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Study on the Reproductive Biology of Brill (*Colistium guntheri* Hutton, 1926) off South Otago, New Zealand

Penelitian Tentang Biologi Reproduksi pada Brill (*Colistium guntheri* Hutton, 1926) di Perairan Otago Selatan, New Zealand

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Abstract

Perkembangan gonad, musim pemijahan dan fekunditas ikan sebelah "brill" (*Colistium guntheri* Hutton, 1926) di perairan Otago Selatan, New Zealand telah diamati selama satu tahun. Sampel ikan ditangkap setiap bulan menggunakan "bottom otter trawl". Indeks gonad, tingkat kematangan gonad dan distribusi frekuensi ukuran telur digunakan untuk menentukan perkembangan gonad dan musim pemijahan. Hasil penelitian ini menunjukkan bahwa perbandingan populasi ikan "brill" jantan dan betina tidak berbeda nyata dari 1 : 1. Pada ukuran panjang tubuh yang sama, ikan betina mempunyai berat badan yang lebih besar dari pada ikan jantan. Hasil analisa histologi menunjukkan bahwa *C. guntheri* termasuk kelompok ikan yang mempunyai perkembangan telur secara *sinkroni*. Berdasarkan perubahan nilai indeks gonad dan perkembangan ovari, jenis ikan ini diketahui mengalami pematangan gonad yang sangat cepat pada akhir musim gugur (Juni), yaitu ditandai dengan peningkatan nilai indeks gonad secara drastis dan ovari didominasi oleh telur pada tingkat kematangan akhir (*final maturation stage*). Musim pemijahan dimulai pada akhir musim dingin (Agustus) sampai musim panas (Januari), ditandai dengan penurunan nilai indeks gonad secara nyata dan ovari didominasi oleh telur yang telah masak (*mature*), hidrasi (*hydrated*) dan paska ovulasi (*postovulatory*). Fekunditas (*batch fecundity*) ditentukan dengan menghitung semua telur yang telah masak, mencakup sekitar 34% dari total telur di dalam ovari. Pada pengamatan ini diketahui bahwa fekunditas (Y) proporsional dengan berat gonad dalam gram (Wg) dengan persamaan regresi $Y = 10^3$ [(4.34 + 20.06 (Wg)], $R^2 = 0.95$, dan fekunditas relatif per gram berat gonad adalah 18.760±1.150 telur.

Key words: *Colistium guntheri*, brill, ovarian development, steroid hormones, fecundity

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Introduction

Many flatfish species form the basis of important demersal fisheries throughout the world and some species have been comprehensively studied. Fourteen species of flatfish are known in New Zealand waters Zealand (Ayling and Cox 1982, Paul and Health 1985). Brill (*Colistium guntheri* Hutton, 1926) is one of the commercially important flatfish species in New Zealand waters, especially the catch of coastal trawlers around South Island of New Zealand (Ayling and Cox 1982; Paul and Health 1985; Setyono 1996).

Brill belongs to the family Pleuronectidae, found in coastal waters off South Otago, on sandy and muddy bottoms, at depth of 30 to 100 m (Graham 1974, Ayling and Cox 1982). This species has both eyes on the right side of the head and lies on the

bottom on their left side.The superior quality of its flesh commands a high market price (Kirk 1988).

Information on seasonal reproductive cycles in the large number of pleuronectid species, either in the Northern hemisphere and/or especially in the Southern hemisphere, in general is relatively scarce (see Setyono 1996). The few studies available have focused generally on biological aspects such as early life history (egg and larval stages), growth, and movement, and little is known about reproductive cycles.

Studies on the Northern Hemisphere pleuronectids showed that flatfish species has a diversity of reproductive strategies. For example, *S. solea* spawn under a wide range of conditions, and spawning times vary, even at the same location (Baynes *et al.* 1993). Timing of spawning in temperate flatfish is governed mainly by seasonal changes in photoperiod and sea temperature (Baynes *et al.* 1993, Harmin *et al*. 1995, Symonds and Rogers 1995).

Despite the commercial importance and aquaculture potential of brill, the only available information on the reproductive biology of this species has been provided by Tait and Hickman (2001) and Poortenaar *et al*. (2001). However, the information on reproductive cycle is incomplete in these paper and is augmented by the present study. Therefore, the objective of this study is to provide additional data to supplement previous studies and to provide more information on reproductive biology, including ovarian development, gonad index, spawning season and batch fecundity.

Materials and Methods

Fish samples were obtained by commercial trawler off South Otago coastal waters, South Island, New Zealand. Trawls were made at a depth of between 30-50 m, and the average duration of a single trawl was 2 hrs. In the laboratory, the fish were weighed and the total length measured, to the nearest 0.1 g and 1 mm, respectively.

Gonads were removed from the body and weighed. Gonad index (GI) was calculated as follows :

GI = (Weight of gonad in g / Weight of fish without gonad in g) x 100%.

GI was calculated for each fish, and the average value was plotted against the time (month) of observation.

A small piece of tissue (approximately 1 cm^2) from the middle part of the gonad was preserved in Bouin's fluid for at least 24 hrs. The tissues were then processed using standard histological procedures (Humason 1979). Tissues were embedded in paraffin wax, sectioned at 6-7 mm with a Cambridge Rotary Microtome, and stained with haematoxylin and eosin.

Gonad sections were examined under a microscope to determine ovarian maturity stages. Ovaries were classified on the basis of the appearance of the most advanced type of oocyte present (Setyono, 1996). Ovaries were divided into four major classes, i.e., previtellogenic, maturing, mature, and postovulatory (see Setyono 1996).

Oocyte diameter was measured in histological sections using an image analysis system, based on the Apple II computer software program VIDS II General Measurement. One hundred oocytes were randomly selected and measured from every gonad sample. Only oocytes sectioned through the nucleus were measured. Calculated mean oocyte diameters were then plotted against the time (month) of observation.

Approximately 3 g of mature gonad samples were taken from the anterior, middle and posterior region of the ovary. The samples were then preserved in Gilson's fluid for about 1 month. A gravimetric method was used to determine batch fecundity. Five replicates of 0.02 g were weighed and washed through a 250 µm sieve. Mature oocytes >250 µm in diameter after preservation in Gilson's fluid were counted to determine batch fecundity. Oocyte counts were done under a binocular microscope and batch fecundity was calculated as follows:

Batch fecundity = (Wg / Ws) x N oocytes.

Where : Wg = weight of the whole gonad (g), Ws = weight of sub sample (g) and N = mean oocyte number in the sub-sample.

Analysis of variance (ANOVA) was performed using software DataDesk 4.1 for Macintosh computer (Data Description Inc., Ithaca, N.Y.) to test the significant differences among the monthly mean values. The Least Significant Difference (LSD) test was used to identify significantly different mean values. The statistical significance level (P) was set at 0.05.

Results

Monthly sampling of 4-16 fish resulted in total number of 36 males and 53 females. However, expected mean number of males (μ_1) and females (μ_2) in monthly sampling was not significantly different (P = 0.0503). It means that the ratio of males to females was not significantly different from 1 : 1. Males ranged from 320 to 480 mm in length and from 300 to 1,300 g in weight, and females ranged from 360 to 500 mm in length and from 700 to 1,800 g in weight. Females were significantly larger than the males (P = 0.0001) in body weight.

Seasonal changes in mean GI and sea surface temperature are shown in Figure 1. GI of female brill varied between about 1-3% for most of the year, except in June when it increased to a mean of approximately 5% (ranged from 1.5% to 10.9%). For the males, GI remained at a constant and low level (<1%). Female gonads were significantly large than the male gonads (P = 0.0001). No data were available for July and August because of the lack of any brill caught during several days of trawling.

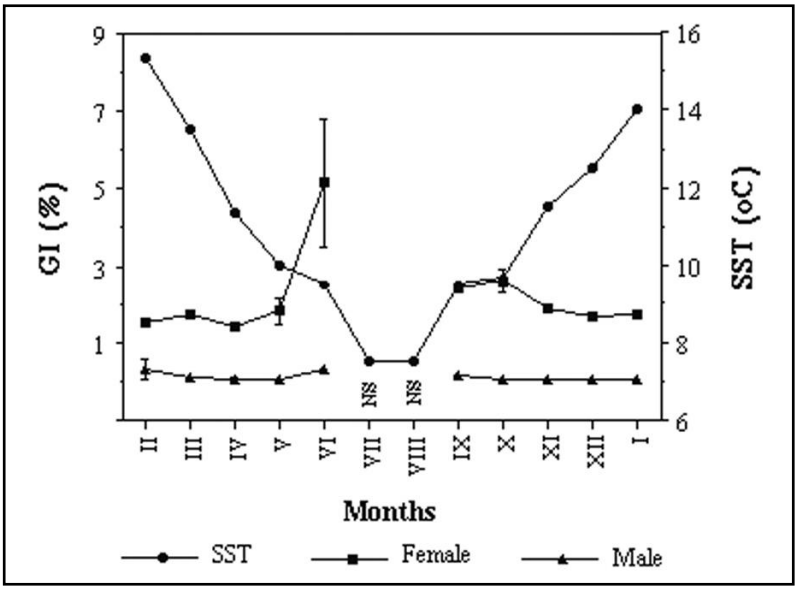

Figure 1. Seasonal changes in gonad index (GI) and sea surface temperature (SST). GI values are mean and standard error. GI values in June is significantly different from the other months (P < 0.05). Number of samples for female (f) and male (m) were: II = 11f, 5m; III = 3f, 1m; IV = 2f, 2m; V = 3f, 7m; VI = 5f, 2m; IX = 4f, 4m; X = 9f, 5m; XI = 6f, 4m; XII = 5f, 2m; and I = 8f, 5m. NS = no sample.

Figure 2 shows seasonal variations in the composition of the ovary, based on the maturation stage of each fish. Vitellogenesis started in February, and by May migratory nucleus stage oocytes dominated the ovaries. Mature ovaries (containing oocytes which had undergone final oocyte maturation) were found in June. In September, ovaries were dominated by mature and hydrated oocytes and postovulatory follicles. Postovulatory ovaries increased significantly from October to November, and previtellogenic ovaries

appeared in samples from December through April. Spermatozoa and spermatids were present in testis samples throughout the year with little evidence of seasonal changes (data not shown).

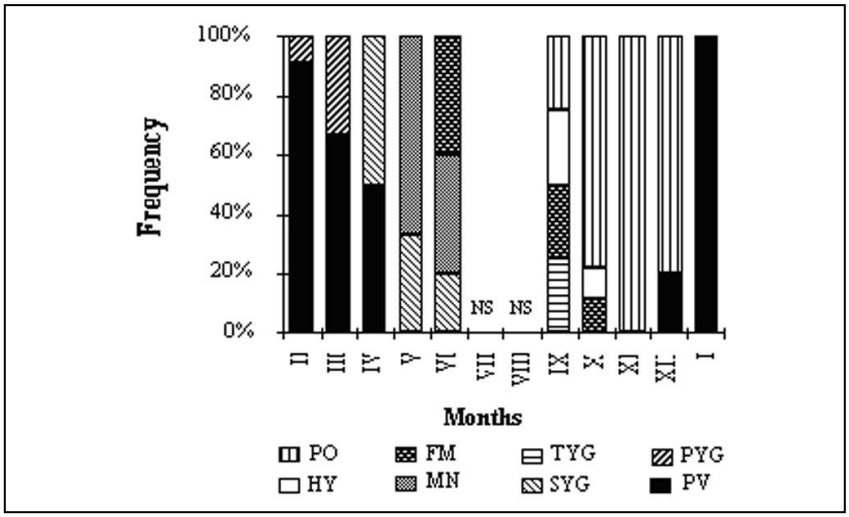

Figure 2. Occurrence of each ovarian stage in monthly samples. PO = postovulatory stage, HY = hydrated stage, FM = final maturation stage, MN = migratory nucleus stage, TYG = tertiary yolk globule stage, SYG = secondary yolk globule stage, PYG = primary yolk globule stage and PV = previtellogenic stage. Number of samples were: II = 11; III = 3; IV = 2; V = 3; VI = 5; IX = 4; X = 9; XI = 6; XII = 5; and I = 8. NS = no sample.

Oocyte size frequency distribution was dominated by a class of small (100 µm) oocytes, but the numbers of these oocytes decreased from June to October as larger size classes increased in frequency. The mean of oocyte diameter increased sharply in June, and decreased to a low value in November, and remained stable at other times (Figure 3).

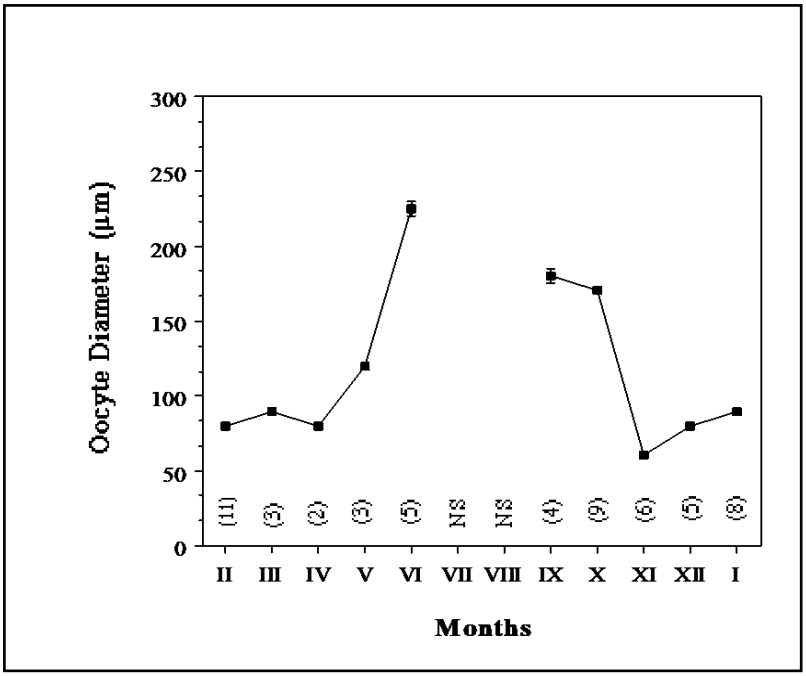

Figure 3. Mean oocyte diameter plotted against the time of observation. One hundred oocytes were measured from each fish. Values are mean and standard error (SE). SE bars are not shown when values were very small. Numbers in parenthesis represent the number of ovaries analysed. NS = no sample.

Figure 4 shows the size ranges for oocytes at a particular ovarian maturation stage. There are overlapping sizes between adjacent stages. Mature ovaries contained oocytes at all stages of development (data not shown). Mature oocytes represented about 34% of the total oocytes, and previtellogenic and maturing oocytes represented 40% and 26%, respectively.

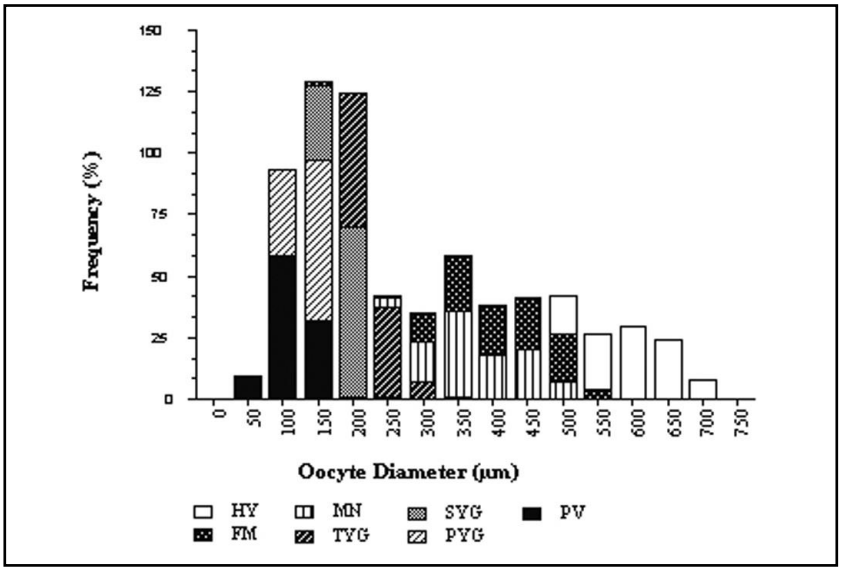

Figure 4. Ranges of oocyte size derived from histological observations for each oocyte stage of brill. At least 300 oocytes were measured for each stage. Data presented as percentage frequency for each oocyte stage. HY = hydrated stage, FM = final maturation stage, MN = migratory nucleus stage, TYG = tertiary yolk globule stage, SYG = secondary yolk globule stage, PYG = primary yolk globule stage and PV = previtellogenic stage.

Batch fecundity (Y) was found to be proportional to the gonad weight (Wg) in grams with a linear regression $Y = 10^3$ [(4.34 + 20.06 (Wg)], $R^2 = 0.95$. However, the linear regression between batch fecundity (Y) and body weight (Wb) was $Y = 10^3$ [(-402.24 + 1.09 (Wb)], $R^2 = 0.68$. Relative batch fecundity per gram of gonad weight (mean and standard error) was 18,760$\pm$1,150 oocytes.

Discussion

The present study revealed that a significant size dimorphism exists between the sexes. The maximum weight attained by males was only approximately 50% that of females. Although 32% more females were caught than males, sex ratio was not significantly different from 1:1, albeit, based on a small sample size. There was also marked size difference between male and female gonad, and the right (upper) side gonad was bigger than the left (lower) side gonad (Setyono 1996, Tait and Hickman 2001).

Setyono (1996) found that GI for female lemon sole, *Pelotretis flavilatus*, and female sand flounder, *Rhombosolea plebeia*, varied between 0.5-1.5%, and 3-6%, respectively. A similar result was found for brill, with mean GIs varying between approximately 1-3% for the females and less than 1% for the males. This finding is also in agreement with Tait and Hickman (2001), who reported that the male brill gonad was only 1 g or less.

For both male and female brill, GIs were relatively unchanging throughout the year except in June when GI for the female increased sharply. There was a lack of data in July and August due to lack of samples. However, Tait and Hickman (2001) and Poortenaar *et al.* (2001) found that GIs were mostly still at a high level during July and August. GI dropped to a low level (about 2.5 for females and 0.5 for males) in September. It can be predicted that this species has a peak spawning season in the late southern winter to early southern summer (August-December) since ovaries containing final maturation stage oocytes were found in early winter (June; present study), and GI was in high level in July-August (Poortenaar *et al.* 2001), and postovulatory ovaries occurred in late winter to early summer (September-December; present study). These both findings (present study and Poortenaar *et al.* 2001) show that spawning season of brill off South Otago waters occurred in a long periods (August-January). Observations on oocyte size frequency distributions support the proposition that brill has a prolonged spawning period from late winter to mid summer when most of the ovaries were in the postovulatory stage, being dominated by oocytes in the smaller size classes.

Data on reproductive cycle were not available for the January-April sampling period in Poortenaar *et al.* (2001). However, the data from the present study show that during these times the brill were in recovery stage and the animals were dominated by ovaries in previtelogenic and primary yolk globule stages. At these times, GIs were relatively stable at a low level, and small oocyte size classes (<100µm) were dominant.

Although male GIs were small, the abundance of spermatids and spermatozoa in testes at all times of observation suggests that spawning in the males could potentially occur throughout the year. The capability of other male pleuronectids from South Otago waters to produce sperm throughout the year was reported by Setyono (1996).

This study also shows that control of gonadal recrudescence, the onset and duration of spawning, and gonadal regression of brill is likely influenced by environmental factors (Figure 1). The drop in sea surface temperature is correlated with large increases in GIs in brill, and also in lemon sole and sand flounder (Setyono 1996) in the same area. The combination of photoperiod and water temperature is likely to be the most important influence (Lam 1983).

Based on ovarian classification, brill were found to have group synchronous ovaries because the mature ovaries contain oocytes at all stages (Figure 4). This result agrees with findings of Poortenaar *et al.* (2001), and other previous reports (deVlaming 1983, Rinchard *et al.* 1993) indicating that members of the family Pleuronectidae have group synchronous oocyte development and are able to spawn several times during a spawning period.

Batch fecundity estimation was based on the counting of all mature oocytes (>250 mm). It was found that oocytes >250 mm ware classified as being in the mature stage (Figure 4). Batch fecundity represents about 34% of the total oocytes, i.e., the clutch of mature oocytes that would be released during the next spawning event. Maturing and previtellogenic oocytes (comprising about 26% and 40% of the total oocytes, respectively) will presumably develop into mature oocytes within a relatively short time and be released in a subsequent spawning event. This batch fecundity is relatively lower than of sand flounder and sole (Setyono 1996) from the same area. This differences may be due to a larger oocyte size in brill.

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