roughy eggs and stage them; data entry.

Technical assistant/Laboratory technician, (25%) (to be named)
Analyse gonadal material for fecundity estimate; histological sectioning and staging to determine reproductive state

16. ADMINISTRATIVE CONTACT

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(Address, telex, fax as above)
Tel: (002) 206 233

17. LITERATURE CITED

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APPENDIX I

The egg survey method for estimating spawning stock biomass.

Following Parker (1985), the relationship between the spawning biomass of a fish stock and its egg production follows from the relationship between egg production (P_O , in numbers), stock biomass (B), the fraction of the stock that is reproductive females (R'), and the fecundity (F), the number of eggs spawned per unit weight of female:

$$P_O = (B R') F \quad (1)$$

For orange roughy, F is relatively easily determined, since they are single batch spawners. R' is composed of two parts: R , the biomass-based sex ratio, and f the fraction of females spawning in a particular year, based upon the incidence of non-reproductive or atretic females (Bell 1989):

$$R' = R f \quad (2)$$

Rearranging Eq. 1 in terms of spawning stock biomass:

$$B = P_O / (R f F) \quad (3)$$

Annual egg production is the integral under the curve of daily egg production, where daily egg production is based upon survey estimates over the course of the spawning season (Fig. 2). Daily egg production is the sum of the number of eggs at stage i (P_i per m^2) at each of n sampling stations j weighted by the area (A_j in m^2) that each station represents and normalized by stage duration (t_j) based upon temperature at station j and adjusted for egg mortality (M):

$$nP_O = \sum (A_j P_i e^{-Mt}) / t_j \quad (4)$$

Egg mortality (M) may be estimated from the slope of the decline in the natural logarithm of abundance with age of the developing eggs, since

$$P_t = P_O e^{-Mt} \quad (5)$$

This is log-transformed to:

$$\ln(P_t) = \ln(P_O) - M t \quad (6)$$

This is in a form suitable for linear regression analysis, where daily egg production ($\ln P_O$) is the intercept and M is the slope.

TABLE 1: PROJECT TIMETABLE

| Dates | 6-8/1990 | 6-8/1991 | 6-8/1992 | 9/92-6/93 |
|------------------------------|--|---|--|-------------------------------|
| Vessel | Charter | S Surveyor | S Surveyor | |
| Activities/Milestones | Initial shipboard experiments (egg devt, density) Field surveys- | Experiments completed Field surveys- | Experiment analysed & written up Field surveys- | Surveys analysed & written up |

FIGURE LEGEND

Figure 1. A chart of the waters around Tasmania showing the proposed survey design. Egg survey stations are shown along 9 transects extending offshore from the 200 m contour. The location of the 1989 spawning aggregation is located on the central transect (open circle). Vertically stratified tows would be conducted over the spawning area and at stations marked with an x. The track of the combined acoustic and trawl survey north and south of the spawning aggregation is shown. The depth contours are in meters.

Figure 2. An idealized curve of egg production over the spawning season as defined by total daily egg production estimated on four cruises. Total egg production is represented by the area under the curve.

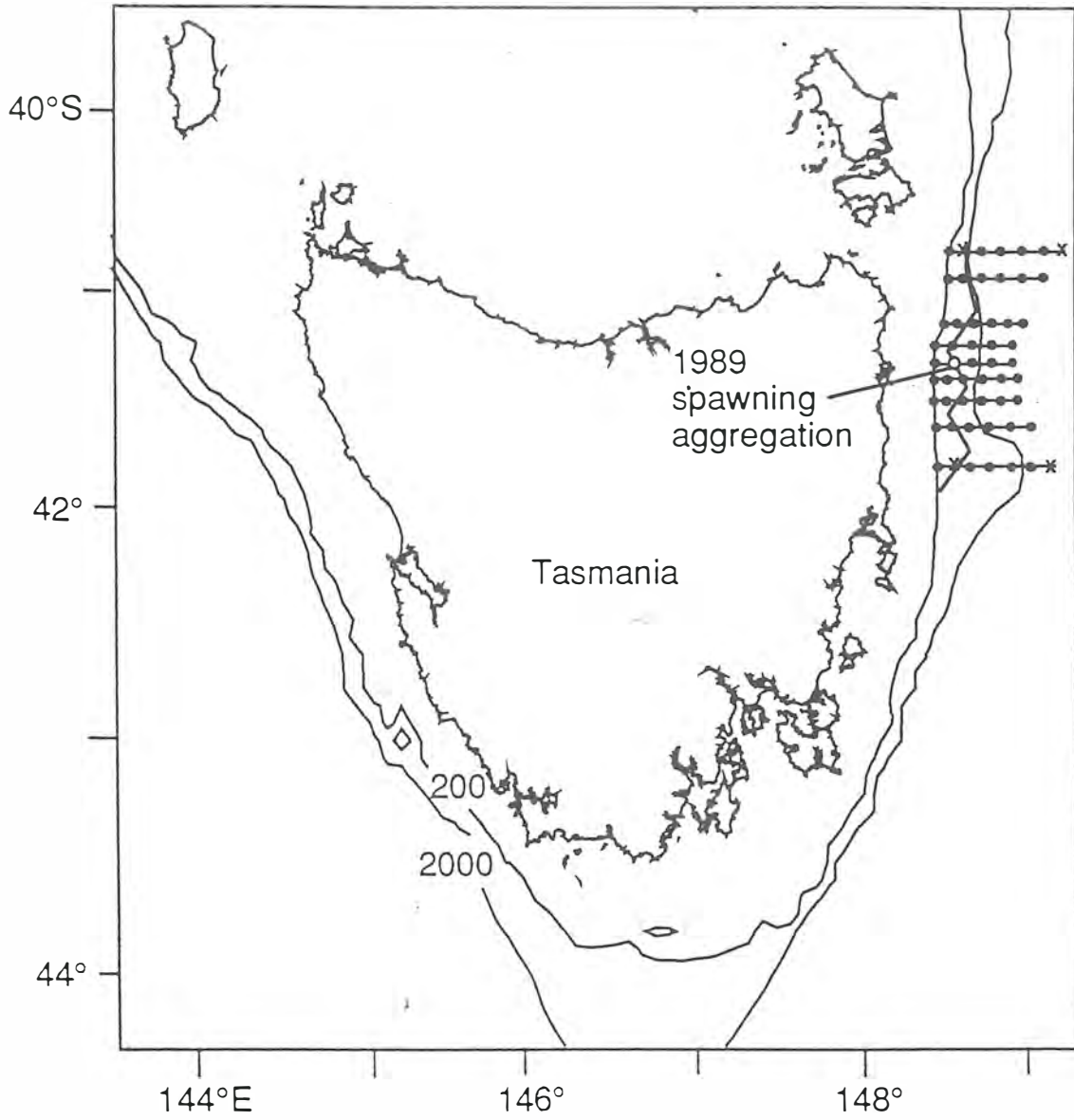


FIGURE 1

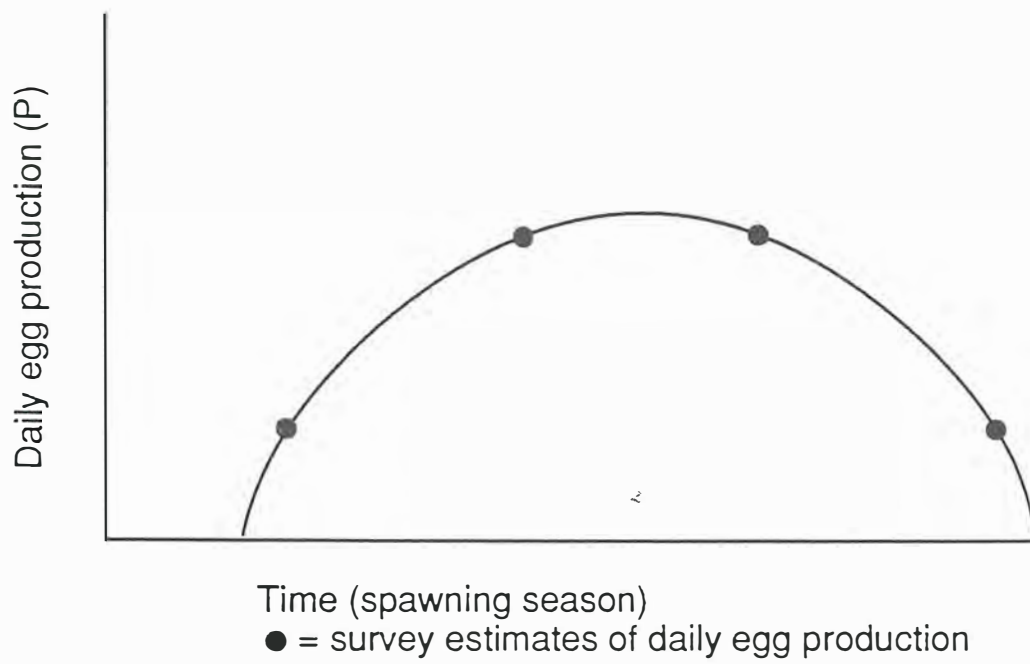


FIGURE 2

PROGRESS REPORT

1991 CONTINUING APPLICATION GRANT



**FISHING INDUSTRY RESEARCH AND DEVELOPMENT COUNCIL
1991 CONTINUING APPLICATION GRANT 90/9**

SECTION 1 - PROJECT TITLE

Development and use of the egg production method to assess the biomass of orange roughy off eastern Tasmania

SECTION 2 - OBJECTIVES

- 1) To assess the standing stock of orange roughy off east Tasmania based upon a survey of the egg production of the spawning aggregation off St. Helens.
- 2) To carry out studies of orange roughy reproductive biology and early life history to determine:
 - a) the temperature-dependent development rate of orange roughy eggs;
 - b) the sex-ratio of orange roughy in the spawning stock;
 - c) the relation between fecundity and body weight; and
 - d) the proportion of non-reproductive fish in the population.

SECTION 3 - PROGRESS REPORT

EGG SURVEY (OBJECTIVE 1)

Three egg collections were carried out over the course of the spawning season: 14 stations early in the spawning season (10-11 July); 20 stations at peak spawning (21-24 July); and 3 stations at the end of the spawning (11 August). Stations ranged 18.5 km (10 nm) north and south of the spawning area and from 200 - 2000 m water depth. Spatial coverage was less than originally planned (> 50 km north and south of the spawning area) due to poor weather.

These were the first quantitative samples of orange roughy eggs. It was necessary this year to determine the distribution of the eggs and the variability between stations in order to assess the optimal size of the sampling grid and number of sampling stations required. The adequacy of different plankton nets and tow types was assessed to obtain adequate samples of the eggs. We initially planned to determine the vertical distribution of the eggs so future sampling could be concentrated on the most suitable depth range, but stratified plankton sampling with an opening-closing plankton net could not be carried out aboard the chartered vessel.

Sufficient numbers of eggs (maxima of > 1000 eggs per tow) were obtained by making a simple vertical tow through the water column from the surface to a maximum of 1000 m, using a ring net with a 2 m² mouth area. A depth pinger was deployed below the net at depths of <1000 m so the net could be lowered to within a few meters of the bottom, thereby ensuring that the entire water column was sampled.

All large egg catches were obtained north of the spawning area, indicating that drift was northward (Fig. 1). A large catch of early stage eggs was obtained on the most northerly transect (~24 km north of the spawning area), indicating that sampling this year was not adequate to encompass the distribution of early stage eggs. An estimate of spawning stock biomass based upon this year's egg survey data may therefore prove conservative.

STAGING AND DEVELOPMENT OF ORANGE ROUGHY EGGS

Orange roughy eggs are readily identified in plankton samples due to their size (~2 mm diam compared with ~1 mm diam for most fish eggs) and unique bright orange oil droplet. The roughy eggs were removed from the plankton samples and enumerated into 11 stages of live eggs (unfertilized; pre-cleavage; 2-cell to pre-gastrulation; gastrulation; etc.) corresponding to standard stages of egg development noted in other fishes (Yusa 1954; Moser and Ahlstrom 1985). As noted in other egg surveys (eg. Moser and Ahlstrom 1985), a significant fraction of the eggs appear either moribund or damaged during collection and preservation, and these were assigned pro rata into the other egg stages obtained from the station.

Attempts were made to incubate orange roughy eggs using eggs and milt from running ripe fish collected aboard the chartered fishing vessel. The eggs failed to develop. Several factors may have been responsible: insufficient oxygenation or temperature control due to the primitive rearing facilities aboard the fishing vessel (ie 1-l jars placed in a fridge); or a pressure-related effect due to rearing embryos adapted to ~1000 m depth at ambient sea-level pressure (Somero et al. 1983). During the next field season, two approaches will be taken to attempt to overcome this difficulty: 1) rearing the eggs in a temperature-controlled room aboard the *Southern Surveyor* at lower densities and in aerated aquaria or a rotating 'plankton wheel'; and 2) rearing eggs in a simple, small high-pressure chamber that we propose to build.

Two yolk-sac orange roughy were collected on 22 July. Based upon the known incidence of spawning, full development could not have taken more than 20 d and most likely occurred over ~15 d, a rate of development similar to that of pollock incubated at a similar temperature (Yusa 1954). This enables us to estimate provisionally the stage duration of the roughy eggs, assuming that the relative proportion of time that orange roughy spend in each stage is similar to observed in other fishes.

ORANGE ROUGHY SEX RATIO

Data on orange roughy sex ratio were routinely collected by ourselves and by J. Lyle (Tasmania Sea Fisheries) both aboard chartered fishing vessels carrying out surveys and in the fish processing plants. Based upon this sampling, the male:female sex ratio was estimated to be 0.59:0.41, which is similar to that recorded last year (J. Lyle, pers. commn.).

ORANGE ROUGHY FECUNDITY

200 fish were collected just prior to the spawning period and processed by J. Bell (FRI, NSW). Based upon presently completed analysis of 120 fish, the following relationship was seen between fecundity (F) and standard length (L) (Fig. 2):

$$\text{Log}(F) = 2.543 \text{Log}(L) + 0.512; r^2 = 0.21; p < 0.01$$

Care was taken that the fish selected were at Stage 4, i.e. just prior to being running ripe, to ensure that no eggs had yet been extruded. The relatively low values for the variance explained is related to the condition of the roughy as defined by their relative weight/length ($r = 0.44$; $p < 0.01$) (Fig. 3). Highly variable fecundity appears to be part of the biology of orange roughy rather than a sampling artifact (Pankhurst and Conroy 1987).

The fecundity of the roughy in eastern Tasmania appeared to differ this year from that observed in 1987 and 1989 (Bell 1989). For example, the predicted fecundity for a 35 cm fish in 1987 and 1989 was 28,000 and was 30,000 this year. Possible differences in fecundity between years will be examined using analysis of covariance when all data are available from the 200 fish collected for fecundity analysis.

PROPORTION OF NON-REPRODUCTIVE ROUGHY

Orange roughy were collected prior to the 1990 spawning season on the spawning grounds. Examination of their ovaries indicated that ~45% of mature females were not proceeding to spawn. This was more than twice the proportion estimated from sampling last year of a smaller number of fish collected over a wider area. Male reproductive development is more difficult to assess. It appears that only 10% of males do not proceed to reproduce, but this estimate is preliminary. The proportion of non-reproductive fish has important implications for estimation of the roughy stock biomass since such fish would not be enumerated by either egg or acoustic surveys. This will be surveyed more intensively next field season.

STOCK BIOMASS

Daily egg production was estimated from the number of Stage 1 and 2 (~1 d old) eggs collected on the three cruises at the beginning, peak, and end of the spawning season (Fig. 4). Total egg production over the spawning season was estimated from the area under the curve defined by these data to be 8.6×10^{11} eggs. 95% confidence limits for this year's limited survey was $\pm 50\%$. The weighted mean fecundity of female roughy on the spawning ground is 31,035 eggs. This implies that the eggs were produced by 2.77×10^7 females. Adjusting this value for the observed male:female ratio (0.41:0.59) and the mean weight of male and female roughy (1.56 and 1.35 kg), the spawning biomass is estimated to be 97.7 mt. Assuming that 10% of males and 25% of females are non-reproductive, total stock biomass is 118,159 mt. If 10% of males and 45% of females are non-reproductive, spawning biomass is 139,019 mt.

In summary, sufficient data were collected during the first field season to demonstrate the feasibility of the method. Several areas were identified that require greater attention. In the 1991 field season, the originally planned sampling density will be covered (Fig. 5) with four cruises through the spawning season; new approaches will be undertaken to incubate the roughy eggs to determine developmental rate; and a survey of the proportion of non-reproductive roughy will be carried out.

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- Yusa, T. 1954. On the normal development of the fish, *Theragra chalcogramma* (Pallas), Alaska pollack. Bull. Hokkaido Reg. Fish. Res. Lab. 10: 1-15.

SECTION 4. TRANSFER OF RESULTS TO INDUSTRY

Results of the project are being disseminated to fishery managers and the industry through the following:

- 1) Results of the first year of the survey, including both the acoustic and egg production methods of resource assessment, were presented the Demersal and Pelagic Fishery Research Group (DPFRG) at their meeting on 4 December 1990; to GITLC at their meeting of 11 December 1990; and at a public seminar organized by GITLC and aimed at the industry on 12 December 1990. These seminars outlined the methods employed, our preliminary estimates of orange roughy stock biomass, the assumptions upon which the estimates are based and plans for future surveys.
- 2) An article was published in the October 1990 issue of *Australian Fisheries* (Smith and Koslow 1990) on the objectives and design of the orange roughy surveys. A further article is planned based upon final results of the survey.

SECTION 5. DURATION OF PROJECT

Commencement Date: 1 July 1990

Duration of project: 3 years

Completion Date: 30 June 1993.

SECTION 6. REQUESTED BUDGET

| | PREVIOUS ESTIMATE FOR 1991/92 \$ | CURRENT BUDGET FOR 1991/92 \$ | CURRENT ESTIMATE FOR 1992/93 \$ |
|--------------------|--|-------------------------------------|---------------------------------------|
| Salaries & Wages | 80,560 | 91,836 | 91,836 |
| Operating Expenses | 9,000 | 15,000 | 4,200 |
| Travel Expenses | - | - | - |
| Capital Items | - | - | - |
| TOTAL | 89,560 | 106,836 | 96,036 |

SECTION 7 EXPLANATION OF VARIATION IN BUDGET

Variation in Salaries is due to changes in CSIRO salary scale. \$1000 additional funds are requested in the operating budget to build a high-pressure chamber to incubate the roughy eggs at ambient deepwater pressure (Appendix A).

\$5000 is requested to purchase a slip-ring swivel, which is required in order to attach a transducer beneath the plankton net. This permits the net to be lowered to within a few meters of the bottom so the entire water column can be sampled for the roughy eggs. Without a swivel, the net and transducer rotate as they are lowered, which causes the conducting cable to kink and fail. Large sections of the cable must then be cut and the electrical connections re-terminated. This occurred repeatedly during the 1990 egg surveys and is not acceptable for the large-scale

surveys planned for 1991. A slip-ring swivel is the simplest solution, since it permits the cable to turn without kinking while maintaining the electrical connection. The swivel is made by Preform Marine Industries.

There are no other changes to the Requested Budget.

SECTION 8 FUNDS SOUGHT FROM OTHER SOURCES

| | |
|--|----------|
| Government-Industry Technical Liaison Committee (GITLC) (to partially cover fuel costs of acoustic & egg surveys) | \$50,000 |
|--|----------|

SECTION 9 FINANCIAL CONTRIBUTION OF APPLICANT

| | 1991/92 | 1992/93 |
|--|----------------|----------------|
| Dr. T. Davis CSOF7 (30%) | 15,940 | 15,940 |
| Mr. C. Liron CSOF4 (20%) | 6,912 | 6,912 |
| Hydrographic technician CSOF3 (20%) | 6,059 | 6,059 |
| Salary sub-total: | 28,911 | 28,911 |
| Salary oncosts | 23,129 | 23,129 |
| | 52,040 | 52,040 |
| OPERATING COSTS | | |
| Full operating costs for FRV <i>Southern Surveyor</i> for 3 weeks at sea, port and harbour dues, normal crew airfares and fuel costs | 496,000 | 496,000 |
| Laboratory facilities and research support | 20,000 | 20,000 |
| TOTAL FUNDS PROVIDED BY APPLICANT | 568,040 | 568,040 |

SECTION 10 BUDGET IN DETAIL

| | PREVIOUS ESTIMATE FOR 1992/93 \$ | BUDGET FOR 1991/92 \$ | CURRENT BUDGET FOR 1991/92 \$ |
|--|--|--------------------------|-------------------------------------|
| SALARIES | | | |
| Dr. J. A. Koslow, CSOF6 (50%) | 22,850 | 25,553 | 25,553 |
| Ms. C. Bulman CSOF4 (25%) | 8,700 | 9,683 | 9,683 |
| Mr. M. Lewis CSOF2 (50%) | 12,057 | 12,840 | 12,840 |
| Tech asst (NSW FRI) (25%) | 6,029 | 6,420 | 6,420 |
| Sub-total | 49,636 | 54,496 | 54,496 |
| Superannuation | 9,133 | 10,028 | 10,028 |
| Comcare | 1,241 | 1,362 | 1,362 |
| Leave loading | 750 | 750 | 750 |
| Marine Survey & overtime 3 sea wks @\$1400/wk x 6 staff | 19,800 | 25,200 | 25,200 |
| Sub-total | 30,924 | 37,340 | 37,340 |
| TOTAL SALARIES | 80,560 | 91,836 | 91,836 |
| OPERATING EXPENSES | | | |
| Plankton nets (Yr 2: 10 EZ nets; Yr 3: 4 replacement nets@ \$800/net) | 8,000 | 8,000 | 3,200 |
| Sample jars, preservatives | 1,000 | 1,000 | 1,000 |
| High-pressure incubation chamber | - | 1,000 | |
| Slip-ring swivel | - | 5,000 | |
| TOTAL OPERATING EXPENSES | 9,000 | 15,000 | 4,200 |
| BUDGET TOTAL | 89,560 | 106,836 | 96,036 |

SECTION 11 ORGANIZATION

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SECTION 12 PROJECT SUPERVISOR

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 CSIRO Division of Fisheries
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SECTION 13 STAFF INVOLVED IN PROJECT

| | | | |
|--|--------|-----|---------------------------|
| J. A. Koslow, Ph.D. | CSOF 6 | 50% | Project supervisor |
| Overall responsibility for project planning and execution; data analysis, interpretation, write-up, and reporting. | | | |
| T. Davis, Ph. D. | CSOF 7 | 30% | Senior Research Scientist |
| Collaboration on planning field survey and laboratory sorting procedures and assistance with field work. | | | |
| C. Bulman, B.Sc.(Hons) | CSOF 4 | 25% | Experimental Scientist |
| Carry out biological experiments (e.g. egg incubation) and field survey; establish laboratory sorting protocols; assist in data analysis, interpretation and write-up. | | | |
| C. Liron | CSOF 4 | 20% | Senior Technical Officer |
| Responsibility for gear and maintenance and assistance with field survey. | | | |
| (To be named) | CSOF 3 | 20% | Hydrographic technician |
| Assist in field surveys. | | | |
| M. Lewis, B.Sc. | CSOF 2 | 50% | Technical assistant |
| Assistance in field sampling; sort plankton samples for roughy eggs and stage them; data entry. | | | |
| (To be named) | CSOF 2 | 25% | Technical assistant |

Analyse gonadal material for fecundity estimate; histological sectioning and staging to determine reproductive state.

Administrative contact:

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FIGURE LEGEND

Figure 1 The numbers of stage 1 and 2 (~1-day old) eggs obtained during the egg survey off northeastern Tasmania of 20-25 July at the approximate peak of orange roughy spawning.

Figure 2 The relationship between fecundity (F) and standard length (SL) based upon \log_e -transformed data.

Figure 3 The relationship between an index of roughy condition factor (the residuals from the regression between weight and length) and relative fecundity (the residuals from the fecundity:length relationship).

Figure 4 Daily egg production during the 1990 spawning season. The area enclosed by the triangle represents the estimated total egg production during the spawning season.

Figure 5 Station plan for the orange roughy egg survey to cover the distribution of one-day old eggs spawned off St. Helens, Tasmania.

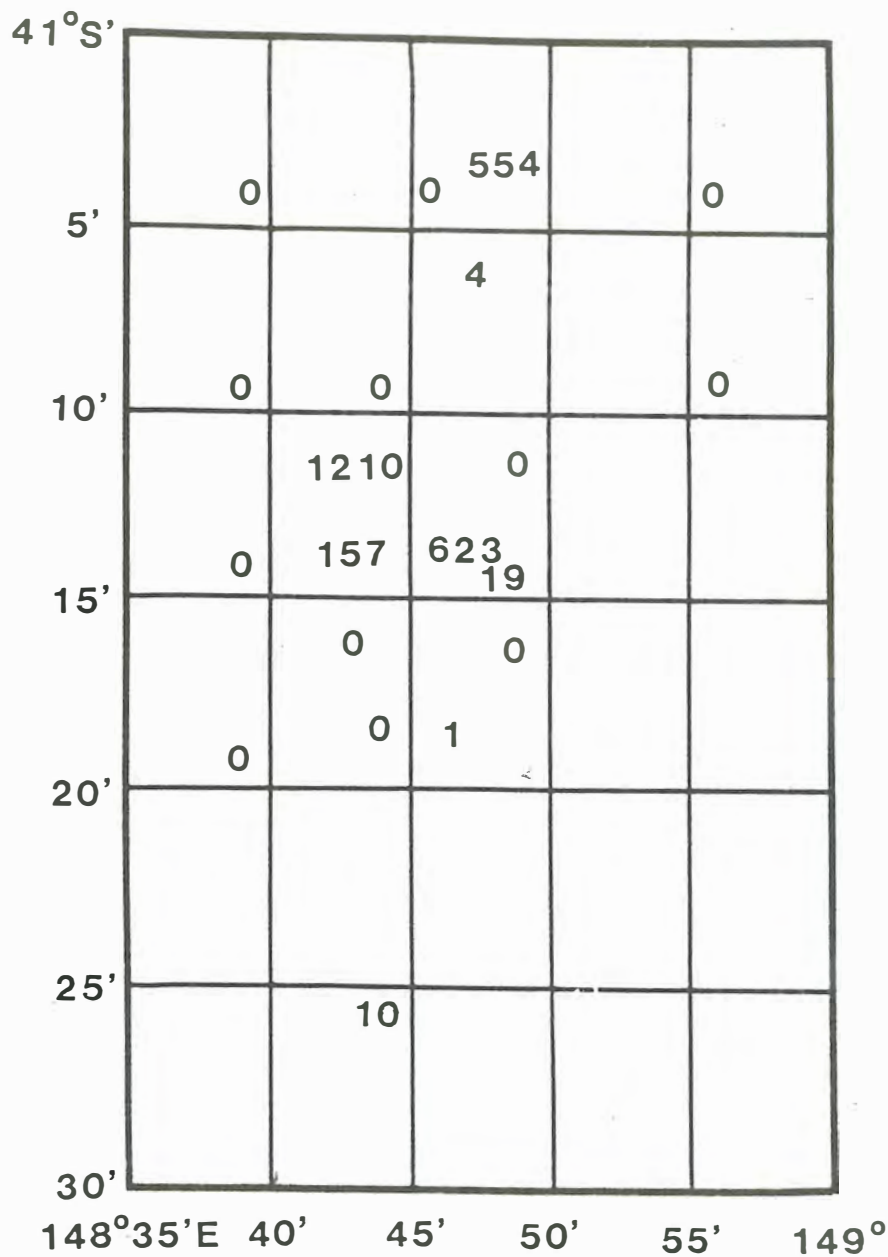


FIGURE 1

Relationship between fecundity and length for orange roughy 1990

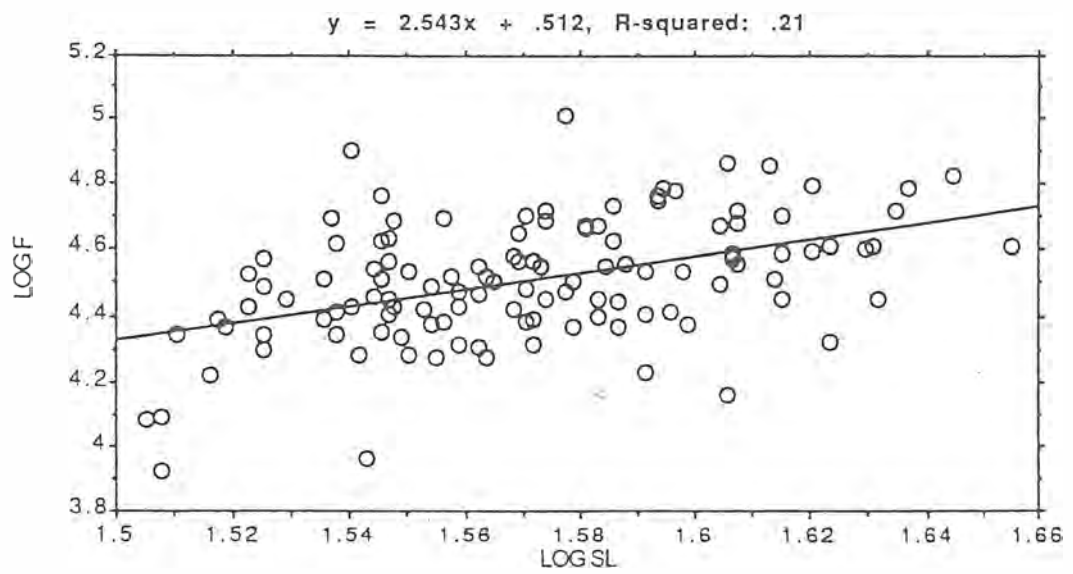


FIGURE 2

Residuals: fecundity/SL vs roughy condition factor

$y = 1.987x + .011$, R-squared: .193

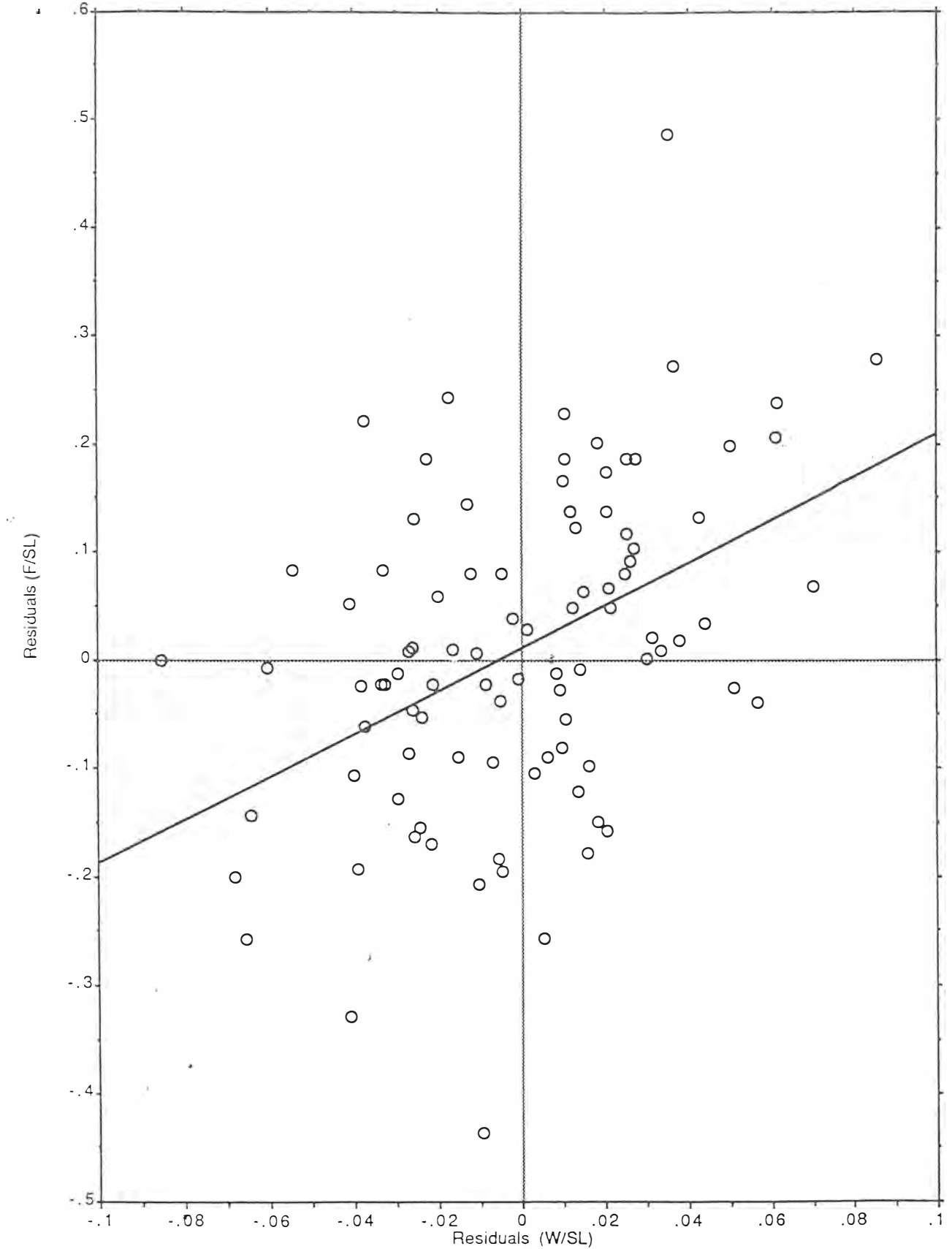


FIGURE 3

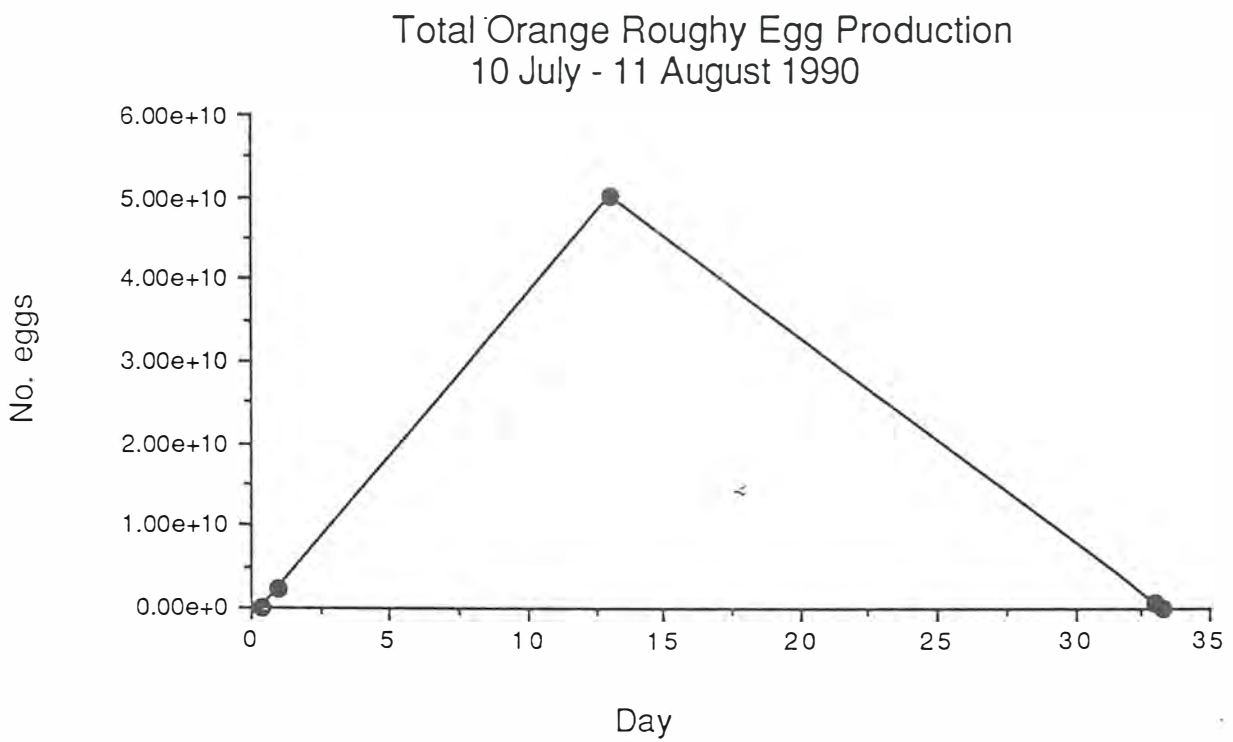


FIGURE 4

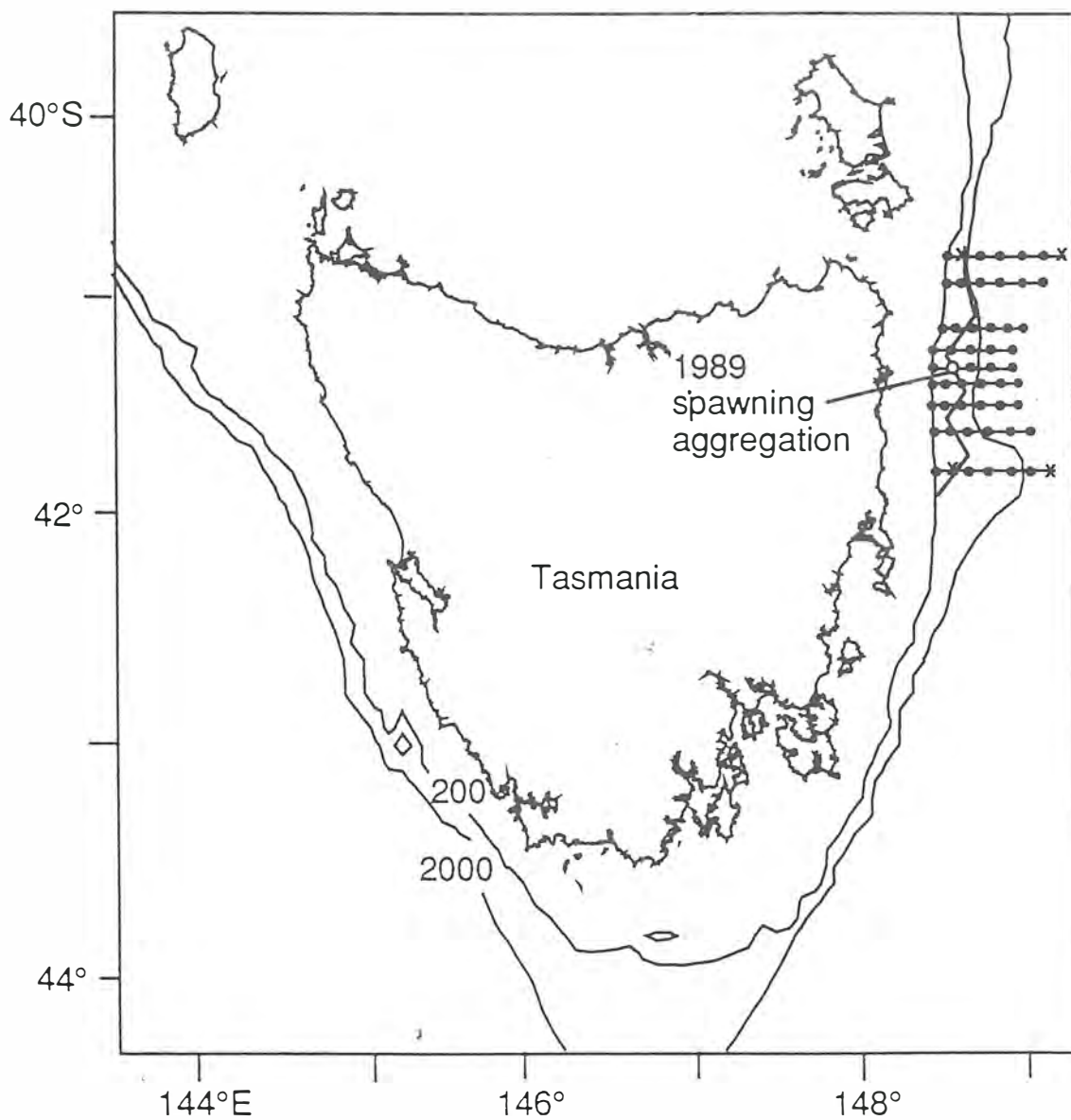


FIGURE 5

PROGRESS REPORT

1992 CONTINUING APPLICATION GRANT



**FISHING INDUSTRY RESEARCH AND DEVELOPMENT COUNCIL
1992 CONTINUING APPLICATION GRANT 90/9****SECTION 1 - PROJECT TITLE**

Development and use of the egg production method to assess the biomass of orange roughy off eastern Tasmania

SECTION 2 - OBJECTIVES

- To assess the standing stock of orange roughy off east Tasmania based upon a survey of the egg production of the spawning aggregation off St. Helens.
- To carry out studies of orange roughy reproductive biology and early life history to determine:
 - the sex-ratio of orange roughy in the spawning stock;
 - the relation between fecundity and body weight; and
 - the proportion of non-reproductive fish in the population.

SECTION 3 - PROGRESS REPORT**Egg Survey (Objective 1)**

Orange roughy egg abundance was estimated during three surveys carried out aboard the chartered *FV Tasmanian Enterprise* TEO1/91 (2-10 July), TEO2/91 (15-23 July), and TEO3/91 (28 July - 5 August). The stations sampled and the stations at which eggs were present are shown in Figure 1. As seen from the estimates of daily egg production (Figure 2), the surveys covered the beginning, peak, and end of the spawning season. This is consistent with observed changes in sexual stage in orange roughy sampled from the spawning aggregation (J. Lyle, Tasmania Department of Sea Fisheries, unpubl. data).

Fecundity was estimated for 120 orange roughy obtained during the first week of July from fish at maturity stage 4. As in 1990, there was a significant relationship between fish weight and fecundity with a relatively low percentage of variation explained (30%) (Fig. 3). Analysis of covariance indicated that the relationship did not vary between the two years. Analyses are underway to examine whether variability in fecundity may be related to condition of the fish

Our best estimate of total stock biomass was 39,500 tonnes. This estimate is based upon the assumptions that 55% of females and 90% of males in the stock spawned and that the ratio of females:males was 0.52. This ratio was 0.41 last year. This difference in sex ratio may have arisen from the combination of vertical segregation of the sexes and differences in the depth distribution of fishing effort during the two years. Total stock biomass calculated using the previous year's sex ratio was 47,000 tonnes.

The coefficient of variation ($CV = sd/mean$) was generally high (~ 0.7), and the 95% upper confidence limits for each cruise were approximately twice the mean. Based upon the observed sampling variance, ~ 50 -100 samples per stratum are required to obtain confidence limits within 30% of the mean, and 20 to 40 samples per stratum are required to obtain confidence limits within 50% of the mean.

Comparing the present egg survey with the survey conducted in 1990, the biomass estimate for last year was considerably higher (139,000 t). However, the egg survey in 1990 was based upon limited sampling (37 stations in total), and its results were viewed as preliminary. Approximately 50% of the 1990 estimate was derived from an exceptionally high catch from a single station, which was ~ 10 nm from the central spawning site. Samples were obtained in the vicinity of this anomalous station during each of the three cruises in 1991, but there was no evidence of enhanced egg densities in the area.

Considerable progress is evident in the 1991 egg survey, particularly with regard to its spatial and temporal coverage of spawning. However, problems in three areas need to be resolved for the method to be fully useful as a survey tool for orange roughy:

- uncertainty concerning egg stage duration
- sampling error (i.e. broad confidence limits)
- uncertainty in the proportion of non-reproductive orange roughy.

Egg stage duration depends upon the temperature-dependent development rate of the eggs. Sampling with an opening-closing EZ plankton net adjacent to the spawning area was successful and indicated that the eggs are predominantly at 700-900 m depth, or at 5-7 $^{\circ}$, based upon temperature profiles obtained from the area. However, until the eggs can be incubated, the duration of stages during early development remains uncertain. It is unclear whether incubation experiments this past year failed due to lack of suitable material (winch failure this past field season limited our ability to obtain ripe-and-running fish) or to inherent difficulties, such as a pressure requirement, associated with rearing orange roughy eggs. A small high-pressure incubation chamber was constructed and trialled but did not seem more successful.

Our estimate of incubation rate at present is based upon obtaining a newly-hatched orange roughy within 10-14 d of the beginning of the spawning period last year. A 10-14 d incubation period is consistent with the expected development rate based upon the size and incubation temperature of orange roughy eggs. However, a high priority will be placed upon successfully incubating the roughy eggs during the 1992 spawning period.

The broad confidence limits associated with this year's survey arise from the high variability of the egg samples. The confidence limits can only be reduced by substantially increased sampling density. It is apparent from this survey that the density of 1-day old eggs declines exponentially with distance from the spawning site. This is due both to dilution of the eggs, which will cause egg densities to decline at a rate inversely proportional to the square of the distance from their source, and to development of the eggs beyond the 1-day stage. At distances greater than 10 nm (18.5 km) from the spawning site, the density of 1-day old eggs approached zero: during peak spawning, egg production beyond this limit accounted for ~1% of egg production, and at other times, no eggs were obtained from this area. In future, therefore, sampling will be restricted to transects within 10 nm of the spawning area, so greater replication can be achieved on the spawning hill and in its immediate environs.

There must also be a greater concentration of sampling effort during the period of peak spawning, which in the last two years has consistently occurred during the later half of July. By reducing the area covered, it should be possible to obtain ~100-150 samples from the central transects during this period.

The proportion of non-reproductive roughy has been assessed from surveys carried out in autumn. Such surveys have been carried out since 1989, but there has not been sufficient sampling to have confidence yet in our estimate. Error in our estimate of the proportion of reproductive fish will directly affect our estimate of total stock biomass. A further survey is planned for autumn, 1992.

In summary, sufficient data were collected this year to define the distribution of early stage orange roughy eggs. The biomass estimate was congruent with results of the acoustic survey, and this was noted by the Demersal and Pelagic Fisheries Research Group to greatly enhance the confidence of the Group in its assessment of orange roughy stock biomass this year. Several areas were identified that require greater attention during the 1992 field season.

Section 4. Transfer of Results to Industry

Results of the project are being disseminated to fishery managers and the industry through the following:

- Results of the 1991 field season were presented to the Demersal and Pelagic Fishery Research Group (DPFRG) during the first week of October (Koslow et al. 1991). A draft report was then prepared within DPFRG, based in part on the survey results, that was presented to the AFS; to the Government-Industry Technical Liaison Committee (GITLC) during a meeting on 13 October; and to the industry and public through a seminar at CSIRO on 14 October.
- An article appeared in *Australian Fisheries* (April 1991, p. 11) that summarized results of the first orange roughy industry seminar (December 1990). Interviews

with the media have been provided regularly to report on progress of the biomass surveys.

- A manuscript will be prepared for publication in a scientific journal (probably the *Australian Journal of Freshwater and Marine Research*) upon completion of the project.
- Koslow, J.A., R. Kloser, C.M. Bulman, J. Bell, J. Lyle. 1991. Assessment of orange roughy (*Hoplostethus atlanticus*) biomass off northeastern Tasmania, winter 1991. A report prepared for DPFGR 10/1991.

SECTION 5. DURATION OF PROJECT

Commencement Date: 1 July 1990
 Duration of project: 3 years
 Completion Date: 30 June 1993.

SECTION 6. REQUESTED BUDGET

| | Previous estimate for 1992/93 (\$) | Current estimate for 1992/93 (\$) |
|-----------------------------|--|---|
| Salaries & Wages | 91,836 | 99,877 |
| Operating Expenses | 4,200 | 4,200 |
| Travel Expenses | | 1,075 |
| Capital Items | | |
| TOTAL (\$) | 96,036 | 105,152 |

Is there a separate supplementary application attached? NO

SECTION 7. EXPLANATION OF VARIATION IN BUDGET

Variation in Salaries is due to changes in CSIRO salary scale. Travel was added to the budget due to the need to report results of the biomass assessment to at least one interstate meeting annually (eg. GITLC, DPFRG). There are no other changes to the Requested Budget.

SECTION 8. FUNDS SOUGHT FROM OTHER SOURCES

SOURCE Government-Industry Technical Liaison Committee (GITLC) or AFMA: small vessel charter to survey the eggs

(Application to be made) \$50,000

SECTION 9 FINANCIAL CONTRIBUTION OF APPLICANT

| Salaries | |
|---|----------------|
| Ms. K. Haskard, CSOF 5 (10%) | 4,370 |
| Mr. M. Sherlock CSOF 4 (10%) | 3,700 |
| Salary on-costs: | 6,450 |
| Total (\$) | 14,520 |
| Operating costs (\$) | |
| Full operating costs for FRV <i>Southern</i> <i>Surveyor</i> for 1 week at sea, port and harbour dues, normal crew airfares and fuel costs | 165,000 |
| Laboratory facilities and research support | 20,000 |
| Total funds provided by applicant | 199,520 |

SECTION 10. BUDGET IN DETAIL

| | Previous estimate for 1992/3 (\$) | Current budget for 1992/3 (\$) |
|--|---|--------------------------------------|
| Salaries (\$) | | |
| Dr. J. A. Koslow, CSOF6 (50%) | 25,553 | 31,092 |
| oncosts | 5,693 | 7,026 |
| Ms. C. Bulman CSOF4 (25%) | 9,683 | 11,530 |
| oncosts | 2,157 | 2,606 |
| Mr. M. Lewis CSOF3 (50%) | 12,840 | 14,477 |
| oncosts | 2,860 | 3,271 |
| Tech asst (NSW FRI) (25%) | 6,420 | 7,239 |
| oncosts | 1,430 | 1,636 |
| Sub-total | | 78,877 |
| Marine Survey allowance & overtime 1 sea wk @\$1400/wk x 7 staff +4 sea wk x 2 staff | 25,200 | 21,000 |
| Total salaries (\$) | 91,836 | 99,877 |
| Travelling costs (\$) | | |
| Industry/Scientific meeting (e.g. GITLC, DPFRG) Airfare (Hobart-Canberra) | | 600 |
| Allowances (3 d @ \$60/d) | | 180 |
| Accommodation (3 d @ \$65/d) | | 195 |
| Vehicle costs (4 airport taxi fares) | | 100 |
| Total Travel (\$) | | 1,075 |

| | | |
|---|---------------|----------------|
| Operating costs (\$) | | |
| Plankton nets (4 replacementnets @ \$800/net) | 3,200 | 3,200 |
| Sample jars, preservatives | 1,000 | 1,000 |
| Total operating expenses (\$) | 4,200 | 4,200 |
| BUDGET TOTAL (\$) | 96,036 | 105,152 |

SECTION 11. FLOW OF BENEFITS

State

- Tasmania 80%
- NSW 10%
- Victoria 10%
- Fishery: Orange roughy 100%

SECTION 12. ORGANIZATION

Dr Peter Young
 Chief, Division of Fisheries
 CSIRO Marine Laboratories
 GPO Box 1538
 Hobart, Tasmania 7001
 Phone: (002) 206264
 Telex: AA 57182
 Fax: (002) 240530

SECTION 13. PROJECT SUPERVISOR

Dr. J. A. Koslow
 Senior Research Scientist
 CSIRO Division of Fisheries
 GPO Box 1538, Hobart, Tasmania 7001
 Phone: (002) 20 6358
 Telex: AA 57182
 Fax:(002)24 0530

SECTION 14. STAFF INVOLVED IN PROJECT

J. A. Koslow, Ph.D., CSOF 6, 50%, Project supervisor
 Overall responsibility for project planning and execution; data analysis, interpretation, write-up, and reporting.

C. Bulman, B.Sc.(Hons), CSOF 4, 25%, Experimental Scientist
 Carry out biological experiments (eg. egg incubation) and field survey; establish laboratory sorting protocols; assist in data analysis, interpretation and write-up.

M. Lewis, B.Sc. , CSOF 2, 50%, Technical assistant
 Assistance in field sampling; sort plankton samples for roughy eggs and stage them; data entry.

(To be named), CSOF 2, 25%, Technical assistant

Analyse gonadal material for fecundity estimate; histological sectioning and staging to determine reproductive state.

Administrative contact:

Mr. Peter Green

CSIRO Marine Laboratories

GPO Box 1538, Hobart, Tasmania 7001

Phone: (002) 20 6233

Telex: AA 57182

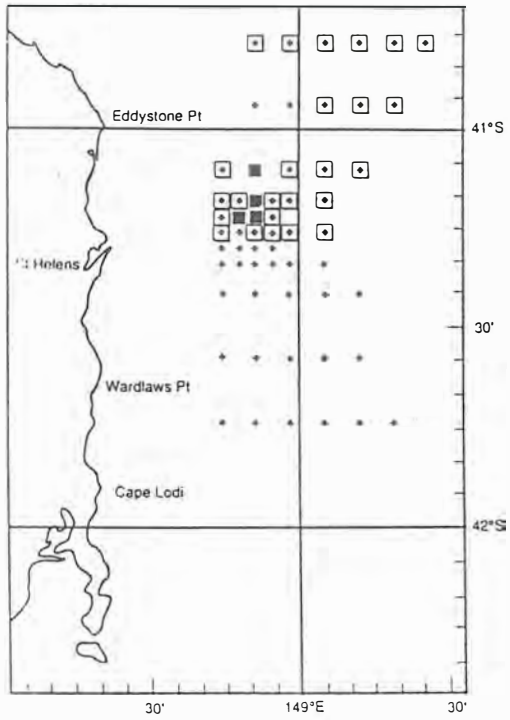
Fax: (002) 24 0530

FIGURE LEGEND

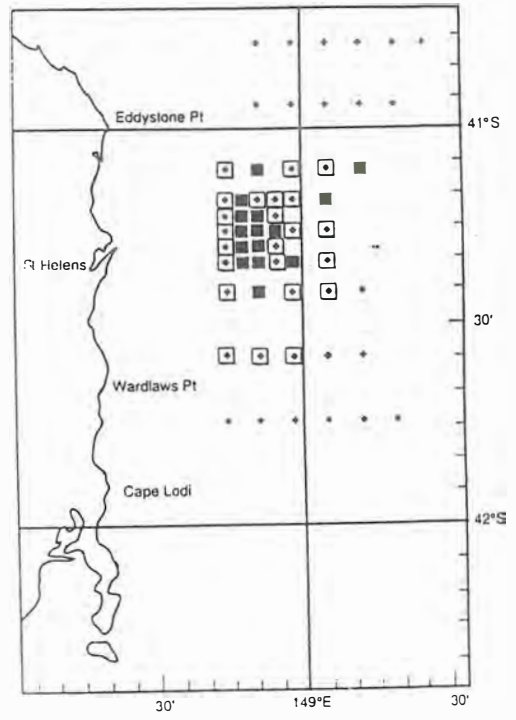
Figure 1. The stations sampled and those with stage 1 and 2 (~1-day old) orange roughy eggs present (solid squares) or absent (open squares) during egg surveys off northeastern Tasmania. Diamonds: station positions not sampled. Cruise 1: 2-10 July; Cruise 2: 15-23 July; Cruise 3: 28 July - 5 August.

Figure 2. Mean daily egg production (+ 1 SE) of orange roughy during the 1991 spawning season. Dates of the beginning and ends of the cruises are shown.

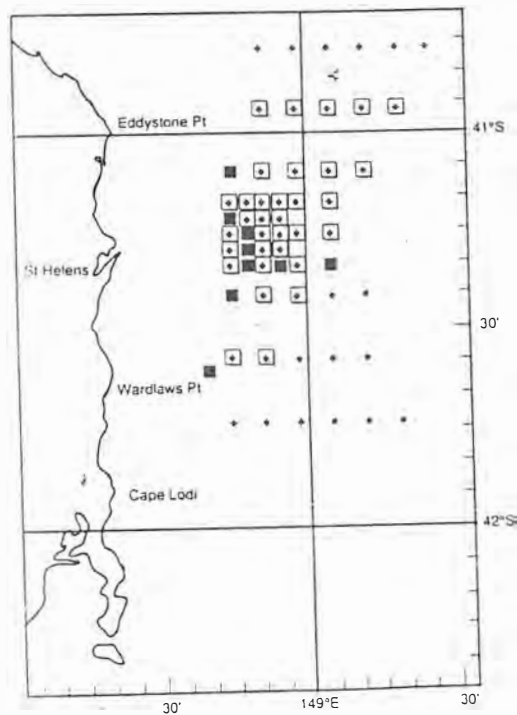
Figure 3. Relationship of orange roughy weight and fecundity for fish from St. Helens in 1990 and 1991.



Cruise 1



Cruise 2



Cruise 3

FIGURE 1

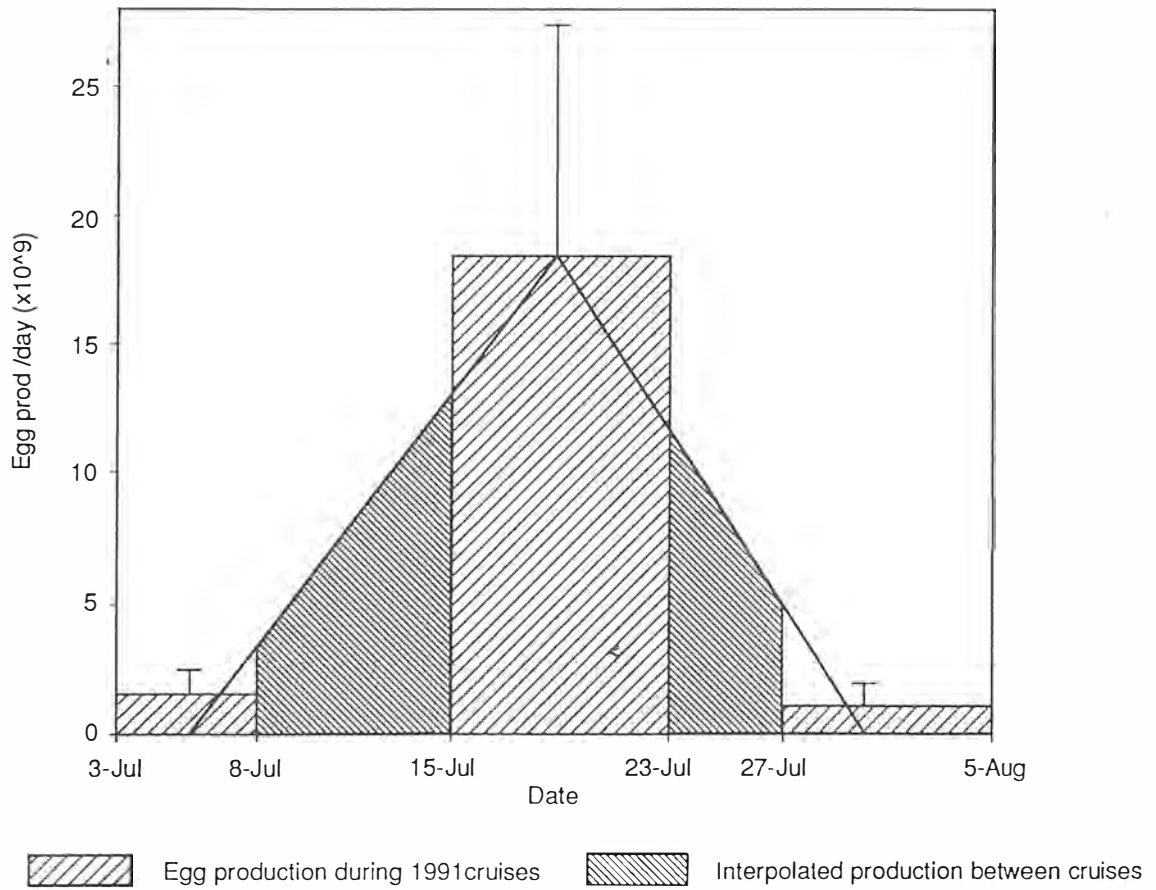


FIGURE 2

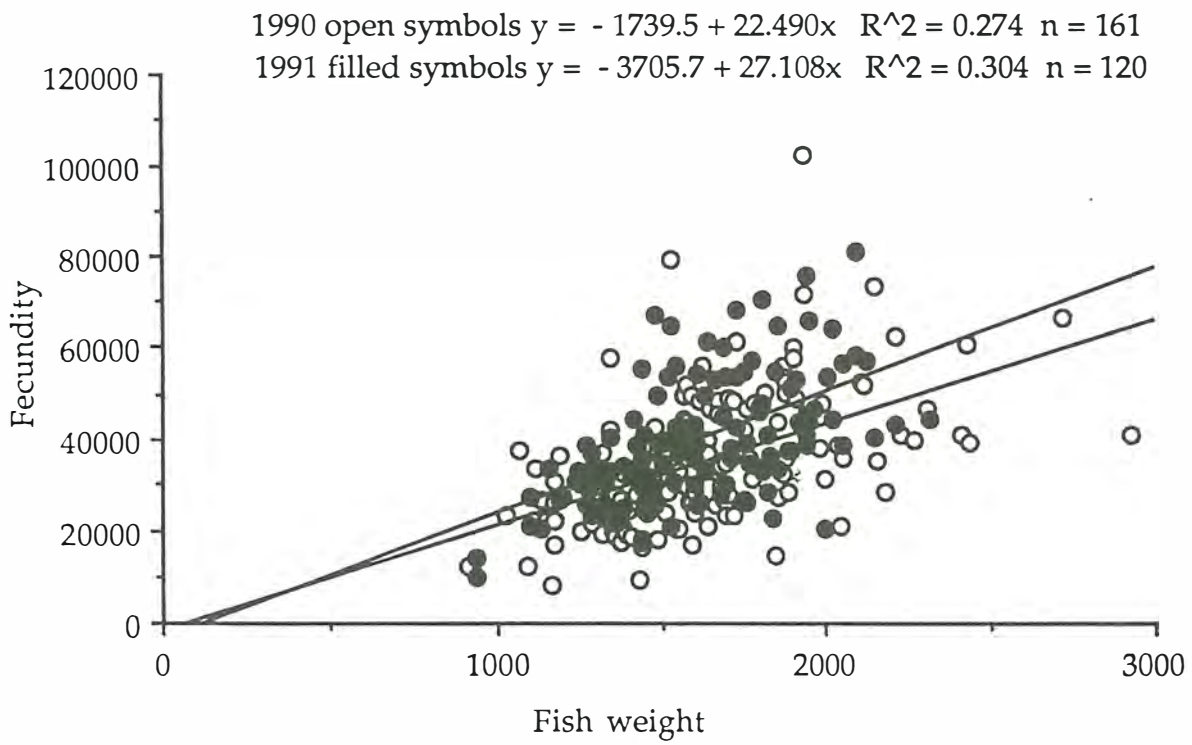


FIGURE 3