THE LIFE HISTORY OF A GIZZARD SHAD, THE BONY BREAM, *NEMATALOSA EREBI* (GUNTHER) (DOROSOMATINAE, TELEOSTI) IN THE LOWER RIVER MURRAY, SOUTH AUSTRALIA.

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CONTENTS

SUMMARY

Throughout Australia, populations of bony bream are characterized by their exceptional abundance. In the highly regulated lower River Murray, commercial catches of this species are increasing while those of other native species are in decline.

Catches of larval bony bream suggest that Lake Alexandrina is favoured for breeding. A drop in catch/effort during winter may indicate a seasonal offshore - onshore migration. The dominance of Lake Alexandrina by bony bream, suggested by the lakes/Coorong commercial catches, is supported by sampling data. However, the relative abundance of the bony bream in the river channel is greatly underestimated by catches in the reach fishery. The sampling data support evidence from the fishery catches for the decline in relative abundance of other large freshwater native species in the lower Murray. The bony bream population is dominated by young-of-year fish with high mortality rates. Adults however have comparatively low mortalities.

Bony bream in the lower Murray show seasonal cycles of condition (more pronounced in smaller fish) which suggest winter starvation. Larger fish show falls in condition

associated with spawning. Growth in young fish slows over winter, and renewed growth becomes apparent in October. Scale - ageing is shown to be valid to age III+, but beyond this probably underestimates true age. Females appear to live longer and grow larger than males. In comparison with other gizzard shads, the bony bream is both large and long-lived.

The reproductive biology of the bony bream resembles that of other gizzard shads. The bony bream matures at a median age of 2-3 years, spawns in December-January at water temperatures of 21-23°C and is highly fecund. Spawning does not appear to be dependent on flooding. Reproductive effort increases with size. The ova (mean diameter 0.83 mm) and larvae (less than 3.5 mm total length at hatch) are small, and the yolk-sac is largely absorbed by 3.5 mm. Development is typical of clupeids except for the fin-ray sequence. Sexual dimorphism does not occur, but females predominate in the largest size-classes.

The lower Murray population of bony bream is subject to an annual epidemic of the oomycete Saprolegnia (principally S. parasitica) and the bacterium Aeromonas hydrophila. The epidemic is species-specific; it affects

mainly adults whose susceptibility may be increased by stress due to winter cold. Mortality rates are not high. Lesions occur on the mid-flank and are characterized by an external mycelium, epidermal erosion, scale loss, hypodermal and muscular oedema, haemorrhage, myofibral degeneration and by the presence of Saprolegnia hyphae at all stages of infection. Although A. hydrophila is common in advanced lesions there is no significant systemic bacterial infection. This appears to be a primary mycotic dermatitis and is noteworthy because Saprolegnia is best-known as a secondary pathogen.

The bony bream in the Murray is therefore long-lived, early maturing, and highly fecund. Flooding is not an essential cue for spawning, and larval mortality is high. Longevity and an exceptional natality are likely to be the major factors in bony bream success.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference or acknowledgement is made in ~~the text of~~ the thesis.

J.T. Puckridge

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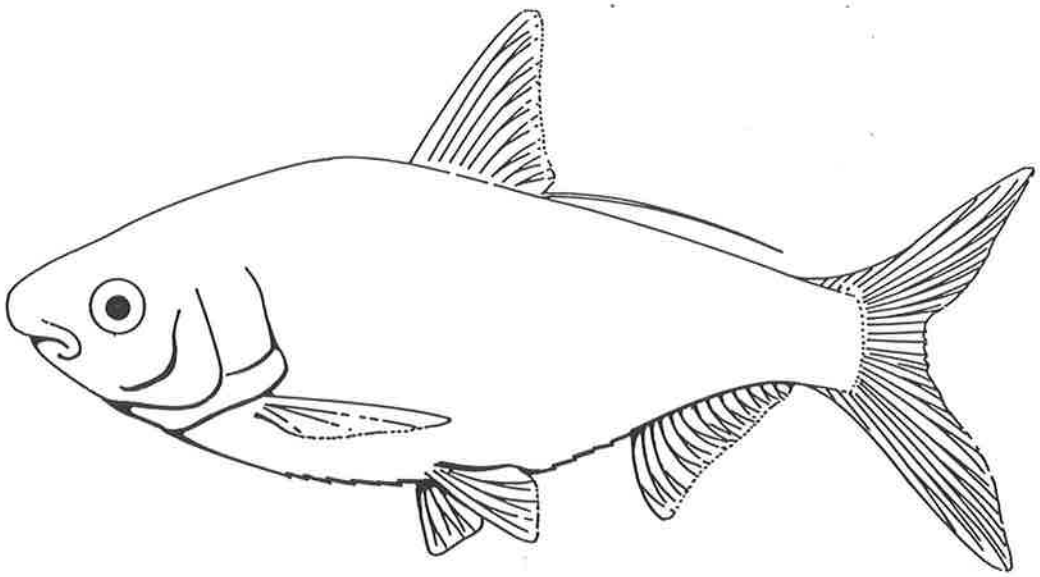


FIGURE 1.1: The bony bream, Nematalosa erebi

CHAPTER 1: INTRODUCTION

1.1 The importance of the bony bream in Australia

The bony bream, Nematalosa erebi (Gunther) (Fig. 1.1), occurs in slow-flowing rivers, lakes and impoundments throughout Australian warm-water catchments (Fig. 1.2 - Lake 1978), and has been described as the most widespread freshwater fish on the continent (Allen, 1982). It is usually abundant (Lake, 1971; Kowarsky & Ross, 1981; Llewellyn, 1983) and may be spectacularly so in the lower reaches of desert rivers (Ruello, 1976; Glover, 1982; Puckridge & Drewien, 1988) and in northern reservoirs (A. Hamlyn, Fisheries Biologist, Queensland Department of Primary Industry, Division of Fisheries Management, pers. comm.). It thrives in both impounded (Gray, 1982; Cadwallader, 1983) and unregulated waters (Hutchins, 1981), in systems which are climatically and hydrologically predictable (Bishop, Allen, Pollard & Cook, in press) and in some which are exceptionally unpredictable even by Australian standards (Puckridge & Drewien, 1988). However, numbers of bony bream have declined markedly in the middle Murray below the Hume and Burrinjuck dams, perhaps because of the lowering of summer water temperatures (Lake, 1971;

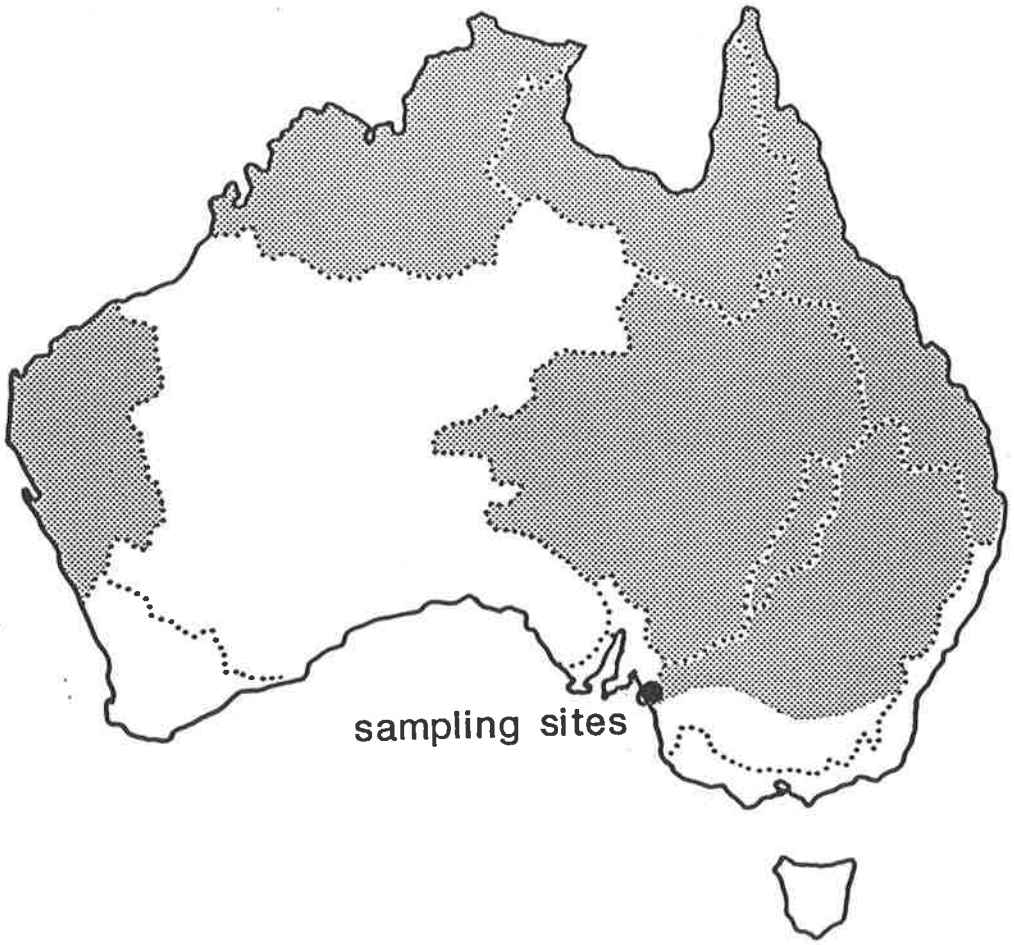


FIGURE 1.2: The distribution of bony bream in Australian catchments (adapted from Merrick & Schmida, 1984).

Cadwallader, 1977). The congeners N. come and N. vlaminghi, and the related Anodontostoma chacunda occur in Australian estuarine and marine waters, and are locally abundant (Beumer, 1978; Blaber, 1980; Chubb, Hall, Lenanton & Potter, 1984). In south-western Western Australia a substantial fishery supplying bait for the crayfish industry is based on N. vlaminghi.

The bony bream has been canned commercially by Uncle Ben's of Australia (Wodonga, Victoria), but its only present commercial use is in the lower Murray, where it is has been caught commercially as crayfish bait since the 1960s. The catch declined in the seventies, but has been steadily increasing since, so that the catch by weight of this species now dominates the lakes and Coorong gillnet fishery (South Australian Department of Fisheries, 1988). In the reach fishery of the river channel, bony bream are less important, but the reliance on drum-nets in this fishery mitigates against the capture of the species. The total lower Murray bony bream catch in 1987/88 was 1000 tonnes, worth $400,000 (Rohan, 1988).

The bony bream is also important as the only freshwater Australian member of the Dorosomatinae, the gizzard shads, an abundant and widespread group. They are principally euryhaline fishes of warm shallow bays and estuaries

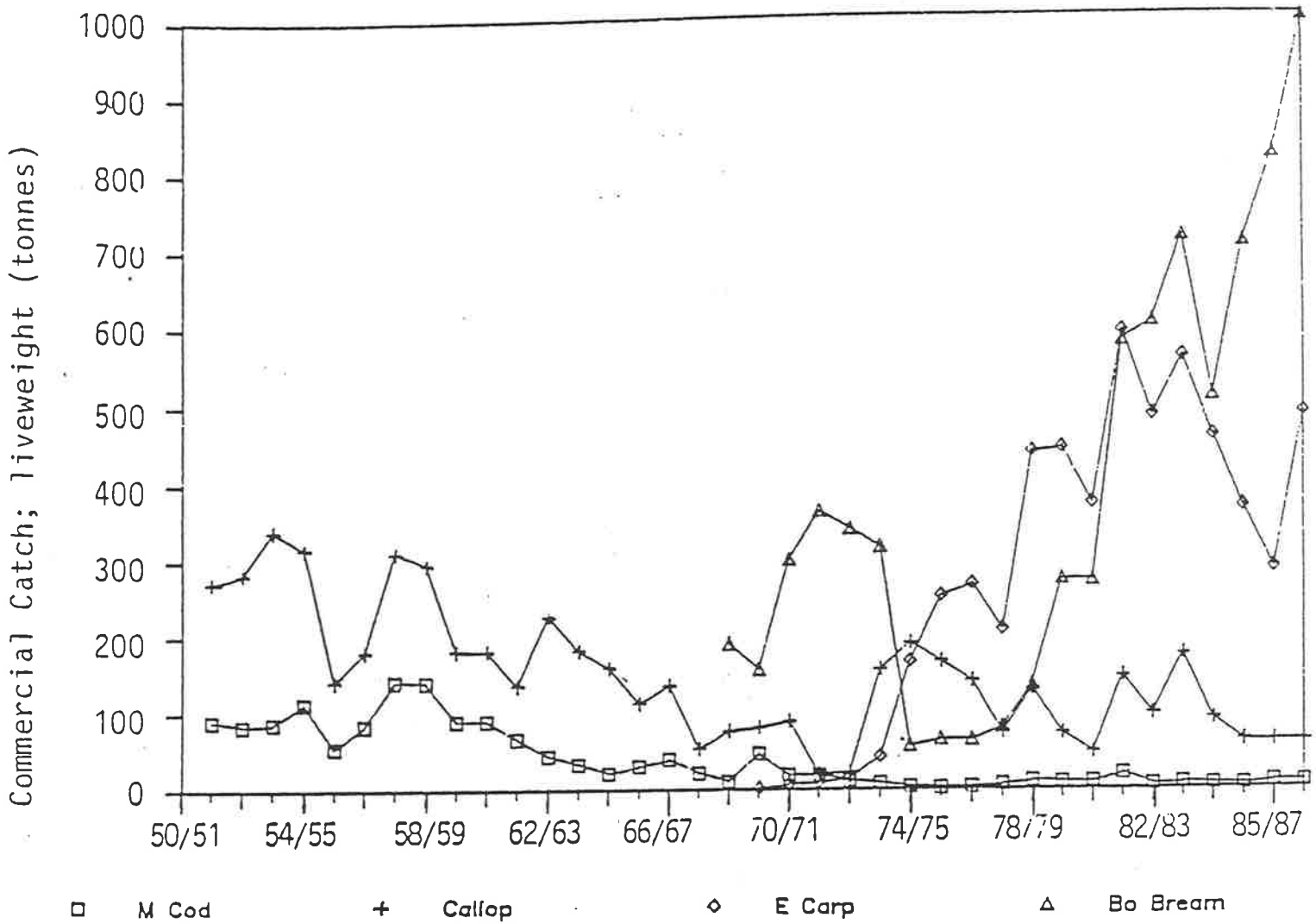


FIGURE 1.3: Total commercial catches of the major River Murray species 1951/52 to 1987/88 (South Australian Department of Fisheries).

throughout the Indo-Pacific (Nelson & Rothman, 1973; Beumer, 1978; Parimala & Ramaiyan, 1980) in slow-flowing and standing fresh waters over much of North America (Miller, 1960; Noble 1981), in Central American lakes (Bussing, 1976) and in both fast and slow-flowing freshwaters of Papua New Guinea (Roberts, 1978). Of the 21 presently recognized species (Nelson & Rothman, 1973; Wongratana 1983), nine normally spend their life-cycles in fresh water, and flourish in warm lakes and slow-flowing streams of North and Central America, India, Burma, Australia and Papua New Guinea. Of the Nematalosa species, all are marine or estuarine except the Australian N. erebi and the Papua New Guinean N. flyensis and N. papuensis. Although most Nematalosa species are sub-tropical, the ranges of N. nasus, N. vlaminghi and N. erebi extend into temperate waters. N. erebi is therefore the only freshwater Nematalosa species with a temperate range. In this respect it is more ecologically comparable with the Dorosoma species of North America, and particularly with Dorosoma cepedianum, than with congeners such as the marine and estuarine N. vlaminghi. The tropical freshwater Papua New Guinean species have been little studied.

Gizzard shads are characteristically abundant (Jones & Sujansingani, 1954; Jester & Jensen, 1972; Martinez, 1976),

and in some cases are the basis for substantial fisheries (Kawanabe Saito, Sunaga, Maki & Azuma, 1968; Jhingran & Gopalakrishnan, 1973; Chubb et al., 1984).
The North American Dorosoma species illustrate this abundance well. Of limnetic larval fish in western Lake Erie, 90% are D. cepedianum or alewife Alosa sapidissima (Mizera, Cooper & Herdendorf, 1981).

The bony bream is also important because it performs a major ecological role. The species has been described as an algal grazer (Allen, 1982), iliophagus (Lake, 1967), detritivorous (Pollard, 1974) a macroinvertebrate feeder (Ruello, 1976) and a microphagic omnivore (Bishop et al., in press). In the lower Murray, larvae and juveniles are zooplanktivorous, adults primarily detritivorous (Atkins, 1984). Adult bony bream therefore feed near the base of the food chain. The bony bream is also an important component in the diets of such commercially valuable fishes as callop Macquaria ambigua (Merrick & Midgley, 1985), Murray cod Maccullochella peeli (Milward, 1965), redfin Perca fluviatilis (Weatherley, 1977), barramundi Lates calcarifer (Mackinnon, 1984) and ariid catfishes (Morrissy, unpub. data). It is also widely reported as a component in the diets of waterbirds (Lake, 1967; Cadwallader & Backhouse, 1983; Morris, Nicholson & Dalziel, 1984). The bony bream

therefore functions ecologically as a major link between lower and upper trophic levels.

All gizzard shads, being principally microphagous, perform a similar role. Marine species such as Anodontostoma chacunda, Clupanodon punctatus and N. nasus are more zooplanktivorous (Chacko, 1948; Rao, 1965; Kawanabe et al., 1968; Beumer, 1978), freshwater species such as D. cepedianum, and D. petenense are more likely to include phytoplankton and detritus with zooplankton (Miller, 1964; Bodola, 1966; Jester & Jensen, 1972). D. cepedianum and D. petenense were introduced to impoundments across the United States as forage for game fish (Range, 1973; Shelton & Grinstead, 1973; Heidinger & Imboden, 1974), and D. cepedianum is a component in the diets of 17 piscivorous species (Miller, 1960).

Increases in the range and abundance of the bony bream as impoundment and regulation proceed are likely. D. cepedianum in North America has extended its range from the Atlantic drainage west into Wyoming, Colorado and Western New Mexico (Jester & Jensen, 1972), up the Missouri into North Dakota (Carufel & Witt, 1963) and north into Lakes Huron and Erie (Miller, 1957). Shallow bays in Kansas reservoirs carry 1123 kg of D. cepedianum per hectare (Cross & Collins, 1975), and

this situation is replicated in impoundments across the United States (Lagler & Van Meter, 1951; Lewis, 1953; Orth, 1980). Further, bony bream may serve an increasingly important forage role in the stocking of warm-water reservoirs, and in polyculture (Mackinnon, 1984). A study of the life-history characteristics of the bony bream, and comparison in particular with those of the American Dorosoma species, should contribute to the formulation of guidelines on the stocking of bony bream and the impoundment of waterbodies in which the species is found.

1.2 Changes in bony bream distribution and abundance in the lower Murray River.

In the lower and middle Murray, the bony bream was apparently abundant before impoundment (Zeitz, 1902; Cadwallader, 1977). In the lower Murray, commercial catches have been increasing steeply over the last decade both in absolute terms and in comparison with catches of other species (Rohan, 1988). The 1987/88 catch was twice the weight of the European carp (Cyprinus carpio) catch, and nearly 20 times that of the catch of callop, the second most abundant native fish (Fig. 1.3). However, the commercial record is not a reliable guide to the relative abundance of bony bream in the lower Murray because both the lakes/Coorong and the river reach are multi-species

fisheries in which effort directed at particular species is difficult to determine. The declared catch is particularly unreliable as an abundance measure because the number of fish kept and declared depends on the seasonal demand for crayfish bait (D. Hall, fisheries biologist, South Australian Department of Fisheries, pers. comm.). More reliable catch - effort data are needed to confirm that the bony bream is increasing in abundance relative to other native species.

Other freshwater gizzard shads appear to have been favoured by impoundment. The canal system, impoundment and deliberate introductions have increased the abundance as well as the range of both D. cepedianum and D. petenense in North America (Carufel & Witt, 1963; Grinstead, Gennings, Hooper, Schultz & Wharton, 1978; Loch, Derkson, Hora & Oetting, 1979). In the 1950s, the dominance of impoundments by Dorosoma species, particularly D. cepedianum, was considered detrimental to game fisheries, and expensive, generally unsuccessful attempts were made at control and even eradication (Parsons, 1957; Hulsh, 1959; Schneider & Little, 1973). The bony bream has a comparable latitudinal range to D. cepedianum, and its success in the impounded lower Murray and in Queensland and New South Wales reservoirs (Rohan, 1988; Cadwallader, 1983) suggests that it has the potential

for similar domination of artificial waterbodies. It seems likely, therefore, that the bony bream has been favoured by impoundment and regulation in the lower Murray.

1.3 Possible explanations for the increase in abundance of bony bream in the lower Murray.

The River Murray has been subject since the 1920s to progressive regulation of the flow regime, and alienation of floodplain habitats from the main channel (Walker, 1985; Cadwallader, 1986). In association with these changes, catches of major commercial fish species such as callop (*Macquaria ambigua*), Murray cod (*Macullochella peelli*) and catfish (*Tandanus tandanus*) have markedly declined (Reynolds, 1976; Rohan, 1988, Fig. 1.3), and this decline has been directly linked to changes in hydrology (Cadwallader, 1978, 1986; Walker, 1983, 1986a). Those species in the lower Murray (Murray cod and catfish) which appear not to synchronize spawning with the hydrological cycle, are believed to be suffering recruitment failure (Cadwallader, 1986; Pierce & Walker, subm.). Even the catches of callop and silver perch (*Bidyanus bidyanus*) - species which do spawn in response to flooding - have suffered some decline. The bony bream is the only large native species for which catches are increasing in the lower

Murray. A study of bony bream life-history may reveal the reasons for this success, and add to our understanding of factors contributing to the decline of other species.

Very little work has been done on the life-history of the bony bream. (General comments only will be made on this work here, as more detail is given in the introductions to the following chapters). Only incidental data are available on age and growth, and no validated age-growth curve is available for the bony bream from any Australian catchment. There have been few studies also of reproduction in bony bream; much of the published information is anecdotal, and appears in general texts or reviews. Two community studies, Puckridge & Drewien (1988) and Bishop et al. (in press) constitute the only primary research. However, neither of these publications was intended to be a definitive study of bony bream reproductive biology. There is no published account of the relationships between size or age and fecundity, size or age and maturity, or of egg and larval development. What primary research is available does not refer to the populations of the lower River Murray.

Certain life-history traits will have a predominant influence on abundance. It has been proposed that larval survival rates depend primarily on the synchrony of spawning and flooding, since only during flooding does zooplankton

succession produce a sufficient density of appropriately sized zooplankters to sustain developing larvae (Welcomme, 1979; Awachie, 1981; Arumugam & Geddes, 1987; Geddes & Puckridge, in press; Pierce & Walker, subm.). If the species does not synchronize spawning and flooding, and high larval mortalities occur, this might not limit population growth if adult mortality is low, as would be expected in a stable system such as an impoundment. But the bony bream is also highly abundant in central Australia under some of the most unpredictable hydrological regimes in the world (Glover & Sim, 1978; Graetz, 1980; Puckridge & Drewien, 1988), and the pre-impoundment Murray also was subject to an erratic hydrological cycle (Walker, 1985). In these circumstances, high adult mortalities must also be common, dependent on the ratio between the areas of flood and drought habitat (Welcomme & Hagborg, 1977). Mean population density sustained over cycles of flood and drought may therefore depend more on the rate of population recovery than on adult food supplies. This rate will depend on the margin between natality and mortality rates. High fecundity, early first maturity, low mean reproductive age and increased longevity all increase natality, although lower mean reproductive age has the strongest effect (Cole, 1954; Caughley, 1967). Meats (1971) however has shown that, for populations with high

pre-reproductive mortality rates (M>0.9), small changes in these rates have greater effects on abundance than any other life-history parameter. So a species living in hydrologically unpredictable environments must be capable of either synchronising spawning and flood cycles to reduce larval mortality, or maturing early with high fecundity to maintain high natality rates.

In environments such as the Lake Eyre catchment, where drought phases may extend for a decade (Tolcher, 1986), repeated recruitment failures are likely. Longevity of the broodstock in this situation may provide a safeguard against local extinction, and ensure that mature adults are available to breed promptly in response to improved conditions. Since the lower Murray was also, before regulation, subject to protracted drought (Walker, 1985), it seems likely that the bony bream of this region is a long-lived species.

Gizzard shads in general are characterized by early maturity (Jacob, 1948; Thomson, 1957; Berry, 1958; Bodola, 1966; Jester & Jensen, 1972; Chubb & Potter, 1986), high fecundity (Rao, 1965; Kilambi & Baglin, 1969b; Chubb & Potter, 1984) and moderate longevity (Jester & Jensen, 1972; Takita, 1978; Chubb & Potter, 1986). Northern hemisphere freshwater gizzard shads follow a regular seasonal spawning cycle even

under the modified hydrological regimes of impoundments (Baglin & Kilambi, 1968; Jester & Jensen, 1972). Flooding is therefore not an obligatory cue for spawning. It is likely that bony bream will also show high natality and a spawning response which is relatively flood - independent. However, life-histories are typically adapted to local conditions (Leggett & Carscadden, 1978; Mann, Mills & Crisp, 1984), and it is important that findings under the modified hydrological regime of the lower Murray be compared with those from other regimes in the species' range (Bishop et al., in press; Puckridge & Drewien, 1988). The latter two studies allow a comparison of reproductive patterns from one of the most predictable and one of the most unpredictable hydrological cycles in Australia. Since the present study site lies at the southern extreme of the species' range (Fig. 1.2), and the above-mentioned studies lie at the northern extreme and centre respectively, comparisons between the three are likely to be informative.

1.4 Fungus disease and its implications for the abundance and range of the bony bream, and for the bony bream fishery.

An annual epidemic of fungus disease in bony bream has been noticed by fishermen in the lower Murray since at least the 1940s (L. Gray, Meningie, S. Aust., pers. comm.). The

condition does not appear to affect other species.

Studies of disease conditions in the bony bream are rare. There is an anecdotal literature, but the only scientific studies are those of Johnston & Bancroft (1921), Langdon, Gudkovs, Humphrey & Saxon (1985), Department of Ports and Fisheries (1986) and Puckridge & Drewien (1988). None of these deals with a disease specific to the bony bream, and only the last refers to what may be a primary mycosis. No previous study has been made of the annual fungus disease in the lower Murray which, unlike the conditions reported above, is exclusive to the bony bream.

A study of the aetiology and epizootiology of the lower Murray fungus disease may indicate which environmental conditions promote the outbreaks, and so potentially limit the range and abundance of the species. It is likely, considering the lower Murray is at the southern edge of the species' latitudinal range, and the species is susceptible to mass mortalities perhaps induced by winter cold (Lake, 1971; Cadwallader & Backhouse, 1983) that cold stress is a major contributing factor. For the fishery, such a study will identify the health risks associated with exploitation of the resource for human consumption and the implications of the introduction of the species to new habitats as a forage fish. It may also predict the effects of future

environmental change upon disease incidence.

1.5 Objectives of the study.

Hypotheses:

1. In the lower Murray, bony bream abundance is increasing relative to that of other large native species.

2. Bony bream are relatively long-lived compared to other gizzard shads.

3. Bony bream either have an exceptional natality, or their spawning is synchronized with flooding.

4. The range and abundance of bony bream is affected by fungus disease, induced by winter cold.

The objectives of this thesis are:

1. to test the above major hypotheses, and a number of minor ones, through fulfilment of the following goals:

    a. Estimate the relative abundance of the bony bream in the lower Murray in relation to the abundance of other lower Murray species.

    b. Describe seasonal cycles in catch per unit effort, and the structure and relative mortality rates of the

lower Murray bony bream population.

c. Evaluate scale-reading as an approach to the ageing of lower Murray bony bream, and describe the age-growth relation and seasonal cycles of body condition.

d. Describe the reproductive biology of the lower Murray bony bream, particularly gonadal cycles, spawning cues, spawning sites, sex ratio, egg and larval development, fecundity, reproductive effort, age and length-maturity relationships.

e. Identify the principal pathogens involved in the saprolegniasis of lower Murray bony bream, describe the pathology and epizootiology of the infection, and determine the environmental factors associated with the initiation of the outbreaks.

2. To determine the implications of the above findings for the bony bream domination of Australian warm waters, and of the lower Murray environment in particular.

3. To compare bony bream life-history to that of other gizzard shads, and decide what this may imply for future trends in the range and abundance of the bony bream.

4. To examine the implications of life-history findings for

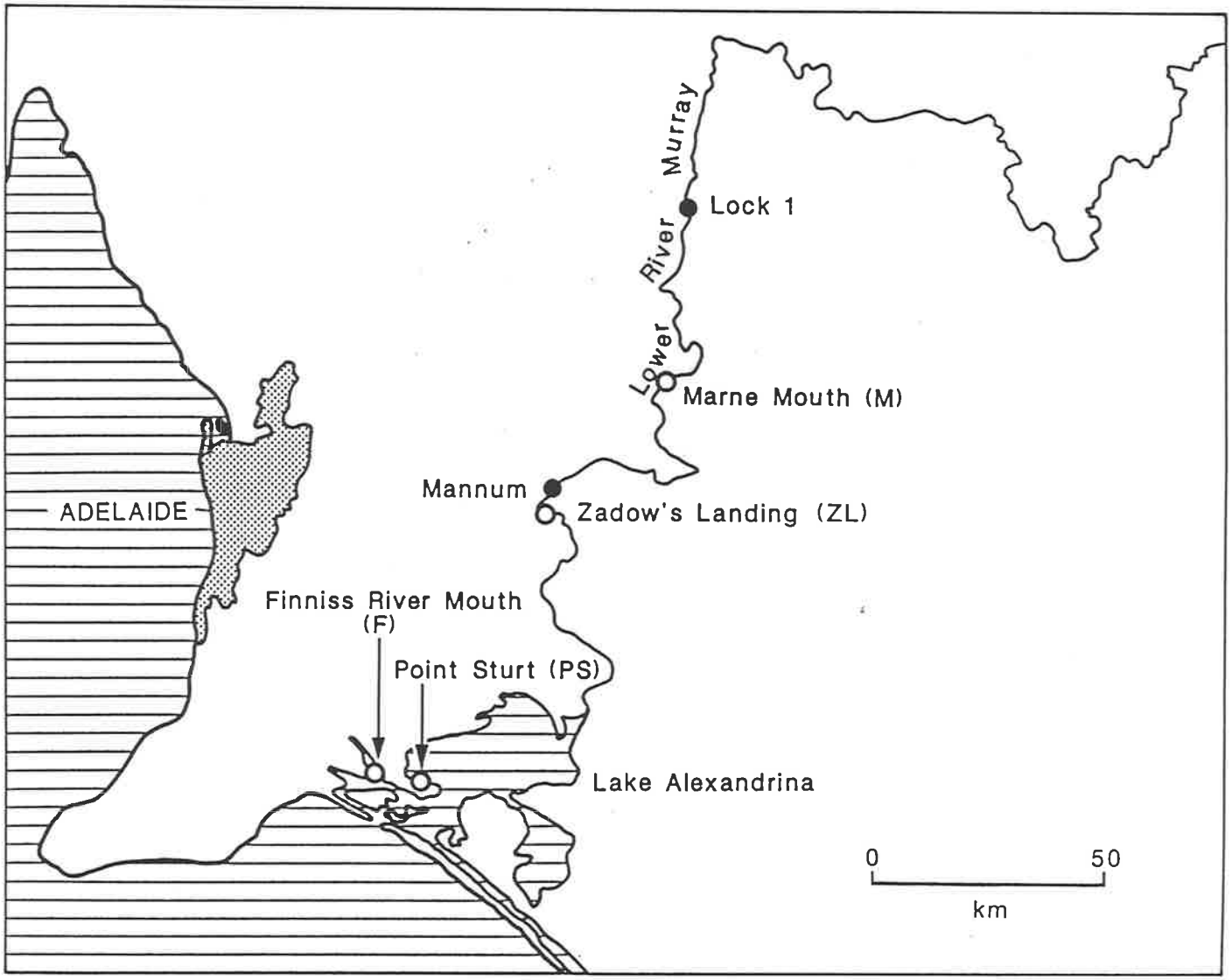


FIGURE 2.4: Sampling sites on the lower River Murray, South Australia.

the bony bream fishery and for the use of bony bream as a forage species.

1.6 Outline of the thesis

The thesis consists of a core of four chapters, each dealing with an area of bony bream life-history. Each of these chapters is presented essentially as a discrete paper, with separate summary, introduction, methods, results and discussion. These papers are integrated through an overall introduction, discussion of general methods, and conclusion. Chapters 5 and 6 are duplicated in their essentials in appendices II and III, which are papers (II submitted, III accepted) derived from these chapters.

CHAPTER 2: GENERAL METHODS

2.1. Description

2.1.1. Sampling sites

Fish were sampled principally at Zadow's Landing (ZL) (Lat. 34°, 58′ Long. 138°, 59′) on the lower Murray and Point Sturt (PS) (Lat.34°, 58′ Long. 139°, 18′) in Lake Alexandrina, but ichthyoplankton trawls were also conducted south of the mouth of the River Marne (M), and at the mouth of the Finnis River (F) South Australia (Fig. 2.4).

ZL is a shallow permanent backwater with a mud-clay substratum bordered with rushes (*Juncus* sp.), broadleaf cumbungi *Typha orientalis* and common reed *Phragmites australis*, and densely vegetated with ribbonweed *Vallisneria spiralis*. Gillnets were set parallel with the edge of the main channel between islands of willows (*Salix* sp.), 18m seines were hauled parallel to the main channel along the river channel border of the backwater, and 2m seines were hauled from 10m offshore into the reedbed border of the backwater. Five ichthyoplankton trawls were made in an upstream direction, five downstream along the main channel 20m offshore parallel to the backwater.

PS, on the northern side of Point Sturt, is a bay protected

from the prevailing winds, but still subject to occasional strong wave action. It has a sand on clay substratum, and only very sparse fringing vegetation - principally Phragmites australis. Gillnets were set at PS parallel to the shore and 200m offshore, 18m seines were hauled parallel to shore and 20m offshore, 2m seines were hauled 10m offshore into shore, and the 130m seine was hauled from 50m offshore onto the beach. Ten ichthyoplankton trawls were made 50m offshore and parallel to the south-western shore of the lake.

Site F is a broad shallow inlet, bordered with dense stands of common reed, and densely vegetated with water milfoil (Myriophyllum sp.). Ten ichthyoplankton hauls were made parallel to the shore and 50m offshore from west to east into the inlet.

Site M is a 2-km reach of the main channel of the Murray immediately south of the Marne mouth, bordered with willows and river red gum (Eucalyptus camaldulensis). Five ichthyoplankton hauls were made in an upstream direction, five downstream parallel to the shore and 20m offshore in the main channel south of the Marne mouth.

2.1.2 Sampling methods

A set of seven 50 m gill nets (mesh sizes 20-110 mm in approximately geometric intervals, after Lagler, 1968), three seines (lengths 2, 18, 130 m; mesh sizes 2, 12, 30/50 mm respectively), and a 0.5m diameter 500 um mesh ichthyoplankton trawl were used. However, only two of the seven gillnets (mesh sizes 90,110) were used at PS, and the 130m seine was not used at ZL. Sites M and F were sampled only with the ichthyoplankton trawl. Gill-netting took place over a 24 to 72 hour period, with nets emptied at a frequency of 0.5 to 8 hours, depending on catch rates. Seines and trawls were conducted in mid-afternoon. The distances hauled per seine were 5m, 20m, and 50m respectively. The ichthyoplankton trawl was hauled at the surface for 3 minutes at 3-4 km/hr. Catch rate of a Smith-Root (GPP 7.5 h) boat-mounted electrofisher was tested at ZL.

Gillnet sampling was conducted monthly at ZL and PS from September 1983 to December 1984, but from then to December 1986 took place only during the fungus disease and breeding seasons (June to December). Ichthyoplankton trawls were conducted from October 1985 to April 1986; 130m seine hauls from February 1985 to February 1986, and 2m and 20m seines from October 1983 to December 1984.

2.1.3. Processing of samples

Depending on catch size, the whole catch or stratified random samples (size-class interval 50 mm total length (TL)) were retained and processed at the PS and ZL field stations. Since there is no obvious sexual dimorphism in bony bream, captured fish were dissected and sexed until at least three fish of each sex in each of eight size-classes from 50 to 450 mm were caught and processed. Although this goal was not always fully attained, the resulting length-distribution facilitated the detection of gradients in sex ratio, condition factor (CF) and gonado-somatic index (GSI) with size, and enhanced the precision of such expressions as the weight-length relation.

$$[CF = 10^5(\text{body weight} - \text{gonad weight})/(\text{total length})^3]$$

$$[GSI = 10^2(\text{gonad weight})/\text{body weight}]$$

However, in GSI and CF analysis, size-classes were grouped to give three categories: large adults (300-450 TL), small adults (150-300 TL) and juveniles (50-150 TL). All larval and juvenile fish below 50mm were identified and measured, but were not further processed. Diseased fish were segregated at catch, and pathology procedures completed before routine processing (see 6.3). All fish were measured (TL, LCF, and body depth to 1 mm), weighed (to 0.1 gm),

dissected, gonads photographed in situ, left and right gonads weighed (to 0.01 gm) and measured (width, to 0.1mm), then preserved in either Gilson's Fluid or 10% buffered formalin (App. I). About ten scales were removed immediately posterior to the tip of the left pectoral fin (after Berry, 1958; Al-Rawi & Toetz, 1972; Bagenal & Tesch, 1978), and in 1986, the left pectoral fin was removed for fin spine sectioning.

2.1.4. Analytical procedures

Data was analysed on Osborne and Sanyo 555 microcomputers, using both programs written by Dr. K.F. Walker (Department of Zoology, University of Adelaide), and NWA Statpak Version 3.1 (Northwest Analytical, Portland Oregon 1984). GSI, CF, gillnet catch/mesh size/hour, mean seine and trawl catch/haul, species composition of catches, and length - frequencies of bony bream catches were calculated.

At each gillnet site on each sampling occasion, wind strength and direction were subjectively estimated, water level measured in metres below a datum on a stake, monthly water temperature read at 0.2m by alcohol-in-glass thermometer and together with dissolved oxygen in ppm at 0.5m depth intervals with a YSI model 51B oxygen meter, pH was determined with a Metrohm model E604 pH meter,

conductivity in uS/cm with a Radiometer CDM3 conductivity meter (converted to TDS in mg/L at 25 C) and Secchi depth was recorded in cm. Dissolved oxygen, pH and conductivity meters were calibrated every three months. Data on modal monthly day length at Adelaide was provided by the Bureau of Meteorology, and the South Australian Engineering and Water Supply Department supplied mean weekly water temperatures at Mannum and Milang, mean daily discharge per month in megalitres at Lock 1 (Blanchetown) and monthly water chemistry data from Mannum (organo-chlorine pesticides, total iron, soluble iron, total aluminium, soluble aluminium, total cadmium, total chromium, total copper, soluble copper, total lead, total manganese, soluble manganese, total zinc, all in mg/L).

2.2 Rationale

Only two sites were routinely sampled, because of transport costs and the time necessary to obtain adequate numbers of fish in each size-class per site. Variances in GSI were both large and variable with season. For example, to obtain a 95% confidence interval for mean large adult GSI within 25% of the mean would have required a catch of >120 females, 3 males in December 1983, 3 females, 10 males in July. Further, both sex ratios and size composition of catches varied strongly with season, which made standard confidence

intervals difficult to maintain. In practice, a minimum catch of three fish of each sex per size-class, with catches of large males sometimes less, was routinely achieved. Larger collections were made for specific purposes, such as determination of age/size at first maturity, size-fecundity relations, or time of scale annulus formation.

A lake and river site (PS and ZL respectively) were chosen to provide informative contrasts and to represent the major habitats of the lower river. The particular sites chosen provided easy access, and at PS, some shelter from prevailing wave action, which could otherwise prohibit sampling. Extra sites M and F, used only for ichthyoplankton trawls, were chosen on the basis of resemblance to spawning habitats of bony bream and Dorosoma species described in the literature (Lambou, 1965; Shelton, 1972; Llewellyn, 1983).

Year-round monthly sampling at both PS and ZL was discontinued in 1985 because effort had to be rationalized, and the spawning and disease cycles were of paramount interest. The full-scale sampling conducted at ZL could not be replicated at PS because of personnel and time constraints, so effort at PS was focussed on sampling of large adults (which showed the clearest gonadal cycles and were most susceptible to fungus disease), and on larvae and

small juveniles, as indicators of spawning and recruitment. That 130m seine and ichthyoplankton trawl samples were not conducted through the intensive sampling period 1983-84 is unfortunate, as this would have provided both useful comparisons with other gear and superior population sampling. However, neither time nor personnel were available to do this.

Gillnets are well-known for a tendency to selectivity (Lagler, 1968; Jester, 1977). For a species without spines or other nettable projections like the bony bream, catch depends largely on body cross-section (although occasional small specimens will bridle in large-mesh nets), and the gear is precisely selective. Such selectivity is obvious in frequency histograms of gillnet catch /hour / mesh size (Fig. 2.5). The larger mesh-sizes show ample overlap in the range of size-classes caught, but there is less overlap between the smaller mesh catches. A closer spacing of mesh sizes would have improved overlap and provided a more efficient tracking of year-classes, but this was not feasible because of financial constraints. However, gillnets had a higher catch rate / person-hour for adult and larger juvenile bony bream than either seining or electrofishing, and this allowed more inter-catch processing time. Further, the fleet of gillnets could be operated by one person - a

critical consideration in a program relying on voluntary personnel. The variation in gillnet immersion times and emptying times was unavoidable, because catch rates varied so greatly on a seasonal basis (Fig. 3.13).

The 2m and 18m seine nets provided adequate sampling of the young-of-year fish (Figs. 2.6, 2.7, 2.8) although no bony bream were caught in the 2m seine at ZL. The 130m seine could only be used at PS, where there was suitable ground, but it caught nearly all size-classes, and gave the best data for the tracking of age-classes and estimates of population size/age structure (Fig. 2.9). Further, as an active method of capture, seining provided a check on the activity component in the passive gillnet captures. However, it was also labour-intensive, and during winter, catches were low. All seining was confined to the afternoon to reduce variation due to diurnal cycles in onshore-offshore movement. Electro-fishing normally provides low-selectivity catches, but tests in winter gave negligible returns, and it was not considered that the method could efficiently provide the size of catches necessary for the purposes of the project.

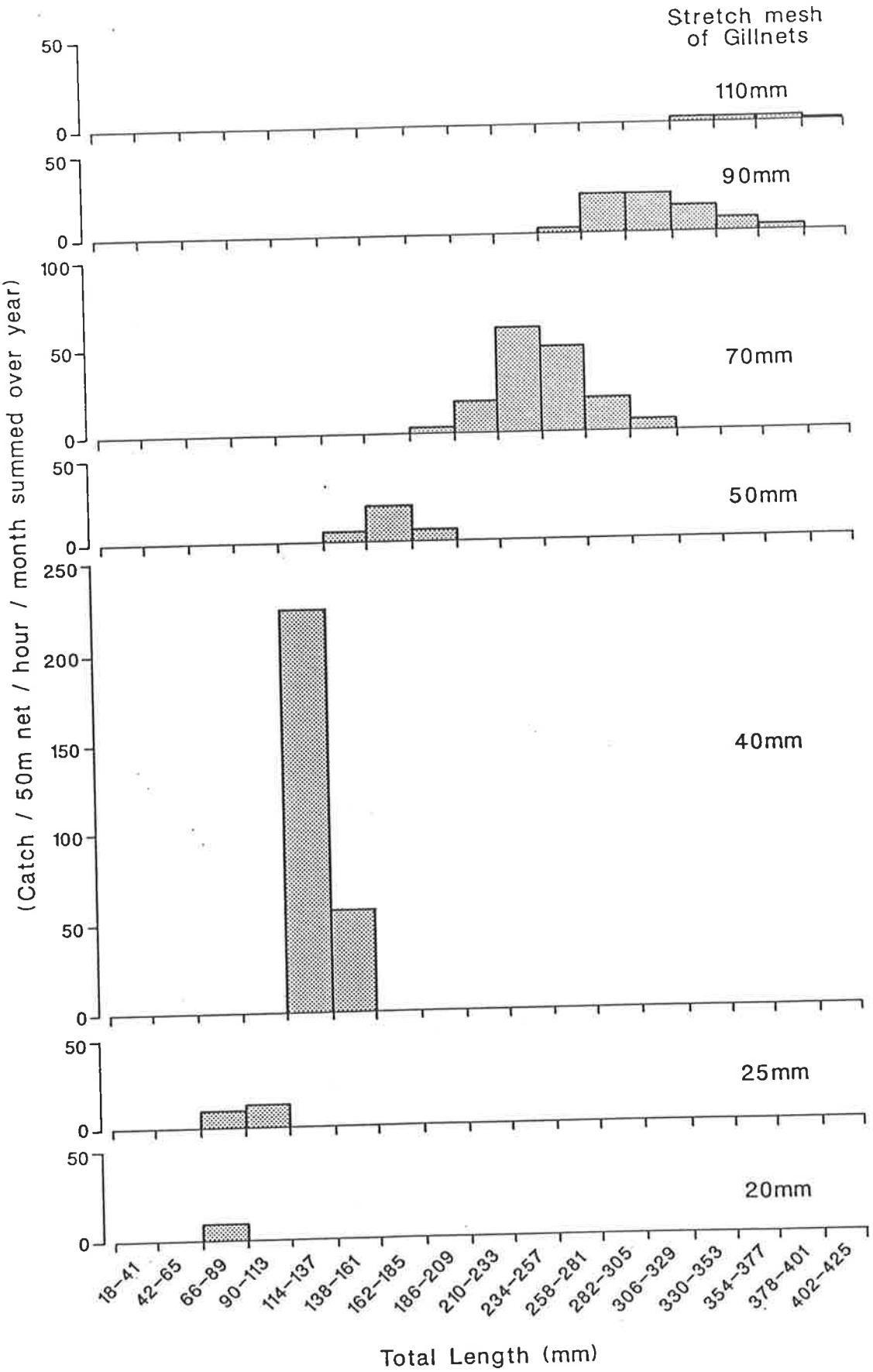


FIGURE 2.5: Size-selectivity of gillnets for bony bream at ZL, 1983-84, as expressed by length-frequencies of catches.

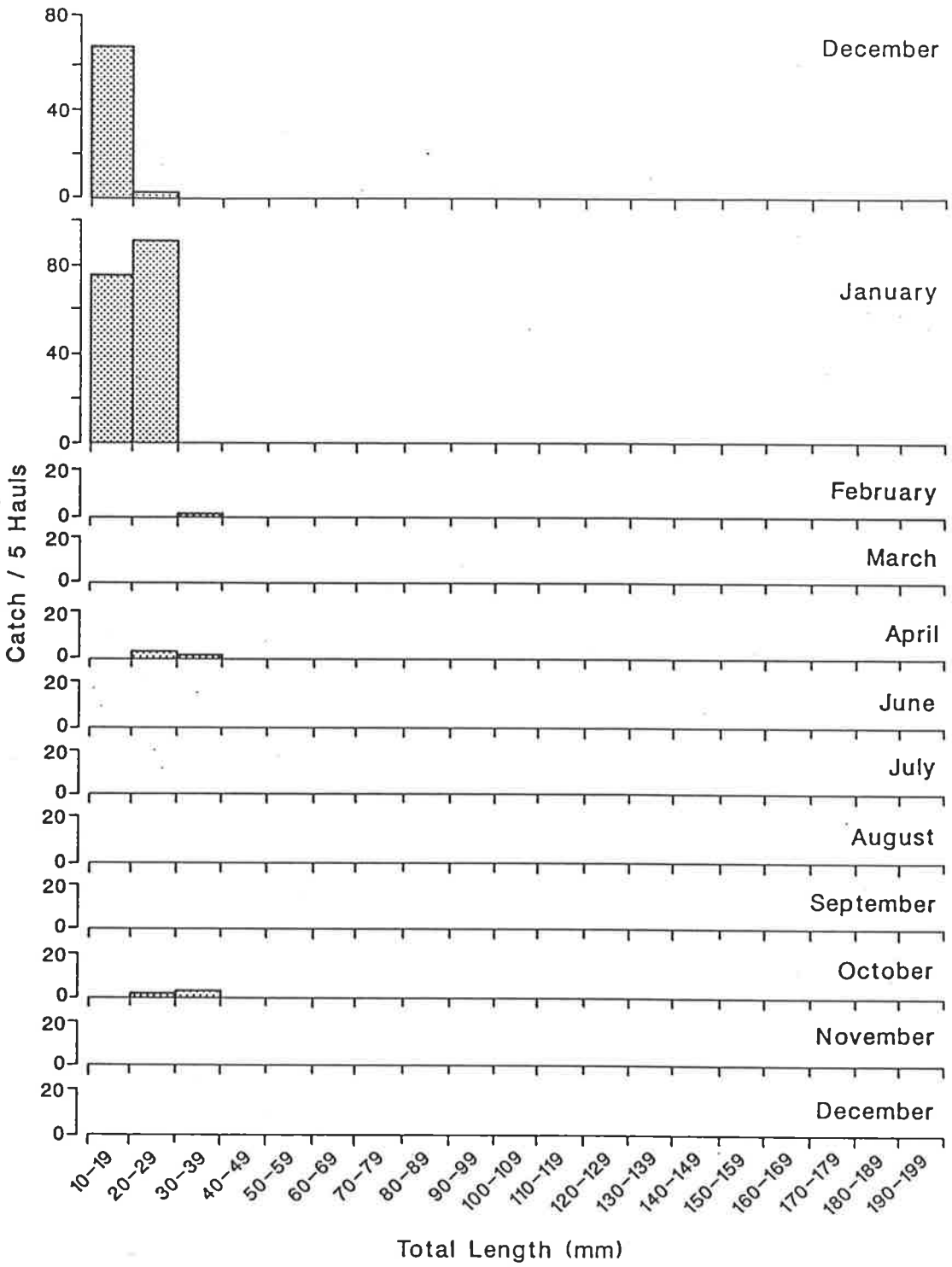


FIGURE 2.6: Length-frequencies of 2m seine catches of bony bream at PS, 1983-84.

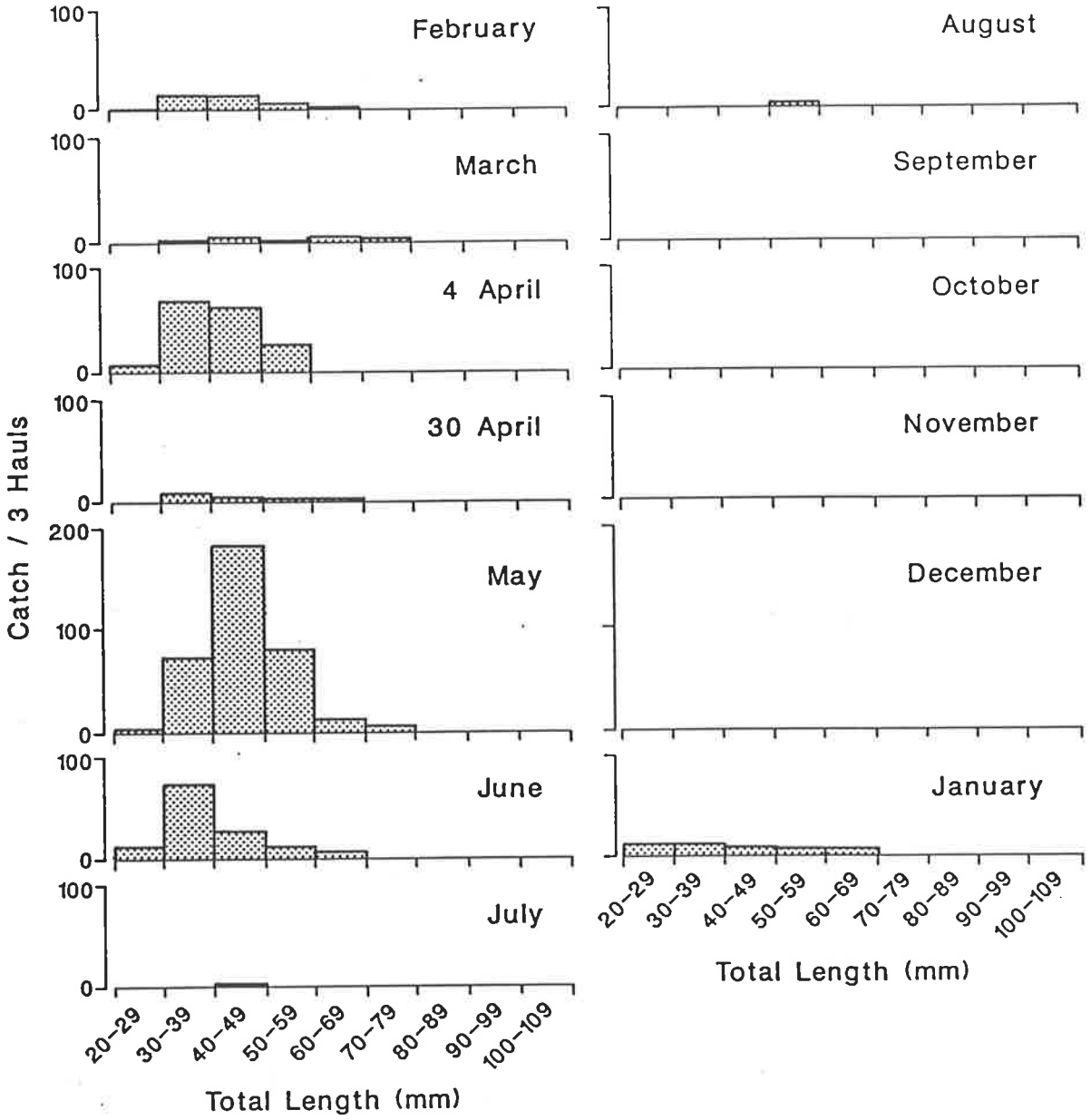


FIGURE 2.7: Length-frequencies of 18m seine catches of bony bream at ZL, 1983-84.

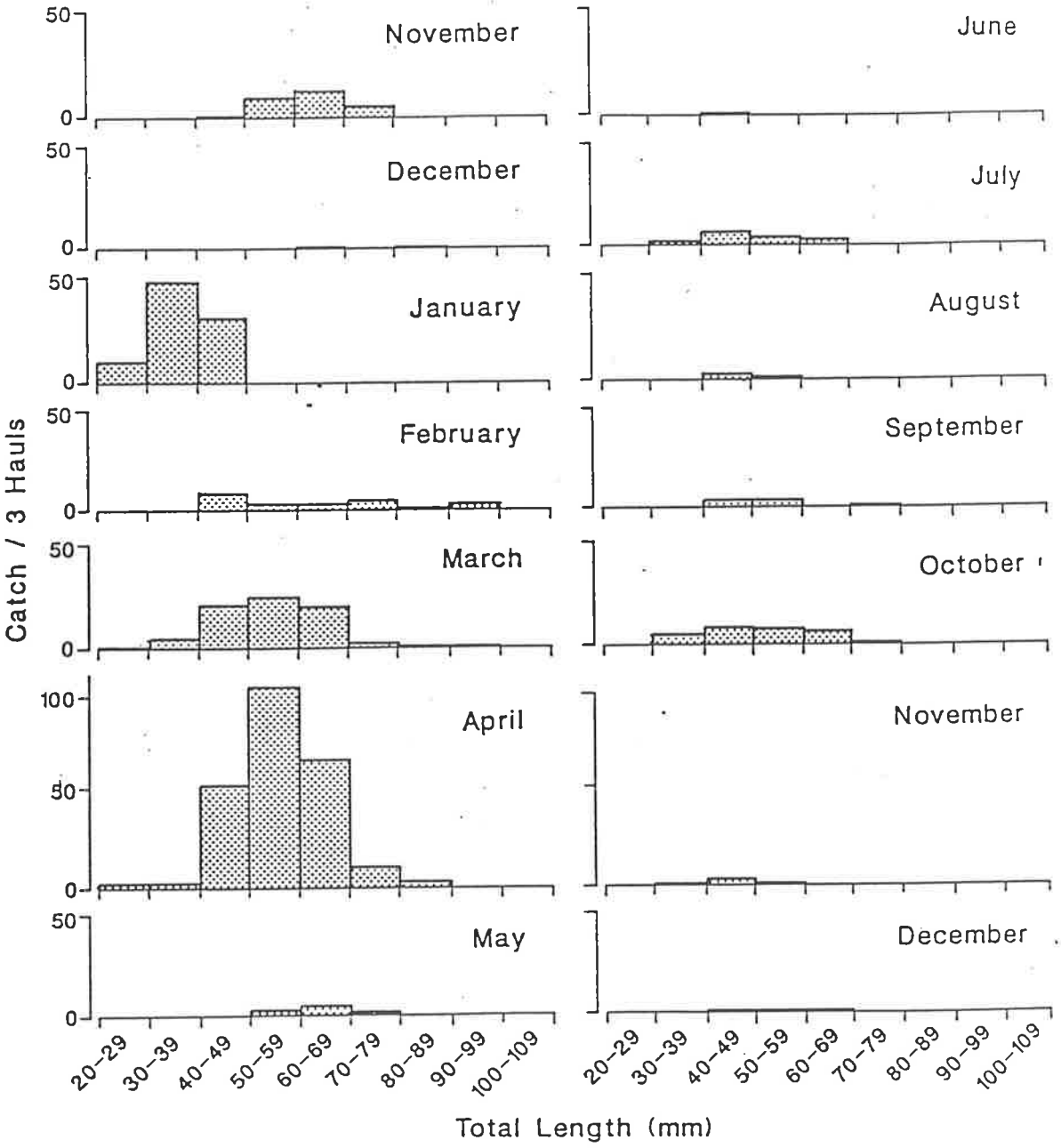


FIGURE 2.8: Length-frequencies of 18m seine catches of bony bream at PS, 1983-84.

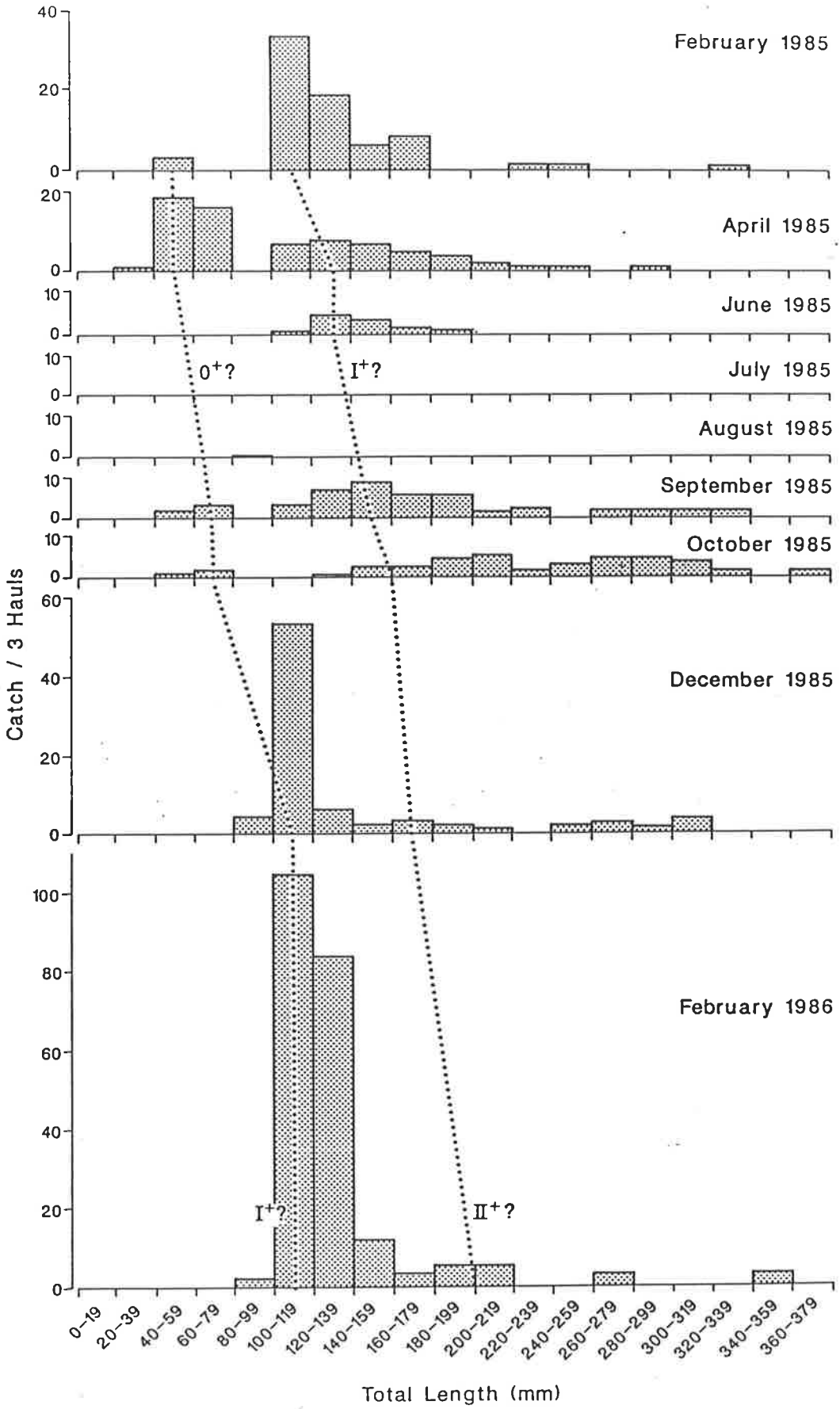


FIGURE 2.9: Length-frequencies of 130m seine catches of bony bream at PS, 1985-86.

CHAPTER 3: DISTRIBUTION, ABUNDANCE, POPULATION STRUCTURE AND MORTALITY

3.1 SUMMARY

Variation in catches of larval bony bream suggest that Lake Alexandrina is favoured for breeding. A drop in catch/effort during winter may indicate a seasonal offshore - onshore migration. The dominance of Lake Alexandrina by bony bream, suggested by the lakes/coorong commercial catches, is supported by sampling data. However, the relative abundance of the bony bream in the river channel is greatly underestimated by catches in the reach fishery. The sampling data support evidence from the fishery catches for the decline in relative abundance of other large freshwater native species in the lower Murray. The bony bream population is dominated by young-of-year fish with very high mortality rates. Adults however have comparatively low mortalities.

3.2 INTRODUCTION

In Chapter 1, the abundance of the bony bream throughout Australia, and commercial catches in the lower Murray were documented (Fig. 1.3). However, the commercial record is not a reliable guide to the relative abundance of bony bream in the lower Murray because both the lakes/Coorong and the river reach are multi-species fisheries in which effort directed at particular species is difficult to determine. The declared catch is particularly unreliable as an abundance measure because the number of fish kept and declared depends on the seasonal demand for crayfish bait (D. Hall, fisheries biologist, South Australian Department of Fisheries, pers. comm.). Catch/effort figures from experimental gill and seine netting are presented below; these are a more reliable guide to the relative abundance of major species, and also provide data on habitat preferences, seasonal behaviour, and population structure of bony bream. It was suggested in Chapter 1 that species which do not synchronize spawning and flooding risk high larval mortalities. The population structure of bony bream in 1983 and 1985 is described here to provide evidence of larval, juvenile and adult mortality rates.

3.3 METHODS

The sites and capture methods used are described in Chapter 2. It was not practicable in the routine sampling program to weigh species other than bony bream. The following abundance estimates are therefore based on numbers of fish caught, in contrast to the fisheries data, which are based on weight. This enhances the proportion of bony bream in the sampling data, since the bony bream population has a relatively lower mean TL and length = weight ratio than the other commercial species. However, the differences in relative abundance are so substantial that effects due to units may be discounted.

3.4 RESULTS

Throughout this text, length measurements are in total length (TL), but the conversion equation to length at caudal fork (LCF) for the lower Murray population is LCF = -1.406 + 0.84(TL) (r^2 = 0.997, n=71, P(F-test)<0.001).

3.4.1. Inter-site comparisons of catches of bony bream

In the lower Murray, mean catch / haul of 10m seines (i.e. of juveniles) was not significantly different at the Lake Alexandrina (PS) and the river backwater (ZL) sites (paired sample t-test of log-transformed data, Fig. 3.10). However, catch/effort of large mesh gillnets (i.e. of large adults)

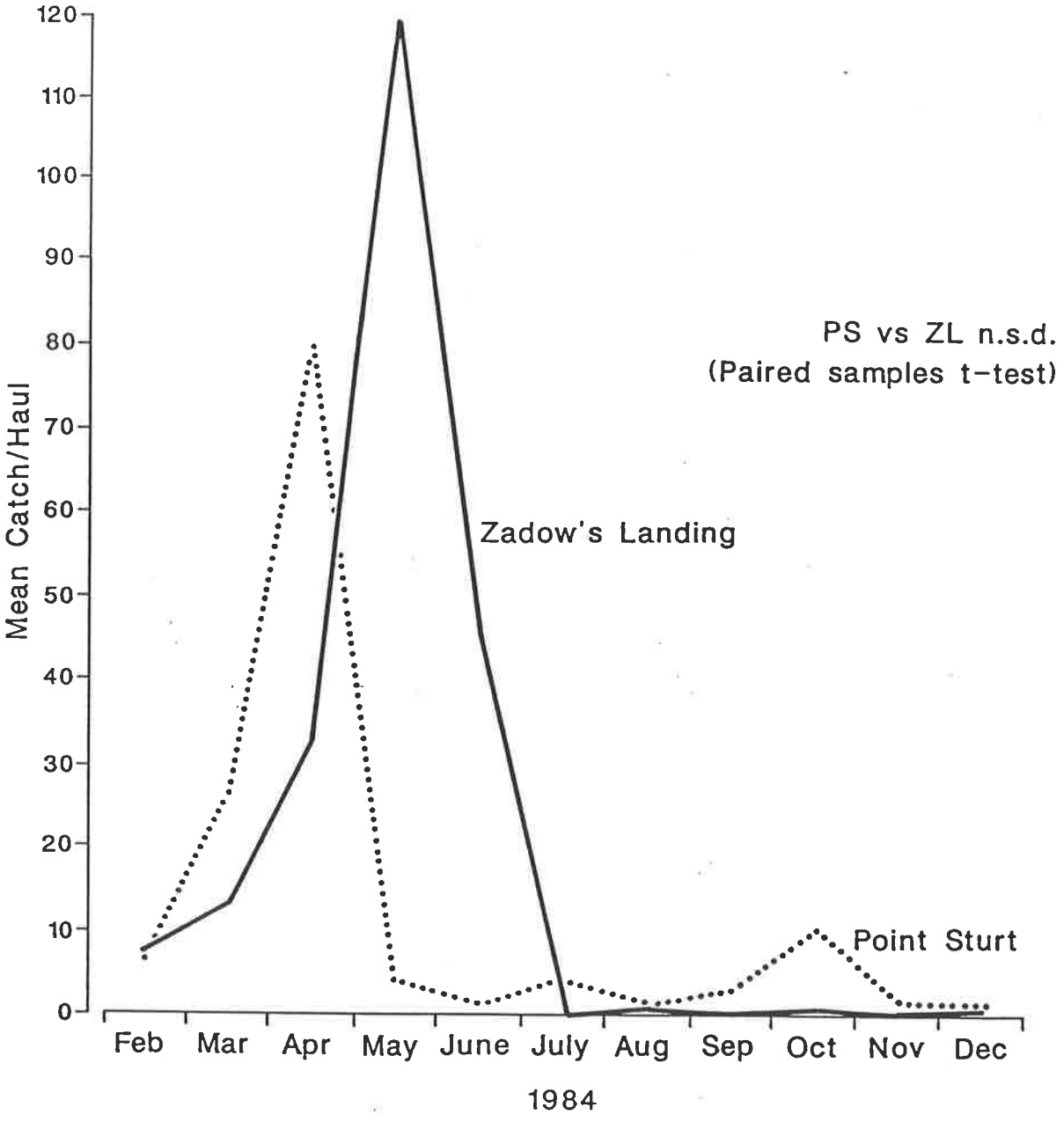


FIGURE 3.10: Monthly 18m seine catch/effort of bony bream at PS and ZL, 1984.

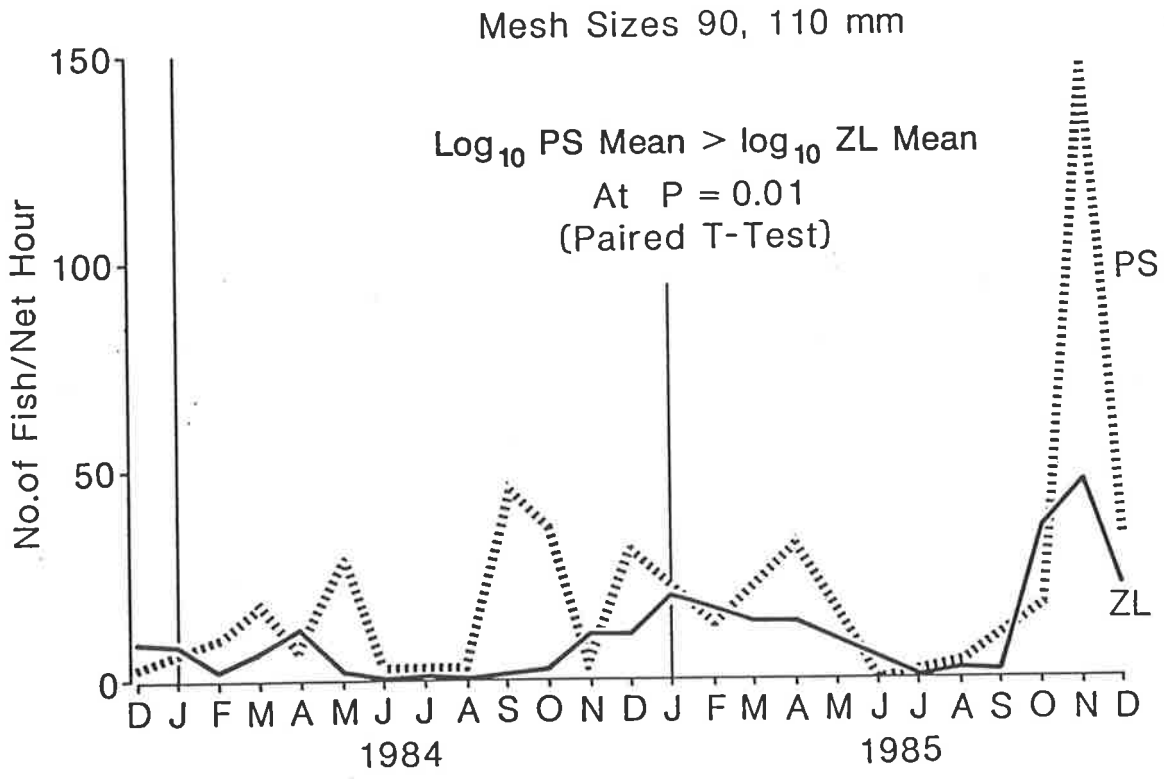


FIGURE 3.11: Monthly gillnet catch/effort of bony bream at PS and ZL, 1984-85.

was significantly higher at PS (paired sample t-test of log = transformed data, P<0.01, Fig. 3.11). Mean catch of bony bream larvae and small juveniles per 2m seine haul was negligible at ZL, but very high at PS (Fig. 3.12).

3.4.2. Seasonal changes in catches of bony bream

A strong decline in catch/effort for both gill and 130m seine nets during winter was noticed at the margins of both the river (ZL) and the lake (PS) (Fig. 3.13).

3.4.3. Species composition of catches

Bony bream dominated gillnet catches at both ZL and PS (Table 3.1, Figs. 3.14, 3.15), with carp about 1/5 and callop about 1/20 of the bony bream catch. Catches of silver perch and catfish were negligible at ZL and zero at PS. No Murray cod were caught at all.

In the 1985 130m seine catches, as in the gillnet catches, bony bream were again most abundant, with carp again 1/5 of the bony bream catch, but juvenile redfin (Perca fluviatilis) were second most abundant, principally because of a particularly strong 1984 year -class (Fig. 3.15).

In the 18m seine catches, bony bream and smelt (Retropinna semoni) had by far the greatest abundance in catches at PS

and ZL. (Figs. 3.14, 3.15).

In the 2m seine catches at ZL mosquitofish Gambusia affinis were the most numerous species, but at PS larval and small juvenile bony bream were most abundant (Figs. 3.14, 3.15).

At PS in December 1983, larval and post - larval bony bream from an early spawning were the most numerous species in the small fish population, as is illustrated by the plot of 2m and 18m seine catches (corrected for area hauled: Fig. 3.16). The mean 1985 130m seine catch at PS (corrected for area hauled) is also shown.

From the peaks in length - frequencies and by comparison with scale - ageing results (Fig. 4.24), the 1984 (I+) and 1983 (II+) cohorts can be identified. Assuming the area-corrected large and small seine catches are comparable, the mortality of the 1983 cohort over the first two years of life is 99.99%. Assuming also that the 1984 cohort was of similar size to that in 1983 - i.e. the larval population at PS was similar in 1984 and 1983 - then mortality in the first year of life was 99.9%. From the third year of life on, mortality remains comparatively low until a TL of at least 300mm. (The impression of low mortality rates may be exaggerated in such data by the overlap of the length-classes of older fish as growth rates slow down). Throughout

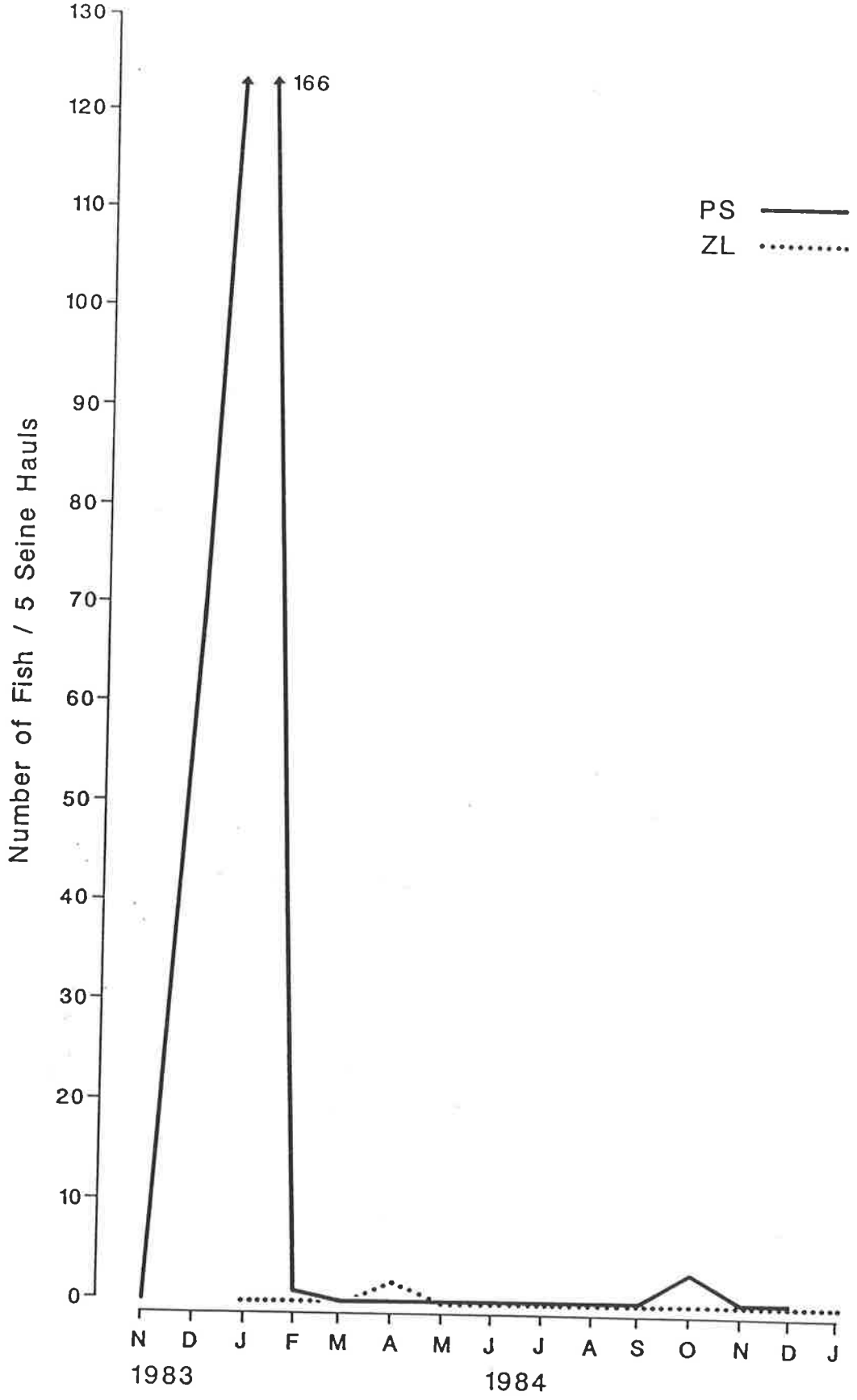


FIGURE 3.12: Monthly 2m seine catch/effort of bony bream at PS and ZL, 1983-84.

90 + 110mm Mesh Gillnets : Catch / 100m Net / Hour
130m Seine : Mean Catch / Haul

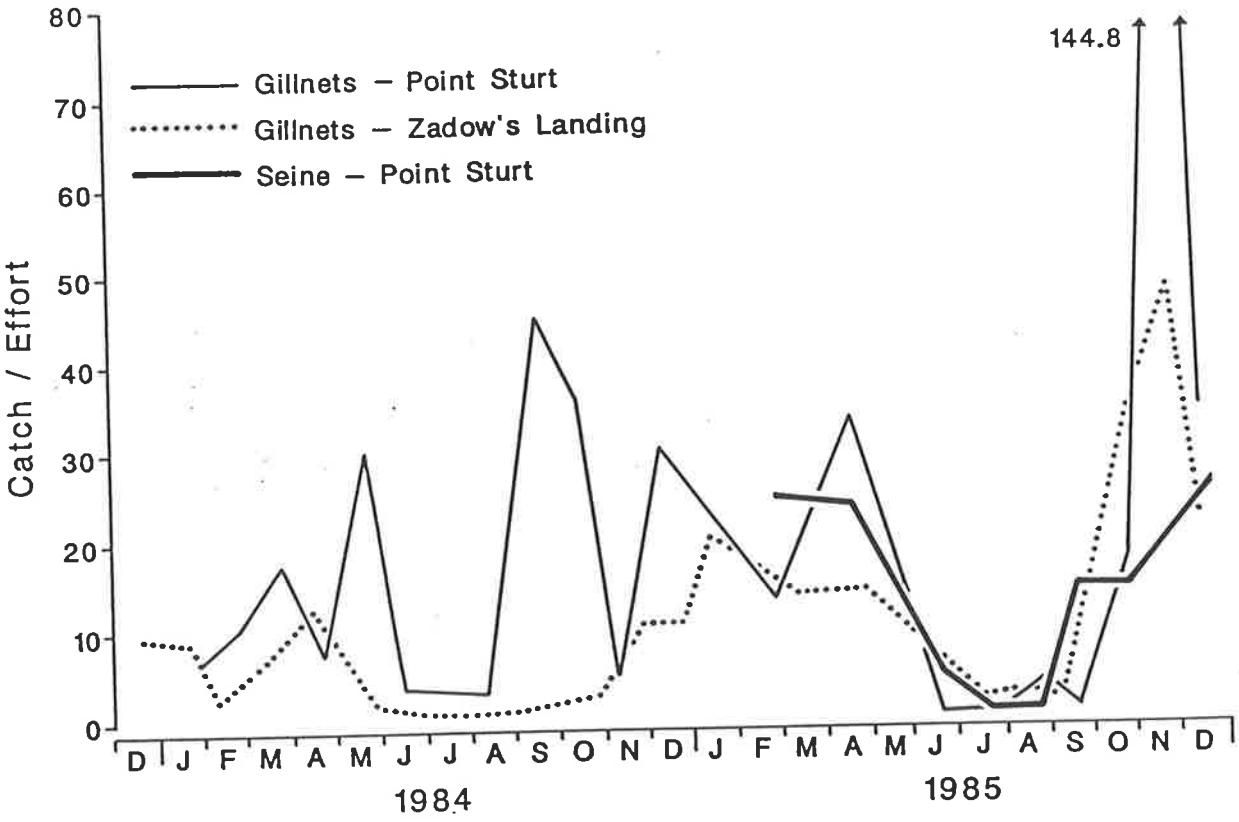


FIGURE 3.13: Seasonal cycles in gillnet and 130m seine catch/effort of bony bream at PS and ZL, 1983-1985.

TABLE 3.1: Key to lower River Murray species.

BB	bony bream	*Nematalosa erebi*
SM	smelt	*Retropinna semoni*
HH	hardyhead	*Craterocephalus eyresii* and *C. stercusmuscarum*
BHG	big-headed gudgeon	*Philypnodon grandiceps* and *P. sp.*
CG	common galaxias	*Galaxias maculatus*
BG	blue-spot goby	*Pseudogobius olorum*
PF	redfin	*Perca fluviatilis*
SS	sandy sprat	*Hyperlophus vittatus*
CON	congolli	*Pseudaphritis urvilli*
MF	mosquito fish	*Gambusia affinis holbrooki*
WCG	western carp gudgeon	*Hypseleotris klunzingeri*
RF	crimson-spotted rainbowfish	*Melanotaenia splendida fluviatilis*
CAR	common carp	*Cyprinus carpio*
C	callop	*Macquaria ambigua*
GF	goldfish	*Carassius auratus*
SIP	silver perch	*Bidyanus bidyanus*
CF	catfish	*Tandanus tandanus*
BT	brown trout	*Salmo trutta*

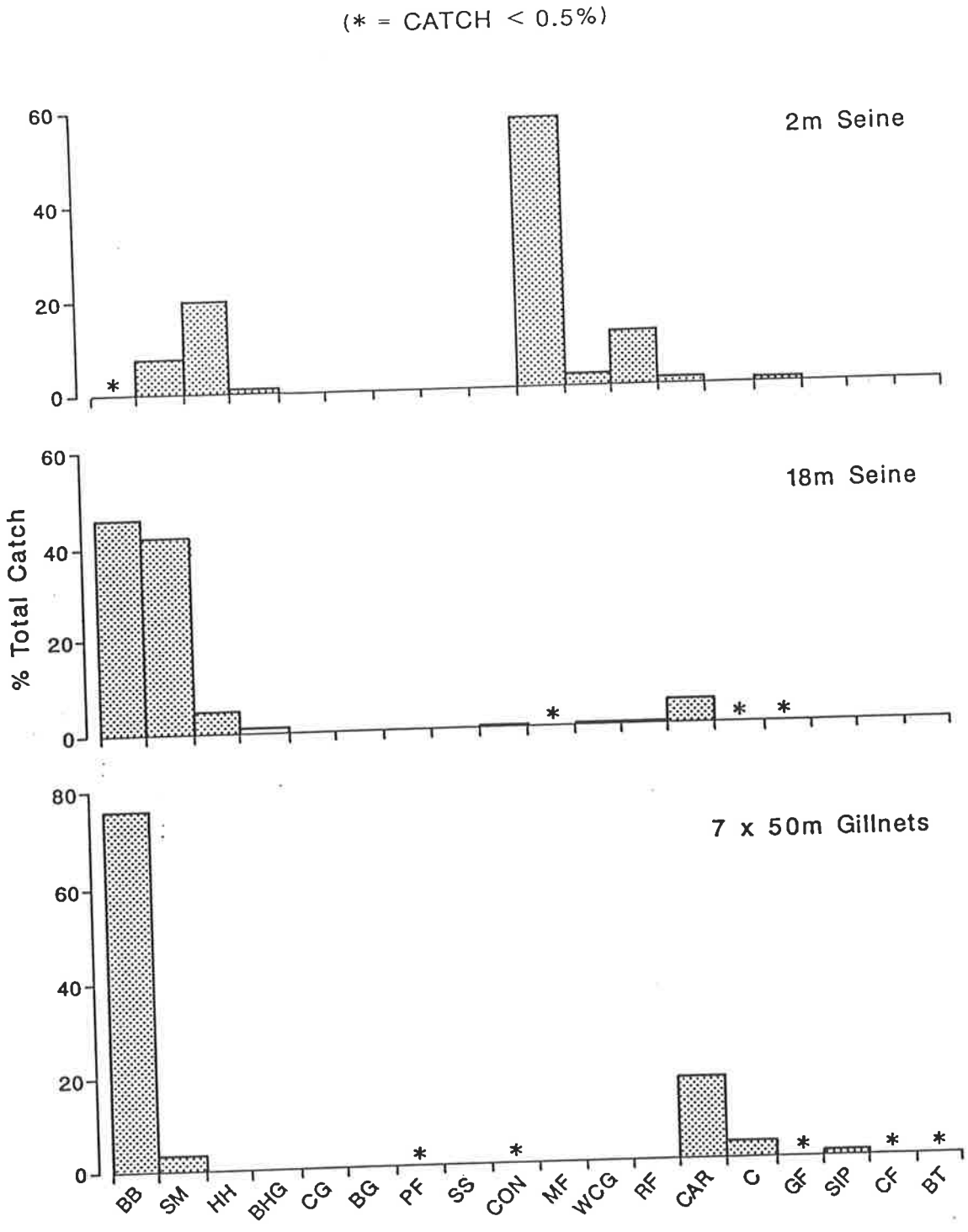


FIGURE 3.14: Species composition of catch for different gears at ZL, 1983-84.

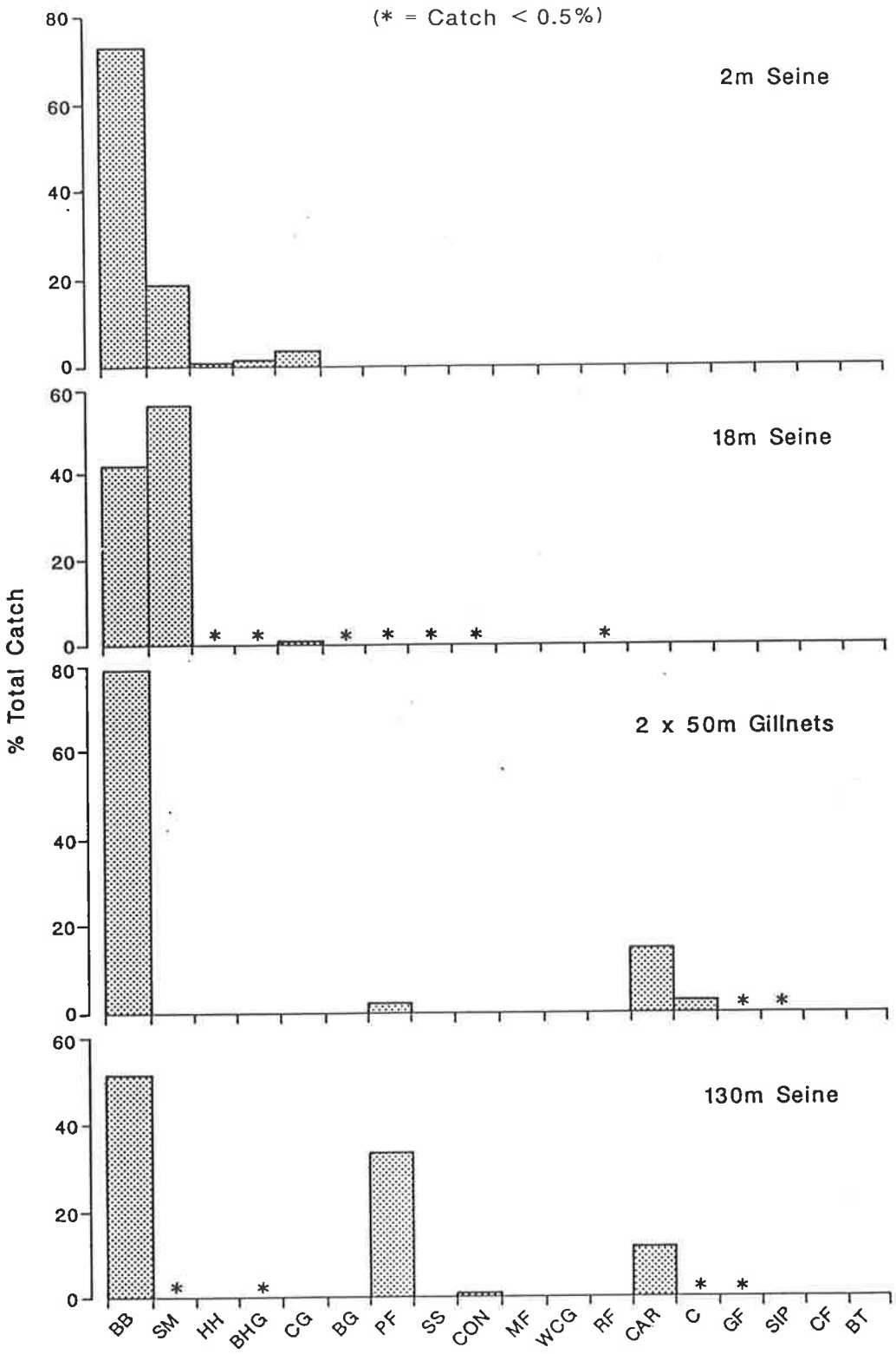


FIGURE 3.15: Species composition of catch for different gears at PS, 1983-85.

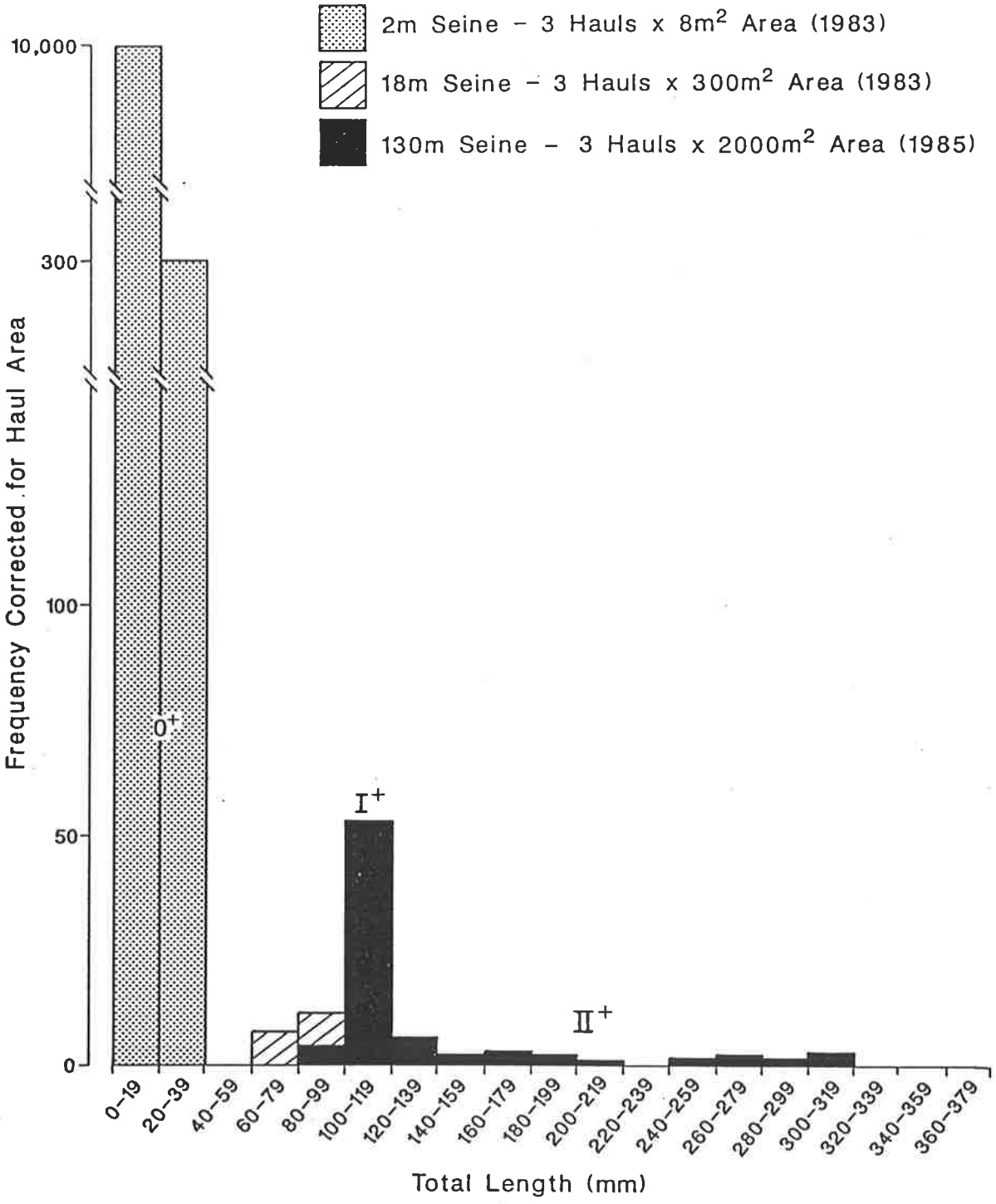


FIGURE 3.16: Length - frequencies of seine catches of bony bream in December at PS.

the year, the population is dominated by young-of-the-year (YOY) fish, as the sum of the year's seine catches demonstrates (Fig. 3.17).

3.5 DISCUSSION

The higher densities of larvae, small juveniles and large adults at PS suggests that lentic conditions may be preferred for breeding, and such conditions perhaps support superior growth rates (see also 5.4). Lloyd and Walker (1986) found juvenile bony bream in all aquatic habitats of the lower Murray, but did not compare the lake and river environments. Dorosoma sp. have been found to flourish particularly in warm turbid shallow lakes (Jester & Jensen, 1972), and bony bream may also (see Hutchins, 1977; Llewellyn, 1983; Bishop et al., in press). However, a much larger range of sites would have to be sampled to define such a tendency.

A decrease in temperature - dependent activity of bony bream during winter could be expected to reduce passive gear (gillnet) catch rates, but this does not explain the parallel decline in seine catches. Juvenile bony bream in Coongie Lake, north-eastern South Australia, have been shown to move between the lake shore and offshore waters on a diurnal basis (Puckridge & Drewien, 1988), and these

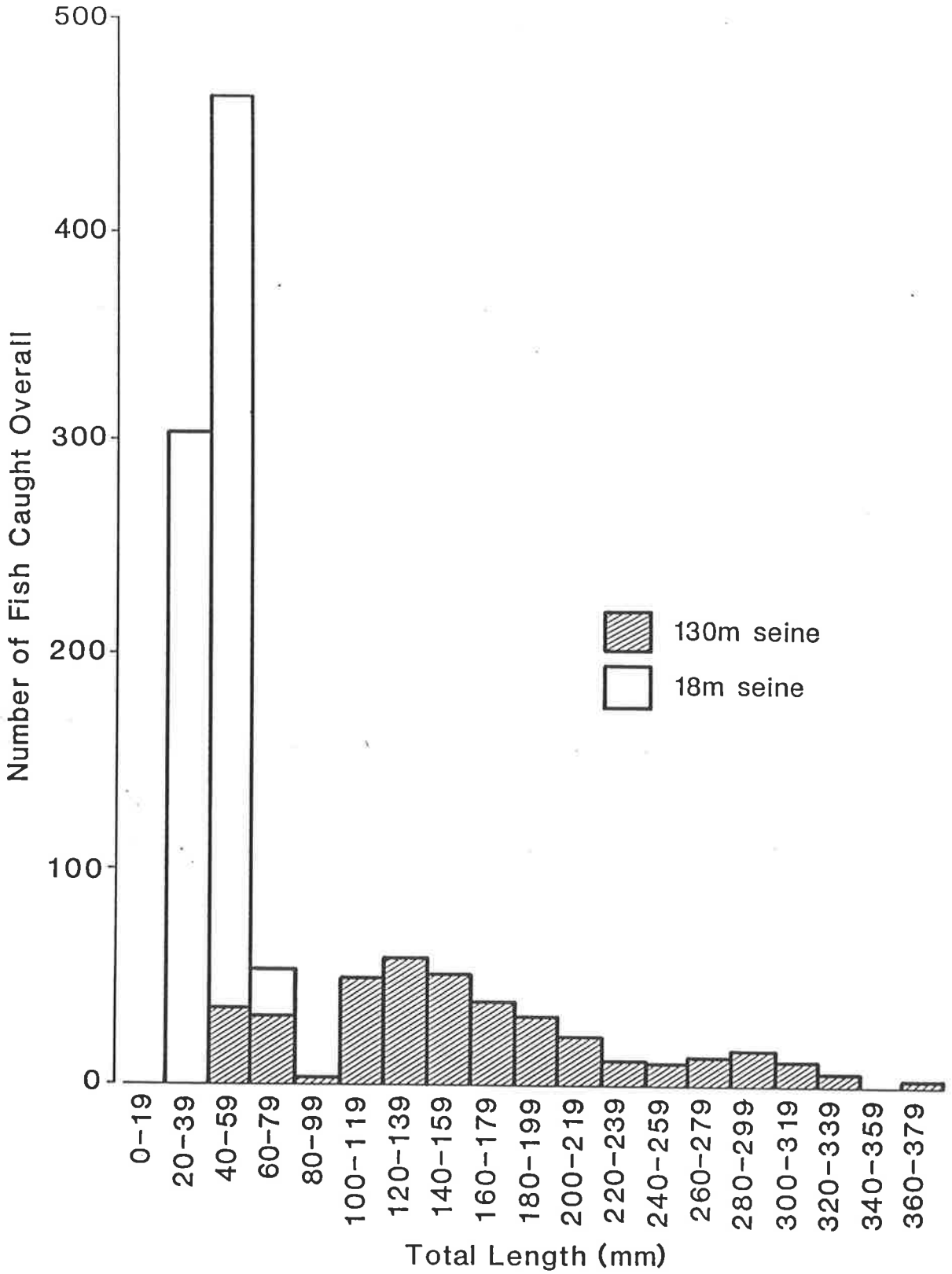


FIGURE 3.17: Length - frequencies of seine catches of bony bream at PS, summed over 1984-85.

movements appear to follow thermal gradients (Puckridge, unpub. data). A winter fall in shallow water catches has been observed with American Dorosoma species in Florida (Moody, 1957), and the same species in cool temperate habitats are known to over-winter offshore (Madden, 1951; Pahl & Willfahrt, 1962; Jester & Jensen, 1972). Such behaviour in lower Murray bony bream would account for the seasonality of catches. The commercial catch data give little information on this matter, since effort is not accurately calculable for the reasons given above (3.2). A seasonal program of comparative offshore and onshore sampling, using trawls and echosounding, would provide relevant data.

The domination of catches by bony bream and the low yields of other freshwater native species reflects the pattern in the lakes/Coorong commercial catches closely (Fig. 3.18). It appears that the latter, despite the lack of correction for effort, reflect the real abundances of these species in Lake Alexandrina. However, the commercial reach catches of bony bream greatly underestimate the abundance of the species in the river channel and backwaters, and confirm that reach fishermen are directing their effort toward other species. Relative catches of carp are considerably lower than the commercial catches, but some proportion of this

discrepancy must be due to the greater weight per individual carp. Overall, the bony bream is clearly flourishing under river regulation, and the low catches of other large native species suggest that their decline in commercial catches reflects real declines in abundance.

The matching abundance of smelt and juvenile bony bream in both the river and the lake reflects findings in the Cooper system (Puckridge & Drewien, 1988), and means that bony bream juveniles may face competition from another abundant, nektonic zooplanktivore.

A mortality curve cannot confidently be derived from the area-corrected seines catches, since the different gears are not strictly comparable. It is possible, for example, that larvae were concentrated closer to shore, where 2m seine hauls were taken. Also, larger fish may be less susceptible to capture, even in the 130m seine, than are larvae in the 2m seine. But even with a 100-fold reduction in larval catches, the pattern of extreme early mortalities would remain. This pattern is similar to that for D. cepedianum, where mortality of young-of-year may exceed 99% (Bodola, 1966; Houser & Bryant, 1967; Jenkins, 1974). Such high early mortalities suggest that the species may be relying more on the magnitude of reproductive output than on

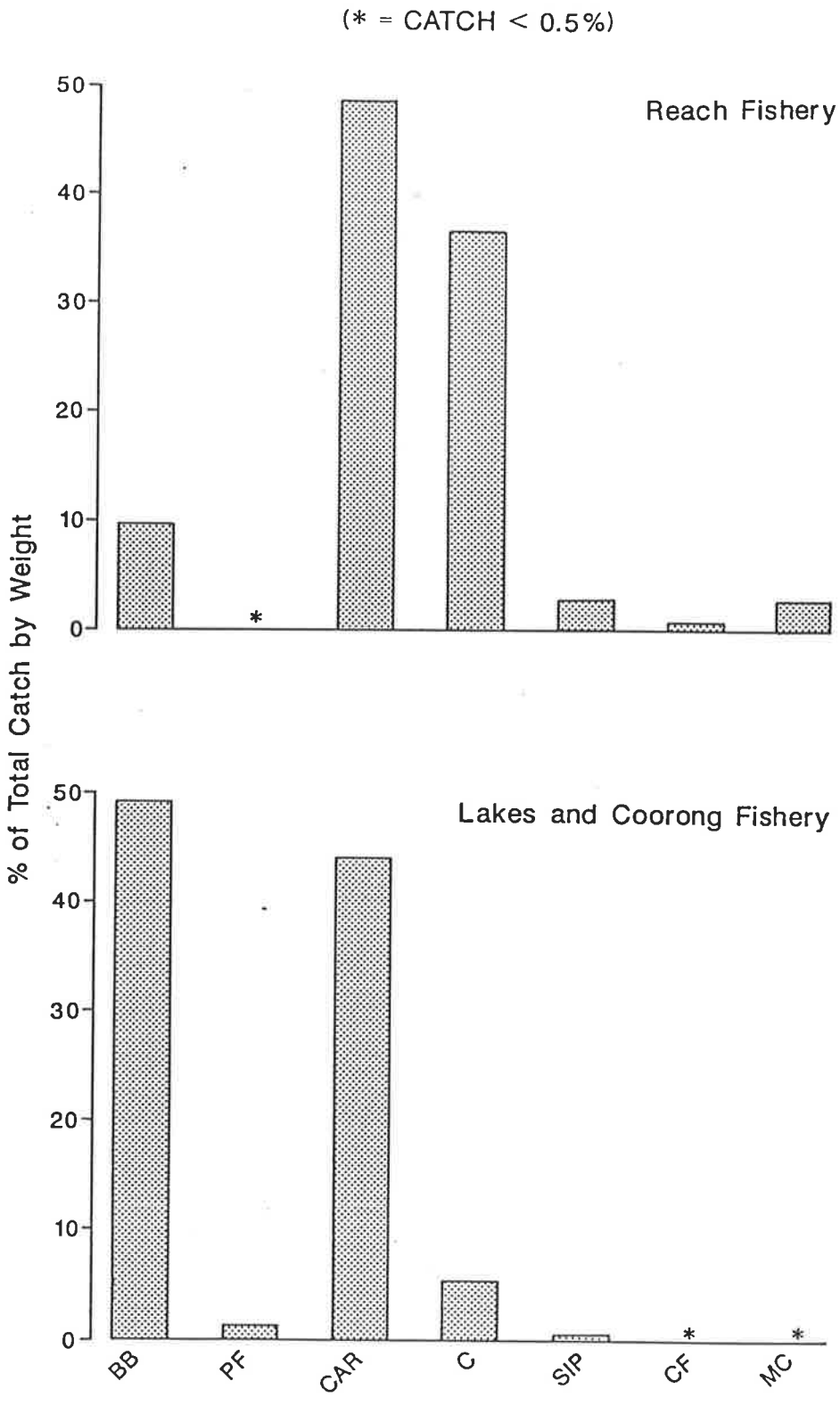


FIGURE 3.18: Species composition of commercial catches in the Reach and Lakes/Coorong fisheries, 1983-84 (South Australian Department of Fisheries, 1985/1986).

the reduction of mortalities by fine-tuning of the spawning cycle to hydrological cues. If this is the case, the lower Murray population should be characterised by early maturity, high fecundity and prolonged iteroparity.

Low mortalities in older fish are to be expected in a fish with a broad trophic base (see Ch. 1), and large adults may also be less susceptible to predation, as is the case for D. cepedianum (Fagan & Fitzpatrick, 1978).Such a broodstock should give resilience to the population, allowing it to survive long intervals of recruitment failure occasioned for example by protracted droughts.

CHAPTER 4: AGE, GROWTH AND CONDITION

4.1 SUMMARY

Bony bream in the lower Murray show seasonal cycles of condition that suggest a winter fast. The cycles are more pronounced in smaller fish. Larger fish show falls in condition associated with spawning. Growth in young fish slows over winter and renewed growth becomes apparent in October. Scale - ageing is valid to age III+, but beyond this may underestimate true age. Females live longer and grow larger than males. In comparison with other gizzard shads, the bony bream is large and long-lived. Longevity may contribute to the abundance of the bony bream in a variety of environments, and in the lower Murray in particular.

4.2 INTRODUCTION

Apart from their intrinsic interest, patterns of growth, ageing and condition are central to life-history, and are major determinants of the ecological roles of species. In accounting for bony bream abundance, a knowledge of the length of reproductive life, and particularly of the relation between first maturity and age, is essential. For management of the fishery it is necessary to know, for example, the mean ages of recruitment and maturity. In regard to the use of bony bream as a forage species, growth rate will determine biomass available to predators, and condition cycles may be used as indicators of stress.

There have been no prior studies devoted to age and growth in the bony bream, but some incidental data are available. Cadwallader (1977) estimated lengths at age in the middle Murray from length - frequency analysis, and Bishop et al. (in press) provide data on seasonal condition in the Magela Creek, Northern Territory. Estimates have been made of growth in the first year and maximum size for populations in north-western and south-eastern Australia respectively (Allen, 1982; Cadwallader & Backhouse, 1983). However, no validated age-growth curve is available for bony bream from any part of Australia.

With respect to other gizzard shads, there is fragmentary information on the subtropical congeners N. nasus and N. come, and on the widespread Anodontostoma chacunda (Thomson, 1957; Rao, 1965; Parimala & Ramaiyan, 1980). However, thorough studies have been made of age, growth and condition in an estuarine congener, N. vlaminghi, in Western Australia (Chubb & Potter, 1986), Dorosoma sp. in North America (Bodola, 1966; Bryant & Hauser, 1968; Jester & Jensen, 1972) and Konosirus punctatus in Japan (Takita, 1978). Generally, gizzard shads in temperate waters are of moderate size and longevity, with rapid early growth and pronounced seasonal cycles in condition (Jester & Jensen, 1972; Takita, 1978; Parimala & Ramaiyan, 1980; Chubb & Potter, 1986).

From the success of the bony bream under a wide variety of hydrological regimes, and particularly under regimes subject to prolonged and unpredictable flood failure, it might be expected that the species would be capable of both prolonged iteroparity and rapid early growth. From the position of the lower Murray at the southern extreme of the bony bream range (Fig. 1.2), and from reports of bony bream winter mortalities (see 6.5), it might be expected that winter cold stress would produce a strong seasonal cycle in condition. Accordingly, this chapter examines bony bream patterns of growth, ageing and condition in the lower

Murray, tests the foregoing hypotheses, and relates the outcomes to the ecological role of the species in the lower Murray and throughout its range.

4.3 METHODS

Fish were collected at ZL and PS over 1983-1986 using the methods described above (2.1). Mean condition per month of fish in gill-net catches through 1983-1984 was plotted for different size-classes and for males and females at PS and ZL. Differences between overall means and variances for males and females and for ZL and PS were tested (paired-samples t-test and F-test) for untransformed and log-transformed data. Untransformed and log - transformed weight-length relations were derived for males and females over all seasons from 1983 to 1985 at ZL, and the slopes compared (d-statistic).

The length-frequency distribution of the sample chosen for ageing corresponded to the distribution in the 130m seine catch, which, on the basis of the frequency distributions of catches, was the least size-selective method used (see Figs 2.5, 2.8). Since scale samples were taken throughout the year, the sample of fish for ageing was so selected that the length distribution of the fish chosen for ageing was matched to the length distribution for the annual catch.

Scales were stored in paper envelopes, examined under a dissecting microscope at 40x, and unless blown (i.e. replacement scales) were taped between microscope slides. The number of annual growth checks (true annuli) were counted on 8-12 unblown scales per fish, and the distance measured from the focus to the anterior margin of the annulus in micrometer units. True annuli were distinguished by the following:

a. Annulus continuous in both fields.

b. Circuli more closely spaced posterior to the annulus than anteriorly.

c. Circuli irregular at annulus and/or circuli cutting over at annulus.

e. More than 50 % of scales show the annulus clearly.

f. All annuli of comparable distinctness.

False (sub-annual) annuli failed to fulfil one or more of these criteria.

A regression of scale length (SL = focus to anterior edge) against total length (TL) was fitted to data for each sex, and the slopes compared (d-statistic). The SL-TL regressions were used to correct TL at each annulus for each fish, the mean TL at the last annulus was calculated and compared to back-calculated weighted means, the age-length curves were

plotted and polynomial and exponential curves fitted. The slopes of log-transformed male and female age - length regressions were compared (d-statistic). A Von Bertalanffy growth curve was fitted to the data for each sex by the method of Rafail (1973).

4.4 RESULTS

4.4.1 Condition

Since gonad weight is deducted from body weight in the calculation of condition factor [CF = 10^5(body weight-gonad weight)/(total length)^3], this factor acts as an index of fatness exclusive of the state of the reproductive organs.

At ZL, smaller bony bream (100<TL<200 mm) show a distinct seasonal cycle, with a low in late winter - early spring and maximum in summer - autumn (Fig. 4.19). In fish of TL 200-300 mm, a similar but less marked cycle occurs. However, fish of TL>300mm show a different pattern, with a short peak in early spring, a steep fall during spawning in November-December, and a rise over late summer - autumn.

Over all months, variability in mean condition of the largest size-group is less than for smaller fish (F-ratio, $n_1=n_2=16$, $P<0.01$). At ZL, there is no significant difference (paired samples t-test) in log-transformed mean monthly

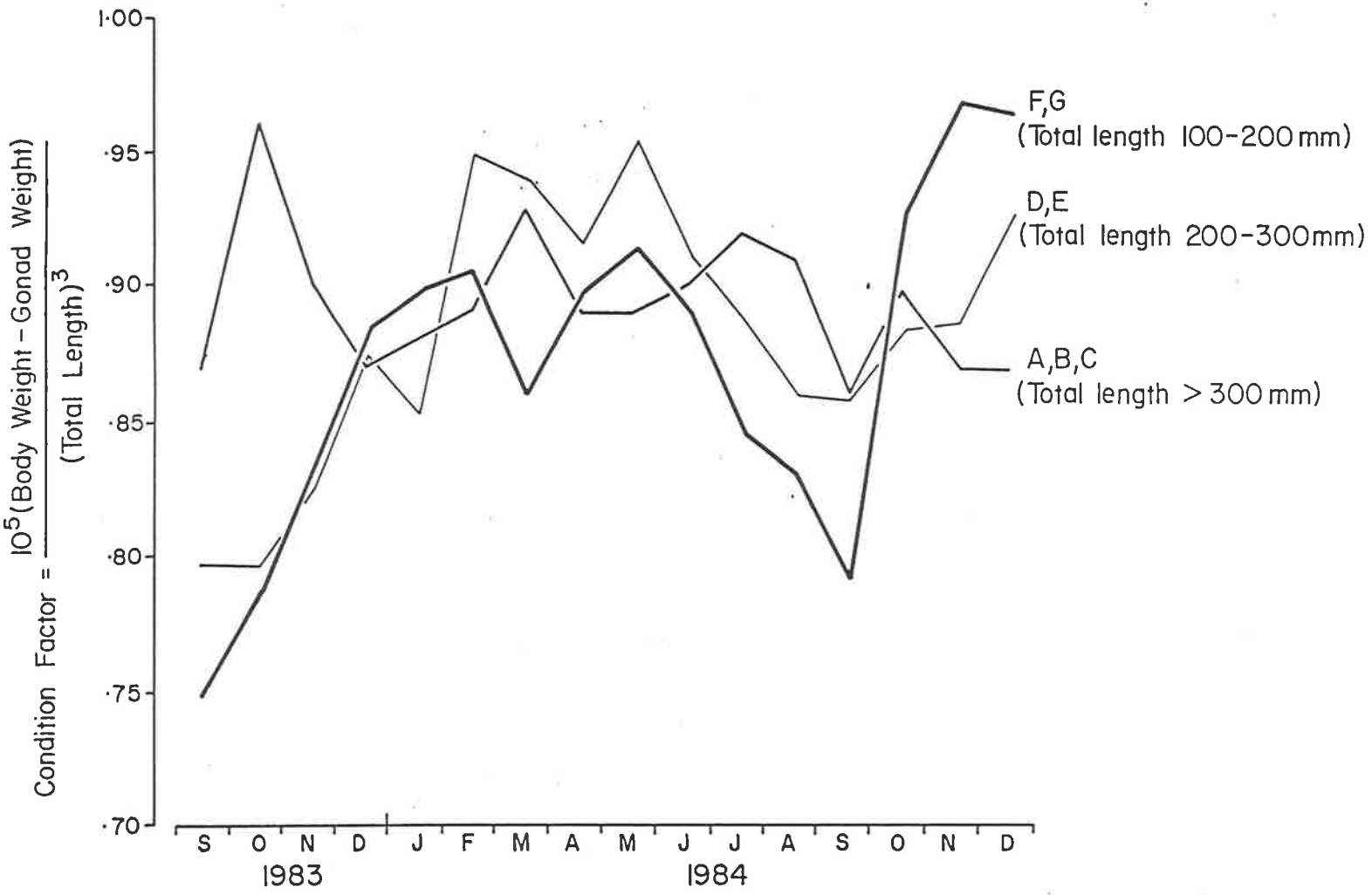


FIGURE 4.19: Condition of three size - groups of bony bream at ZL, 1983-84 (both sexes).

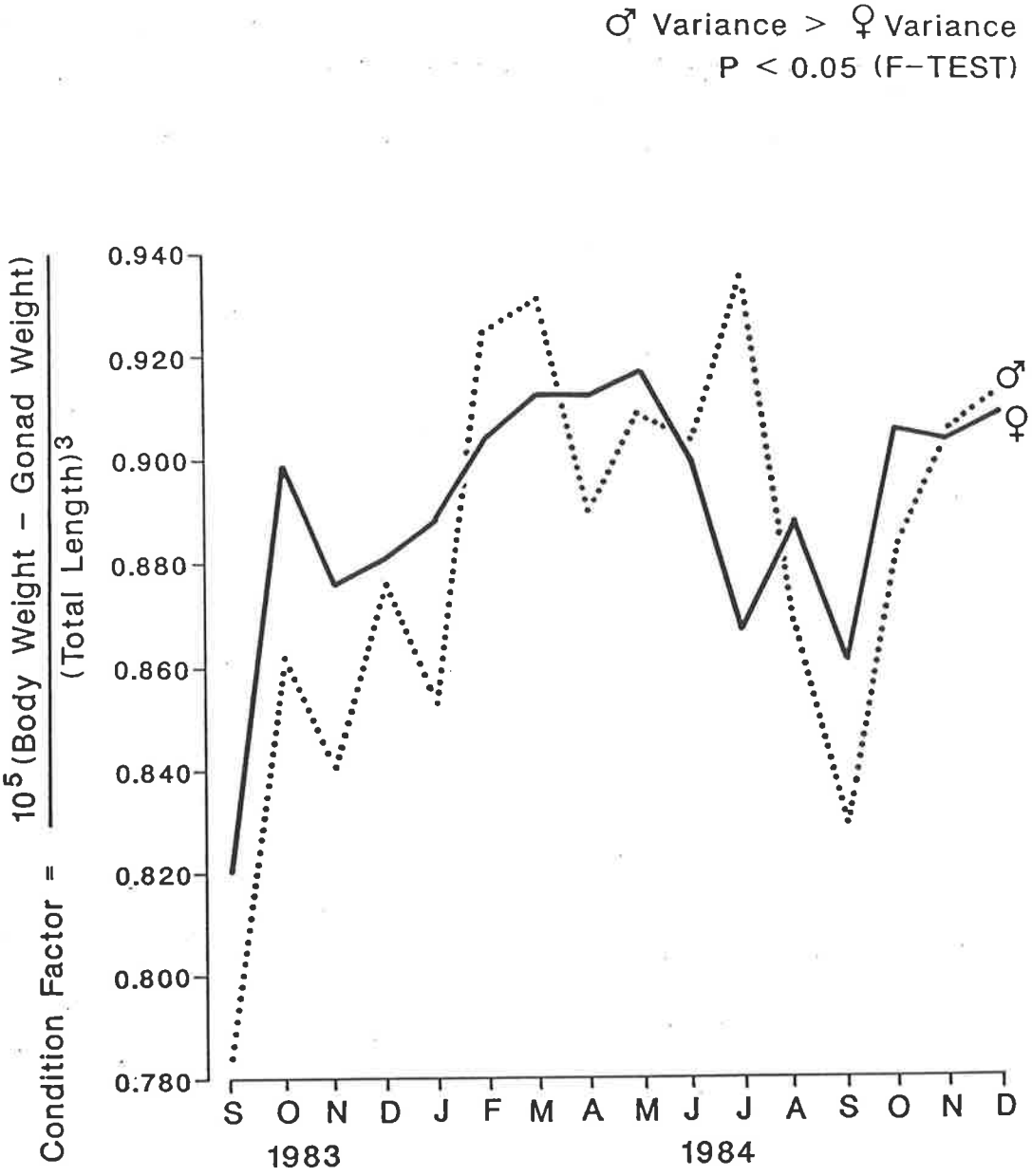


FIGURE 4.20: Condition of male and female bony bream at ZL (all size-classes).

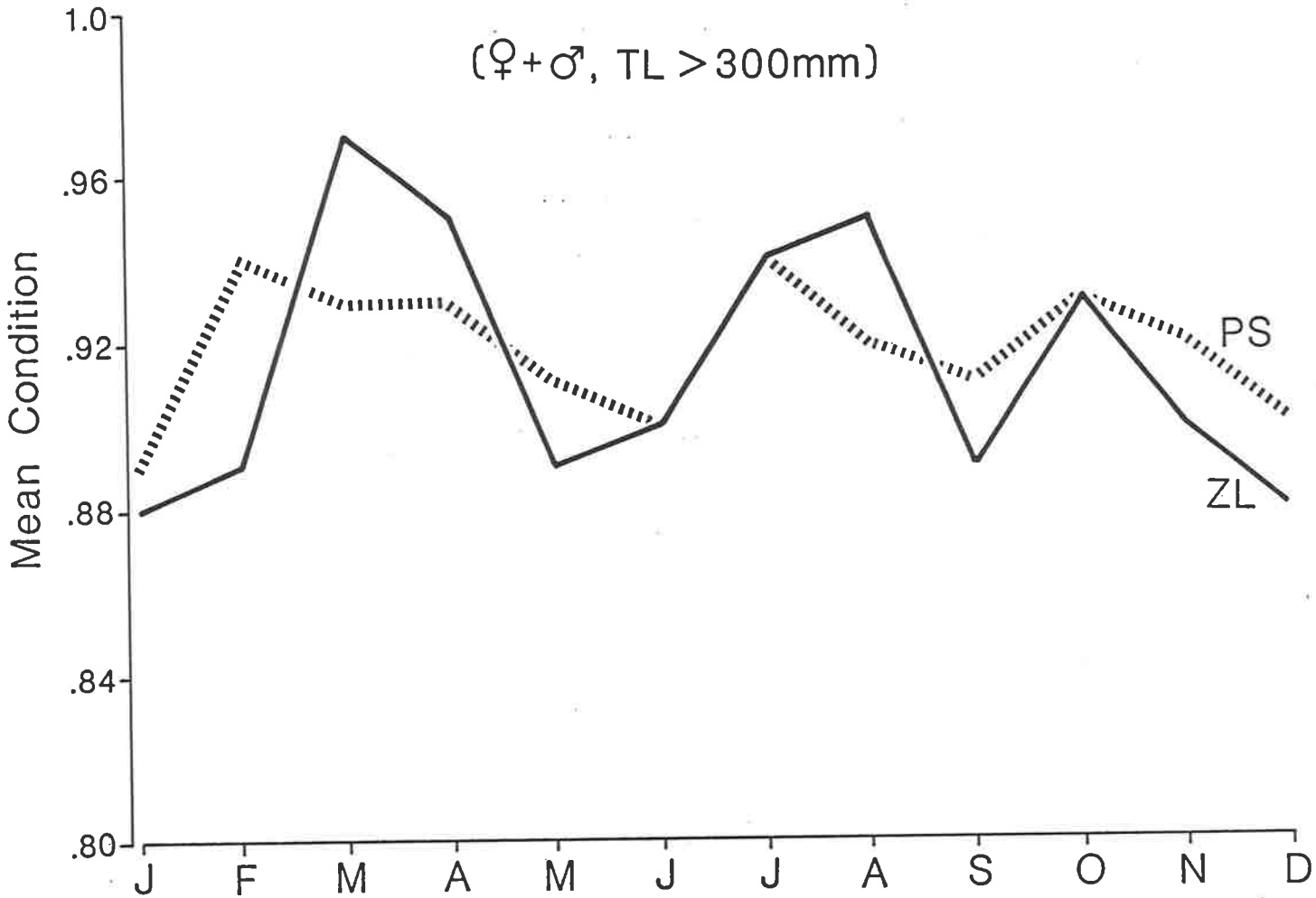


FIGURE 4.21: Mean monthly condition of large adult bony bream at ZL and PS (1983-85).

condition between the sexes for all size-classes together (Fig. 4.20). Males do have a significantly higher variability in untransformed mean condition than females on a monthly basis (F-ratio, $n_1=n_2=16$, $P<0.05$). The log (x+1)-transformed mean condition of larger fish (TL>300mm) at PS and ZL is identical (Fig. 4.21), but the variance of condition is higher at ZL than at PS (F-ratio, $n_1=511$, $n_2=340$, $P<0.001$).

4.4.2. Length-weight relationship

The slopes of the $\log_{10}$weight - $\log_{10}$total length relations for males and females for all months combined (1983 - 85) are not significantly different (d statistic). Data for both sexes combined gave the following relationship:

$\log_{10}$ body weight (W) = -5.290 + 3.104 $\log_{10}$ TL

(F-test, n=1524, $P<0.001$).

4.4.3. Age and growth

Bony bream have large readily shed cycloid scales, with well-marked annuli. False annuli occur, particularly between the first and second true annuli, but they differ from true annuli chiefly in forming an incomplete and less distinct mark, and in being absent on more than 50% of scales. The first annulus is often less distinct, but provided it was

unbroken in both the anterior and posterior fields, it was accepted as a true annulus. Occasional deep scoring also occurs, and was not accepted as a year-mark. Blown or regrown scales have a large area around the focus either transparent and smooth or with a cross-hatched effect. These invalidated some samples, particularly in older fish.

The plot of mean scale length beyond the last annulus shows a clear annual cycle in I+ and II+ fish, with the new annulus appearing in October-November (Fig. 4.22). Growth beyond this annulus is rapid through summer, and declines in winter, with evidence of growth cessation and even scale resorption in June-July. The pattern for III+ fish is similar, but the period of annulus formation is less clearly marked. Nevertheless the annulus is a valid year-mark for fish to age III+. Because annulus formation takes place in October, and spawning in December-January, there is a discrepancy between length at annulus and length at age. Beyond III+, there is no clear annual pattern of growth and renewal of the post-annulus scale (Fig. 4.23).

The scale length (SL) vs total length (TL) regressions are highly significant for both sexes (F-test, n(females)=208, $P<0.001$; n(males)=203, $P<0.001$) and the slopes of these regressions are significantly different (d statistic, $P>0.001$). The final plots of total length at age (corrected

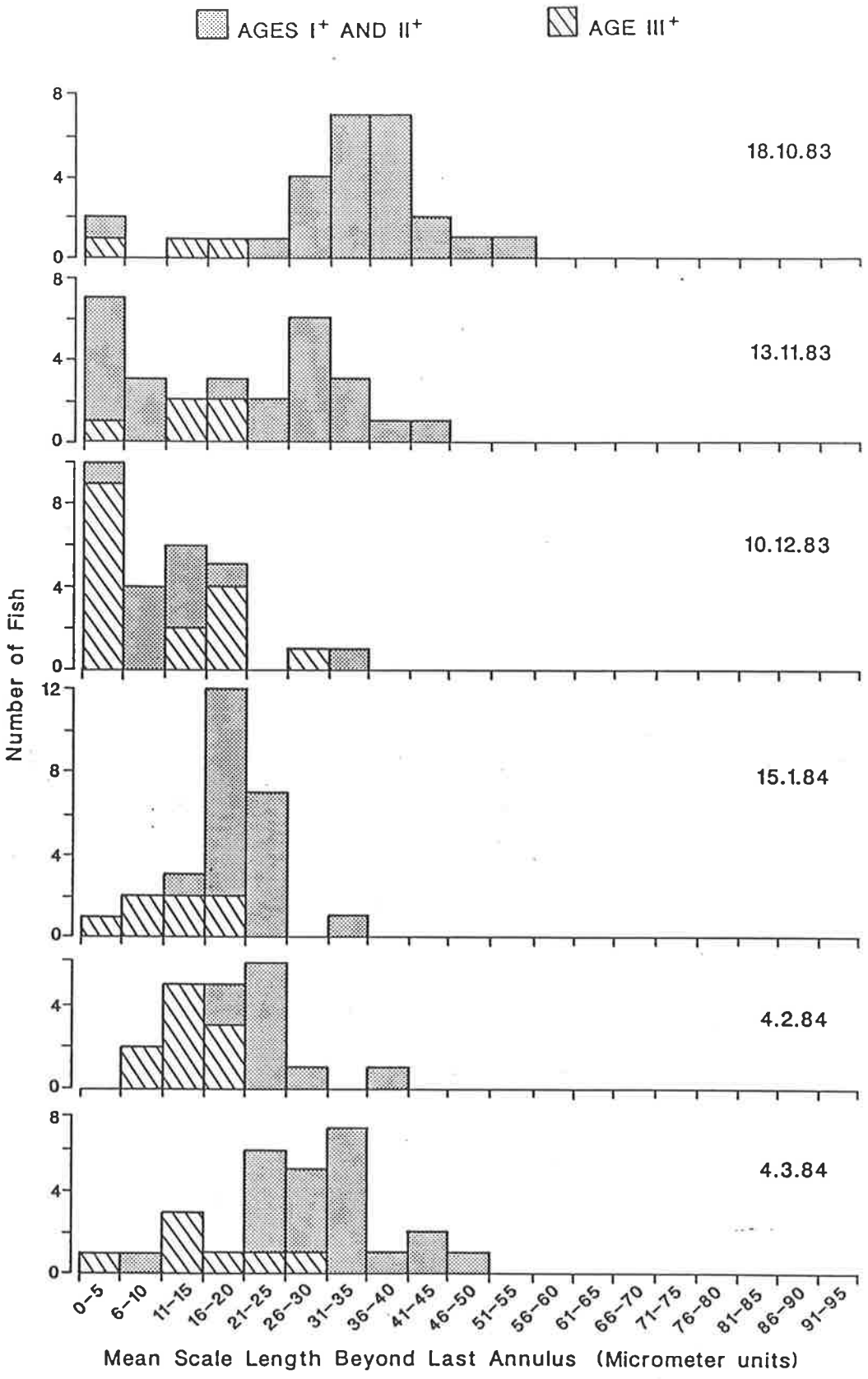


FIGURE 4.22: Frequency per month of bony bream scale lengths beyond the last annulus at ZL (ages I+ - III+).

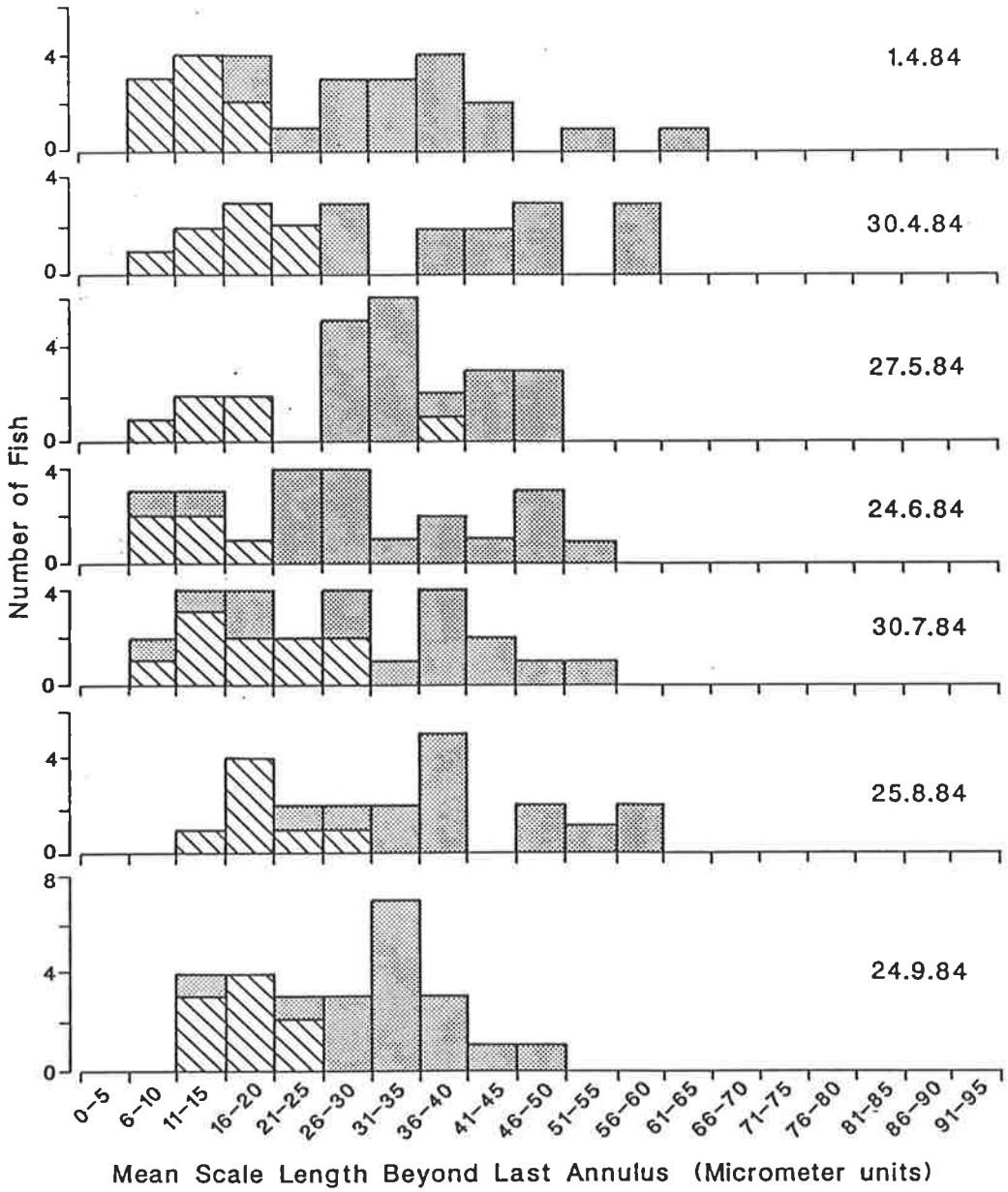


FIGURE 4.22 (cont.).

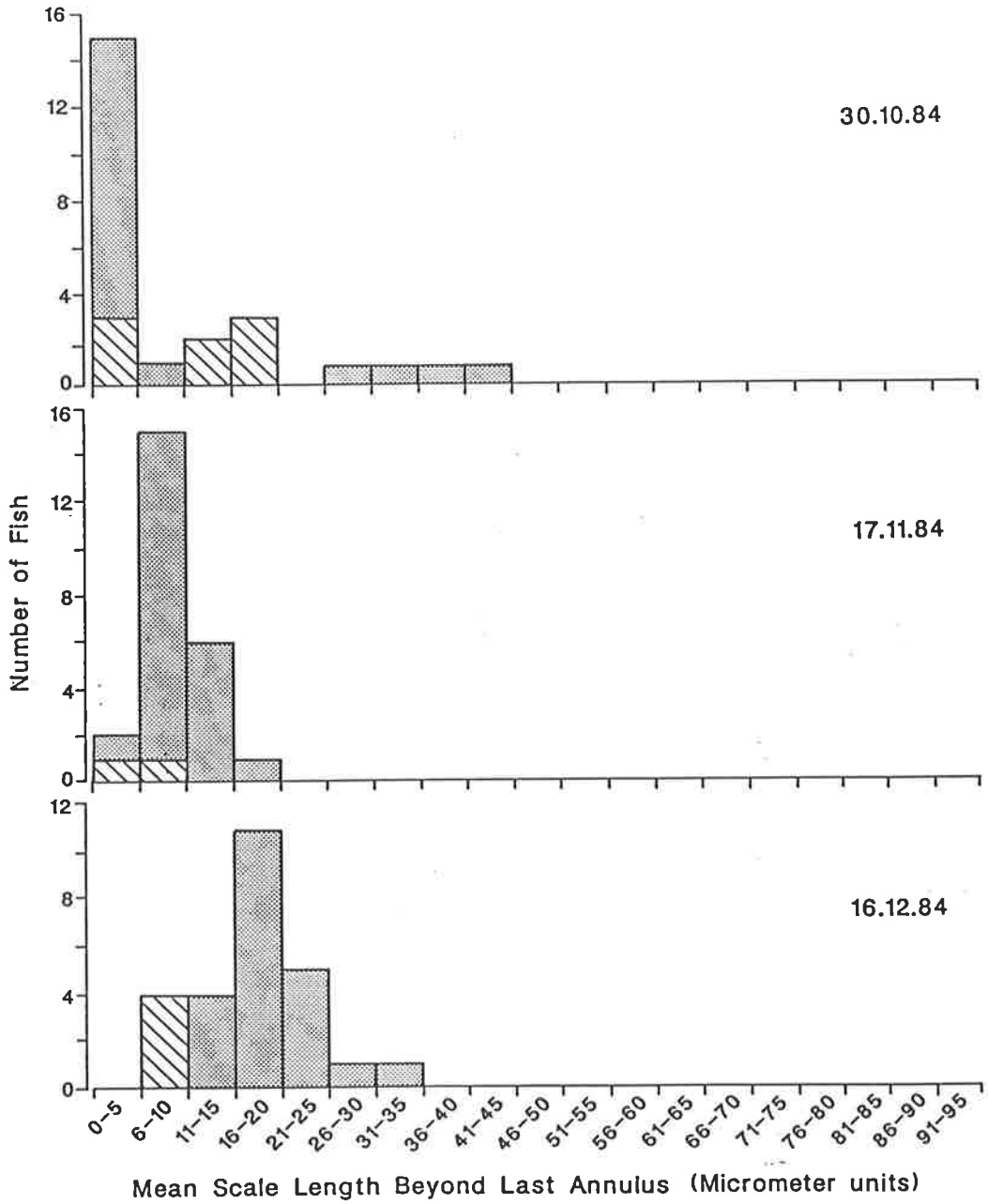


FIGURE 4.22 (cont.).

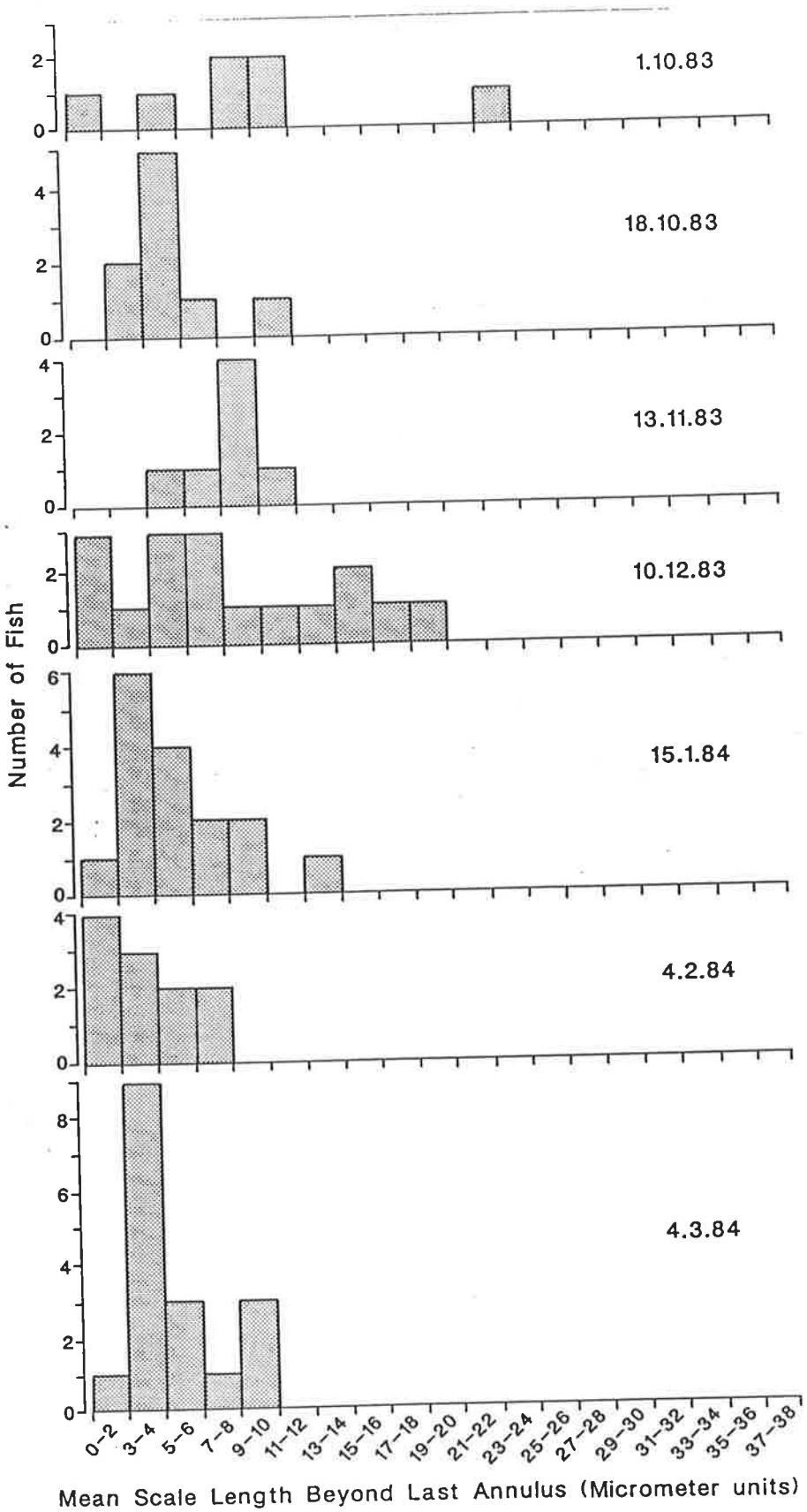
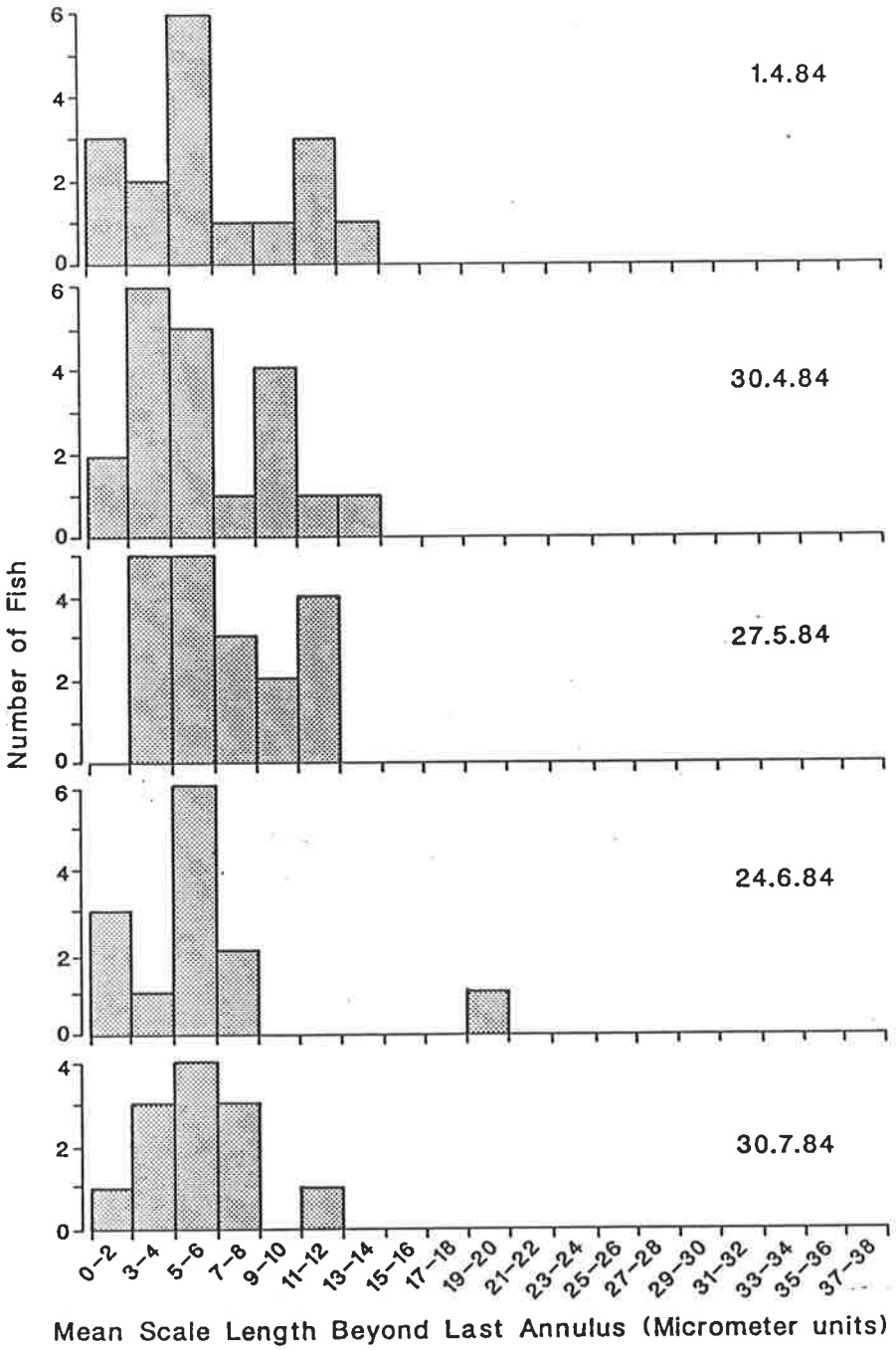


FIGURE 4.23: Frequency per month of bony bream scale lengths beyond the last annulus at ZL (ages IV+ -).



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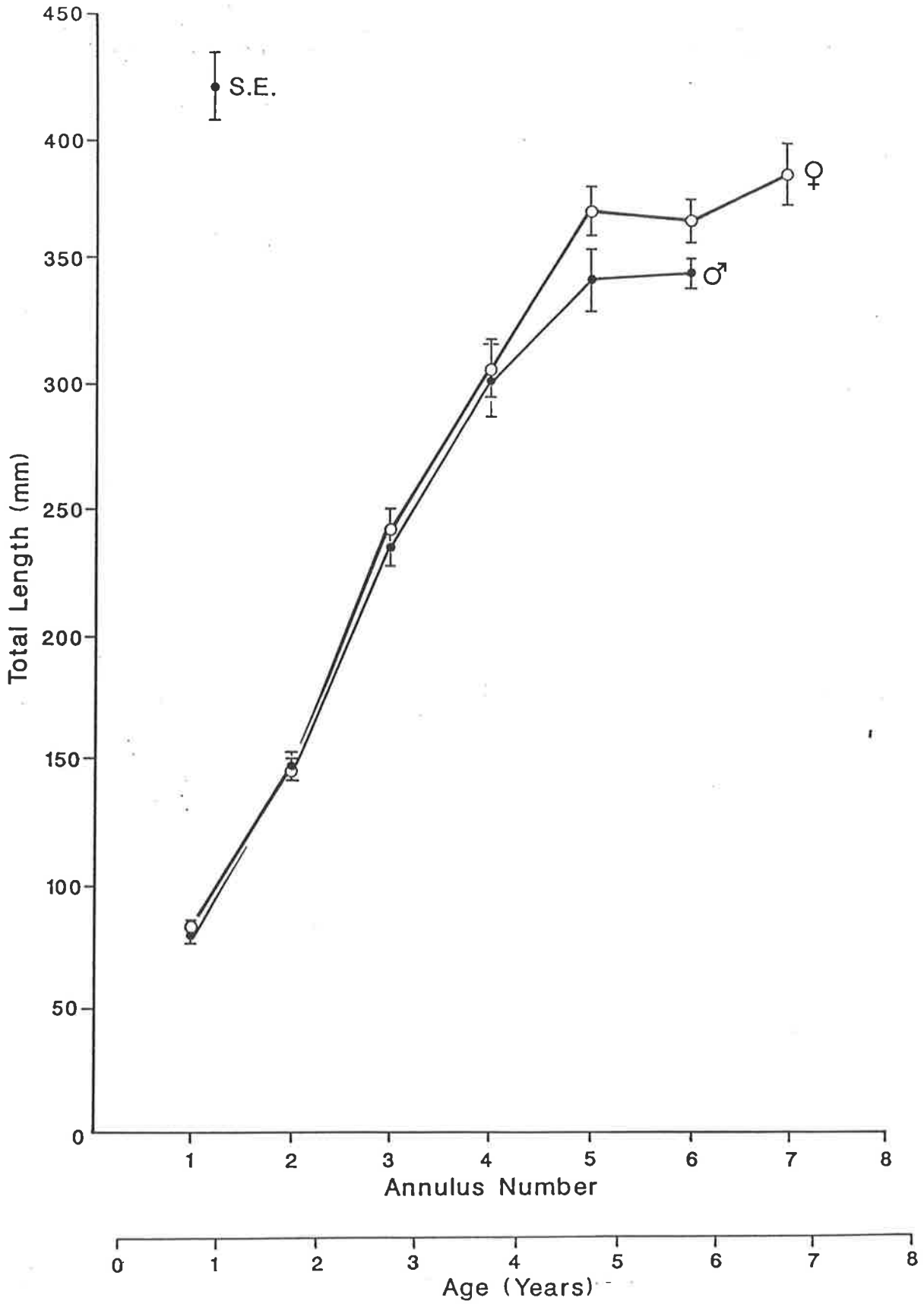


FIGURE 4.24: Growth curves for male and female bony bream at ZL, 1983-84.

from the respective male and female TL-SL regressions) give assymptotic curves (Fig. 4.24). Female growth is rapid and almost linear from I+ to V+, then slows abruptly. Male growth is similar to IV+, declines in year V+, then levels out. Growth continues beyond age VII+, but sample numbers are too low to provide reliable estimates of rates.

Length - frequency plots of monthly gillnet catches at ZL for 1983-84 do not show a clear progression of peaks which could be identified as age-classes (Fig. 4.25). However, on the length - frequency plots of 130m seine hauls for 1985-86 (Fig. 4.26) the growth of O+ and I+ fish may be traced as a progression of peaks. The October mean lengths of these age-classes (70 mm at O+, 160 mm at I+) approximate to the lengths at annulus formation (in October) derived from scale analysis (length at annulus I = 81 mm, at II = 148 mm). Since only the first three annuli have been confirmed as year-marks, and only the first two years' growth checked by length-frequency analysis, the rest of each curve is provisional. However, weighted mean back-calculated total lengths at each annulus (Table 4.2) approximate to directly observed lengths at all ages except II.

For both sexes, the best fit to the age-TL data is provided by quadratic equations; for females TL = -44.9 + 123.3A -

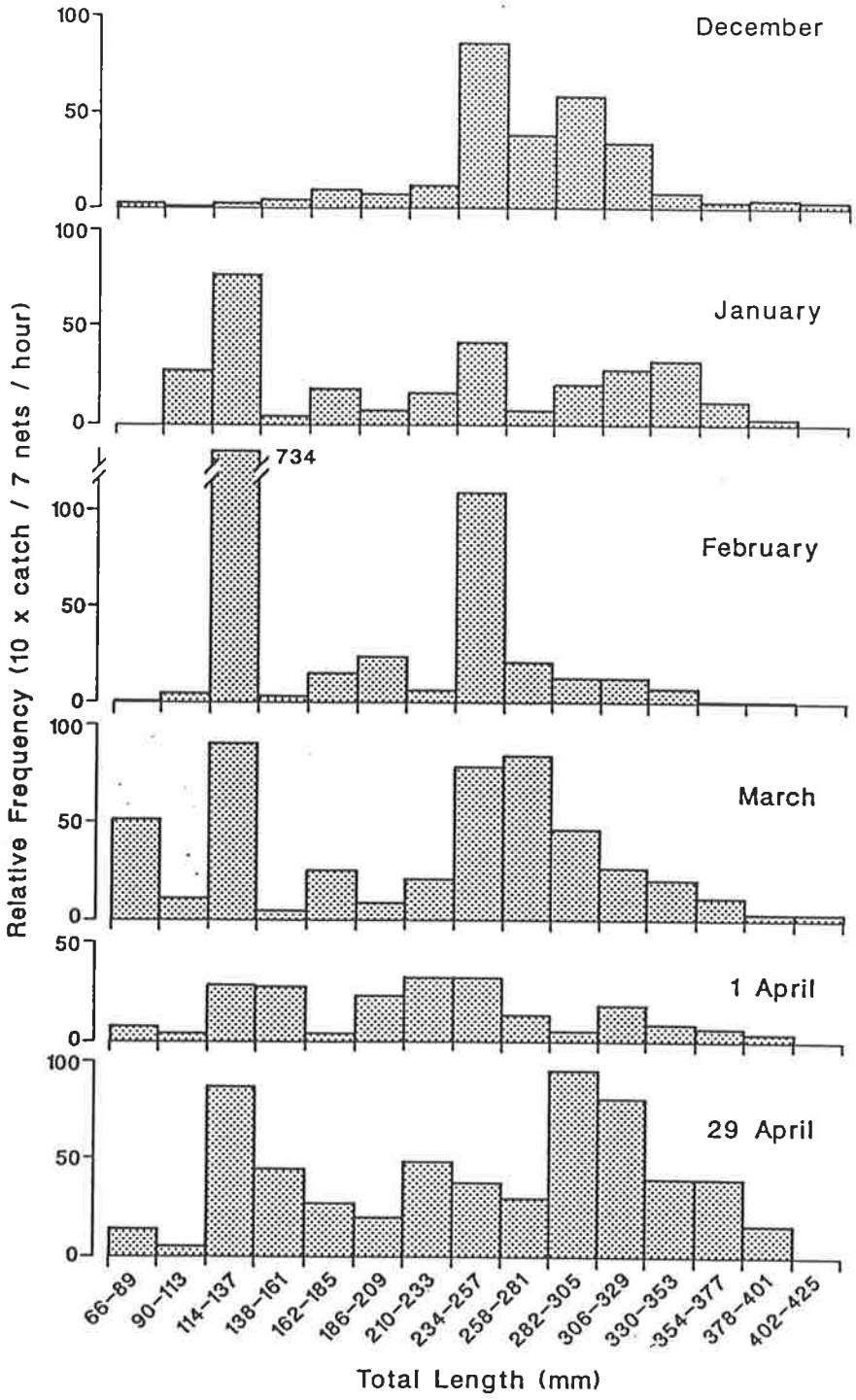


FIGURE 4.25: Length - frequencies of monthly gillnet catches of bony bream at ZL, 1983-84.

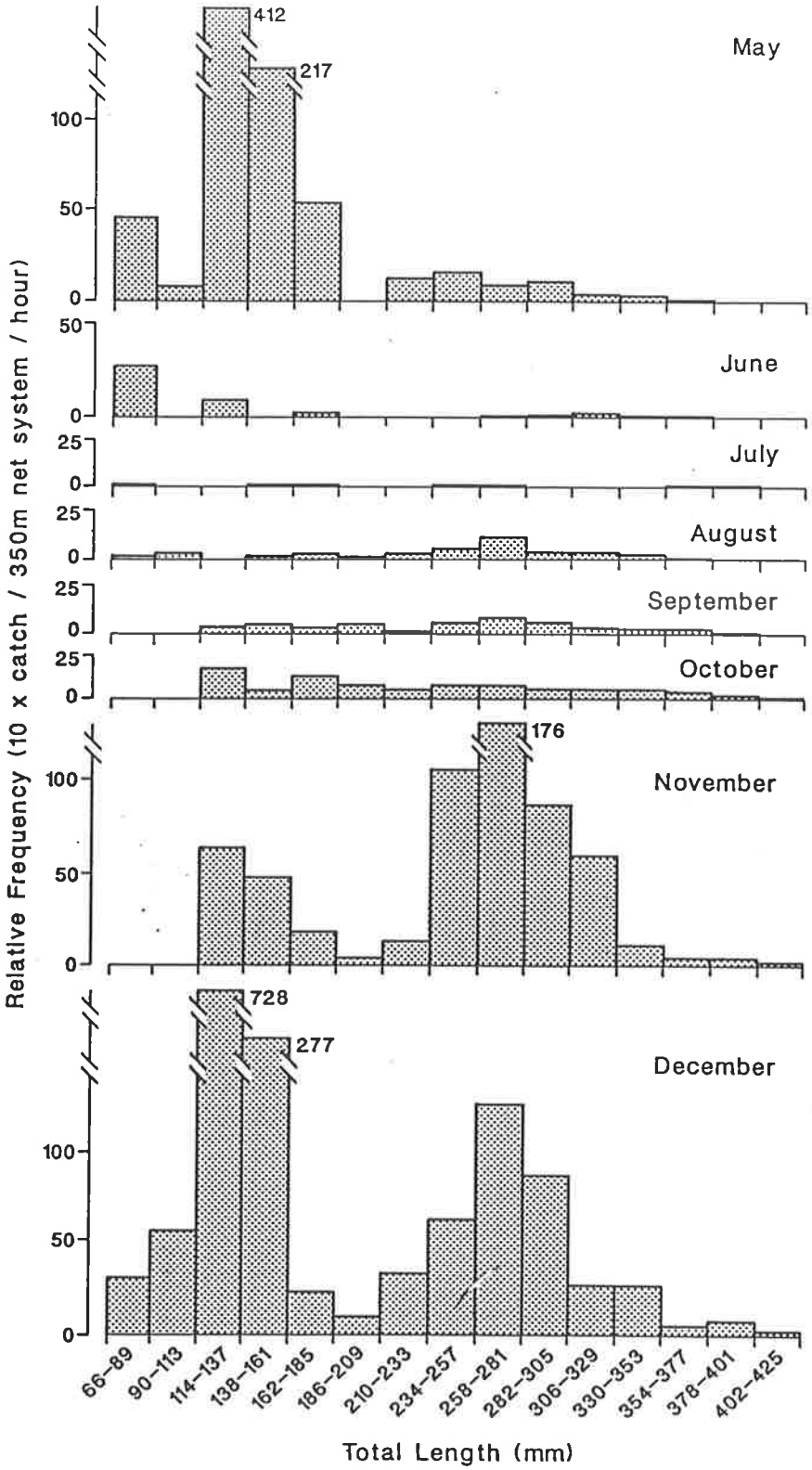


FIGURE 4.25 (cont.).

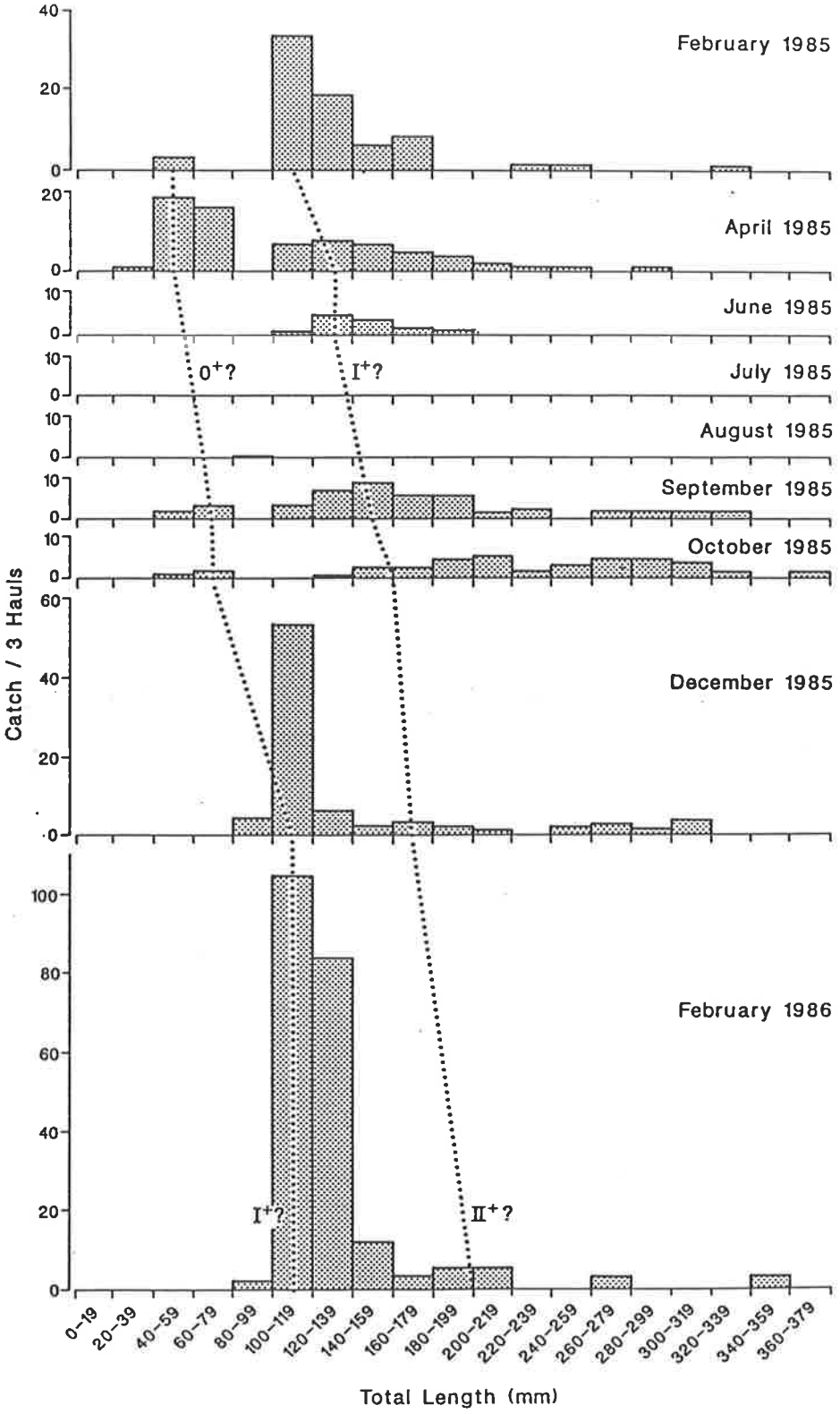


FIGURE 4.26: Length - frequencies of 130m seine catches of bony bream at PS, 1985-86.

8.7A2 (r^2 = 0.98, n=7, P(F-test)<0.001), and for males TL = =44.3 + 123.6A - 9.6A2 (r^2 = 0.99, n=7, P(F-test)<0.001). Fitted Von Bertalanffy curves are highly significant (males, n=7, P(Chi-square)<0.001; females, n=7, P(Chi-square)<0.005) (Table 4.3). The parameters K and Lmax are 0.44 and 369.5mm for males and 0.11 and 745.9 for females. Actual maximum ages and lengths observed (but not included in the age-length curve) were 10 years and 480mm for females and 8 years and 405 mm for males. Overall, female mean lengths at age are significantly higher than male (paired samples t-test, P<0.05), but a comparison of the slopes of the male and female linear log-log regressions shows no significant difference (d-statistic).

TABLE 4.2: Mean back-calculated total lengths at each scale annulus.

AGE	N	MEAN BACK-CALCULATED TL (mm) AT ANNULUS						
		1	2	3	4	5	6	7
FEMALES								
1	72	81.7						
2	49	78.5	147.3					
3	22	83.9	175.6	244.6				
4	8	98.2	191.1	269.5	309.2			
5	4	118.7	196.9	269.1	315.3	372.0		
6	7	88.1	166.8	240.0	295.4	335.9	369.9	
7	3	93.9	186.9	264.1	314.5	337.8	360.4	388.8
WEIGHTED MEANS		83.2	162.6	252.0	306.6	346.6	367.1	388.8
MALES								
1	81	80.2						
2	42	79.2	148.4					
3	23	84.9	175.6	236.7				
4	5	98.8	192.9	268.0	302.6			
5	6	95.9	170.5	241.8	293.0	344.0		
6	3	83.4	153.6	220.1	270.8	313.1	347.5	
WEIGHTED MEANS		81.8	161.0	240.4	291.7	333.7	347.5	

TABLE 4.3: Fit of total lengths at age predicted by the Von Bertalanffy curves to the lengths at age observed.

SEX	AGE	OBS. TL	EXP. TL
MALE	1	80.2	51.7
	2	148.4	164.4
	3	236.7	237.2
	4	302.6	284.1
	5	344.0	314.4
	6	347.5	334.0
	7	349.0	346.6

$$Lt = 370[1-e^{(-.44(t-.66))}],\ P(\text{Chi-squared})<0.001$$

FEMALE	1	81.7	105.2
	2	147.3	171.9
	3	244.6	231.6
	4	309.2	285.1
	5	372.0	333.1
	6	369.9	376.0
	7	388.8	414.5

$$Lt = 746[1-e^{(-.11(t-.38))}],\ P(\text{Chi-square})<0.005$$

4.5 DISCUSSION

4.5.1. Condition

The pronounced seasonal cycle in condition of young bony bream suggests a winter fast, probably in response to low temperatures. In the Magela Creek, Northern Territory, condition peaks in late wet-early dry after the period of maximum feeding which follows flooding, and falls in the late dry (Bishop et al., in press). This cycle is governed more by hydrological than thermal changes. I+ N. vlamingbi and 0+ - III+ D. cepedianum show a pattern similar to that of young bony bream in the lower Murray (Pierce, Wissing, Jakorski, Givens & Megrey, 1980; Chubb & Potter, 1986). Low winter temperatures may be stressful to bony bream, as disease incidence typically peaks in August/September, after the July temperature minimum (see 6.4), and Dorosoma petenense is subject to cold stress at comparable winter temperatures (Griffith, 1978). The absence of a pronounced late winter low in condition of large bony bream may be related to the greater metabolic efficiency of larger shad (Pierce, Wissing & Megrey, 1981) and their superior tolerance of cold (Adams, McLean & Huffman, 1982). The presence of a low in condition of larger bony bream during spawning may be due to the greater demands reproductive output makes upon this size-class (see 5.4). The occurrence

of greater stresses in mature males than in females is suggested by greater variability in condition evident in male fish, the reduced incidence of males in the larger size-classes (see 5.4), and higher mortality in older males (see below). Prolonged spawning by males (see 5.4), may account for higher male stress levels.

4.5.2. Age and growth

The identity of the length-weight relations for the sexes suggests that the preponderance of females in the larger length-classes is not due to a sexual difference in body shape of older fish. The length - weight relation for bony bream in the lower Murray may be compared with the equation $\log_{10} W = -1.92 + 3.12 \log_{10} LCF$ for bony bream in the Magela Creek (Bishop et al. in press.), which gives a similar slope but a different intercept (because LCF is used instead of TL). The equation $\log_{10} W = -5.1 + 3.04 \log_{10} TL$ for D. cepedianum (Jester & Jensen, 1972) is very close in both intercept and slope, and demonstrates the uniformity of gizzard shad body shape.

A clearly marked period of annulus formation in older bony bream may be absent either because increased reproductive stress prolongs annulus formation (see 5.4), or because the growth increments are small and resorption at the scale edge

is considerable. Annulus formation in older fish may only occur in seasons of exceptional growth. It seems likely that scale ageing underestimates true age for older bony bream, but a larger sample and calibration against another ageing technique are needed to confirm this. [Dr John Harris (N.S.W. State Fisheries) has undertaken to provide estimates of age from pectoral fin-spine sections to compare with the scale - ages presented here.]

The significant difference in the slopes of the male and female body length - scale length regressions is unusual and difficult to explain in a species where sexual dimorphism is otherwise slight (see 5.4). It may be an artifact of the large numbers involved in the regressions. The steep slope of the early growth curve may reflect a need to outgrow predators, as for D. cepedianum (Fagan & Fitzpatrick, 1978), or to maximize efficiencies of size (Beitinger, Thommes & Spigarelli, 1977; Pierce, 1977).

Although there is no overall difference in male and female growth rates, females appear to both live longer and to continue growth longer than males. This is reflected in the difference in Von Bertalanffy parameters for male and female bony bream. The values of these parameters may be compared with K = 0.17 and Lmax = 381 mm for N. vlaminghi,

both sexes combined (Chubb & Potter, 1986). Male bony bream appear to reach maximum size faster, females slower than this cogener. Male and female N. vlamingbi show no significant difference in mean lengths at age or in the slopes of the scale radius - total length regressions. There is also no difference in the slopes of the growth curves of males and females of D. petenense, but males have a higher mortality rate (Bryant & Houser, 1968; Johnson, 1970). In western Lake Erie, female D. cepedianum grow faster but there is no evidence of differential mortality (Bodola, 1966).

The lengths at age, maximum lengths and maximum ages derived in this study may be compared with those suggested for bony bream in the middle Murray (Cadwallader, 1977) and in the Magela Creek (Bishop et al., in press), and with data from other dorosomatids (Table 4.4). . The present study in the lower Murray gives a slower overall growth rate and greater longevity for bony bream than do either Cadwallader (1977) for the middle Murray or Bishop et al. (in press) for the Magela Creek. The Perth herring has more rapid early growth than the bony bream, but grows more slowly after age II. Both D. petenense and Konosirus punctatus appear to be shorter-lived species. However, the great variation in growth rate of D. cepedianum from different parts of the

United States illustrates how labile dorosomatid growth rates may be. Bony bream in the present study were found to be as long-lived and to grow nearly as large as the largest, most long - lived population of D. cepedianum. Low adult mortality is also suggested by the size - structure of the bony bream population (see 3.4).

It was argued earlier (1.4) that the abundance of the bony bream in the regulated lower Murray (where catches of other native species have declined), is more likely to be due to a potential for rapid population increase than to longevity. Certainly the native species for which catches have declined most drastically - Murray cod and catfish - are also long-lived (Davis, 1977; Cadwallader & Backhouse, 1983). However the resilience provided by a long-lived broodstock may, in the case of the bony bream, supplement a high potential for increase, particularly where an unfavourable hydrological regime causes repeated recruitment failure (Walker, 1983; Cadwallader, 1986; Pierce & Walker, subm.).

TABLE 4.4: Lengths at age, maximum lengths and maximum ages of members of the Dorosomatinae compared with the results of the present study.

SEX	MEAN LENGTHS AT ANNULUS (TL/SL)								MAX AGE	MAX SIZE	
	I	II	III	IV	V	VI	VII	VIII	AMAX	LMAX	WMAX
D. cepedianum (Jester & Jensen, 1972, Miller, 1960), TL											
M+F	94	151	183	219	254	273	291	324	10	521	
D. cepedianum (Bodola, 1966), SL											
M	141	273	313	343	349						
F	140	285	335	364	386						
D. petenense (Bryant & Houser, 1968), TL											
M	62	117	123	141							
F	66	118	134								
K. punctatus (Takita, 1978a), SL											
M+F	148	189	210								
N. vlaminghi (Chubb & Potter, 1986), TL											
M+F	90	162	172	200	218	271	311	286		361	555
N. erebi (Cadwallader, 1977), TL											
M+F	67	173	245	340	387					470	2000
N. erebi (present study), TL											
M	80	148	237	303	344	348	349	388	8+	405	574
F	82	147	245	309	372	370	389	437	10+	480	953

CHAPTER 5: REPRODUCTIVE BIOLOGY

5.1 SUMMARY

The reproductive biology of the bony bream resembles that of other gizzard shads. The bony bream matures at a median age of 2-3 years, spawns in December-January at water temperatures of 21-23°C and is highly fecund. Spawning does not appear to be dependent on flooding. Reproductive effort increases with size. The ova and larvae are small, the yolk-sac stage is brief and development is typical of clupeids except for the fin-ray sequence. Sexual dimorphism does not occur, but females predominate in the largest size-classes. The success of the bony bream appears to be due to an exceptional capacity for population recovery rather than to synchronisation of the spawning cycle and flooding.

5.2 INTRODUCTION

A paper based on this chapter has been submitted for publication in the Australian Journal of Marine and Freshwater Research (Puckridge & Walker, subm., Appendix II).

In Chapter 1 it is argued that the characteristic abundance of the bony bream, and its success in the lower River Murray, indicate either an exceptional reproductive output, or close synchrony of spawning and the flood cycle. This chapter examines these features of bony bream life history.

There have been no major studies of reproduction in the bony bream. Most information is anecdotal, in general texts and reviews. Cadwallader (1977) provides estimates of the age at first maturity of the bony bream in the middle reaches of the Murray, and Lake (1967) gives anecdotal information on the breeding season, the size of ova, approximate fecundity, probable size and age at maturity and sexual dimorphism of the species in the Murray-Darling system. Llewellyn (1983), referring to the New South Wales Murray-Darling populations, adds anecdotal notes on maximum size and weight, breeding sites and ovum buoyancy. McDowall (1980) and Cadwallader & Backhouse (1983) provide similar notes for the upper and

middle Murray populations. A comment on breeding season is provided for the lower Murray populations by Scott, Glover & Southcott (1980). However, two community studies, Puckridge & Drewien (1988) and Bishop et al. (in press) constitute the only primary research. Bishop et al. provide preliminary information on fecundity, and more extensive data on maturity, reproduction and condition in relation to hydrological and seasonal cycles, sex ratios and spawning sites of bony bream in Magela Creek, tropical northern Australia. Puckridge & Drewien (1988) provide data on reproductive cycles in relation to season and hydrology from the North-west Branch of Cooper Creek, central Australia. These two studies allow a comparison of reproductive patterns from one of the most predictable and one of the most unpredictable hydrological cycles in Australia. However, neither was intended to be a definitive study of bony bream reproductive biology. The work of Bishop et al. is based on a per season sampling frequency, and Puckridge & Drewien based their accounts of reproductive cycles on catches of larvae and running ripe fish. There is no published account of the relationships between size or age and fecundity, size or age and maturity, or of egg and larval development. What primary research is available does not refer to the populations of the lower River Murray.

Several other gizzard shads have been thoroughly studied. There are comparative data on ovum development, fecundity and spawning movements of the estuarine Perth herring, N. vlaminghi, in Western Australia (Chubb & Potter, 1984). Ovum and larval characters are described in detail for the Indian Anodontostoma chacunda (Thangaraja & Ramamoorthi, 1980), and there are numerous reports on the biology of Japanese and North American gizzard shads (e.g. Kilambi & Baglin, 1969a, 1969b; Johnson, 1971; Jester & Jensen, 1972; Takita, 1978a, 1978b; Kim & Lee, 1984).

In this chapter gonadal cycles, spawning sites, sex ratio, egg and larval development, fecundity, reproductive effort and age/length-maturity relationships are examined for bony bream in the lower River Murray. Comparisons are also made with data for other gizzard shads to identify the particular attributes of bony bream, and with data for other native species to elucidate reasons for the comparative abundance of bony bream in the regulated Murray environment.

5.3 METHODS

5.3.1. Sampling

Sampling sites and general methods are described in Chapter 2.

5.3.2. Reproduction

Gonads were staged (virgin, maturing virgin, recovering, spent/developing, mature ripe and spent) according to colour, size, texture and shape (modified from Pollard, 1972); the stages were also validated against Gonado-Somatic Index (GSI) and Ovum-Diameter Frequency (ODF) cycles.

Ovum diameters were determined from material preserved in Gilson's Fluid. About 200 ova were measured for eight randomly selected females each month for one year. The ova were freed by shaking, diluted in suspension to 1l, remixed and a 10 ml subsample taken for viewing with a Leitz Diavert compound microscope fitted with a video camera and monitor. Ova were measured (largest diameter) with electronic calipers recording from the monitor screen to an Osborne 1 microcomputer via an Arlec MPF-1 microprocessor.

5.3.3. Spawning and larval development

Monthly between November 1985 and February 1986, and again in April 1986, ten 3-minute surface hauls of the ichthyoplankton trawl were made at 3-4 km h^{-1} in mid-afternoon on consecutive days at the four sampling sites. Samples were fixed in 10% buffered formalin for two weeks then preserved

in 70% ethanol. Later the larvae were immersed in glycerine, measured and drawn under polarised light. In this way a series of specimens was developed (from embryos to readily identifiable juveniles), which facilitated identification of the earlier stages.

5.3.4. Fecundity

The mature ovaries of 27 fish (c. 4 fish per 50mm size-interval from 150mm upward) captured between late October and early December 1983-85 were preserved in Gilson's Fluid. Fecundity was determined by the volumetric method (Mason, Beamish & McFarlane, 1983). Ova of >250 um diameter were counted as ODF analysis showed that only these were likely to be spawned in the current season. Subsamples were repeated until the 95% confidence intervals for the mean were <10% of the mean.

5.3.5. Age and length vs maturity

The sexual maturity of fish was assessed from gonad appearance and mean GSI (calculated monthly for each sex and size-class), and validated by ODF analysis. Records of log mean GSI per month were compared for females >300 mm TL from PS and ZL, using fish selected from the October-December catches when the distinction between maturity and immaturity

was clearest. Age determinations were based on scale readings supported by length-frequency analysis (see 4.4). The frequency distributions of size-classes of fish used were based on those of the 130 m seine catches for this period, because these most closely approached a random sample. The age vs percent maturity and class midlength vs percent maturity relations for each sex were analysed following Leslie, Perry & Watson (1945).

5.4 RESULTS

5.4.1 Gonad Morphology

The gonads of both sexes are paired and elongate, sometimes encased in fat deposits, enclosed by the peritoneum, and open externally through short, paired gonadal ducts. The left lobe normally is the larger in both sexes. The slope of the regression of left vs right gonad weight was significantly higher for females than males [n(females)=411, n(males)=310, *d*-statistic, P<0.001], indicating that the left lobe is relatively heavier in females.

5.4.2 Seasonal Cycle

In 1983-85 GSI of bony bream (both sexes combined) at ZL showed a distinct seasonal cycle with a peak in November, at water temperatures of 19-20^{o}C (Fig. 5.27A) a day-length of

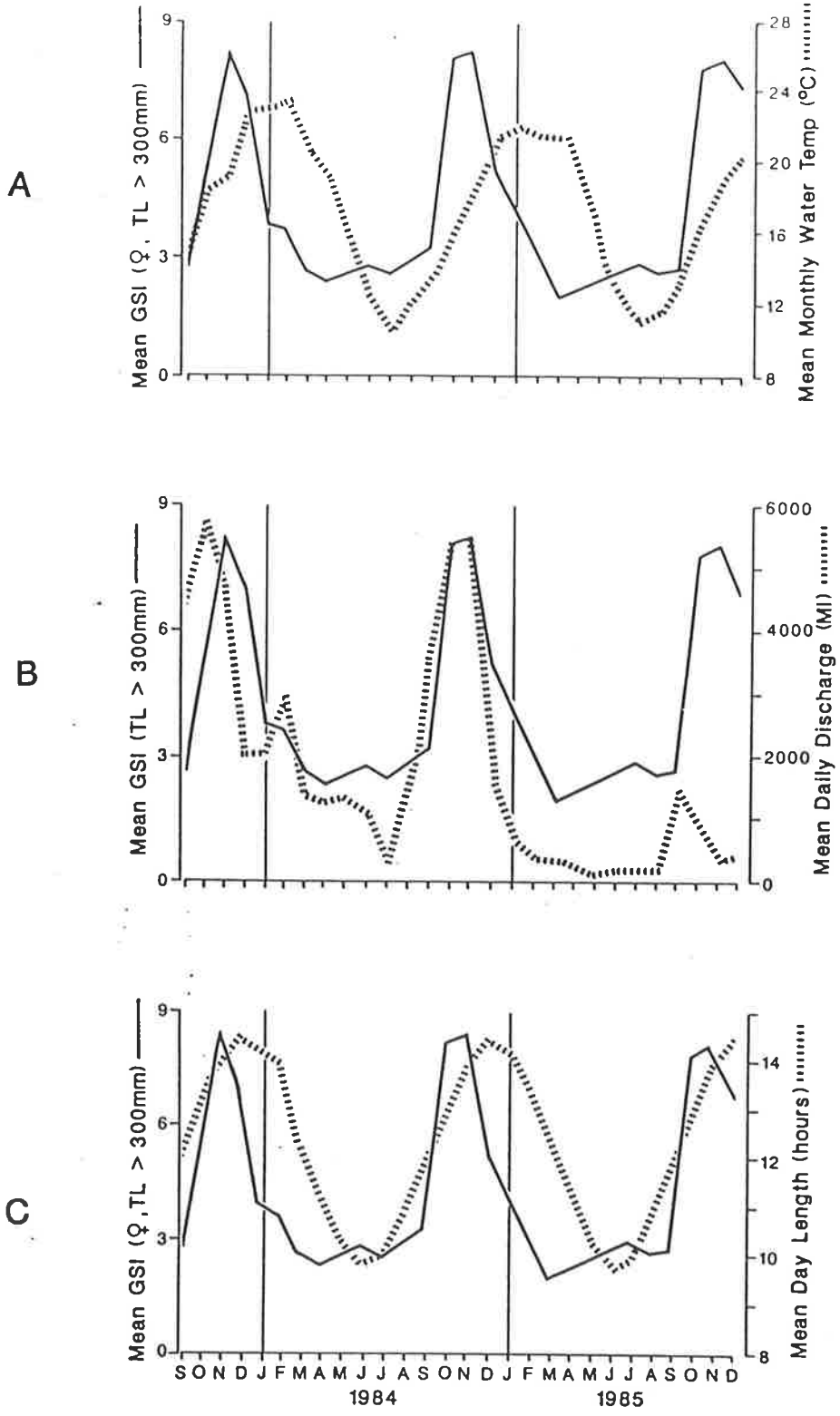


FIGURE 5.27: Mean monthly GSI of large adult bony bream at ZL in relation to **A**: mean monthly water temperature at Mannum **B**: mean daily discharge at lock 1, Blanchetown **C**: mean day length at Adelaide.

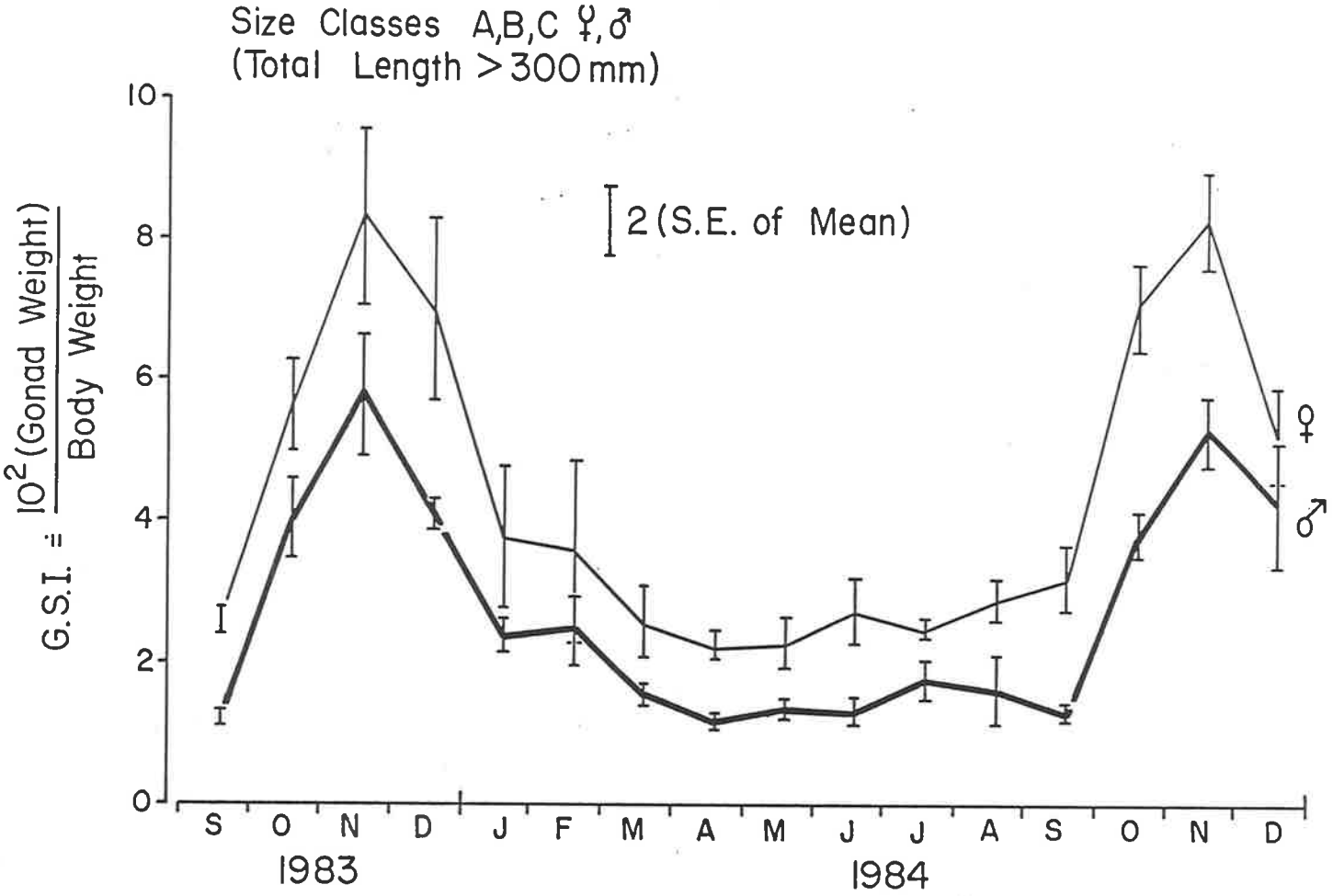


FIGURE 5.28: Mean GSI of male and female large adult bony bream at ZL, 1983-84.

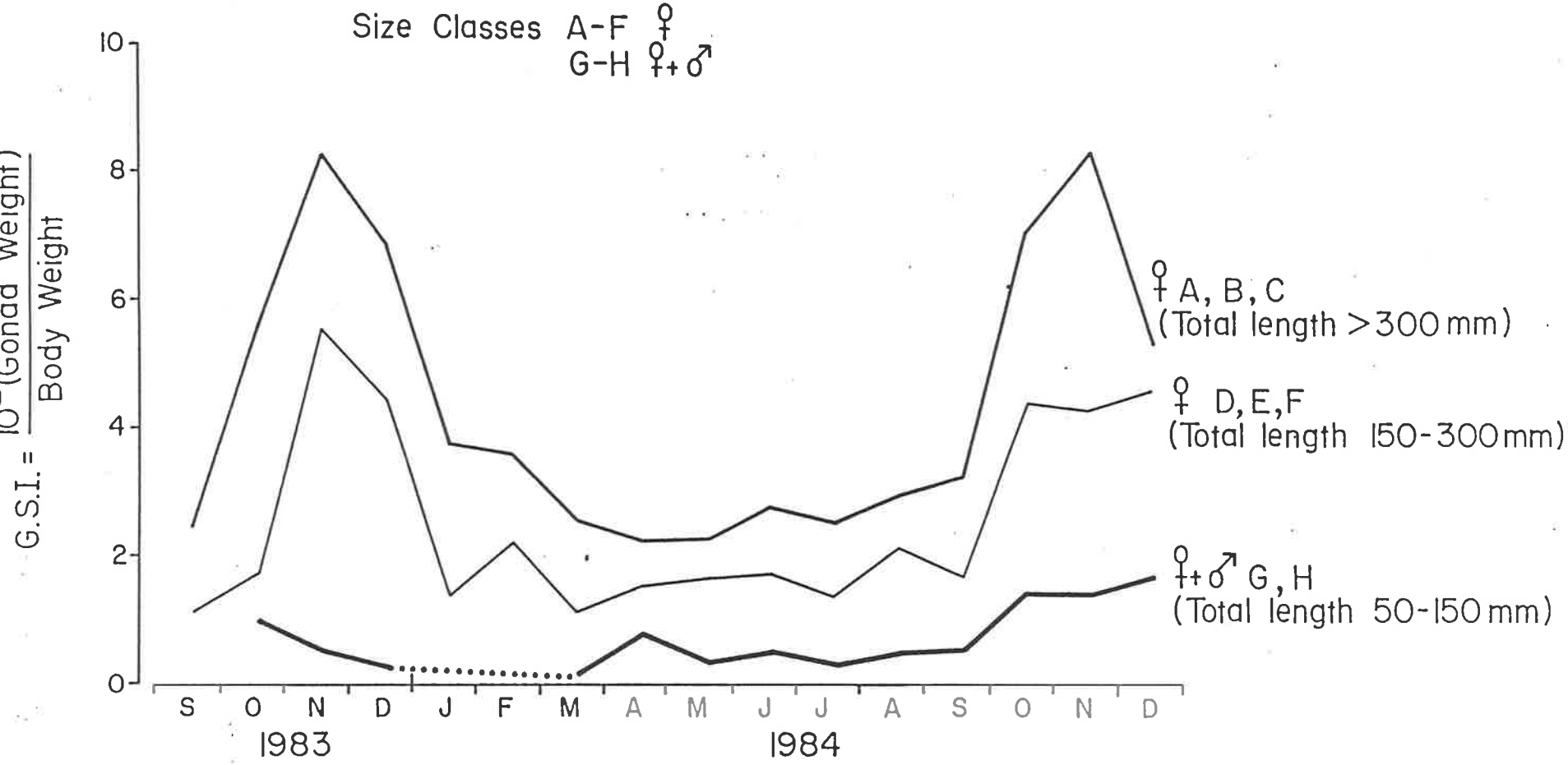


FIGURE 5.29: Mean GSI of female bony bream of three size-groups at ZL, 1983-84.

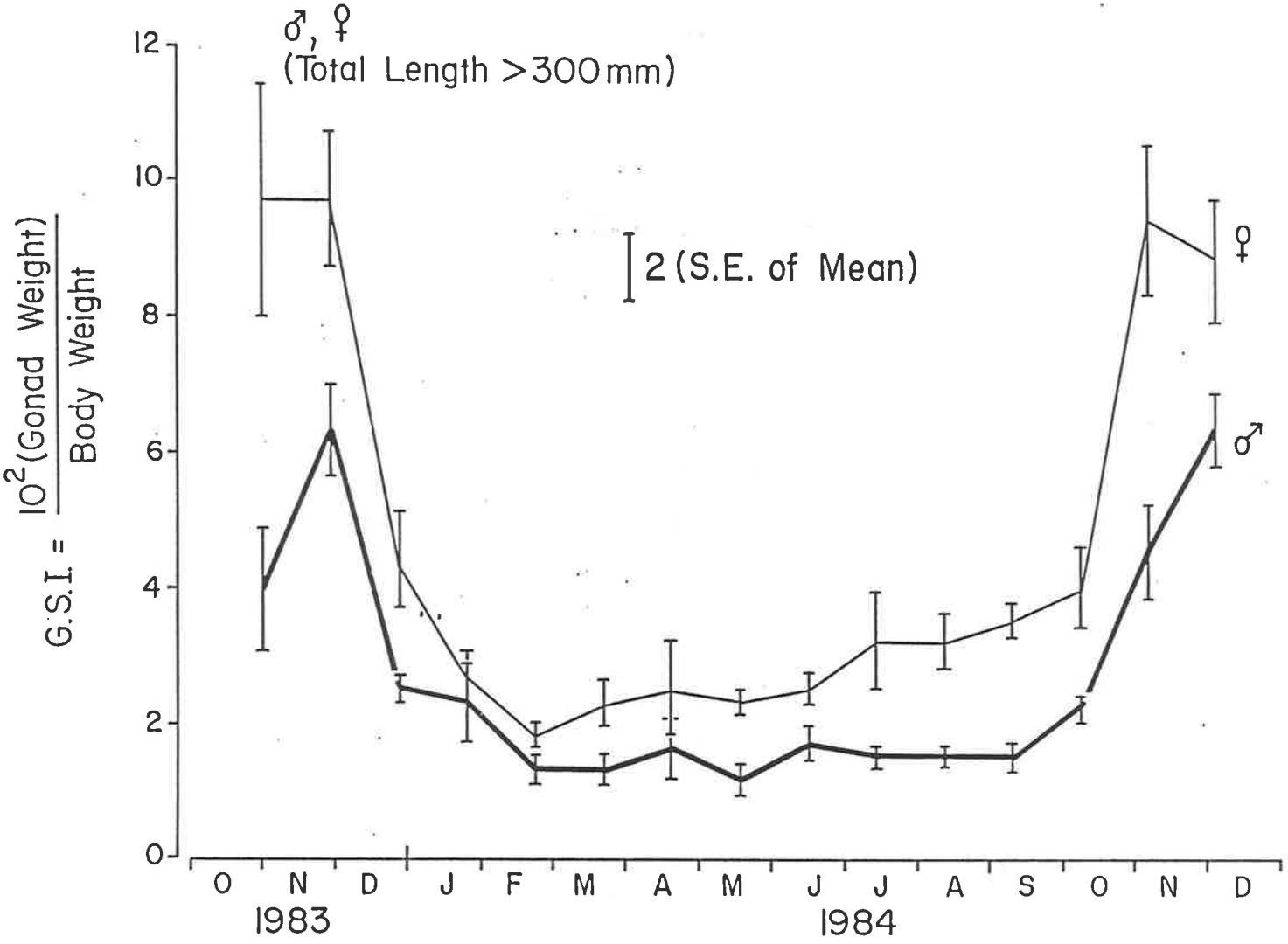


FIGURE 5.30: Mean GSI of male and female large adult bony bream at PS, 1983-84.

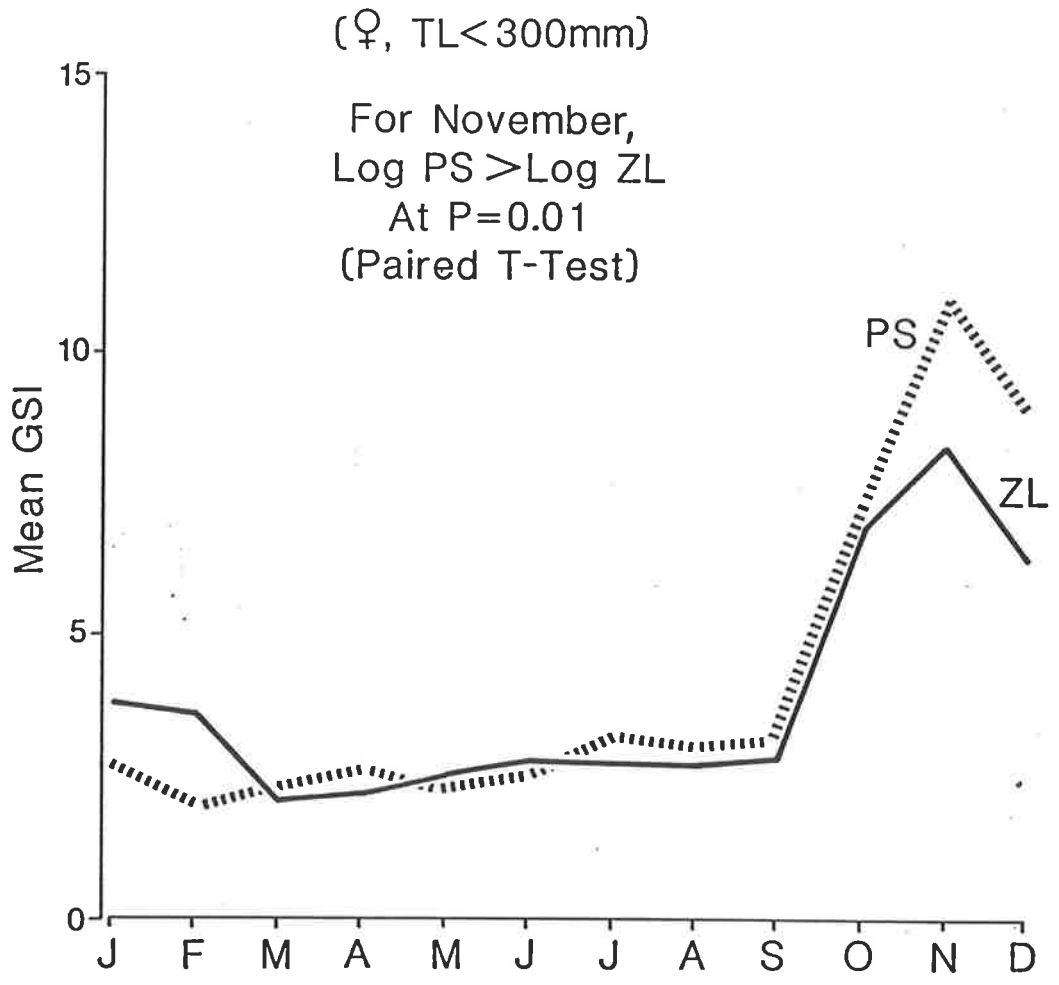


FIGURE 5.31: Comparison of mean monthly GSI of large adult female bony bream at ZL and PS, 1983-85.

14 h (Fig. 5.27C), and up to two months after the seasonal flood peak (Fig. 5.27B). The pattern was synchronized (to within a month) both for the sexes (Fig. 5.28) and the different size-classes (Fig. 5.29). The pattern was repeated at PS (Fig. 5.30). However, for fish of matched TL, the peak log female GSI at PS was significantly higher than at ZL (Fig. 5.31, n=29, paired *t*-test, P <0.01). At neither site was there any apparent adjustment of the GSI cycle in response to flood timing and intensity (Fig. 5.27B).

Vitellogenesis apparently begins in September-October (spring); maturity is attained in November-December and spawning occurs in December-February at water temperatures of 21-23^{o}C (Table 5.5). ODF analysis confirms this interpretation (Fig. 5.32). From the background of primary oocytes (mean diameter 104 um), vitellogenic ova (diameter >250 um) first appear in substantial numbers in October. In December, spawning begins to deplete the mature eggs and this process is substantially completed by January. Only one major cohort of ova separates from the reservoir of primary oocytes during the cycle, although a few mature-size oocytes are present in some individuals much later in the year (March, July and August).

TABLE 5.5: Gonad states of bony bream in 1983-84 at ZL. Note that appearance is recorded *in situ* and colours differ when the gonads are dissected free of the peritoneum.

Stage	Sex	Months	Appearance	Gonad Length/ Body cavity
Virgin	F	All year	Grey, translucent thin strips	2/3
	M	All year	Colourless to grey-white strips	2/3
Maturing Virgin	F	Apr-Aug	Pink-grey, translucent, firm	3/4
	M	Apr-Aug	Creamy pink, opaque, firm	3/4
Recovering Spent/	F	Apr-Sep	Orange-pink, translucent, firm, quilted	4/5-1.0
Developing	M	Apr-Sep	Creamy pink, firm, smooth, opaque	4/5-1.0
Mature	F	Oct-Dec	Yellow-orange, bloodshot, granular, opaque, quilted, distended	1.0
	M	Oct-Dec	Creamy white, turgid, blocky	1.0
*Ripe	F	Dec-Jan	Translucent in patches, light yellow, quilted, distended, ova distinct	1.0
	M	Dec-Jan	Creamy white, turgid, blocky	1.0
Spent	F	Dec-Mar	Grey-pink, watery, semi-translucent, flaccid	1.0
	M	Dec-Mar	Pink to dark pink, bruised, flaccid	1.0

*Gonad products extruded on pressure

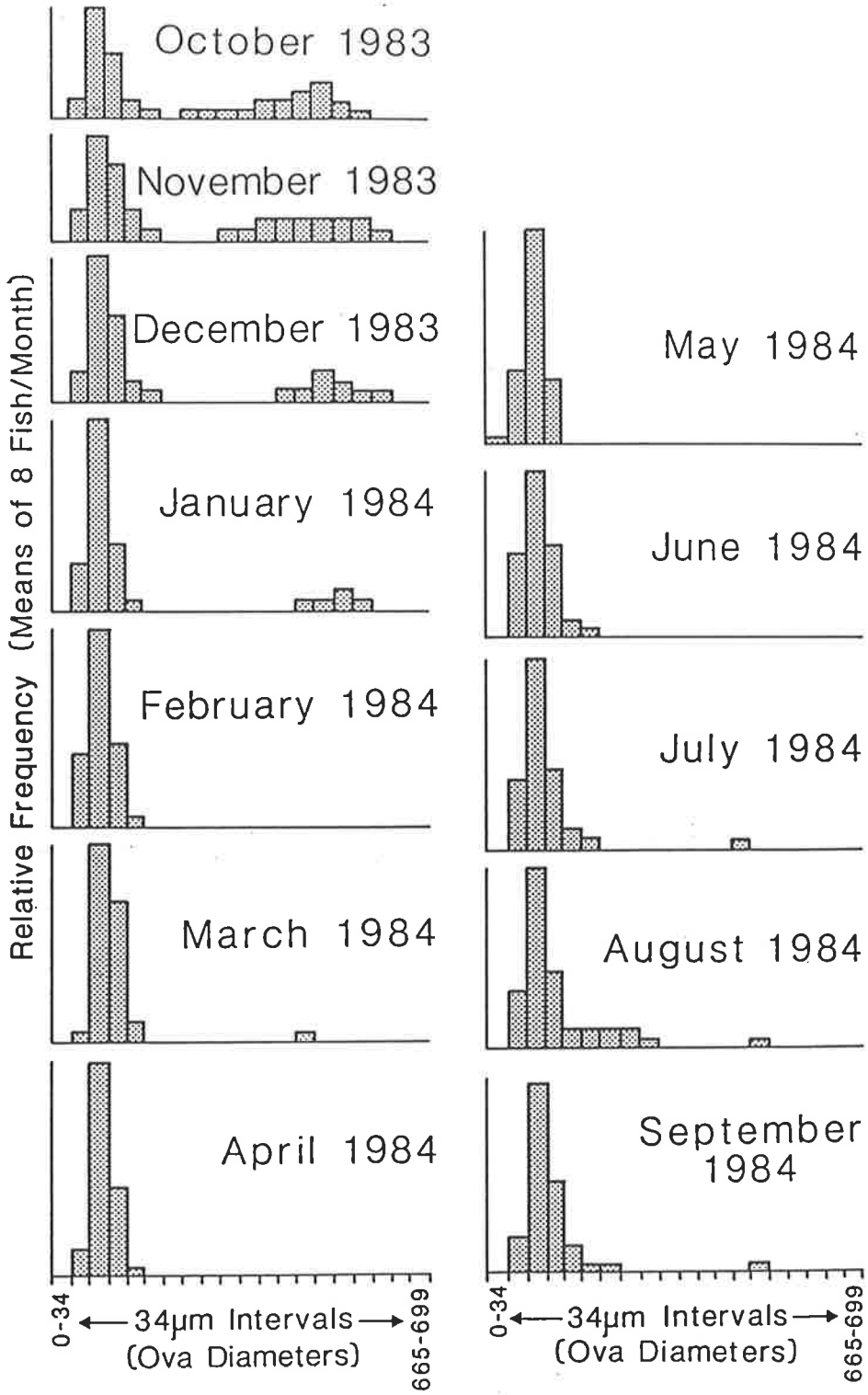


FIGURE 5.32: Monthly relative frequencies of bony bream ova diameters at Z1 and PS combined.

5.4.3 Spawning

Spawning was not directly observed and attempts to rear artificially-fertilized ova were unsuccessful. Female fish were rarely encountered in ripe condition, but most males maintained this condition throughout the breeding season. At only one site did ichthyoplankton trawls yield substantial numbers of ova; this was off a sandy shore west of PS early in January 1986 (Fig. 5.33). The catch/effort of bony bream larvae there was significantly higher than at ZL (Mann - Whitney, P=0.001) (Fig. 5.34). No larvae were caught at sites M and F.

5.4.4 Sex Ratio

The sexes in bony bream are externally indistinguishable. There is no significant difference in the slopes of the $\log_{10}$ body length vs $\log_{10}$ body depth curves for the two sexes [n(females)=401, n(males)=341, *d*-statistic; P >0.05].

In the 1105 fish examined at both PS and ZL (Fig. 5.35) the ratio of males to females was 0.86 and significantly different from unity (Chi-squared, P <0.05). Females were significantly more abundant in the largest size-class (TL >350 mm) than overall (n=263, Chi-squared, P <0.001). However, they were significantly less abundant than males in the middle size-class (250 <TL <350 mm, n=361, Chi-squared,

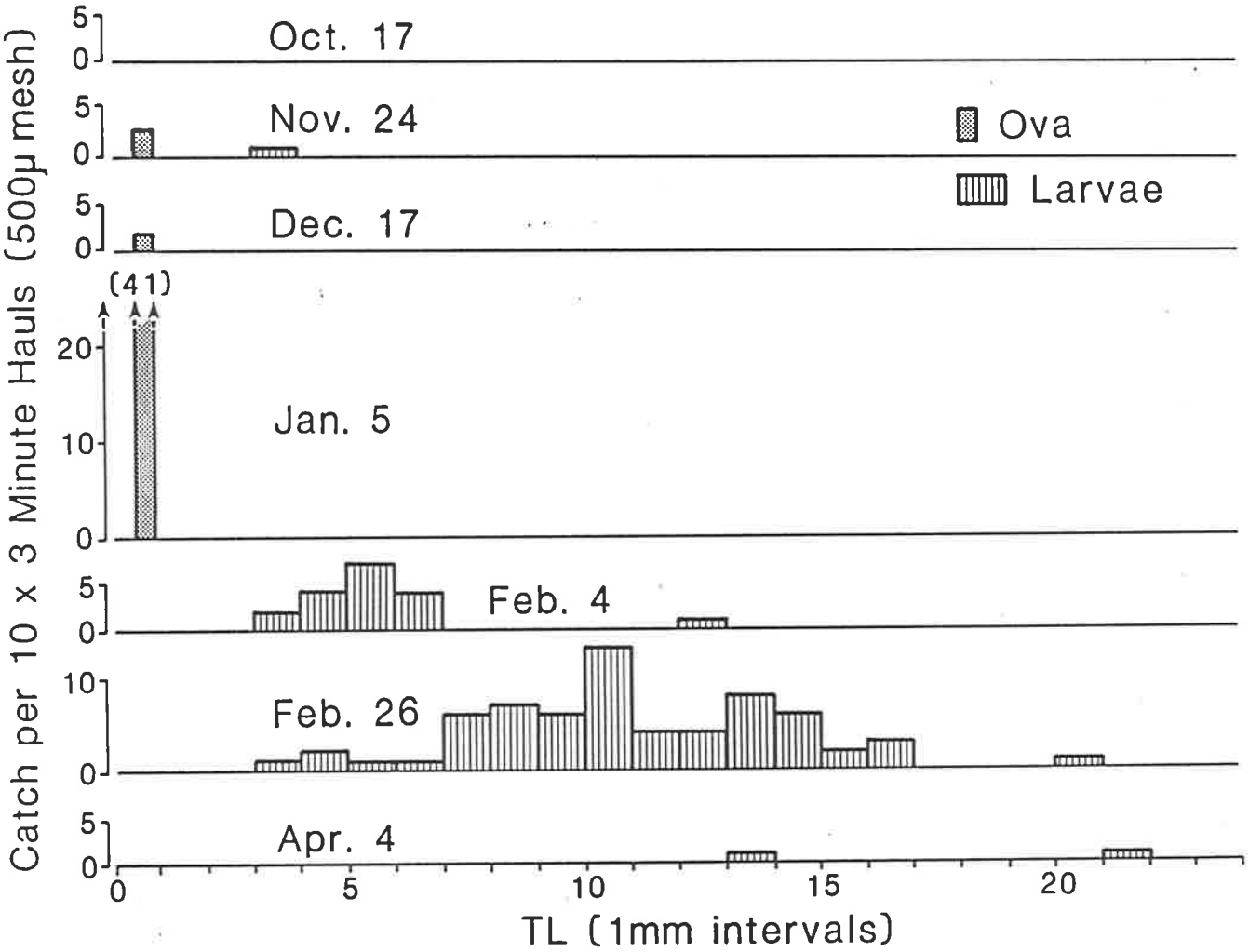


FIGURE 5.33: Monthly catch/effort of bony bream ova, and length - frequencies of bony bream larvae trawled at PS, 1985-86.

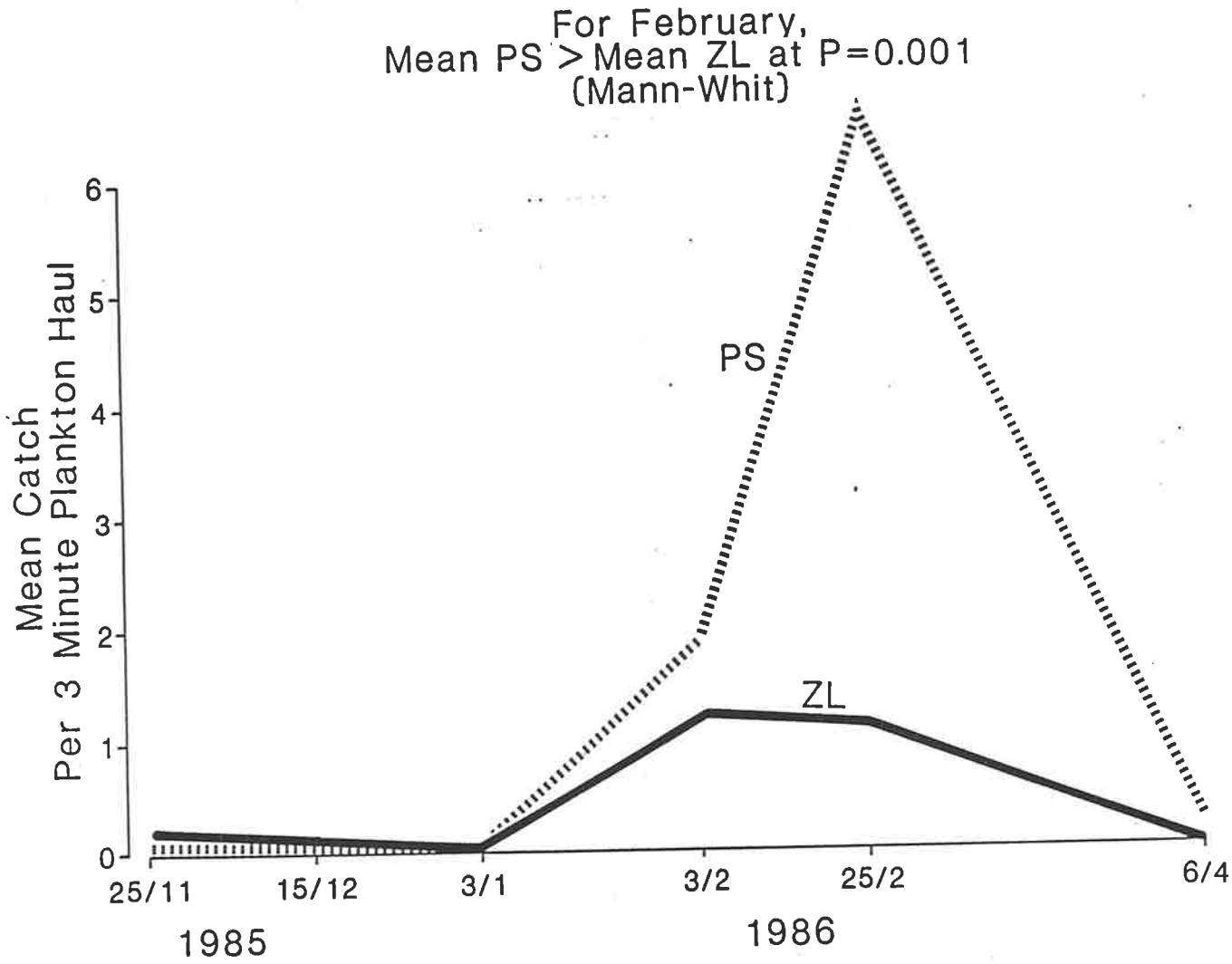


FIGURE 5.34: Comparison of monthly trawl catches of bony bream larvae at PS and ZL, 1985-86.

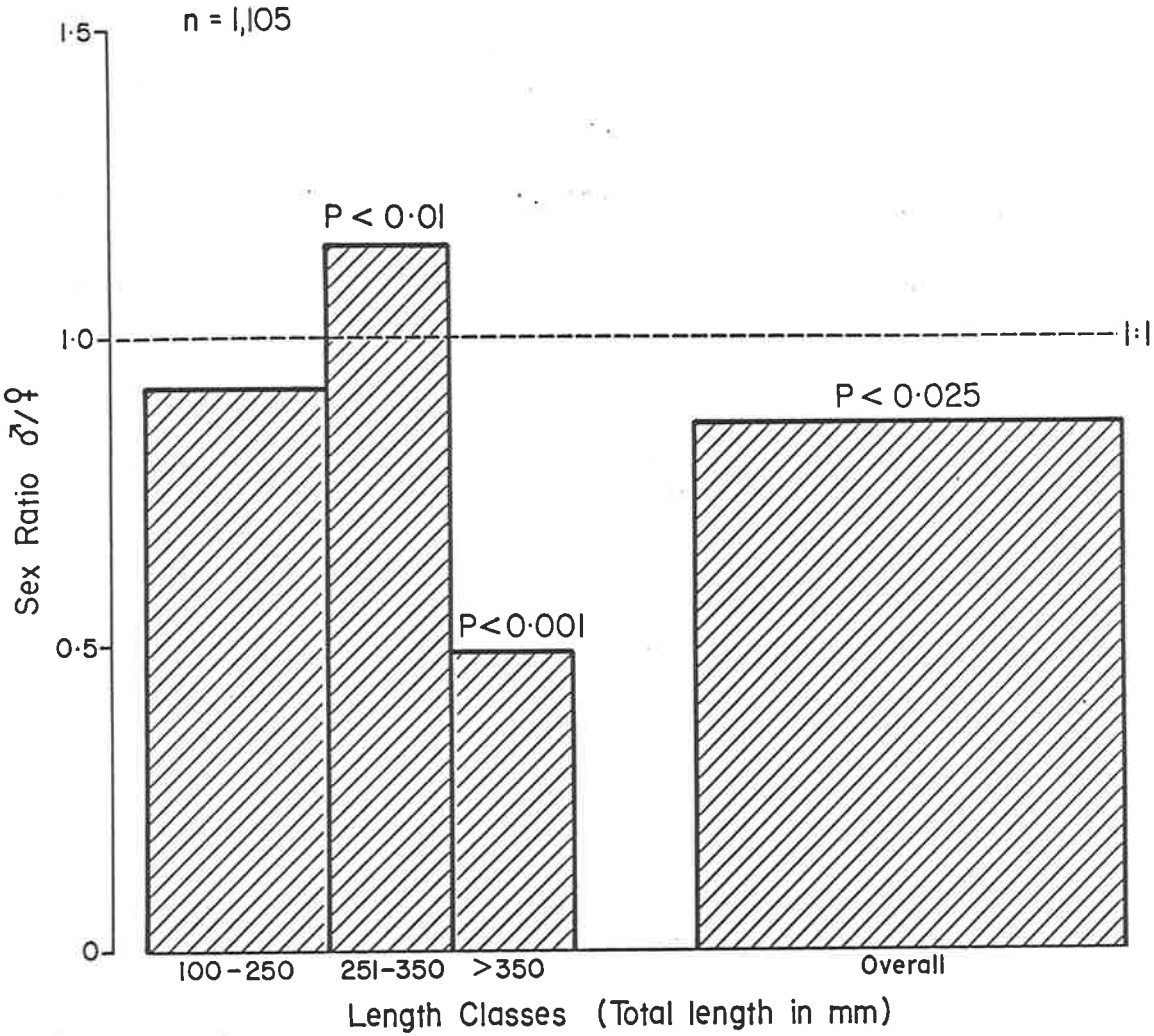


FIGURE 5.35: Sex ratios of size-groups of bony bream in gillnet catches at ZL, 1983-84.

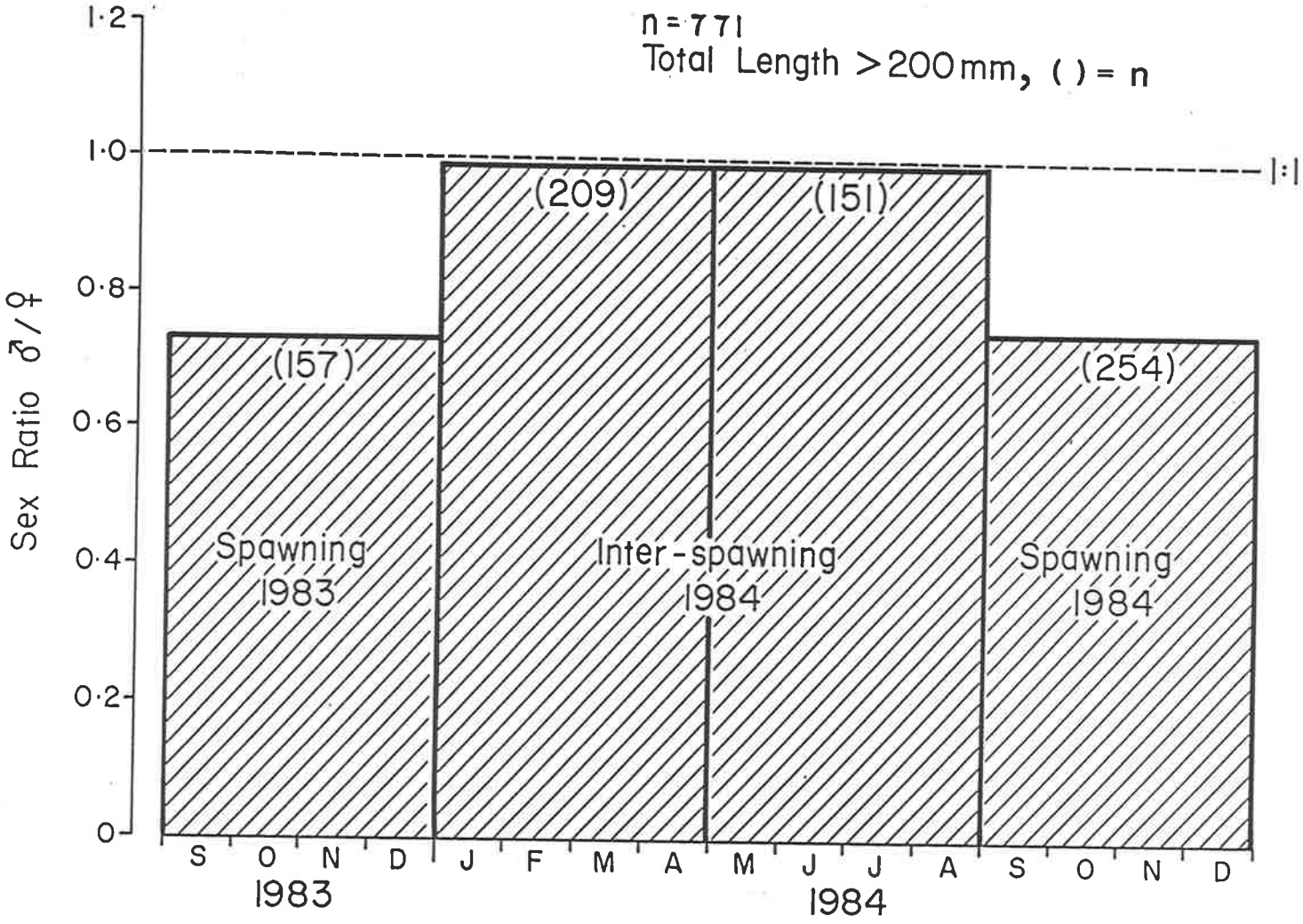


FIGURE 5.36: Sex ratios of adult bony bream in gillnet catches during spawning and inter-spawning periods at ZL, 1983-84.

P (0.01) and abundances were not significantly different in the lower size-class (100 (TL (250 mm, n=481, Chi-squared, P >0.05). The female to male ratios for the pre- and non-spawning periods were significantly different (n=771, Chi-squared, P (0.001), with higher catches of females in the pre-spawning period and higher catches of males in the remainder of the year (Fig. 5.36).

5.4.5 Egg and Larval Development

Bony bream ova are spherical, with a smooth chorion and finely-segmented yolk. The water-hardened ova collected by ichthyoplankton trawl (Fig. 5.37) had a mean diameter of 0.829 mm (± 0.016 95% CI, n = 41); these were apparently semi-buoyant, as they were trawled from surface waters. Newly-stripped water-hardened ova were of similar diameter (0.834 ± 0.036 mm, n = 16), but were demersal and adhesive.

In the trawled ova the yolk diameter was 0.54 mm and the chorion thickness c. 0.010 mm, leaving a perivitelline space of 0.14 mm. The ova typically contained a single large oil-droplet (diameter 0.26 mm) with the micropyle clearly visible alongside (Fig. 5.37A). An extra-chorionic layer was revealed by attached sand grains. Twenty percent of the trawled ova had clearly recognizable embryos and the more highly developed showed eye pigmentation and a ventro-

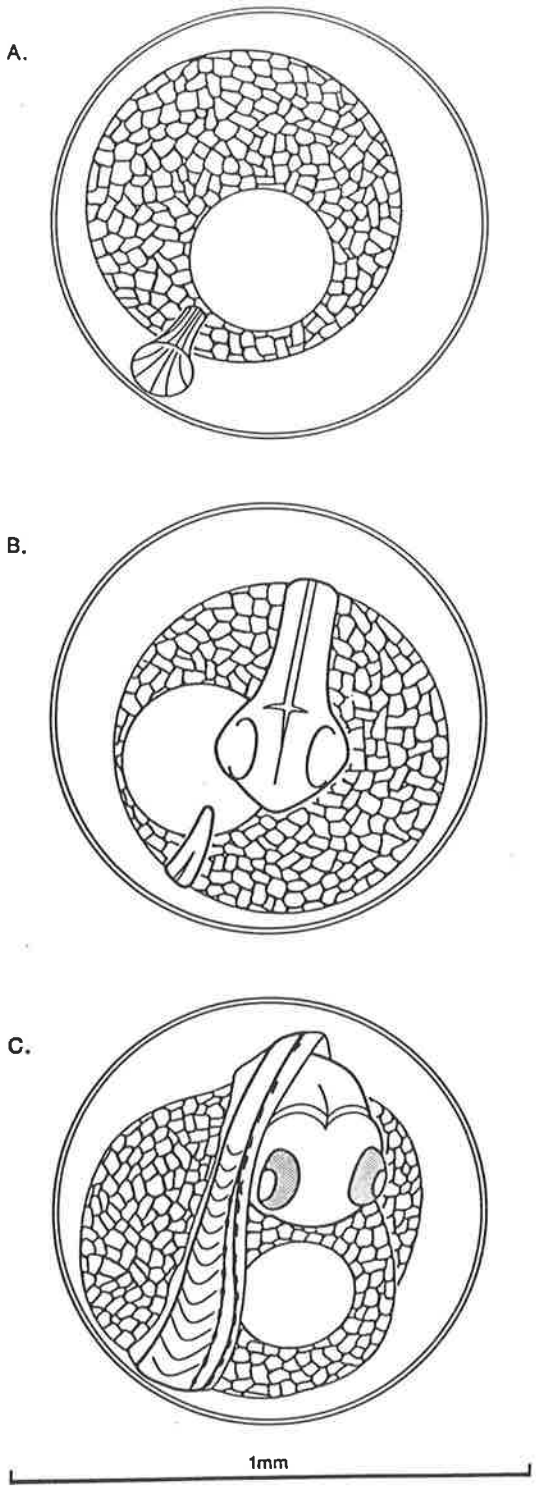


FIGURE 5.37: Bony bream ova and embryos from trawl catches at PS, Jan. 1986.

lateral line of melanophores (Figs. 5.37B,C). The largest embryo found was 2.5 mm TL and the smallest free larva was 3.5 mm TL; the size at hatching therefore probably lies between these figures.

The larvae of bony bream, smelt (Retropinna semoni) and western carp gudgeon (Hypseleotris klunzingeri) comprised almost all of the trawled catch. Western carp gudgeon larvae can be distinguished from those of bony bream by body shape and mean myomere count (31 ± 0.97, n = 8; cf. 44.7 ± 0.53 for bony bream, n = 33, with pre-anal count 38.5 ± 0.83). The larvae of smelt have an eel-like form similar to that of bony bream larvae (Fig. 5.38), and both absorb the yolk sac early; hence discrimination of these must be based on mean myomere counts (53.6 ± 0.95 for smelt, n = 9). After dorsal fin development has begun smelt and bony bream can be readily distinguished by the relative positions of that fin and the anal primordium along the anterior-posterior axis (in smelt, the dorsal overlaps the anal fin; in bony bream it does not). Although this description is incomplete because yolk-sac larvae were not collected, the sequence of major changes in larval development is clear:

(1) The yolk sac is largely absorbed by 3.5 mm TL.

(2) The caudal and pectoral fins develop rays first at

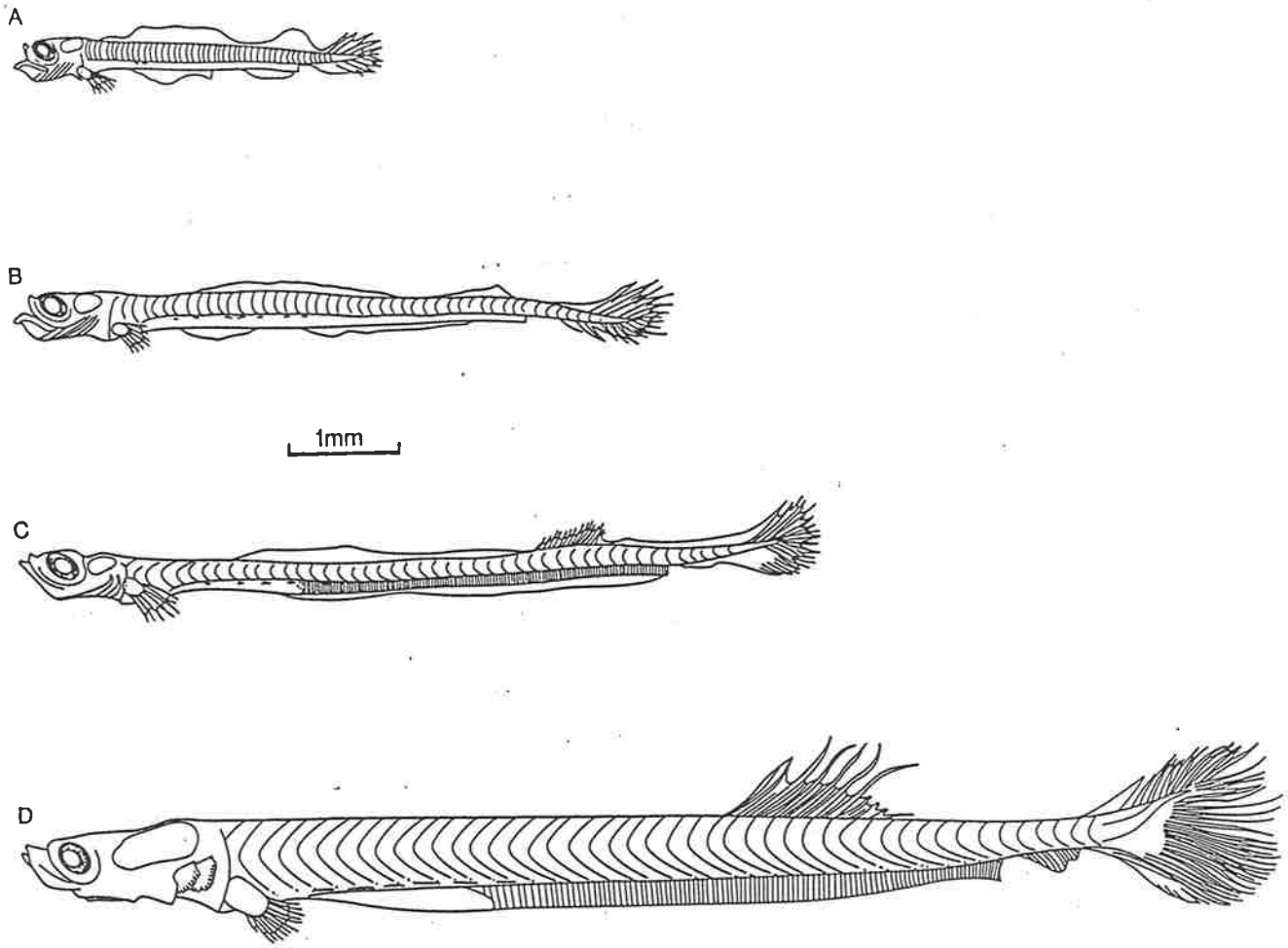


FIGURE 5.38: Bony bream larvae from trawl catches at PS, Feb. 1986.

approximately 3 mm TL. The dorsal fin follows at 7 mm, the anal fin at 11 mm and the pelvic fin at 16 mm.

(3) Throughout larval development melanophores are present in a line along the dorsal border of the gut. After 6 mm TL a single melanophore becomes obvious ventrally and immediately anterior to the cleithrum, and a pair appears ventrally immediately posterior to the cleithrum. A fine mid-ventral line of melanophores also appears along the hindmost 2/3 of the gut.

(4) Caudal flexion of the notochord begins at 10 mm TL.

(5) Anterior migration of the anus and the dorsal and anal fins is noticeable at 17 mm.

(6) First scalation appears along the lateral line at 26 mm and is complete by 35-40 mm.

5.4.6 Fecundity

Fecundity (F) rises from 33,000 for a fish of 199 mm TL (body weight 88.9 g) to 880,000 for a fish of 403 mm TL (595.4 g) (Figs. 5.39,5.40). These estimates are derived from the equations

$$\log_{10} F = -3.923 + 3.725 (\log_{10} TL)$$

$$(n = 27, r^2 = 0.88, \text{F-test}, P<0.001)$$

and $$\log_{10} F = 2.188 + 1.290 \log_{10} (\text{body weight})$$

$$(n = 27, r^2 = 0.91, \text{F-test}, P<0.001).$$

A log-log equation best describes the relation between fecundity and gonad weight, but the goodness of fit is little better than that for the equation based on untransformed data (r = 0.92). Thus

$$\log_{10} F = 3.822 + 1.131 \log_{10} (\text{gonad weight})$$
$$(n = 27, r^2 = 0.95, \text{F-test}, P < 0.001).$$

There is a significant relation between fecundity, body weight (exclusive of gonad weight) and TL, viz.

$$F / (\text{body weight} - \text{gonad weight}) = 117.58 + 2.54\ TL$$
$$(n = 27, r^2 = 0.28, \text{F-test}, P < 0.01).$$

Log-log plots of GSI vs TL (Fig. 5.41) yield highly significant regressions for males and females (respectively,

$\log_{10}GSI = -2.462 + 1.253\log_{10}TL$

[$n = 87, r^2 = 0.32$, F-test, $P < 0.001$)];

$\log_{10}GSI = -5.615 + 2.547\log_{10}TL$

[$n = 154, r^2 = 0.68$, F-test, $P < 0.001$)].

The slope is significantly greater for females (d-statistic, $P < 0.001$), indicating a more rapid rise in GSI with body size for females compared with males.

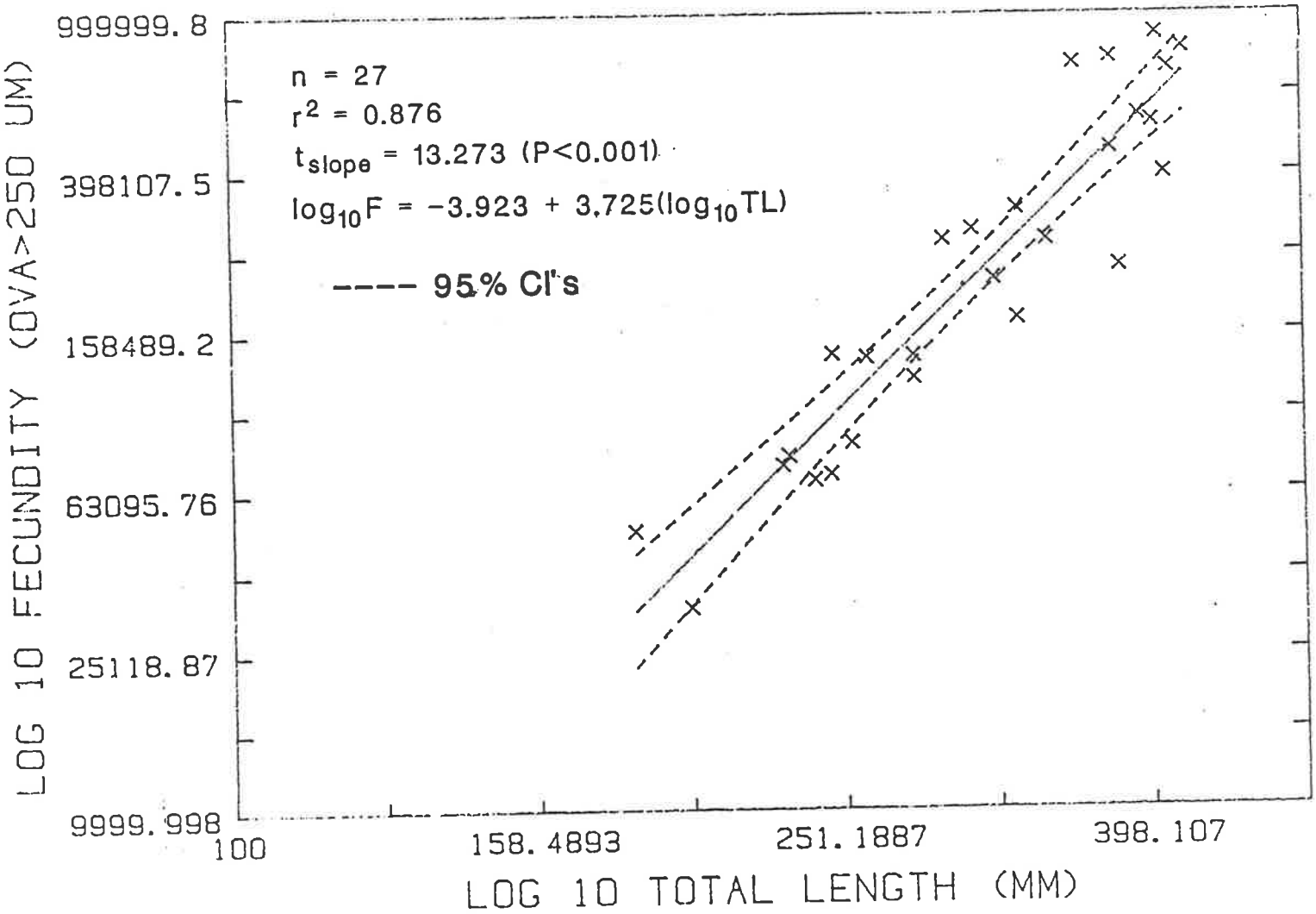


FIGURE 5.39: The log fecundity vs log total length relation in bony bream at ZL, 1983-85.

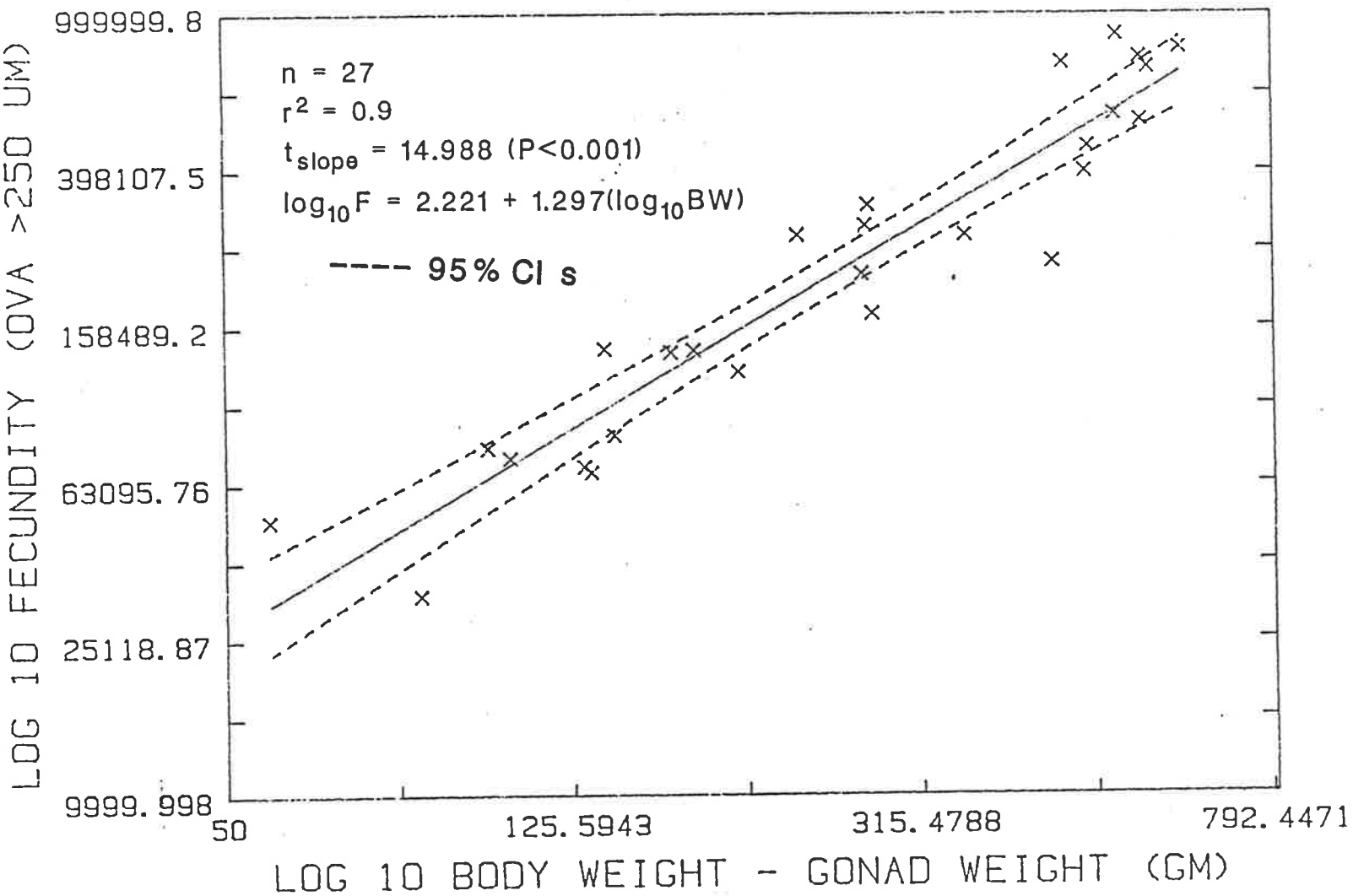


FIGURE 5.40: The log fecundity vs log (body weight-gonad weight) relation in bony bream at ZL, 1983-85.

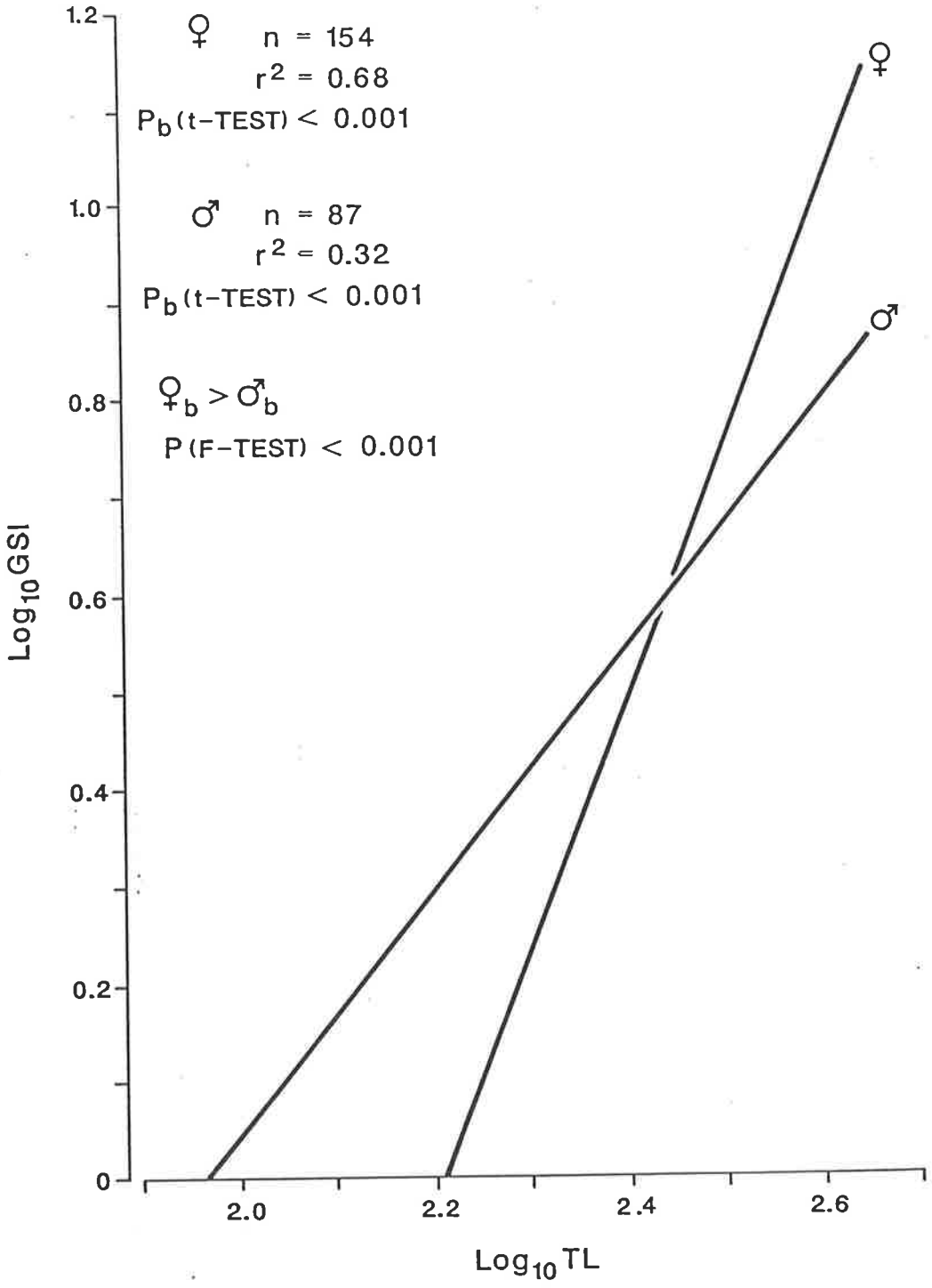


FIGURE 5.41: The relation between reproductive effort (log GSI) and log TL in male and female bony bream at ZL, Nov. - Dec. 1983-85.

5.4.7 Length and Age at Maturity

For both sexes, plots of percent mature fish in a length-group against the midlength of that group produce sigmoidal curves (Fig. 5.42A). Many males mature at a smaller TL than females, but immature males are also found to a greater length. Probit analysis shows a significant difference between the intercepts of the regressions for males and females (Chi-square, n(males)=n(females)=13, P<0.005), but not between the slopes (Chi-square), (Fig. 5.42B). The median lengths at maturity for males and females are 159 mm TL (n=108, 95% confidence limits 144-175) and 199 mm TL (n=121, 95% confidence limits 180-234) respectively.

Similarly, in a plot of percent mature fish against age (Fig. 5.43A), males mature earlier and consequently both mature and immature males can be found over a greater range of ages. In this case, however, probit analysis shows the regressions for the sexes differ in both intercept (Chi-square, n(males)=5, n(females)= 3, P<0.05) and slope (Chi-square, P<0.05) (Fig. 5.43B). The median ages at maturity are 2.4 y (n=212, 95% confidence limits 1.6-3.4) for males and 2.7 y (n=231, 95% confidence limits 2.4-3.6) for females. Length at first maturity (LFM) for males was 126 mm TL (age 0+) and for females 155 mm TL (age I+).

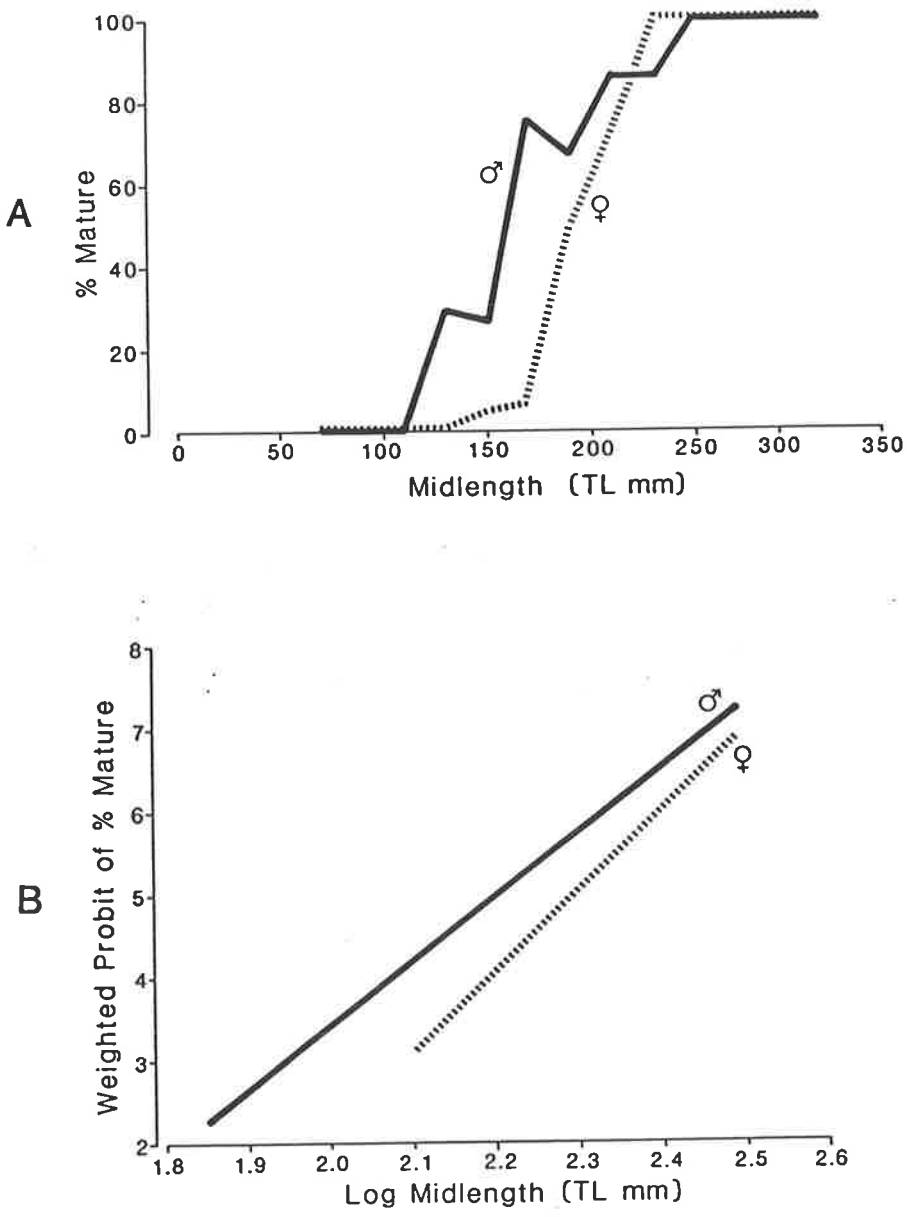
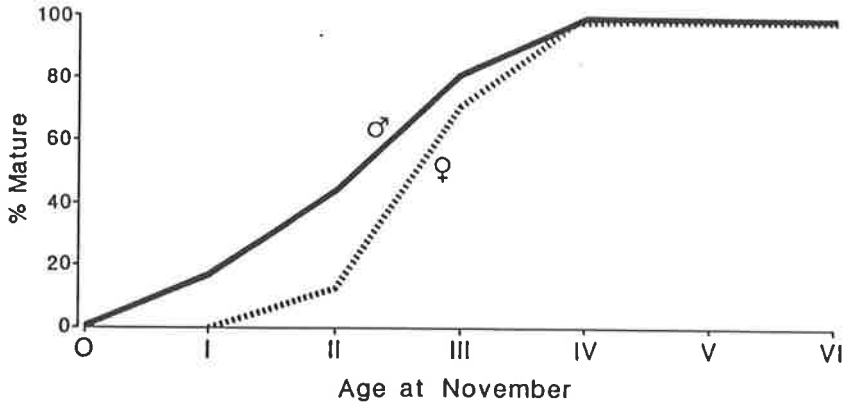


FIGURE 5.42: Percentage of mature bony bream vs midlengths of total length classes at ZL and PS, Oct.- Dec. 1983-85: A: untransformed data. B: probit-log transformed data.

A



B

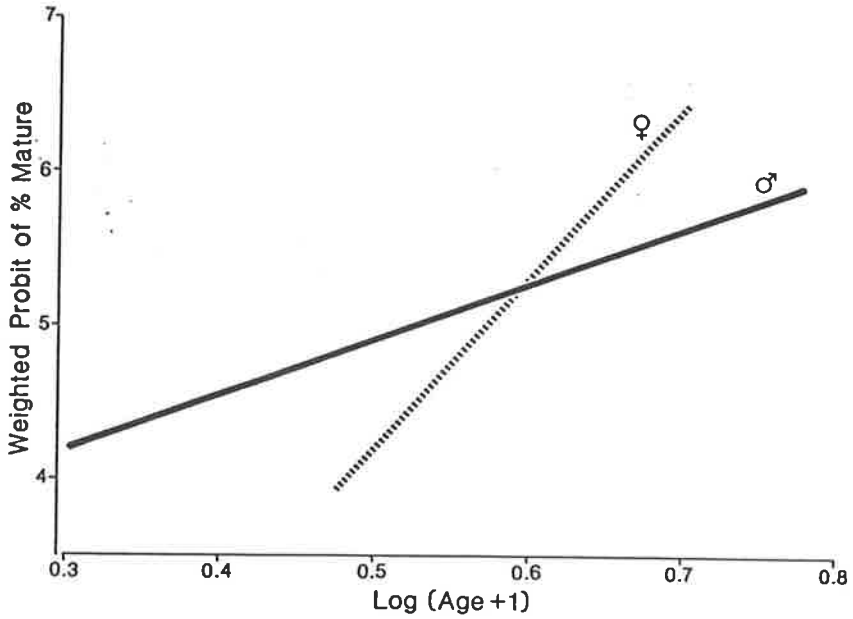


FIGURE 5.43: Percentage mature bony bream vs age at ZL and PS, Oct - Dec 1983-85: **A:** untransformed data. **B:** probit-log transformed data.

5.5 DISCUSSION

In bony bream, as in most gizzard shads, the sexes are externally similar (Miller, 1960; cf. Bodola, 1966). The male:female ratio of 0.86:1 reported here contrasts with that of 1:1 reported for bony bream in northern Australia (Bishop et al., in press), and with 1.6:1 for D. cepedianum (Jester & Jensen, 1972). In both bony bream and D. cepedianum, however, there are more females in the largest size-ranges, suggesting that the sexes may have different growth or mortality rates. The reduced incidence of males in spawning - season catches may arise from seasonal behavioural differences that cause them to outnumber females on the spawning grounds, as for Dorosoma spp. (Warner, 1941; Bodola, 1966; Jester & Jensen, 1972). The observation that males were running ripe throughout the breeding season (as in D. petenense, Johnson, 1971) suggests that multiple matings by males may be common. During the spawning season this would deplete by emigration the male population of non-spawning areas like ZL. Unfortunately an equivalent size-distribution (and hence a comparison of the sex ratio) is not available for PS, which is close to a known spawning ground.

The fecundity of bony bream is high (cf. Lake, 1967; Merrick

& Schmida, 1984; Bishop et al., in press) and exceeds that of other gizzard shads (Rao, 1965; Kilambi & Baglin, 1969b; Chubb & Potter, 1984) except D. cepedianum (Kilambi & Baglin, 1969a). The bony bream also shows an exceptional rate of increase in fecundity with body size. Egg size evidently does not change significantly with body size - hence the trivial difference between the untransformed and log-log equations for fecundity vs gonad weight (cf. Mann & Mills, 1985). Other relationships demonstrate that reproductive effort increases with size, and that female effort increases more rapidly than that of males. The rate of change of reproductive effort with size evidently depends on local conditions and/or stocks; some populations of D. cepedianum show an increase (Fagan & Fitzpatrick, 1978) and others a decline (Bodola, 1966; Jester & Jensen, 1972; Shelton, 1972). The steep increase of reproductive effort with size in the lower Murray population of bony bream is consistent with the species' pattern of size - specific mortality, which means that larger (and presumably older) fish provide a more secure reproductive investment than smaller (and younger) fish (Murphy, 1968). Also, early high fecundity may involve a cost in terms of reduced later fecundity and even survival (Bell, 1980). However, an early peak in fecundity is more effective in raising natality than a much higher fecundity later (Meats, 1971), so in this

respect bony bream life history favours security over potential rate of population increase.

Bony bream in the lower Murray spawn in December-January, later than in New South Wales (October-December: Llewellyn, 1983), but at the same time as N. vlamingbi in the Swan estuary (Chubb & Potter, 1984). Bony bream in tropical Australia show little spawning seasonality, although there is a peak in the early wet season which probably represents a response to flooding (Bishop et al., in press). Flooding is not an essential cue to spawning in the River Murray populations, as it is for certain other Murray species (cf. Cadwallader, 1986). Indeed, bony bream are known to spawn in isolated water-bodies remote from the influence of floods (Lake, 1978; Puckridge & Drewien, 1988). The present data provide no clear evidence of synchrony, in either amplitude or timing, of the GSI and flood cycles. However, an intense May-June spawning occurred in the northern lakes of the Northwest Branch of Cooper Creek in 1988 (Puckridge, unpub. data), following an exceptional flood peak in April. This spawning took place four months later than that recorded in the previous year (Puckridge & Drewien, 1988), and at water temperatures 10°C lower. It was confined at the time of sampling to the most recently flooded of the lakes. Evidently the spawning behavior of bony bream - at least in

the lower Cooper - is responsive to certain flood events; in the absence of such events, spawning occurs on a seasonal basis. It may be that flooding is not as important a spawning stimulus in the lower Murray, where the scale of hydrological variation is much less than in the Cooper. Also, to describe a species' spawning behaviour as flood-dependent or not (*sensu* Cadwallader 1986) may be too simplistic; it is more likely that the spawning behaviours of species vary along a spectrum in their sensitivity to flood stimuli. Flood-induced spawning may also occur intermittently in the lower Murray population; but the nature and hence the frequency of flood events needed to induce such spawning in the lower Murray is unknown.

For bony bream, Lake Alexandrina (PS) may be a better breeding environment than the main channel of the Murray (ZL); this is suggested by the higher peak female GSI and catch/effort of larvae at the lake site. Open, sandy shallows appear to be the preferred spawning habitat in the lower Murray, but in reaches of the Murray-Darling system in New South Wales spawning reportedly occurs in schools in shallow backwaters (Llewellyn, 1983). In northern Australia spawning may be concentrated in muddy lowland lagoons (Bishop *et al.*, in press). *D. cepedianum* and *D. petenense* also spawn in backwaters or lake coves (Jester & Jensen,

1972; Shelton, 1972; Littlejohn, Holland, Jacobson, Huston & Hornung, 1985). Unlike D. petenense (Gerdes & McConnell, 1963), the bony bream does not appear to spawn near aquatic vegetation.

Some gizzard shads, like the bony bream, spawn only a single batch of ova (Annigeri, 1967; Chubb & Potter, 1984), others spawn serially (Annigeri, 1967; Baglin & Kilambi, 1968; Johnson, 1971;). The major stages in gonadal development for bony bream show typical diagnostic features (e.g. Pollard, 1972), and resemble the histologically-based staging described for N. vlaminghi (Chubb & Potter, 1984). The presence of mature-sized oocytes in some individuals late in the year (March, July and August) suggests either a capacity for opportunistic spawning (perhaps in response to exceptional flood events) or incomplete atresia (some specimens were found with hardened egg-masses).

The smooth chorion and finely-segmented yolk of bony bream ova is typical of clupeids (McGowan & Berry, 1984). Although Llewellyn (1983) suggested that the ova were semi-buoyant, they apparently have an early demersal and a later buoyant phase making bony bream, like D. cepedianum, litho-pelagophils sensu Balon (1975). It has been suggested (Reynolds, 1983) that the upstream spawning migration of

callop and silver perch in the River Murray safeguards against the loss of pelagic eggs to the sea. There is no evidence for upstream spawning migration in adult bony bream. The combination of an early demersal and later buoyant phase in bony bream eggs may be a compromise between the need for dispersal and the risk of drift into the sea.

The pigmentation patterns, body shape, gap between dorsal and anal fins and forward migration of the anus in bony bream larvae are also typically clupeid (McGowan & Berry, 1984). However, the rate of absorption of the yolk-sac in bony bream after hatching is exceptional (complete by 3.5 mm TL), as D. cepedianum and D. petenense retain it to at least 5 mm TL (Shelton & Stephens, 1980) and A. chacunda retains it until 5 mm TL (Thangaraja & Ramamoorthi, 1980). This may indicate a relatively rapid development rate in bony bream embryos and pro-larvae, but this awaits confirmation. The early development of the pectoral fin-rays in bony bream larvae is also unusual among clupeids (McGowan & Berry, 1984), although observed in A. chacunda (Thangaraja & Ramamoorthi, 1980).

It has been suggested that larval callop, because of their small total length at first feeding and their consequent poor prey capture ability, are particularly dependent at this time on high zooplankton densities (Arumugam & Geddes,

1987). The development of larval callop in this model would pass through a 'critical period' resembling that proposed for the development of marine fish larvae (Hjort, 1914). Bony bream larvae exhaust their yolk supply at a smaller TL than any other commercial Murray species, including callop (Arumugam & Geddes, 1987). If the callop model is applicable to other species, the survival of bony bream larvae must depend even more acutely on zooplankton density at first feeding. Further, if the model of flood-induced zooplankton succession is correct (Arumagam & Geddes, 1987; Pierce & Walker, subm.), then an adequate density of appropriate zooplankters can only be provided by substantial flooding. According to these models, the survival rate of larval bony bream will depend on the degree of synchrony between flooding and spawning. In this respect, the models resemble the "match-mismatch" hypothesis proposed to explain variation in recruitment of North Sea commercial species (Cushing, 1972). In these species the timing of spawning for each stock is relatively constant, but climatic events which determine the onset of the spring production cycle are variable. It is hypothesised that larval survival is dependent on the degree of synchrony between spawning and the production cycle.

However, the dependency of bony bream larvae on zooplankton densities at first feeding has not been tested, and the 'critical period' concept has been challenged (May, 1974). Further, the initiation of zooplankton succession by flooding has been demonstrated in hatchery ponds, not in the Murray. Overall densities of zooplankters in the main channel of the lower Murray respond to a variety of parameters, and do not show a clear relationship to either season or flows (Shiel, Walker & Williams, 1982). In Lake Alexandrina however, although total microcrustacean densities are relatively stable, a decline does occur during periods of low flow, and this is reversed by flooding (Geddes, 1988). Information is lacking about zooplankton responses to flooding in lower Murray floodplain habitats.

Although it is common in fish biology to use an "age at first maturity" index (AFM/LFM), this index says little about the age (or length) - maturity relationship. Moreover, the index is sensitive to sample size. Probit analyses offer useful supplementary information, as they allow statistical comparisons between regression relationships and provide a median length (MLM) or age at maturity. Generally, however, only AFM and/or LFM data are available for other gizzard shads. The LFM values of 126mm TL for

males, and 155 mm TL for females in the lower Murray may be compared with LFM estimates for bony bream in the Murray in New South Wales, which include an improbably low 70-80 mm TL in the first year (Lake, 1967; Cadwallader, 1977; Cadwallader & Backhouse, 1983). In comparison, Bishop et al. (in press) reported LFM values of 156 mm for males and 168 mm for females in their first year in Magela Creek, Northern Territory. Comparisons with LFM and AFM data for other Dorosomatinae (Jacob, 1948; Thomson, 1957; Berry, 1958; Bodola, 1966; Jester & Jensen, 1972; Chubb & Potter, 1986) show that bony bream conform to the gizzard shad pattern of early maturity.

The bony bream in many respects is a typical gizzard shad. It matures early, has small ova and larvae, no external sexual dimorphism, and favours warm, shallow waters. It is unusual in certain features of larval development and in its exceptional fecundity.

In Chapter 1 it was argued that, because of its abundance under a variety of hydrological regimes, the bony bream must be capable of either flood-cued spawning or exceptional reproductive output. On the basis of the above profile of reproductive biology, including the unpublished data from Cooper Creek, it appears that the species is capable of both. In general terms, bony bream abundance may be

attributed to these capabilities. However, the particular combination of a species' potentialities which is expressed will vary between environments (Persov, 1972; Carscadden & Leggett, 1975; Mann, Mills & Crisp, 1984).

In the lower Murray bony bream population, spawning is not usually flood-cued, and may occur 2-3 months after the flood peak. In this respect the bony bream resembles the two native species most severely threatened in the lower Murray -the Murray cod and catfish. Further, bony bream larvae are small, have a very brief interval of yolk nutrition, and their survival is likely to be strongly flood-dependent. However, the bony bream matures early, and is exceptionally fecund. Both Murray cod and catfish mature late [age IV-V for cod (Cadwallader, 1977); III-V for catfish (Davis, 1977b)] and have low fecundity (cf. Lake, 1959; Davis, 1977b) and so cannot recover rapidly after prolonged recruitment failure. It seems likely therefore that bony bream success in the lower Murray is due to an exceptional natality. However, given the flexibility of spawning response observed on Cooper Creek, it is also possible that certain flood events may induce bony bream spawning, and such events would support exceptional recruitment.

CHAPTER 6: DISEASE

6.1 SUMMARY

The lower Murray population of bony bream is subject to an annual epidemic of the oomycete Saprolegnia (principally S. parasitica) and the bacterium Aeromonas hydrophila. The epidemic is species-specific; it affects mainly adults whose susceptibility may be increased by stress due to winter cold. Mortality rates do not appear to be high. Lesions occur on the mid-flank and are characterized by an external mycelium, epidermal erosion, scale loss, hypodermal and muscular oedema, haemorrhage, myofibral degeneration and by the presence of Saprolegnia hyphae at all stages of infection. Although A. hydrophila is common in advanced lesions there is no significant systemic bacterial infection. This appears to be a primary mycotic dermatitis and is noteworthy because Saprolegnia is best-known as a secondary pathogen.

6.2 INTRODUCTION

[A paper (Puckridge, Walker, Langdon, Daley & Beakes, in press. Journal of Fish Diseases) based on the material of this chapter was accepted for publication on 8th November, 1988, and is bound as Appendix III.]

The most abundant large fish species in the lower Murray, the bony bream, is the only species subject to regular, widespread epidemic disease. Fishermen in the region have noticed fungal infections in late winter or early spring since at least the 1940s (L. Gray, Meningie, S. Aust., pers. comm.). In the following the principal pathogens are identified, the pathology and epizootiology of the infection are described, and implications for the role of the bony bream in the lower Murray are explored.

Oomycetes of the genus Saprolegnia include a number of facultative pathogens responsible for saprolegniasis in fish. Most outbreaks follow bacterial or viral infection (Egusa & Nishikawa 1965; Willoughby 1970; Bekesi, Kovaks-Gayer, Ratz & Turkovics 1984), injury to the epidermis (White 1975; Pickering & Willoughby 1977) or conditions associated with captivity (Willoughby & Pickering 1977; Copland & Willoughby 1982). These infections normally are single or sporadic, although there are reports of regular

infections in wild salmonids subject to spawning stress (Neish 1977; Richards & Pickering 1978; Pickering & Christie 1980). S. parasitica is the dominant saprolegniacean in these infections (Willoughby 1978; Wood, Willoughby & Beakes 1988).

The bacterium Aeromonas hydrophila may be a secondary invader of lesions (Humphrey 1985; Menasveta 1985) or a primary pathogen in systemic and integumentary infections of fish subject to spawning, thermal or low-oxygen stress (Richards & Roberts 1978; Esch & Hazen 1980; Nieto, Corcobado, Toranzo & Barja 1985).

Where both A. hydrophila and Saprolegnia spp. have occurred in an infection primacy usually has been attributed to A. hydrophila (Egusa 1965; Thorpe & Roberts 1972; Inman & Bland 1981; Callinan 1985). In the one reported exception known to me -- a case where A. hydrophila was associated with an otherwise typical salmonid saprolegniasis (Richards & Pickering 1978) -- bacterial samples were not taken from integumentary lesions. This chapter reports a further exception that occurs regularly in a wild population of a non-salmonid species - the bony bream of the lower River Murray.

6.3 METHODS

6.3.1 Sampling

The general sampling program has been described (2.1).

6.3.2 Mycology

Mycelial samples from lesions were cultured on chloramphenicol-cornmeal agar (250 mg chloramphenicol per litre agar) and duplicate samples were placed in sterilized river water. The lesion was photographed, mapped on a gridded fish outline, dissected free to a depth of 1 cm and preserved in 10% buffered formalin. Hyphal tips from the agar colonies were subsampled repeatedly until bacteria-free. The mycelia kept in sterilized river water were examined before sub-culturing to check the selectivity of the agar medium (cf. Willoughby 1978). For 60 isolates the mycelial clumps from sterilized water and hyphal tips from agar cultures were transferred to sterile distilled water prior to observations of zoospore release. Fifteen isolates were maintained in the dark at 7°C on sterilized hemp seed in filtered and autoclaved Murray water to allow observations of the formation of sexual structures (cf. Willoughby 1978).

Selected isolates were also examined by Dr G.W. Beakes (University of Newcastle-upon-Tyne) under the transmission electron microscope to check their cyst coat morphology (Appendix III).

6.3.3 Bacteriology

In September 1986 bacterial sampling was included in the protocol for 34 infected and 32 uninfected fish. These samples were taken with a flamed loop, streaked on DIFCO nutrient agar in a 90-mm petri dish and spread with a sterile swab dipped in sterile saline. If mycelia were present on a lesion part of the mycelial mat was lifted with sterilized forceps and the loop touched on the exposed tissue. If mycelia were absent the ulcerated surface was sampled directly. A sample was taken also from unaffected skin on the mid-flank. The flank was then seared and opened along the swim-bladder using sterilized scissors. The liver and anterior kidney were incised with a sterilized scalpel and the incision sampled. A control plate also was streaked with the sterilized loop and spread as above.

Each infected fish was paired with a similar-sized uninfected fish caught at the same time and place; these were treated in the same way, excepting the lesion sample. The culture plates were stored for three days at about river

temperature (12-18°C).

Preliminary identification of bacterial isolates was performed by C. Daley of the Institute of Medical and Veterinary Science, Adelaide. Final identification of presumptive A. hydrophila isolates was performed by Dr Dawn Austin of the Department of Brewing & Biological Sciences, Heriot-Watt University, Edinburgh (Appendix III).

6.3.4 Virology

In September 1986 three healthy, three slightly infected and three severely infected fish were frozen on dry ice and sent to Dr J.S. Langdon of the Australian Fish Health Reference Laboratory at Benalla, Victoria, who tested the specimens for the presence of viral agents (Appendix III).

6.3.5 Histology

Skin tissue samples from nine infected and three apparently uninfected specimens were subjected to histological examination by Dr J.S. Langdon (Appendix III).

6.4 RESULTS

6.4.1 Epizootiology

The epizootic typically begins in June-July, when temperatures are lowest for the year (Fig. 6.44A) and

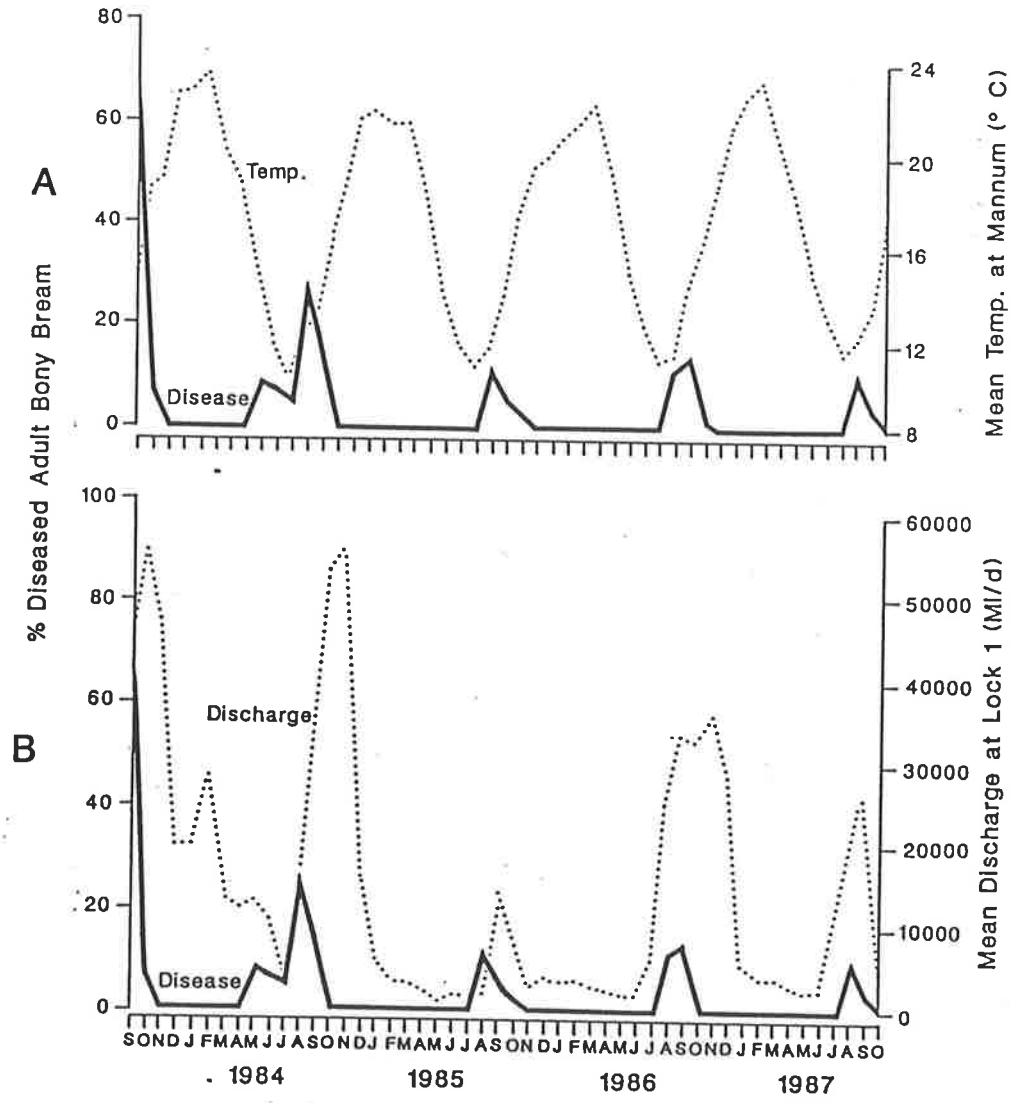


FIGURE 6.44: Percentage incidence of dermatitis in adult bony bream 1983-1987 compared to A: mean monthly water temperature at Mannum B: mean daily discharge at lock 1, Blanchetown.

flooding has not commenced (Fig. 6.44B). This is two months before the annual low in the body Condition Factor (Fig. 6.45A), three months before there is a significant rise in GSI and five months before spawning (Fig. 6.45B). The peak monthly incidence of infection is significantly negatively correlated with the mean July-August water temperature ($r = -0.9$, Spearman Rank $Z = -2.08$, $n = 5$, $P < 0.05$), but not with mean daily discharge. Incidence reaches a peak in August and September and involves 10-64% of the adult population. Juvenile fish (TL <150 mm) are rarely affected (incidence <0.4%). There is no significant difference in the incidence of infection in the two sexes corrected for population sex ratio (Chi-squared, $n = 166$). There are no significant differences in mean Condition Factor ($\log(X+1)$-transformed) or mean GSI for infected and uninfected fish (paired samples *t*-test, $n = 86$ pairs), and no significant correlation between condition and area of the lesion (Spearman Rank).

The water quality data suggest that, over the sampling period, no heavy metals or organo=chlorine pesticides occurred in the lower Murray in concentrations likely to be toxic to fish. Levels of organo-chlorines were in fact below limits of detection by Engineering and Water Supply Department techniques. Nor were there changes in pH,

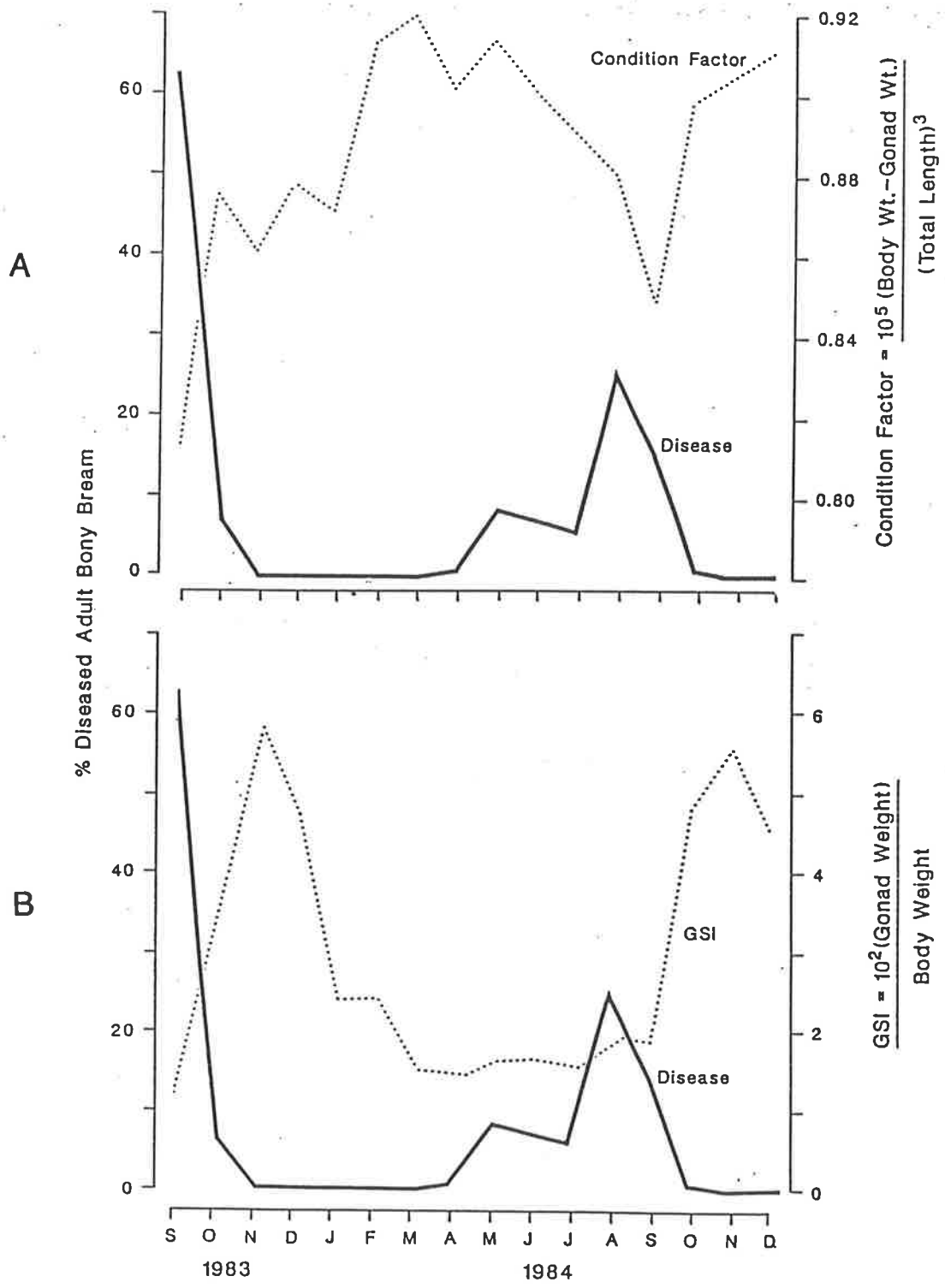


FIGURE 6.45: Percentage incidence of dermatitis in adult bony bream in 1983-1984 compared to A: mean monthly condition factor B: mean monthly GSI.

conductivity or dissolved oxygen likely to cause stress to the bony bream. No other fish species showed signs of infection.

6.3.2 Gross Pathology

The proportions of infected grid cells (among 168 per fish) in four body regions (fins, head, mid-flank and posterior flank) on the left and right sides of the body were analyzed by Stepwise Logistic Regression (BMDP Statistical Software 1987, program LR). The potential effects due to differences between individual fish, body region and side (left or right) were included as main effects in the logistic model, together with a side vs region interaction. The area of lesions is significantly higher on the anterior mid-flank (F-test, P <0.001); the head and gills, fins and posterior flank are rarely affected. Curiously, there is also a significantly greater mean area of infection on the left than the right flank (F-test, P <0.05). There is no significant interaction between side and region (F-test). The mean area of lesion is 5.3% of the body surface (SD = 3.5%, n = 91) and the maximum is 16%. The least severe and probably earliest lesions appear as a thin fuzz of mycelia over skin with no obvious haemorrhage or inflammation (Fig. 6.46A). Distinct lesions

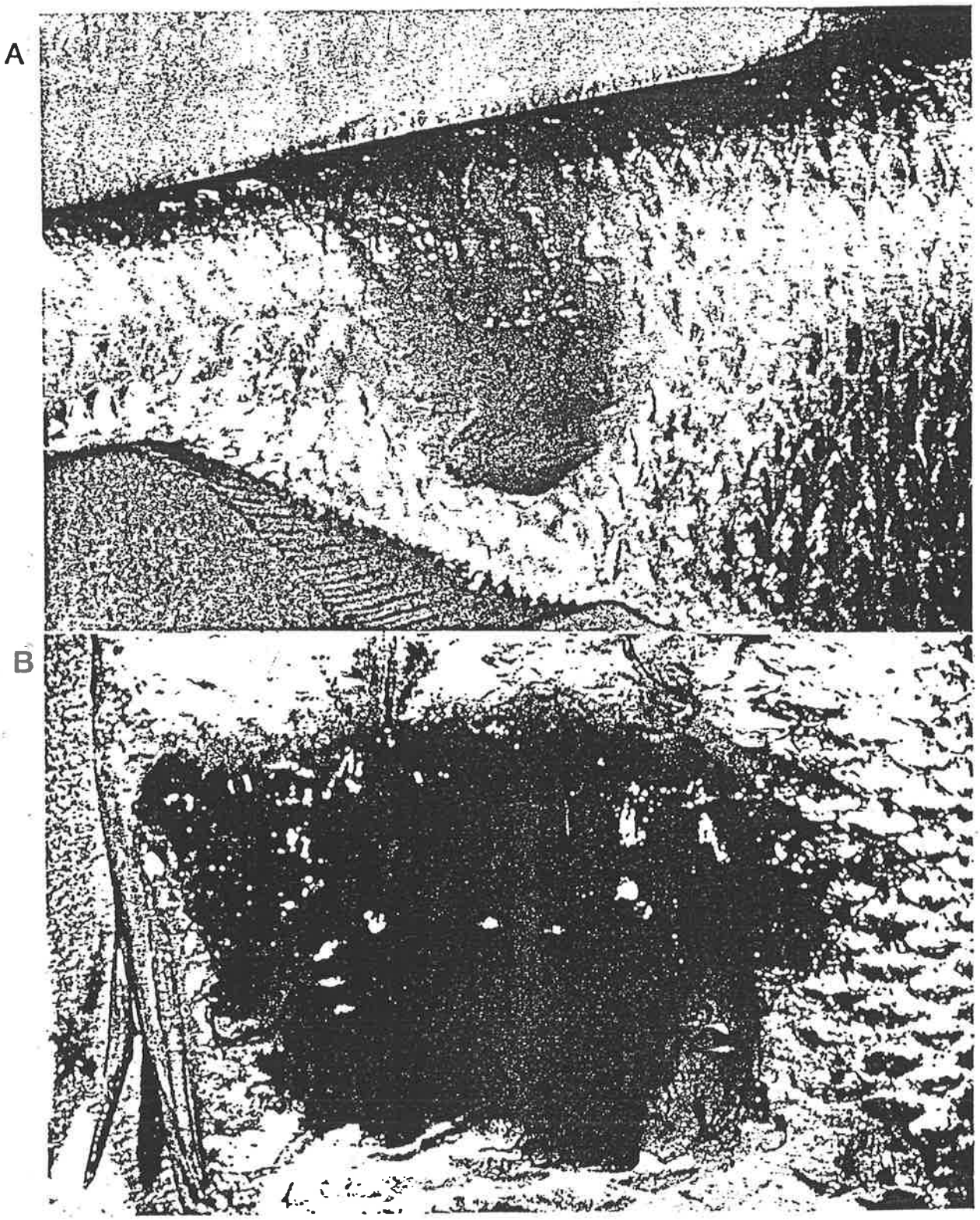


FIGURE 6.46: Mycotic lesions on the skin of bony bream, A: slight B: moderate.

without fungus infection rarely were observed; there was occasional reddening of the skin and elevation or loss of scales, but this is typical of the reaction of bony bream to capture and handling.

Lesions progress by an increase in the density of investing mycelia, erosion of the epidermis and protrusion then loss of scales, with increasing peripheral haemorrhage and erythema (Fig. 6.46B). Intermyotomal haemorrhage and myomalacia occur in advanced lesions. One specimen only was found with scar tissue and pigmentation suggestive of healing, and only two moribund specimens were captured. With few exceptions the internal organs of fish with external lesions appeared normal.

6.4.3 Mycology

In mycelia transferred directly from lesions to sterile water there were occasional hyphomycetes, but the dominant organisms were non-septate oomycetes and all, apart from one specimen of *Leptomitus* sp., belonged to the Saprolegniales.

In 60 of 62 isolates of Saprolegniales the primary zoospores cleaved within the zoosporangium and were released terminally. Ten isolates observed at the moment of zoospore release all demonstrated active dispersal of the primary zoospores from the sporangium mouth, as is typical of

Saprolegnia. In only one of 61 isolates (Achlya sp.) were primary zoospores found encysting at the sporangial opening. Of 34 fish with lesions collected in 1986, 31 yielded Saprolegnia isolates.

Six of the 15 isolates (code numbers 1289, 1356, 1376, 1431, 1494, 1570) maintained on hemp seed developed oogonia within six months. The isolate which most readily reproduced sexually (1494) was identified as S. ferax (Gruith.) Thuret and the other five as S. diclina Humphrey (after Seymour 1970). Electron microscopy provided supporting evidence for these identifications, and for the referral of the asexual isolates to S. parasitica (Appendix III).

6.4.4 Bacteriology

The following groups were identified tentatively in isolates from diseased and healthy fish: Aeromonas, Pseudomonas, Alcaligenes, Flavobacterium, Chromobacterium and "oxidase-negative gram-negative bacillus". Aeromonas hydrophila was identified in 38/48 suspected Aeromonas isolates. A. hydrophila was isolated from 22/34 skin lesions and 3/31 skin samples from healthy fish. Only 4/34 fish with lesions yielded A. hydrophila from liver and/or kidney. Seven samples from uninfected skin areas of 34 diseased fish gave A. hydrophila isolates (Fig. 6.47). There was no difference

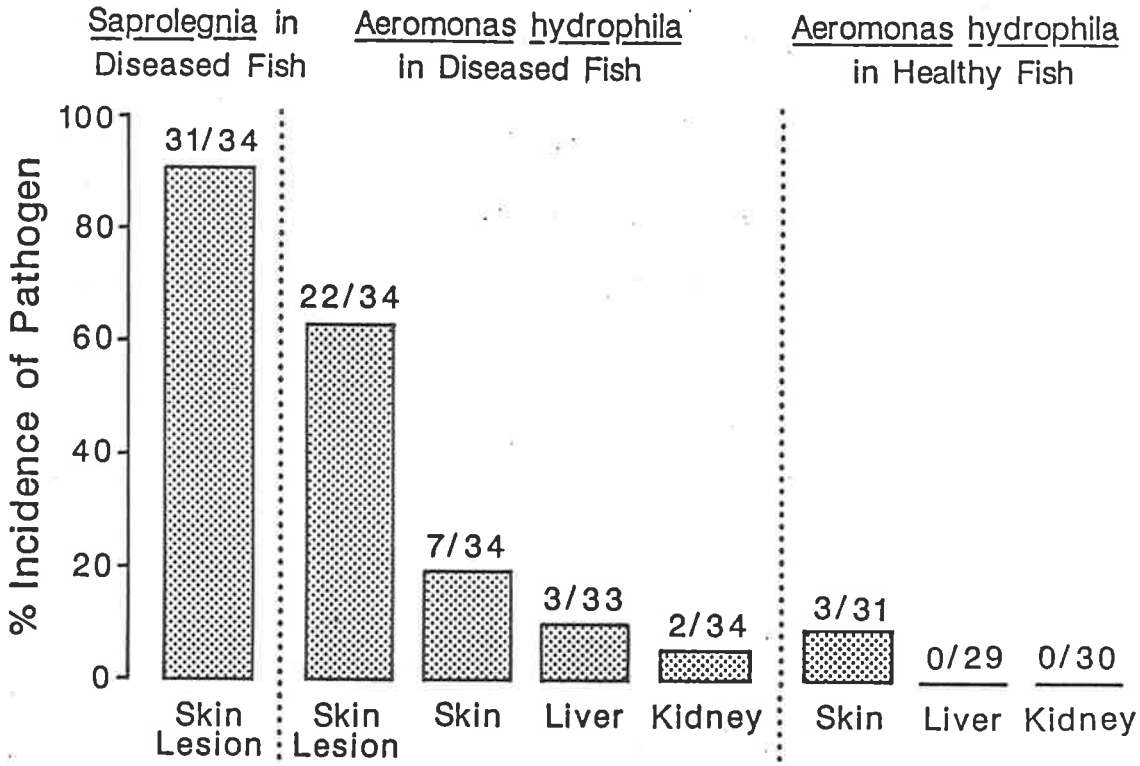


FIGURE 6.47: Percentage incidence of Saprolegnia and Aeromonas hydrophila isolates in infected and healthy tissues of bony bream.

between the incidence of Saprolegnia and A. hydrophila in lesions (Chi-squared, P >0.05), but of the 3/34 fish that did not yield Saprolegnia, one did not yield A. hydrophila, one showed an integumentary infection of A. hydrophila and the third had obvious Aeromonas septicaemia.

6.4.5 Virology

No cytopathic agents were isolated from the kidney, liver, spleen or skin lesions of diseased fish.

6.4.6 Histopathology

The principal lesions involved erosion of the epidermis and loss of scales, hypodermal and muscular oedema, haemorrhage and myofibral degeneration. The earliest obvious infections consisted solely of fungal hyphae penetrating the epidermis and dermis, often in scale pockets (Fig. 6.48A). This progressed to erosion of the epithelium and hypodermal oedema, as previously described for saprolegniasis (Copland & Willoughby 1982). Bacterial invasion also was commonly observed at this stage in development of the lesion.

Extensions to the underlying musculature occurred in severe cases, inducing haemorrhage and oedema, particularly in the intermyotomal connective tissue. Fungal hyphae were not visible in sections stained with haematoxylin and eosin, but

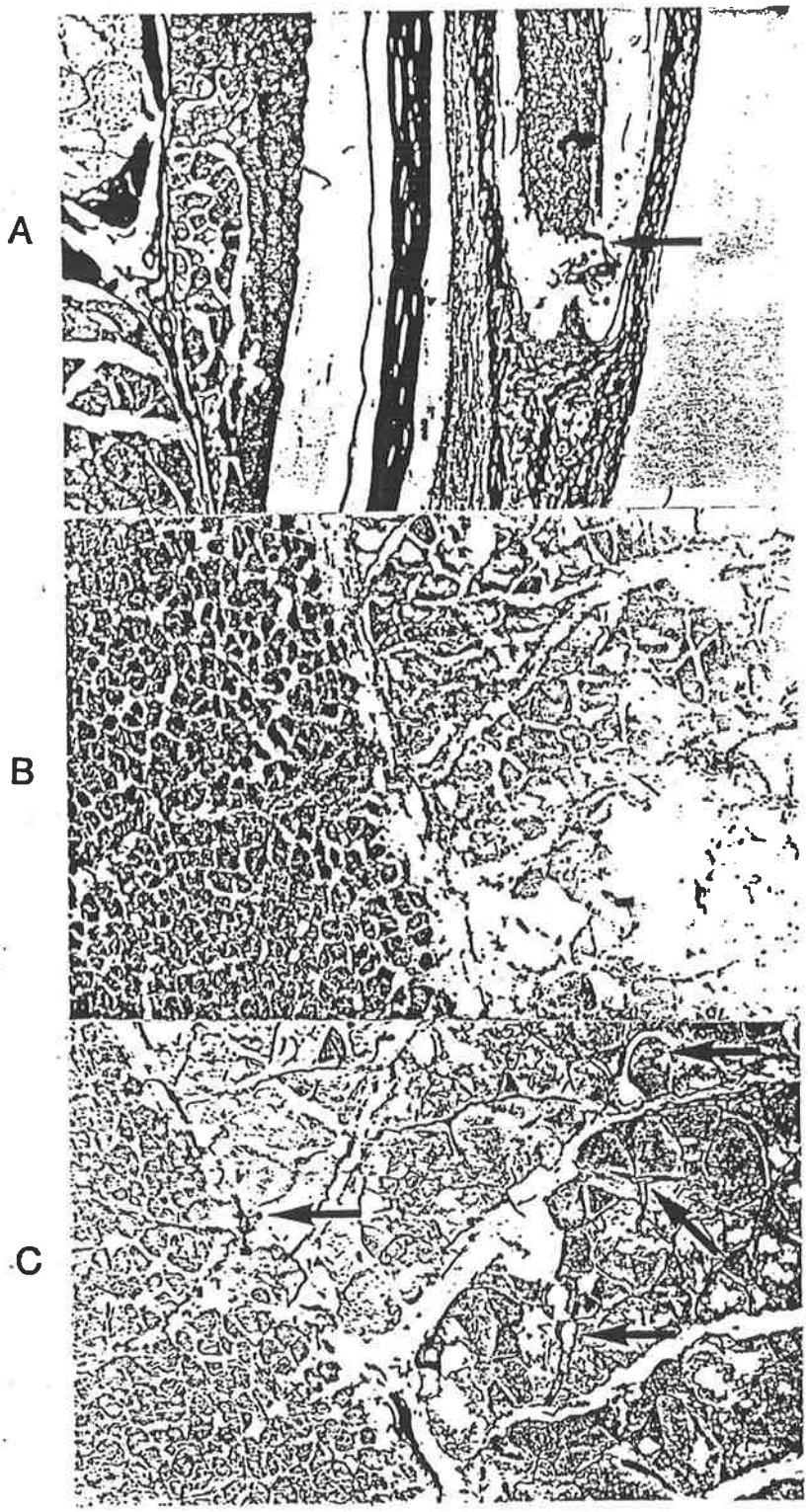


FIGURE 6.48: Histopathology of mycotic dermatitis in bony bream. A: Fungal hyphae (arrow) in scale pocket in earliest stage of infection (methenamine silver, X130). B: Haemorrhage and oedema of intermyotomal connective tissues at junction of pink lateral and white muscle, and white myofibral degeneration (H+E, X100) C: Section similar to 5B but silver-stained to reveal fungal hyphae (arrows) within haemorrhagic foci and myofibres (methenamine silver, X130)

methenamine silver staining revealed one or more hyphae, but never numerous hyphae, within each haemorrhagic focus (Figs. 6.48B, 6.48C). Individual myofibres often contained a single hypha within the sarcoplasm, with or without sarcoplasmic degeneration. Bacteria were rarely visible in these deeper lesions.

No stage of the lesions showed leucocytic inflammatory response to the fungal elements. Lesions with substantial bacterial involvement displayed mononuclear inflammatory infiltration at the sites of invasion.

6.5 DISCUSSION

Irruptions of Saprolegnia and Aeromonas hydrophila commonly occur in late winter and spring (Jester & Jensen 1972; De Figueiredo & Plumb 1977; Porak & Tranquilli 1981). It appears that low winter temperatures may act directly to lower antibody production and blood proteins and thereby immunity, or indirectly through inhibition of feeding (Roberts 1975; Cipriano, Bullock & Pyle 1984; Brenden & Huizinga 1986a). The rise of temperatures in spring also may lower immunity through stress, particularly in the case of A. hydrophila infections (Esch & Hazen 1980; Nieto, Corcobado, Toranzo & Barja 1985). Sexual maturation may contribute but the mechanism is unclear (Pickering &

Pottinger 1985, Pickering 1986).

The general preference of bony bream for warmer waters is consistent with the winter-stress model; the lower Murray lies near the southern extreme of the species' range. Bony bream possibly are sensitive to low temperatures and low oxygen concentrations (Cadwallader 1977; Allen 1982), as these conditions are implicated in reported infections by bacteria and Saprolegnia (Johnston & Bancroft 1921), Chilodonella (Langdon, Gudkovs, Humphrey & Saxon 1985) and rhabdovirus and Aeromonas (Department of Ports & Fisheries 1986) in this species. An exception to this pattern is the mycosis (involving Achlya sp.) of bony bream in the Northwest Branch of Cooper Creek, which occurs under a variety of conditions, including both high temperatures and high dissolved oxygen concentrations (Puckridge, unpub. data).

The timing of the epizootics in the lower Murray also is consistent with the winter-stress model. Peak incidence occurs after the winter temperature minimum in July, and the level of incidence is correlated with the mean July-August water temperature. However, in 1984 the incidence of the lower Murray disease peaked before body condition reached its minimum. There was no difference between the condition

of healthy and diseased fish, and condition was not significantly correlated with the area of lesions. If winter-cold stress is acting as an initiating factor in the disease cycle, it seems likely that it is doing so directly through suppression of immunity rather than by lowering body condition through inhibition of feeding.

Several authors have commented on the lack of a leucocytic inflammatory response to invading hyphae, and the presence of haemorrhage in the inflammation induced by the hyphae of Saprolegnia sp., particularly in deeper lesions (Bootsma 1973; Wolke 1975; Nelsh 1977; Copland & Willoughby 1982). The lack of such a response in bony bream could arise from temperature-mediated inhibition, or it may reflect the fungal species involved (there is some cellular inflammatory response to bacterial invasion). This contrasts with the marked granulomatous inflammatory response shown by fish infected by Aphanomyces sp. (Noga & Dykstra 1986), Phoma herbarium (Ross, Yasutake & Leek 1975), Aphanomyces piscicida (Hatai, Takahashi & Egusa 1984) and Exophiala psicophila (Langdon & McDonald 1987).

The fact that adults, rather than juveniles, are affected may arise from differences in the feeding habitats of the two (Atkins, 1984), which could cause differential exposure to the pathogen. It also suggests that hormonal changes

associated with sexual maturation may increase susceptibility to the disease. In fact pre-maturational changes are indicated because the infection becomes intense two months before the onset of vitellogenesis. Similarly, the annual flooding of the Murray usually occurs too late to affect the initiation of infection. However, it may be instrumental in the cessation of the epidemic.

Captive salmonids and eels succumb rapidly to saprolegniasis (Copland & Willoughby 1982; Pickering & Willoughby 1982), and it is possible that wild populations also suffer high mortalities (White 1975). In the present case the scarcity of healing or dead bony bream is difficult to explain. However, the absence of large numbers of dead or moribund fish suggests that mortality rates due to the disease are low. Both actual mortality rates and effects on reproductive output, however, should be examined experimentally, perhaps in river enclosures.

The specificity of the lower Murray disease for bony bream contrasts with the generality of a dermatitis attributed to Achlya sp. in fish of the Coongie Lakes region of Cooper Creek in central Australia, where bony bream, callop, desert rainbowfish (Melanotaenia splendida tatei) and goldfish (Carassius auratus) are affected (Puckridge & Drewien 1988).

It seems likely that S. parasitica is the principal fungal pathogen involved in bony bream dermatitis, as it is in British salmonid saprolegniasis. But in Japan S. diclina also has been implicated as a salmonid pathogen (Hatai, Willoughby & Beakes, in press), so a definite answer must await a study of the pathogenicity of both taxa.

In saprolegniasis the localization of surface lesions is distinctive for particular host-pathogen relationships. The pattern seen in bony bream, favouring the mid-flank region, differs from that in salmonids (White 1975; Neish 1977; Richards & Pickering 1978) where head, dorsal surface and fins are affected. It differs also in being not consistent with initiation of infection by injury. The significant difference in infection area between the two sides of the fish is not simply explained, and may be an artifact of large sample size. However, there is evidence of assymetry in parasitic infections (Moser, Sakanari, Wellings & Lindstrom 1984; Rohde 1984), and bony bream have significant gonad assymmetry, with the larger gonad on the more severely infected side (see 5.4).

The Chi-square analyses reported by Richards & Pickering (1978) are at best marginal indicators of environmental and biological effects, whereas the method employed here is

multivariate in nature and has potential for comprehensive studies of the relations between such effects and the distribution and intensity of infection.

The task of establishing primacy in a complex disease process is difficult, and conflicting evidence is common. This is well-shown by work on Japanese "fungus disease" of eels (Egusa 1965), UDN of salmonids (Carbery 1968) and the Asia-Pacific ulcer disease (Roberts, MacIntosh, Tonguthai, Bronyaratpalin, Tayapatch, Phillips & Millar 1986). In the present situation, it is possible that inconspicuous prefungal A. hydrophila lesions occur initially in the lower Murray disease, and that Saprolegnia invades these before they become obvious. However, there are several lines of evidence to suggest that this is a case of primary saprolegniasis:

(1) Saprolegnia occurs in the earliest detectable lesions.

(2) Gross haemorrhage and inflammation are absent in early lesions.

(3) External and internal symptoms of systemic bacteraemia are absent.

(4) The yield of A. hydrophila cells from internal organs is low (this is not uncommon (Thorpe & Roberts 1972; Snieszko 1974) and the slightly enhanced levels that occur in fish weakened by Saprolegnia lesions are to be expected).

(5) About a third of lesions (11/34) yielded Saprolegnia but not A. hydrophila.

(6) Few lesions (2/34) yielded A. hydrophila but not Saprolegnia.

(7) Saprolegnia hyphae occur in the scale pockets in early lesions, and deep in the muscle in advanced lesions.

(8) Viral agents were not apparent, at least in preliminary investigations.

The evidence presented here suggests that the lower Murray bony bream epizootic is an addition to the comparatively few known instances in fish of primary pathogenicity among aquatic fungi (Ross & Yasutake 1973; Ross, Yasutake & Leek 1975; Hatai, Takahashi & Egusa 1984; Noga & Dykstra 1986). In addition, this appears to be the first reported case of primary saprolegniasis in a wild, non-salmonid fish population.

Mycotic dermatitis may be an expression of stresses experienced by a species at the margin of its range. Although neither such stresses nor the disease appear to cause substantial mortalities, marginal changes in the lower Murray environment could precipitate major changes in the severity of the epidemics. The possible susceptibility of bony bream to low temperatures and the association of the mycotic dermatitis epidemics with winter temperature minima

suggests that change in the thermal regime could be critical. For example, the projected Greenhouse temperature rise of 2-4^{o}C by the year 2030 (Graetz, Walker & Walker, 1988), could eliminate the outbreaks entirely.

CHAPTER 7: CONCLUSION

7.1 Life History and Abundance

Bony bream are the most abundant large fish species in the lower River Murray. Other large native species form only minor proportions of the fish population, and these proportions, particularly of the Murray cod and catfish, are declining.

In adult bony bream mortalities appear to be low, although this should be tested experimentally. Annual epidemics of mycotic dermatitis are confined to adults, and do not appear to result in high mortalities. However, a susceptibility to low water temperatures is suggested by the winter decline in condition and growth of young fish, the movement of all size-classes away from thermally variable onshore waters in winter, and the occurrence of peak disease - incidence in August-September. This may explain the decline in abundance of bony bream in the middle Murray, where impoundment has lowered water temperatures (Lake, 1971; Cadwallader, 1977; Walker, Hillman & Williams, 1978). The higher mortality rate in age V+ males suggests that spawning activity also may be stressful.

The large size of adult bony bream compared to other gizzard shads may mean lower mortality through greater metabolic efficiency, reduced susceptibility to cold stress, and immunity from all but the largest predators. The increase in reproductive effort with size may be advantageous in a species with low adult mortality.

Larval and juvenile bony bream mortalities appear to be very high. High larval mortalities may be due to the small size at first feed, the brief period of yolk-derived nutrition and the fact that spawning is not usually flood-cued. A model may be proposed in which larvae are critically dependent on high densities of small zooplankters at first feed, and such densities are often unavailable because spawning is not synchronized with flooding, which resets zooplankton succession. The altered flood regime of the lower Murray, and the alienation of much of the floodplain from the main channel for all but exceptional floods, mean that flood-induced zooplankton succession (if it does occur) occurs more rarely and with different timing than under the natural regime. To test this model, flood-induced zooplankton succession on the lower Murray floodplain and the dependency of bony bream larvae on such a succession must be examined. Further study is needed also into the role of hydrological cues in spawning, given the evidence for

flood-cued spawning of bony bream on the Northwest Branch of Cooper Creek (5.5).

Given high larval mortalities, the abundance of bony bream in the lower Murray and perhaps elsewhere is likely to be based on an exceptional natality arising from early maturity and high fecundity. In the stable environment of the regulated lower Murray, however, low adult mortalities and the steep increase in fecundity with size may also play a part by contributing to a large broodstock with high reproductive potential. In habitats like Cooper Creek, where protracted drought may lead to high adult as well as larval mortalities, potential longevity may not be realised.

Since the bony bream is able to flourish in regulated waters, further regulation and impoundment in warm-water catchments throughout Australia is likely to extend the range and abundance of the species. The species is likely also to be favoured by the enhancement of flows in central Australia and the rise in temperatures in southern Australia predicted as a consequence of the Greenhouse Effect (Pittock, 1983; Graetz, Walker & Walker, 1988).

Bony bream are in many respects typical gizzard shads. They feed low in the food chain, have high reproductive potential and high early mortality. They favour warm, shallow waters,

are stressed by winter cold, and are characteristically abundant. None of the freshwater gizzard shads demonstrates flood-dependent spawning, and all species in regulated - flow environments recruit successfully. However, in some respects bony bream (at least in the lower Murray) are atypical. Their maximum fecundity and their rate of increase of fecundity with body size are exceptional. They are exceeded in maximum size only by D. cepedianum, and probably are equally long-lived. Unlike most freshwater gizzard shads, bony bream flourish under highly variable and unpredictable hydrological regimes. An element in their success over a wide range of conditions may be flexibility in spawning response - the ability to spawn successfully with stable water levels, and to spawn in response to floods when opportunity arises.

Some important tasks remaining in the study of bony bream life-history are as follows:

a. Validate scale-ageing against another technique - for example mark-recapture in a small confined population.
b. More accurately estimate mortality rates, principally by enlarging sample size, reducing selectivity of gear, and increasing replication to provide estimates of variance.
c. Improve techniques of stripping gonadal products from ripe fish, in vitro fertilisation and rearing of larvae,

to clarify early development patterns, particularly in the yolk sac stage.

d. Describe in relation to flooding the succession of zooplankters important in the diet of larval bony bream.

e. Examine feeding and survival of bony bream larvae at different developmental stages in relation to successional stages of zooplankton assemblages.

f. Test the spawning response of bony bream to hydrological cues, in particular to the timing, intensity and duration of flooding.

g. Describe the effects of mycotic dermatitis epidemics on bony bream mortality rates and reproductive output, perhaps by using in - river enclosures.

h. Using echosounding and trawls, examine the inshore-offshore movements of the bony bream population in response to seasonal and diurnal gradients in mean water temperature and temperature variance.

7.2 The Fishery

The high mortality in the 0+ year-class suggests that if the fishery were to expand substantially - for example to produce a canned or minced product - consideration should be given to focussing effort on this age-class, perhaps using surface trawls.

To determine the effects of the fishery on the adult bony bream population, age-structure would have to be monitored over some years. Mortality in larger/older fish is relatively low at present; but population stability is likely to depend on the maintenance of a brood stock of large fish, which are highly fecund, and will tide over years of poor recruitment.

The fishery is heavily dependent on the lakes environment both for the bulk of the catch and because of major spawning off the lake shores. Greenhouse - induced sea level rise over the next 50 years (Barth & Titus, 1984) may necessitate the withdrawal of the barrages, perhaps to Wellington. Bony bream are at present abundant in the upper Coorong (Hall, 1986), and are known to tolerate sea - water salinities (Ruello, 1976; Glover, 1982). Such a restoration of estuarine conditions could be an effective way of converting bony bream biomass into biomass of more commercially valuable estuarine species, such as mulloway (Argyrosomus hololepidotus), which at present utilize bony bream in the Coorong (Hall, 1986). However, salinity tolerances of bony bream eggs and larvae and the effects of salinity on spawning behavior are unknown. The lakes spawning ground might be lost, and a large piscivore like the mulloway could substantially reduce the adult broodstock of bony bream.

The lakes and Coorong fishery uses minimum gillnet mesh sizes of 50 mm, which catch bony bream of TL = 138-209 mm. The median lengths at maturity are 159mm for males, 199mm for females. Some reproduction therefore is taking place in fish below the capture size, but pre-reproductive fish are also being caught. Proportions of fish caught commercially in the different mesh sizes through the year need to be determined, and if the pre-reproductive catch is high, consideration should be given to lifting the minimum mesh size to 70 mm.

A research program into some of the questions raised by this study should be considered, for example:

a. What is the overall pattern of spawning grounds in the lower Murray, including the Coorong?
b. What are the salinity tolerances of bony bream eggs and larvae, and the effects of high salinities on spawning?
c. What are the seasonal and diurnal patterns of movement of bony bream juveniles and adults in the lower Murray?
d. Are the lake and river populations genetically distinct stocks?
e. How does the size-structure of the commercial catch relate to the length - maturity relation?
f. What are the natural and fishing mortality rates in the

population?

g. What are the present and future prospects for higher-value commercial uses (e.g. canning, pickling) of this resource?

7.3 Bony bream as a forage fish

Criteria for evaluating the suitability of potential forage species have been developed for the United States impoundment stocking programs (Ney 1981). Suitable species must be:

a. Prolific: Bony bream, with early maturity and high fecundity, have exceptional reproductive potential.

b. Stable in abundance: Bony bream catch rates have been relatively stable in the last five years. The longevity of bony bream provides stability to the broodstock, and conditions in the impoundment environments intended for stocking are likely to be stable.

c. Trophically efficient - i.e. feed at a low trophic level: Adult bony bream, more than almost any other Australian freshwater fish, fulfil this requirement.

d. Vulnerable to predation: Juvenile bony bream form a major component of the diets of many of the major freshwater commercial species. However large adult bony bream are only vulnerable to top predators like Murray cod, mulloway, barramundi and ariid catfishes. Situations

of over-abundance of larger, predator-free gizzard shads in United States impoundments are now dealt with by stocking top predators (Noble 1981).

e. Non-emigrating: Bony bream, particularly juveniles, are certainly mobile in unregulated river systems, and are effective colonizers (Kowarsky & Ross 1981, Puckridge & Drewien, 1988). However, since they are naturally widespread in nearly all Australian warmwater catchments, such colonization should not normally be cause for concern.

f. Innocuous to other species: Larval and juvenile bony bream are zooplanktivores and so may compete for food with the young of other species. Effects on the survival of larval game fish from competition by Dorosoma species have been debated (Jenkins, 1957; Morris & Follis, 1979; Wydoski & Bennett, 1981). However, several of the angling species stocked in Australian warmwater reservoirs do not breed under impoundment conditions, so larval interactions for those species are irrelevant. Further, bony bream have the advantage of being indigenous to most catchments, and so the larvae of most local predator species should have adapted to compete with larval bony bream. Adult bony bream, being unusual in Australian freshwaters as microphagic omnivores, are rarely in

trophic competition with other indigenous species.

In addition,

a. Bony bream are pre-adapted to impounded waters. Their spawning is not flood-dependent, and impoundment provides an enlarged and stable sediment surface upon which to feed (see also Miller 1960, Jacobs & Swink 1983). Bony bream are apparently not dependent on high densities of aquatic macrophytes, and flourish in the open, less vegetated waters often found in impoundments.

b. Bony bream are relatively free of parasites, although they are found in the lower Murray with occasional trematode cercaria (Puckridge, unpub. data). However, they are subject to attack by the fungi Saprolegnia and Achlya. The former is species-specific, but the latter, which is common in warmer northern waters, affects a range of species, and could be a problem in sub-optimal conditions. Aeromonas hydrophila, which is common in mycotic skin lesions of bony bream, is an important fish pathogen in American reservoirs (Hazen, Fliermanns, Hirsch & Esch, 1978; Hazen, 1979), and may also infect a wide range of vertebrate species, including humans (Shotts, Gaines, Martin & Prestwood, 1972; Brenden & Huizinga, 1986).

c. Adult bony bream are not readily maintained in captivity

(Puckridge, unpub. data). Their feeding modes are skimming or pecking the substratum and mid-water filter-feeding, usually in schools. They are highly mobile, and probably require large feeding territories. They show no interest in fine particulate artificial diets; intensive aquaculture of the species would probably require new approaches to nutrition. However they flourish in earthen ponds and dams.

APPENDIX I: Composition of preservatives.

Buffered formalin

Conc. formaldehyde	100 ml
Distilled water	900 ml
Sodium dihydrogen phosphate (anhydrous)	3.5 gm
Disodium hydrogen phosphate (anhydrous)	6.5 gm

Modified Gilson's Fluid

60% alcohol	100 ml
Distilled water	880 ml
80% nitric acid	15 ml
Glacial acetic acid	18 ml
Zinc chloride	20 gm

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Reproductive biology of a gizzard shad, Nematalosa erebi (Gunther) (Dorosomatinae: Teleosti), in the River Murray, South Australia.

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ABSTRACT

The reproductive biology of the native Australian "bony bream", Nematalosa erebi, resembles that of other gizzard shads. The bony bream matures at a median age of 2-3 years, spawns (independently of flooding) in December-January at water temperatures of 21-23°C and is highly fecund. Reproductive effort increases with size. The ova and larvae are small, the yolk-sac stage is brief and development is typical of clupeids except for the fin-ray sequence. Sexual dimorphism does not occur, but females predominate in the largest size-classes. In the regulated lower River Murray,

catches of bony bream are increasing where those of other native species are in decline. This is more likely to be due to an exceptional capacity for population recovery than to adjustments of the spawning cycle in relation to environmental cues.

I. INTRODUCTION

The "bony bream", Nematalosa erebi (Günther), occurs in slow-flowing rivers, lakes and impoundments throughout the warmer parts of Australia. It is a native member of the gizzard shads (Clupeidae: Dorosomatinae), a group common in coastal shallows and estuaries throughout the sub-tropics (Miller, 1960; Nelson & Rothman, 1973) and in slow-flowing and standing waters over much of North America (Miller, 1960; Noble 1981). Gizzard shads are typically highly fecund and early maturing (Rao, 1965; Kilambi & Baglin, 1969a, 1969b).

The bony bream population of the lower River Murray, South Australia, is of ecological interest because it may be the only large native fish species that has not declined in abundance since the advent of flow regulation (cf. Cadwallader, 1978, 1986; Walker, 1983, 1986a, 1986b). This is despite the fact that the population is subject to annual epidemics of mycotic dermatitis (Puckridge et al., in press) that do not affect the co-occurring species. The bony bream sustains a lobster-bait fishery with an annual catch of 830 tonnes (South Australian Department of Fisheries, 1988) and is important also as a forage species (e.g. Milward, 1965; Weatherley, 1977). Prior studies of the biology of the

species (Lake, 1967; Cadwallader, 1977; Llewellyn, 1983; Bishop et al., in press) have been incidental.

In this paper we examine gonadal cycles, spawning sites, sex ratio, egg and larval development, fecundity, reproductive effort and age/length-maturity relationships for bony bream in the lower River Murray. We also make comparisons with data for other gizzard shads to identify the particular attributes of bony bream, and with data for other native species to elucidate reasons for the comparative abundance of bony bream in the regulated Murray environment.

II. MATERIALS AND METHODS

SAMPLING

Samples were taken from the River Murray at Zadows Landing (ZL) (34° 58'S, 138° 59'E), at the mouth of a shallow backwater, and from an exposed shore near Point Sturt (PS) (34° 58'S, 139° 18'E) in Lake Alexandrina. Ichthyoplankton trawls were made also in the Murray below the entry of a small, intermittent tributary, the River Marne (M), and in a shallow inlet at the mouth of the Finnis River (F), a small tributary to Lake Alexandrina (Fig. 1).

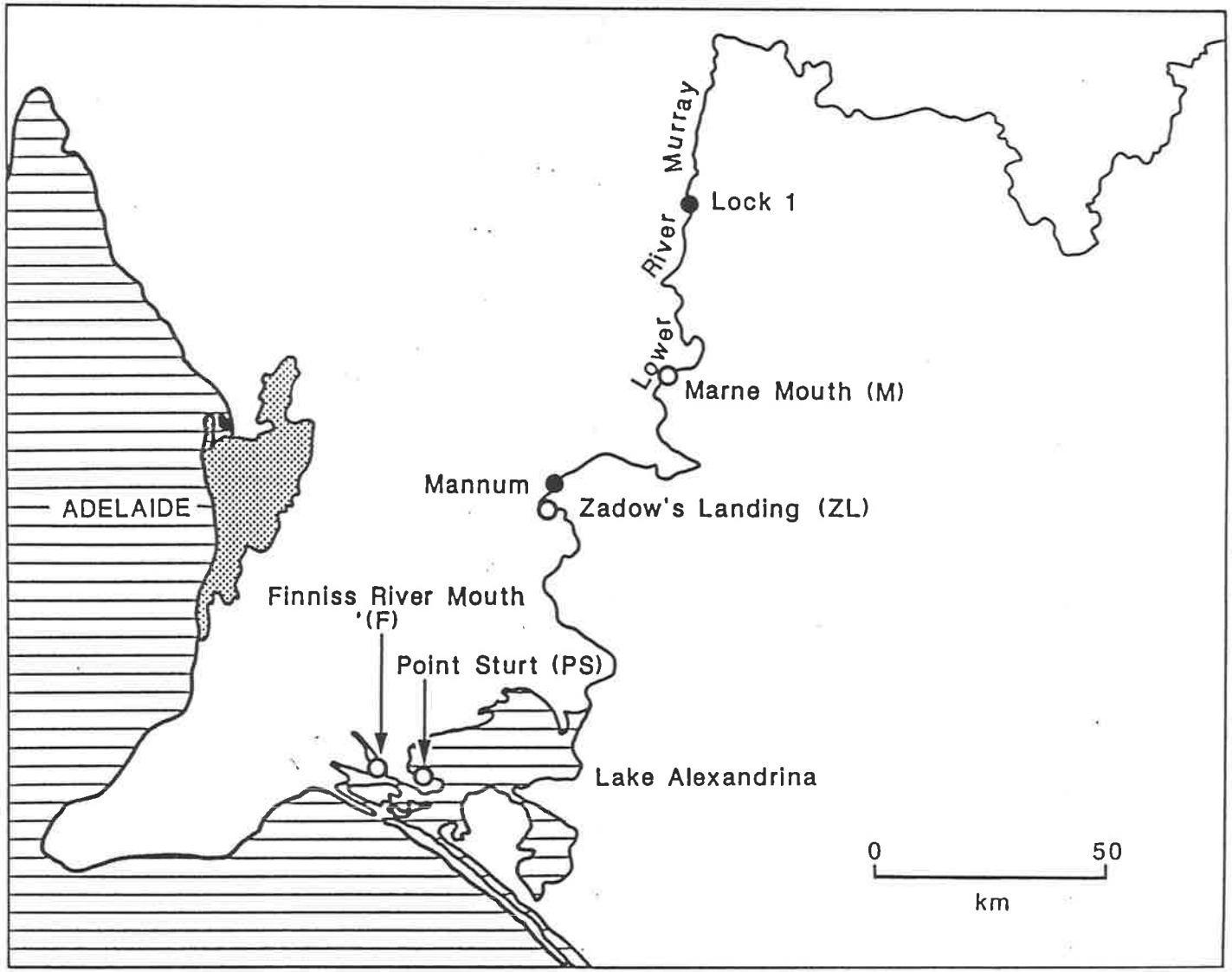


Fig. 1: Sampling sites on the lower River Murray, South Australia.

Sampling was monthly at ZL and PS from September 1983 to December 1984 and from June to December in 1985 and 1986. Seven 50 m gillnets (20-110 mm mesh), three seines (2, 18 and 130 m lengths; 2, 12 and 30/50 mm mesh) and an ichthyoplankton trawl (0.5 m diameter, 500 um mesh) were used. Only two of the seven gillnets (90, 110 mm) were used at PS and the 130 m seine was not used at ZL. Depending on the size of the catch, either the entire catch or stratified random subsamples (size-class interval 50 mm total length (TL)) were retained and processed on site. Fish were measured (TL, length to caudal fork LCF and body depth, accurate to 1 mm) and weighed (0.1 g) before dissection. The left and right gonads were photographed in situ and their weight (0.01 g) and width (0.1 mm) determined before transfer to Gilson's Fluid.

Routine records were kept of wind movements, water level, water temperature, pH, conductivity, Secchi transparency and dissolved oxygen. Day-length data were obtained from the Commonwealth Bureau of Meteorology, Adelaide and records of water chemistry and daily river temperatures and discharges were obtained from the Engineering & Water Supply Dept, Adelaide. The data were analysed using Microsoft BASIC computer programs written by the authors or included in NWA Statpak 3.1 (Northwest Analytical, Portland, Oregon).

REPRODUCTION

Gonads were staged (virgin, maturing virgin, recovering, spent/developing, mature ripe and spent) according to colour, size, texture and shape; the stages were also validated against gonado-somatic index (GSI) and ovum-diameter frequency (ODF) cycles.

Ovum diameters were determined from material preserved in Gilson's Fluid. About 200 ova were measured for eight randomly selected females each month for one year. The ova were freed by shaking, diluted in suspension to 1 l, remixed and a 10-ml subsample taken for viewing with a Leitz Diavert compound microscope fitted with a video camera and monitor. Ova were measured (largest diameter) with electronic calipers recording from the monitor screen to an Osborne 1 microcomputer via an Arlec MPF-1 microprocessor.

Spawning and larval development

Monthly between November 1985 and February 1986, and again in April 1986, ten 3-minute surface hauls of the ichthyo-plankton trawl were made at 3-4 km h^{-1} in mid-afternoon on consecutive days at the four sampling sites. Samples were fixed in 10% buffered formalin and preserved in 70% alcohol. Later the larvae were immersed in glycerine, measured and drawn under polarised light. In this way

series were developed from embryos to identifiable juveniles.

Fecundity

The mature ovaries of 27 fish (c. 4 fish per 50mm size-interval from 150mm upward) captured between late October and early December 1983-85 were preserved in Gilson's Fluid. Fecundity was determined by the volumetric method (Mason et al., 1983). Ova of >250 um were counted as ODF analysis showed that only these were likely to be spawned in the current season. Subsamples were repeated until the 95% confidence intervals for the mean were <10% of the mean.

Age and length vs maturity

The sexual maturity of fish was assessed from gonad appearance and the mean gonadosomatic index (GSI = 100 X (gonad weight/body weight)), calculated monthly for each sex and size-class and validated by ODF analysis. Records of log mean GSI per month were compared for females >300 mm TL from PS and ZL, using fish selected from the October-December catches when the distinction between maturity and immaturity was clearest. Age determinations were based on scale readings supported by length-frequency analysis (Puckridge & Walker, unpublished). The frequency distributions of size-classes of fish used were matched to

those of the 130 m seine catches for this period, because these most closely approached a random sample. The age vs percent maturity and class midlength vs percent maturity relations for each sex were analysed following Leslie, Perry & Watson (1945).

III. RESULTS

GONAD MORPHOLOGY

The gonads of both sexes are paired and elongate, sometimes encased in fat deposits, enclosed by the peritoneum, and open externally through short, paired gonadal ducts. The left lobe normally is the larger in both sexes. The slope of the regression of left vs right gonad weight was significantly higher for females than males (*d*-statistic, $P<0.001$), indicating that the left lobe is relatively heavier in females.

SEASONAL CYCLE

In 1983-85 GSI of bony bream at the river site (ZL) showed a distinct seasonal cycle with a peak in November, at water temperatures of 19-20^{o}C (Fig. 2A) and a day-length of 14 h (Fig. 2B), and up to two months after the seasonal flood peak (Fig. 2C). The pattern was synchronized (to within a month) both for the sexes and the different size-

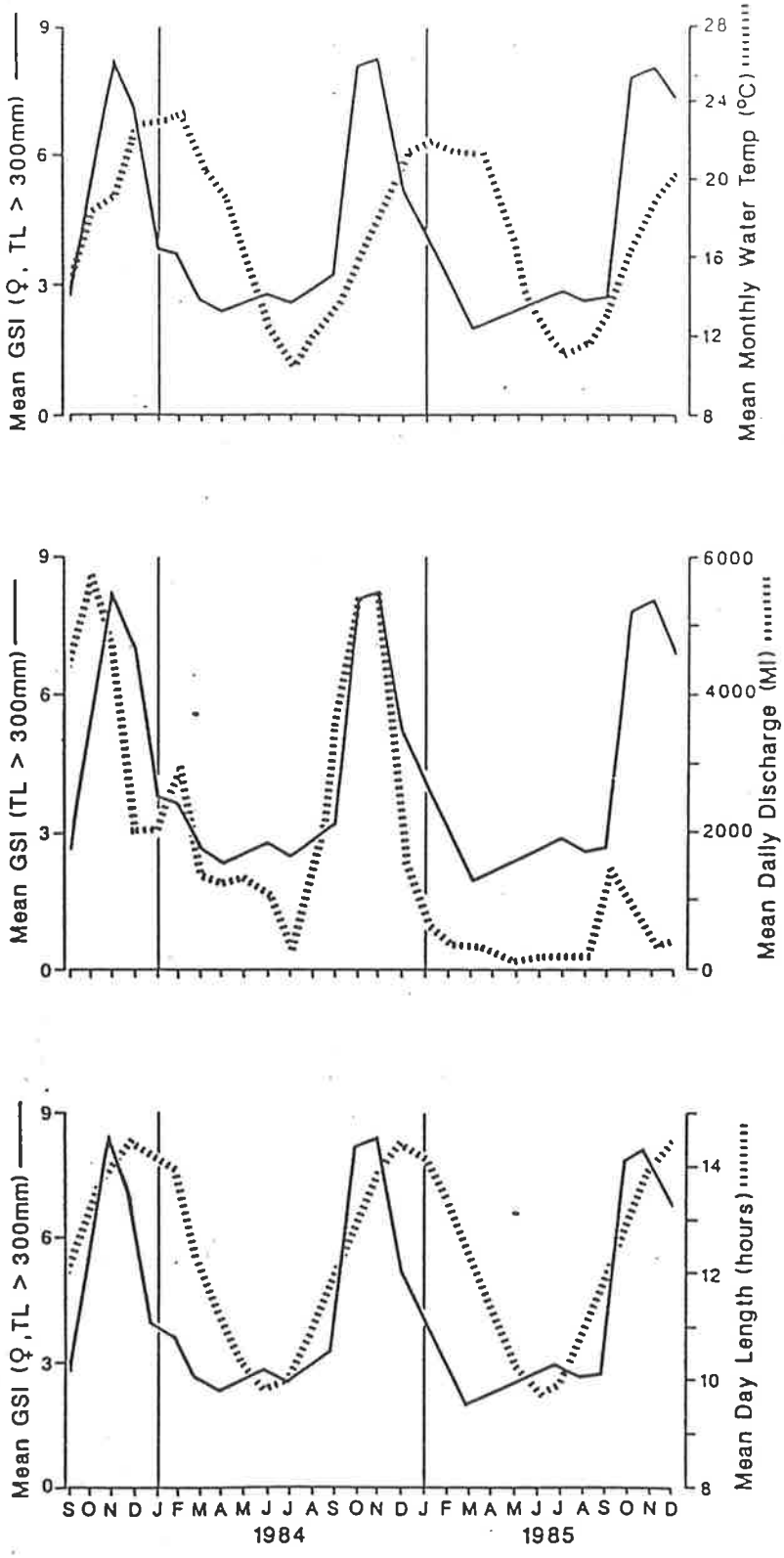


Fig. 2: Mean GSI of adult bony bream (Zadows Landing, 1983-84) plotted against data for sites nearby:

(a) vs mean monthly water temperature at Mannum.

(b) vs mean daily discharge at Lock 1, Blanchetown.

(c) vs mean day length at Adelaide.

classes. It was repeated at the lake site (PS), although the peak log female GSI was significantly higher (paired *t*-test, P <0.01). At neither site was there any apparent adjustment of the GSI cycle in response to flood timing.

Vitellogenesis apparently begins in September-October (spring); maturity is attained in November-December and spawning occurs in December-February at water temperatures of 21-23°C (Table 1). ODF analysis confirms this interpretation (Fig. 3). From the background of primary oocytes (mean diameter 104 um), vitellogenic ova (>250 um) first appear in substantial numbers in October. In December, spawning begins to deplete the mature eggs and this process is substantially completed by January. Only one major cohort of ova separates from the reservoir of primary oocytes during the cycle, although a few mature-size oocytes are present in some individuals much later in the year (March, July and August).

SPAWNING

Spawning was not directly observed and attempts to rear artificially-fertilized ova were unsuccessful. Female fish were rarely encountered in ripe condition, but most males maintained this condition throughout the breeding season. At only one site did ichthyoplankton trawls yield

TABLE 1: Gonad states of bony bream in 1983-84 at ZL.

Note that appearance is recorded *in situ* and colours differ when the gonads are dissected free of the peritoneum.

Stage	Sex	Interval	Appearance	Length/ Body cavity
Virgin	F		Grey, translucent thin strips	2/3
	M		Colourless to grey-white strips	2/3
Maturing Virgin	F	Apr-Aug	Pink-grey, translucent, firm	3/4
	M	Apr-Aug	Creamy pink, opaque, firm	3/4
Recovering Spent/ Developing	F	Apr-Sep	Orange-pink, translucent, firm, quilted	4/5-1.0
	M	Apr-Sep	Creamy pink, firm, smooth, opaque	4/5-1.0
Mature	F	Oct-Dec	Yellow-orange, bloodshot, granular, opaque, quilted, distended	1.0
	M	Oct-Dec	Creamy white, turgid, blocky	1.0
*Ripe	F	Dec-Jan	Translucent in patches, light yellow, quilted, distended, ova distinct	1.0
	M	Dec-Jan	Creamy white, turgid, blocky	1.0
Spent	F	Dec-Mar	Grey-pink, watery, semi-translucent, flaccid	1.0
	M	Dec-Mar	Pink to dark pink, bruised, flaccid	1.0

*Gonad products extruded on pressure

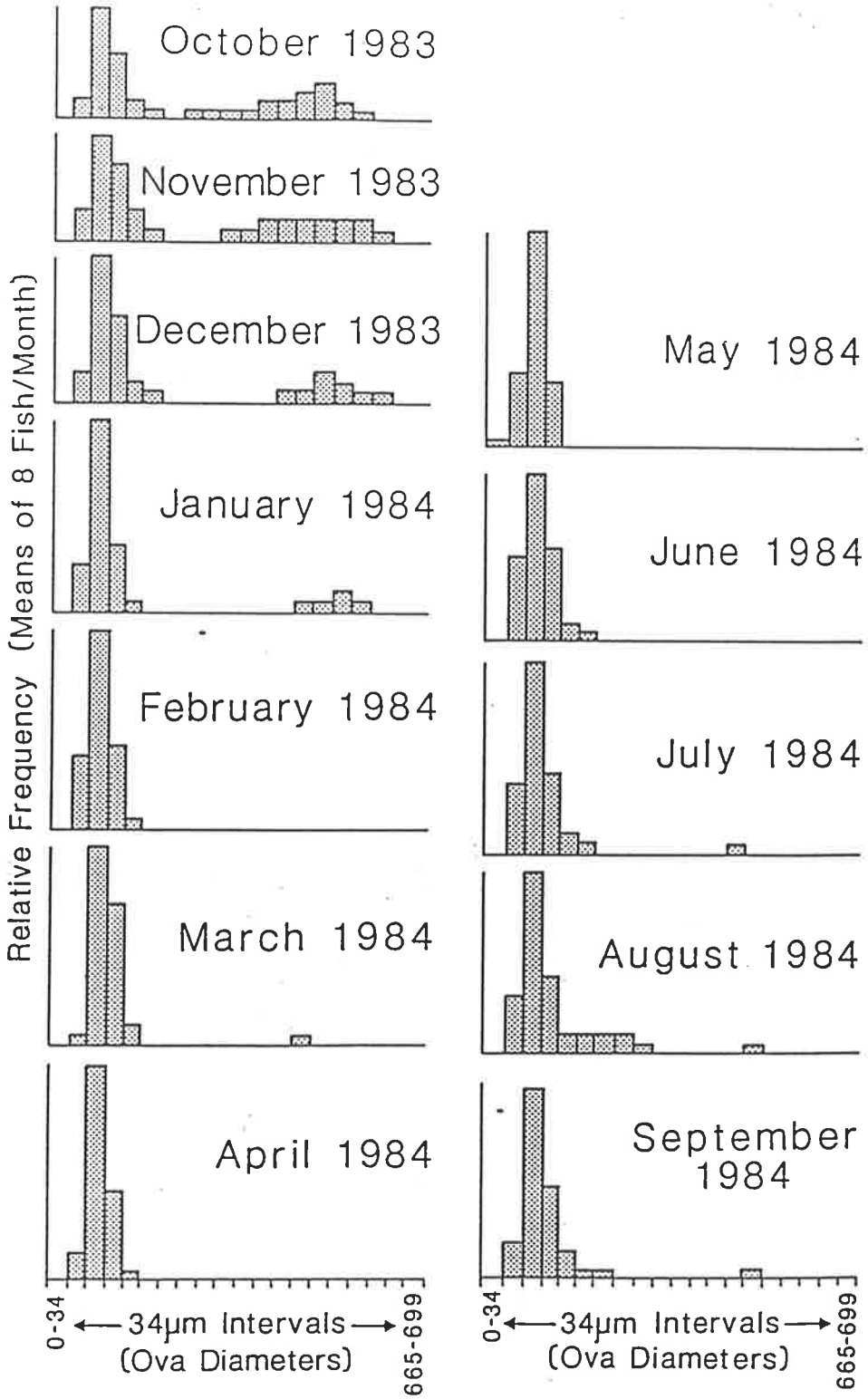


Fig. 3: Size-frequencies of bony bream ova (Zadows Landing and Point Sturt, 1983-84).

substantial numbers of ova; this was off a sandy shore west of PS early in February 1986 (Fig. 4). The catch/effort of bony bream larvae there was higher than at either of the river sites or at the other lake site.

SEX RATIO

The sexes in bony bream are externally indistinguishable. Nor is there a significant difference in the slopes of the log body length vs log body depth curves for the two sexes (d-statistic; P >0.05).

In the 1105 fish examined the ratio of males to females was 0.86 and significantly different from unity (Chi-squared, P <0.05). At both PS and ZL females were significantly more abundant in the largest size-class (TL >350 mm) than overall (Chi-squared, P <0.001). However, they were less abundant than males in the middle size-class (250 <TL <350 mm, Chi-squared, P <0.01) and of similar abundance in the lower size-class (100 <TL <250 mm, Chi-squared, P >0.05). The female to male ratios for the pre- and non-spawning periods are significantly different (Chi-squared, P <0.001), with higher catches of females in the pre-spawning period and higher catches of males in the remainder of the year.

EGG AND LARVAL DEVELOPMENT

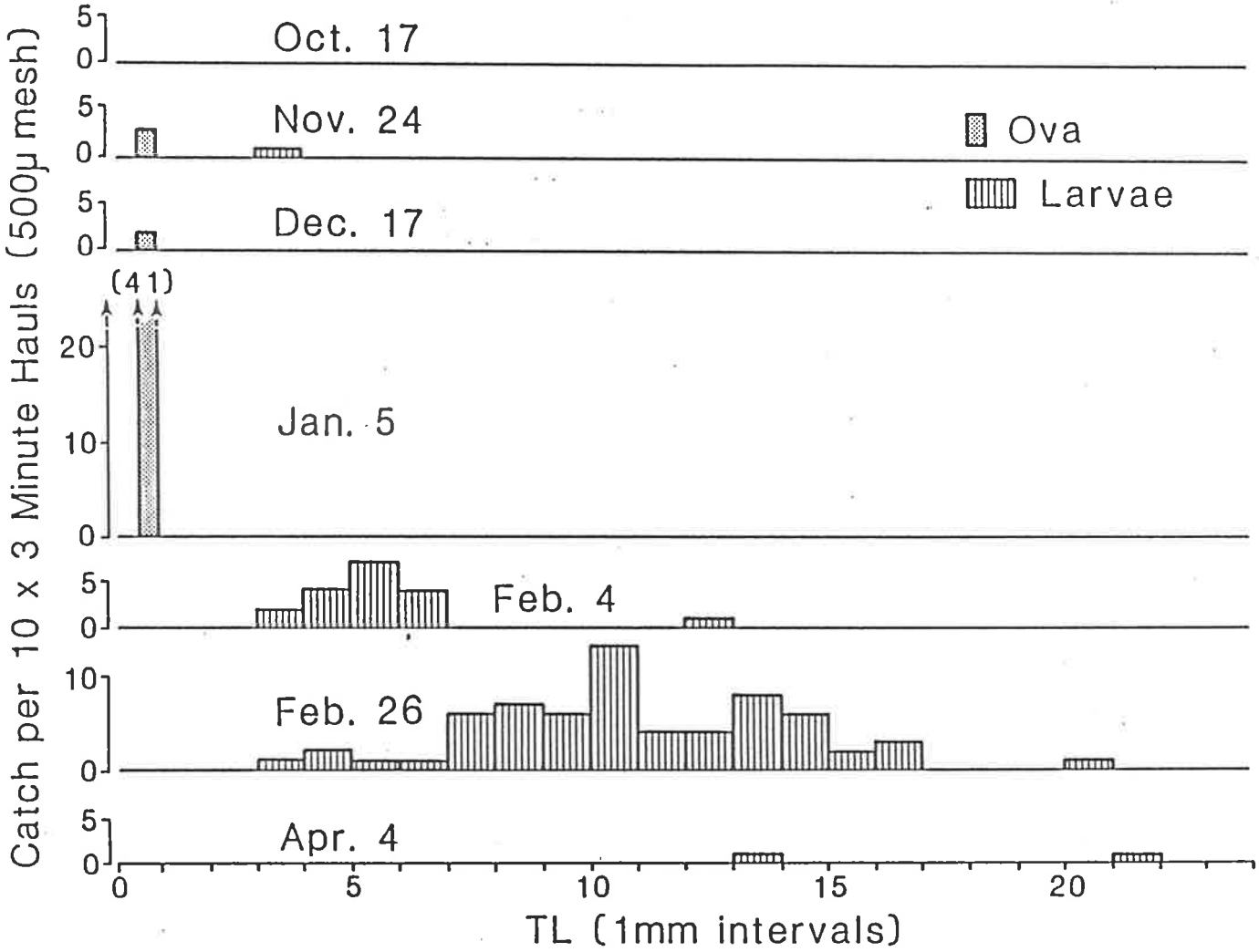


Fig. 4: Catches of bony bream ova and length-frequencies of larvae in ichthyoplankton trawls (Point Sturt, 1985-86).

Bony bream ova are spherical, with a smooth chorion and finely-segmented yolk. The water-hardened ova collected by ichthyoplankton trawl (Fig. 5) had a mean diameter of 0.829 mm (± 0.016 95% CI, n = 41); these were at least semi-buoyant, as the trawl was from the surface. Newly-stripped water-hardened ova were of similar diameter (0.834 ± 0.036 mm, n = 16), but were demersal and adhesive. In the trawled ova the yolk diameter was 0.54 mm and the chorion thickness c. 0.010 mm, leaving a perivitelline space of 0.14 mm. The ova typically contained a single large oil-droplet (diameter 0.26 mm) with the micropyle clearly visible alongside. An extra-chorionic layer was revealed by attached sand grains. Twenty percent of the trawled ova had clearly recognizable embryos and the more highly developed showed eye pigmentation and a ventro-lateral line of melanophores (Fig. 5C). The largest embryo found was 2.5 mm TL and the smallest free larva was 3.5 mm TL; the size at hatching therefore probably lies between these figures.

The larvae of bony bream, smelt (Retropinna semoni) and western carp gudgeon (Hypseleotris klunzingeri) comprised almost all of the trawled catch. Western carp gudgeon larvae can be distinguished from those of bony bream by body shape and mean myomere count (31 ± 0.97, n = 8; cf. 44.7 ± 0.53 for bony bream, n = 33, with pre-anal count

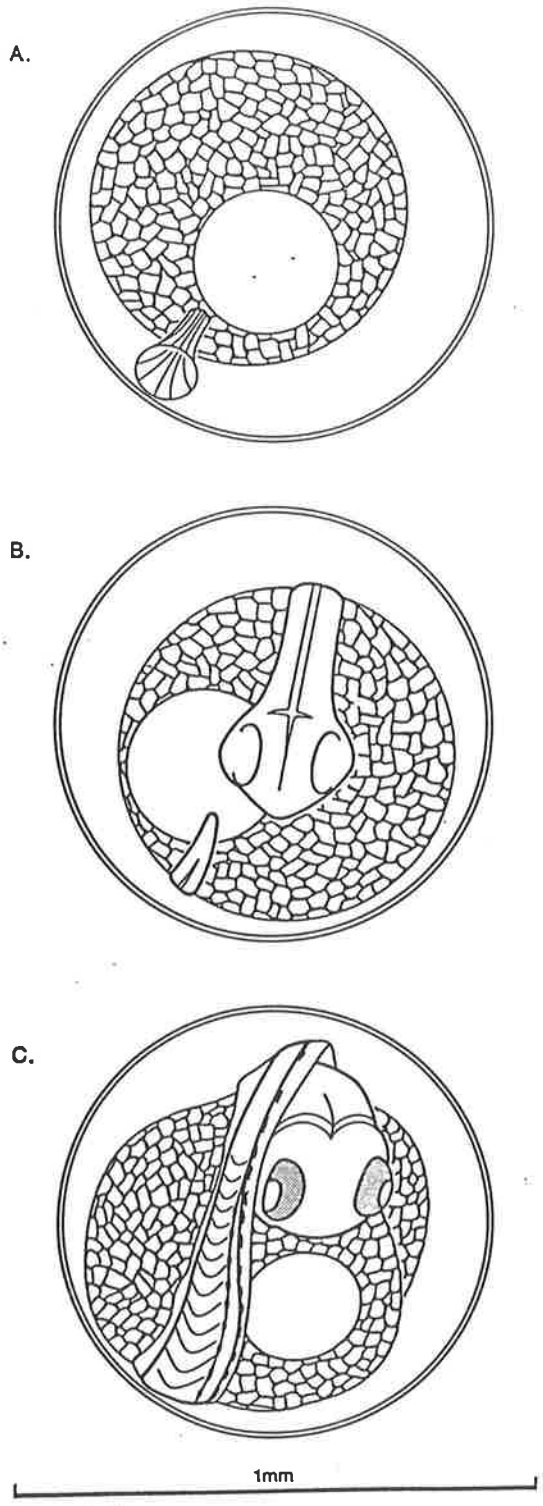


Fig. 5: Bony bream ova and embryos from ichthyoplankton trawls (Point Sturt, February 1986).

38.5 ± 0.83). The larvae of smelt have an eel-like form similar to that of bony bream larvae (Fig. 6), and both absorb the yolk sac early; hence discrimination of these must be based on mean myomere counts (53.6 ± 0.95 for smelt, n = 9). After dorsal fin development has begun smelt and bony bream can be readily distinguished by the relative positions of that fin and the anal primordium along the anterior-posterior axis (in smelt, the dorsal overlaps the anal fin; in bony bream it does not). Although this description is incomplete because yolk-sac larvae were not collected, the sequence of major changes in larval development is clear:

(1) The yolk sac is largely absorbed by 3.5 mm TL.

(2) The caudal and pectoral fins develop rays first at approximately 3 mm TL. The dorsal fin follows at 7 mm, the anal fin at 11 mm and the pelvic fin at 16 mm.

(3) Throughout larval development melanophores are present in a line along the dorsal border of the gut. After 6 mm TL a single melanophore becomes obvious ventrally and immediately anterior to the cleithrum, and a pair appears ventrally immediately posterior to the cleithrum. A fine mid-ventral

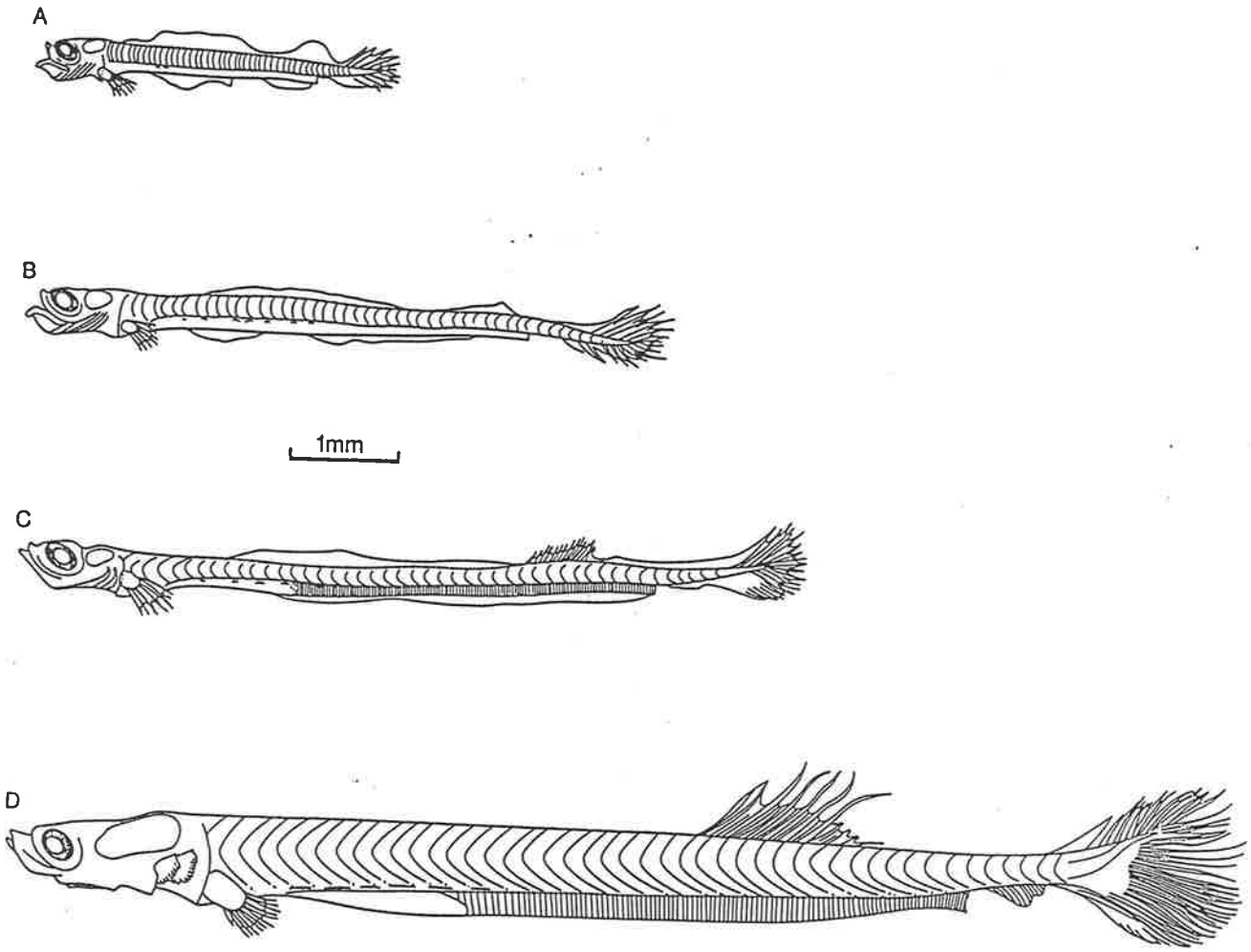


Fig. 6: Bony bream larvae from ichthyoplankton trawls (Point Sturt, February 1986).

line of melanophores also appears along the hindmost 2/3 of the gut.

(4) Caudal flexion of the notochord begins at 10 mm TL.

(5) Anterior migration of the anus and the dorsal and anal fins is noticeable at 17 mm.

(6) First scalation appears along the lateral line at 26 mm and is complete by 35-40 mm.

FECUNDITY

Fecundity (F) rises from 33,000 for a fish of 199 mm TL (body weight 88.9 g) to 880,000 for a fish of 403 mm TL (595.4 g). These estimates are derived from the equations

$$\log_{10} F = -3.923 + 3.725 (\log_{10} TL)$$
$$(n = 27, r^2 = 0.88, \text{F-test}, P < 0.001)$$

and $\log_{10} F = 2.188 + 1.290 \log_{10}$ (body weight)
$(n = 27, r^2 = 0.91, \text{F-test}, P < 0.001)$.

A log-log equation best describes the relation between fecundity and gonad weight, but it is little different from the equation for untransformed data ($r = 0.92$). Thus

$$\log_{10} F = 3.822 + 1.131 \log_{10} (\text{gonad weight})$$
$$(n = 27, r^2 = 0.95, \text{F-test}, P < 0.001).$$

There is a significant relation between fecundity, body weight exclusive of gonads and TL, viz.

$$F / (\text{body weight} - \text{gonad weight}) = 117.58 + 2.54\ TL$$

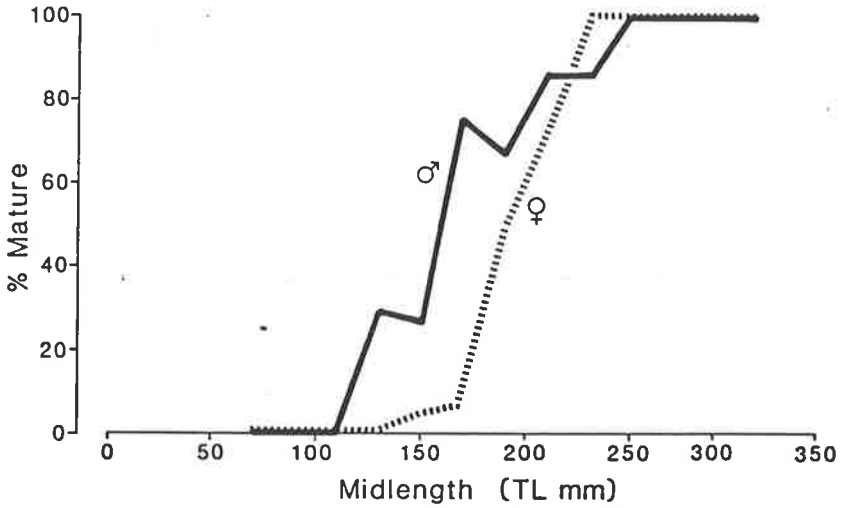
$(n = 27,\ r^2 = 0.28,\ F\text{-test},\ P < 0.01)$.

Log-log plots of GSI vs TL yield highly significant regressions for males and females (respectively, $n = 87$, $r^2 = 0.32$, F-test, $P < 0.001$; $n = 154$, $r^2 = 0.68$, F-test, $P < 0.001$). The slope is significantly greater for females (d-statistic, $P < 0.001$), indicating a more rapid rise in GSI with body size.

LENGTH AND AGE AT FIRST MATURITY

For both sexes, plots of percent mature fish in a length-group against the midlength of that group produce sigmoidal curves (Fig. 7A). Many males mature at a smaller TL than females, but the males also mature over a greater range of TL. Probit analysis shows a significant difference between the intercepts of the curves for males and females, but not between the slopes (Fig. 7B). The median lengths at maturity for males and females are 159 (95% confidence limits 144-175) mm TL and 199 (180-234) mm TL respectively.

Similarly, in a plot of percent mature fish against age



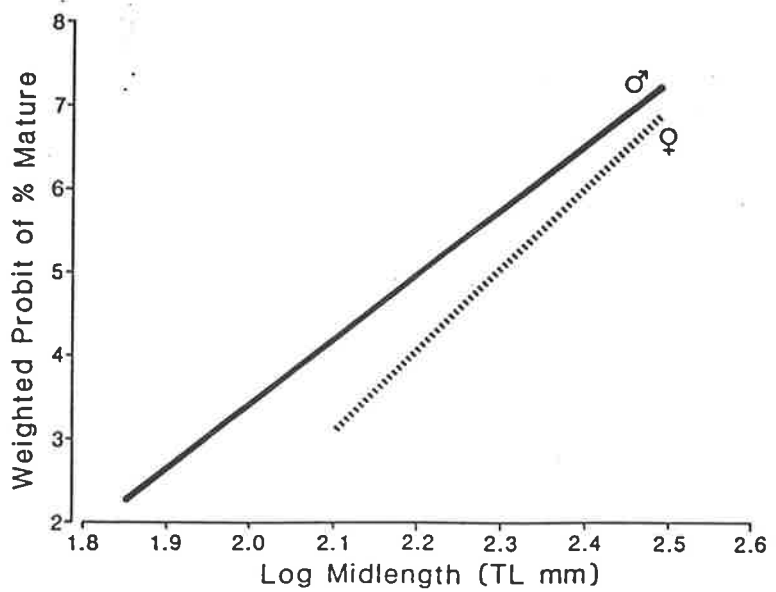


Fig. 7: Percentage mature bony bream vs midlengths of total length classes (Zadows Landing and Point Sturt, October - December 1983-85): (a) untransformed data and (b) probit-log transformed data.

(Fig. 8A), males mature earlier but male maturation extends over a greater range of ages. In this case, however, the probit-transformed data for the sexes differ in both intercept and slope (Fig. 8B). The median ages at maturity are 2.4 (95% confidence limits 1.6-3.4) y for males and 2.7 (2.4-3.6) y for females. Even so, one male matured in the first year at a total length (LFM) of 126 mm, and one female in the second year at 155 mm.

IV. DISCUSSION

In bony bream, as in most gizzard shads, the sexes are externally similar (Miller, 1960; cf. Bodola, 1966). The male:female ratio of 0.86:1 reported here contrasts with that of 1:1 reported for bony bream in northern Australia (Bishop et al., in press), and with 1.6:1 for D. cepedianum (Jester & Jensen, 1972). In both bony bream and D. cepedianum, however, there are more females in the largest size-ranges, suggesting that the sexes may have different growth or mortality rates. The predominance of males in pre-spawning catches may arise from seasonal behavioural differences that cause them to outnumber females on the spawning grounds, as for Dorosoma spp. (Warner, 1941; Jester & Jensen, 1972; Bodola, 1966). The presence of males running ripe throughout the breeding season (as in

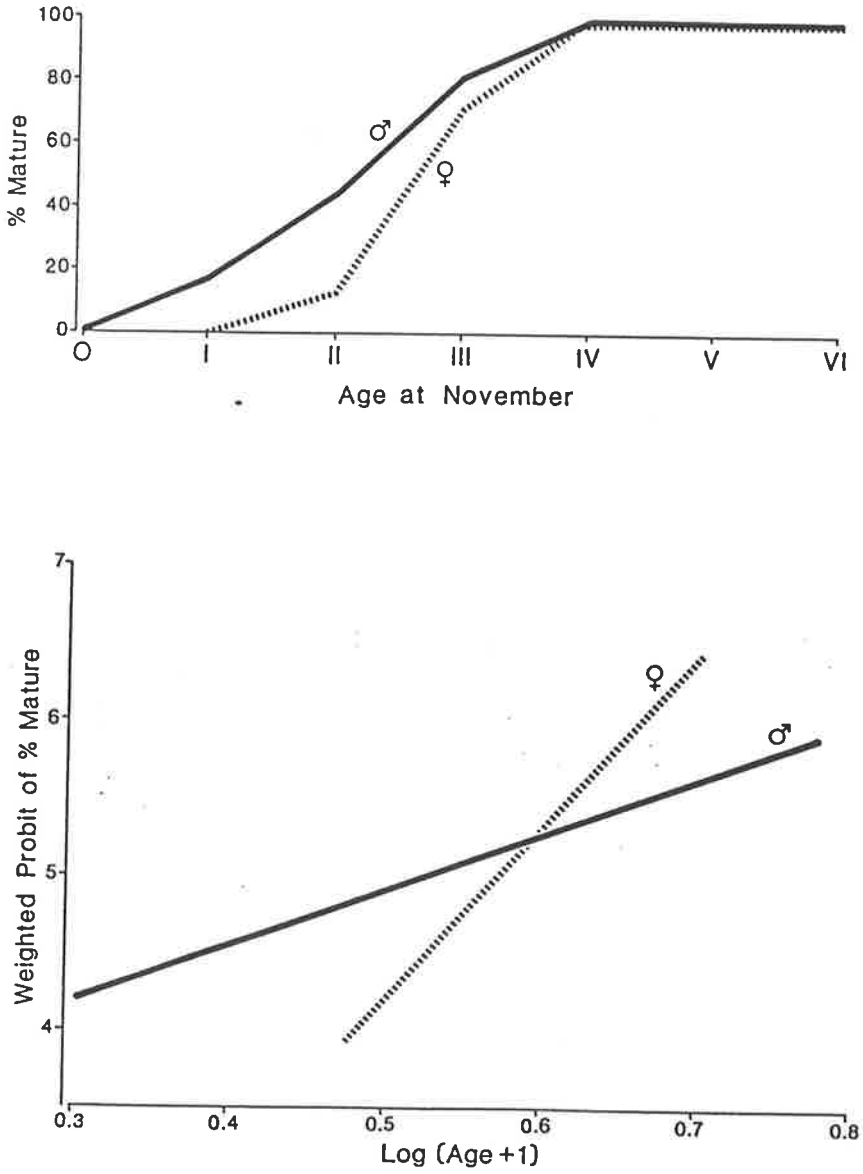


Fig. 8: Percentage mature bony bream vs age (Zadows Landing and Point Sturt, October - December 1983-85): (a) untransformed data and (b) probit-log transformed data.

D. petenense, Johnson, 1971) suggests that multiple matings by males may be common. This would deplete the male population of non-spawning areas like ZL. Unfortunately an equivalent size-distribution (and hence a comparison of the sex ratio) is not available for PS, which is close to a known spawning ground.

The fecundity of bony bream is high (cf. Lake, 1967; Merrick & Schmida, 1984; Bishop et al., in press) and exceeds that of other gizzard shads (Chubb & Potter, 1984; Rao, 1965; Kilambi & Baglin, 1969b) except D. cepedianum (Kilambi & Baglin, 1969a). The bony bream also shows an exceptional rate of increase in fecundity with body size. Egg size evidently does not change significantly with body size -- hence the trivial difference between the untransformed and log-log equations for fecundity vs gonad weight (cf. Mann & Mills, 1985). Other relationships demonstrate that reproductive effort increases with size, and that female effort increases more rapidly than that of males. The rate of change of reproductive effort with size evidently depends on local conditions and/or stocks; some populations of D. cepedianum show an increase (Fagan & Fitzpatrick, 1978) and others a decline (Bodola, 1966; Jester & Jensen, 1972; Shelton, 1972).

Bony bream in the lower Murray spawn in December-January,

later than in New South Wales (October-December: Llewellyn, 1983), but at the same time as N. vlamingbi in the Swan estuary (Chubb & Potter, 1984). Bony bream in tropical Australia show little spawning seasonality, although there is a peak in the early wet season (Bishop et al., in press). Flooding is not an essential cue to spawning, as it is for certain other Murray species (cf. Cadwallader, 1986). Indeed, bony bream are known to spawn in isolated water-bodies remote from the influence of any floods (Lake, 1978; Puckridge & Drewien, 1988). The present data suggest no adjustment of the GSI cycle to flood timing. Flooding may enhance recruitment, however, by expanding and improving juvenile feeding habitat (Puckridge & Drewien, 1988).

For bony bream, Lake Alexandrina (PS) may be a better breeding environment than the main channel of the Murray (ZL); this is suggested by the higher peak female GSI and catch/effort of larvae at the lake site. Open, sandy shallows appear to be the preferred spawning habitat in the lower Murray, but in reaches of the Murray-Darling system in New South Wales spawning reportedly occurs in schools in shallow backwaters (Llewellyn, 1983). In northern Australia spawning may be concentrated in muddy lowland lagoons (Bishop et al., in press). D. cepedianum and D. petenense also spawn in backwaters or lake coves (Jester & Jensen,

1972; Shelton, 1972; Littlejohn *et al.*, 1985). Unlike *D. petenense* (Gerdes & McConnell, 1963), the bony bream does not appear to spawn near aquatic vegetation.

Some gizzard shads, like the bony bream, spawn only a single batch of ova (Annigeri, 1967; Chubb & Potter, 1984), others spawn serially (Baglin & Kilambi, 1968; Johnson, 1971; Annigeri, 1967). The major stages in gonadal development for bony bream show typical diagnostic features (e.g. Pollard, 1972), and resemble the histologically-based staging described for *N. vlaminghi* (Chubb & Potter, 1984). The presence of mature-sized oocytes in some individuals late in the year (March, July and August) suggests either a capacity for opportunistic spawning or incomplete atresia (some specimens were found with hardened egg-masses).

The smooth chorion and finely-segmented yolk of bony bream ova is typical of clupeids (McGowan & Berry, 1984). Although Llewellyn (1983) suggested that the ova were semi-buoyant, they apparently have an early demersal and a later pelagic phase making bony bream, like *D. cepedianum*, litho-pelagophils *sensu* Balon (1975).

The pigmentation patterns, body shape, gap between dorsal and anal fins and forward migration of the anus in bony bream larvae are also typically clupeid (McGowan &

Berry, 1984). However, the rate of absorption of the yolk-sac in bony bream after hatching is exceptional (complete by 3.5 mm TL), as D. cepedianum and D. petenense retain it to at least 5 mm TL (Shelton & Stephens, 1980) and A. chacunda retains it until 5 mm TL (Thangaraja & Ramamoorthi, 1980). This may indicate a relatively rapid development rate in bony bream embryos and pro-larvae, but this awaits confirmation. The early development of the pectoral fin-rays in bony bream larvae is also unusual among clupeids (McGowan & Berry, 1984), although observed in A. chacunda (Thangaraja & Ramamoorthi, 1980). As the larvae are small and the yolk-sac stage is brief, the survival of bony bream larvae must depend acutely on zooplankton density at first feeding (c.f. Arumugam & Geddes, 1987).

Although it is common in fish biology to use an "age at first maturity" index (AFM/LFM), this index says little about the age (or length) - maturity relationship. Further, the index is sensitive to sample size. Probit analyses offer useful supplementary information, as they allow statistical comparisons between regression relationships and provide a median length (MLM) or age at maturity. Generally, however, only AFM and/or LFM data are available for other gizzard shads. Comparative LFM estimates for bony bream in the Murray in New South Wales include an improbably

low 70-80 mm TL in the first year (Lake, 1967; Cadwallader, 1977; Cadwallader & Backhouse, 1983). In comparison, Bishop et al. (in press) reported LFM values of 156 mm for males and 168 mm for females in their first year in Magela Creek, Northern Territory. Comparisons with LFM and AFM data for other Dorosomatinae (Jacob, 1948; Thomson, 1957; Berry, 1958; Bodola, 1966; Jester & Jensen, 1972; Chubb & Potter, 1986) show that bony bream conform to the gizzard shad pattern of early maturity.

The bony bream in many respects is a typical gizzard shad. It matures early, has small ova and larvae, no external sexual dimorphism, and favours warm, shallow waters. It is unusual in certain features of larval development and in its exceptional fecundity.

Interesting comparisons may be drawn between bony bream and other species native to the lower Murray. It has been suggested (Cadwallader, 1986; Pierce & Walker, in press) that the decline of populations of Murray cod (Maccullochella peelli) and freshwater catfish (Tandanus tandanus) is due to failures in recruitment. Although spawning by these species is not flood-cued, larval survival is flood-dependent and almost certainly there is extensive larval mortality due to changes in flow. Populations of

callop (Macquaria ambigua) and silver perch (Bidyanus bidyanus), in which spawning is flood-cued, have adjusted more successfully. Curiously then, the bony bream -- in which spawning is not flood-cued, which has small larvae having a very brief yolk dependency and which spawns 2-3 months after the usual flood peak -- is flourishing in the regulated flow environment of the lower Murray. This is likely to reflect a superior capacity for population increase. Both Murray cod and catfish mature late (age IV-V for cod (Cadwallader, 1977); III-V for catfish (Davis, 1977)); they also have low fecundity (cf. Lake, 1959; Davis, 1977) and so cannot recover rapidly after prolonged recruitment failure.

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Figures

Sturt, October - December 1983-85): (a) untransformed data and (b) probit-log transformed data.

Fig. 8: Percentage mature bony bream vs age (Zadows Landing and Point Sturt, October - December 1983-85): (a) untransformed data and (b) probit-log transformed data.

APPENDIX III

Mycotic dermatitis in a freshwater gizzard shad, the bony bream Nematalosa erebi (Günther), in the River Murray, South Australia

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Abstract. The lower Murray population of bony bream is subject to an annual epidemic of the oomycete Saprolegnia (principally S. parasitica) and the bacterium Aeromonas hydrophila. The epidemic is species-specific; it affects mainly adults whose susceptibility may be increased by stress due to winter cold. Lesions occur on the mid-flank and are characterized by an external mycelium, epidermal erosion, scale loss, hypodermal and muscular oedema, haemorrhage, myofibral degeneration and by the presence of Saprolegnia hyphae at all stages of infection. Although

A. hydrophila is common in advanced lesions there is no significant systemic bacterial infection. This appears to be a primary mycotic dermatitis and is noteworthy because Saprolegnia is best-known as a secondary pathogen.

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Introduction

Oomycetes of the genus Saprolegnia include a number of facultative pathogens responsible for saprolegniasis in fish. Most outbreaks follow bacterial or viral infection (Egusa & Nishikawa 1965; Willoughby 1970; Bekesi, Kovaks-Gayer, Ratz & Turkovics 1984), injury to the epidermis (White 1975; Pickering & Willoughby 1977) or conditions associated with captivity (Willoughby & Pickering 1977; Copland & Willoughby 1982). These infections normally are single or sporadic, although there are reports of regular infections in wild salmonids subject to spawning stress (Neish 1977; Richards & Pickering 1978; Pickering & Christie 1980). S. parasitica is the dominant Saprolegniacean in these infections (Willoughby 1978; Wood, Willoughby & Beakes 1988).

The bacterium Aeromonas hydrophila may be a secondary invader of lesions (Humphrey 1985; Menasveta 1985) or a primary pathogen in systemic and integumentary infections of fish subject to spawning, thermal or low-oxygen stress (Richards & Roberts 1978; Esch & Hazen 1980; Nieto, Corcobado, Toranzo & Barja 1985).

Where both A. hydrophila and Saprolegnia spp. have occurred in an infection primacy usually has been attributed to A. hydrophila (Egusa 1965; Thorpe & Roberts 1972; Inman & Bland 1981; Callinan 1985). In the one reported exception known to us -- a case where A. hydrophila was associated with an otherwise typical salmonid saprolegniasis (Richards & Pickering 1978) -- bacterial samples were not taken from integumentary lesions.

This paper reports a further exception that occurs regularly in a wild population of an Australian freshwater fish, the bony bream (Nematalosa erebi (Günther)). This species, a gizzard shad (Dorosomatinae), is the most abundant fish in the River Murray in South Australia, where it is netted as bait for a marine rock-lobster fishery. Fishermen in the region have noticed fungal infections in late winter or early spring since at least the 1940s (L. Gray, Meningie, S. Aust., pers. comm. to JTP). Here, we identify the principal pathogens and describe the pathology

and epizootiology of the infection.

Methods

(1) Sampling

Bony bream were obtained from Zadows Landing on the lower Murray (34° 58'S, 138° 59'E). Sampling was monthly and continuous from September 1983 to December 1984, but thereafter confined to the epizootic and breeding seasons (June to December each year until October 1987). Seven 50-m gill nets (mesh 20-110 mm) and three seines (lengths 2, 18, 130 m; meshes 2, 12, 30/50 mm) were used. Samples comprising either the entire catch or stratified random subsamples (total length (TL) size-class interval 50 mm) were retained. Infected and healthy fish were handled separately. All fish were measured (TL to 1 mm), weighed (0.1 g), and the gonads dissected and weighed (0.01 g). Analyses employed computer-based statistical packages (BMDP Statistical Software 1987; NWA Statpak 3.1, North-Western Analytical, Washington) and our own programs in Microsoft BASIC. The Gonado-Somatic Index

$$GSI = 100\ (\text{gonad weight})/(\text{body weight})$$

and a Condition Factor

$$10^5\ (\text{body weight-gonad weight})/(\text{total length})^3$$

were routinely calculated.

Water temperature, pH, conductivity, Secchi depth, dissolved oxygen and river levels were monitored on each sampling occasion, and supplementary records of temperature, discharge, water chemistry and organo-chlorine pesticide concentrations were obtained from the Engineering & Water Supply Dept, Adelaide.

(2) Mycology

Mycelial samples from lesions were cultured on chloramphenicol-cornmeal agar (250 mg chloramphenicol per litre agar) and duplicate samples were placed in sterilized river water. The lesion was photographed, mapped on a gridded fish outline, dissected free to a depth of 1 cm and preserved in 10% buffered formalin. Hyphal tips from the agar colonies were subsampled repeatedly until bacteria-free. The mycelia kept in sterilized river water were examined before sub-culturing to check the selectivity of the agar medium (cf. Willoughby 1978). For 60 isolates the mycelial clumps from sterilized water and hyphal tips from agar cultures were transferred to sterile distilled water prior to observations of zoospore release. Fifteen isolates were maintained in the dark at 7°C on sterilized hemp seed in filtered and autoclaved Murray water to allow observations of the formation of sexual structures (cf. Willoughby 1978).

Selected isolates (Table 1) were also examined under the transmission electron microscope to check their cyst coat morphology (see Beakes and Ford 1983; Hatai, Willoughby & Beakes in press). Colonies were grown in a liquid glucose peptone salts medium (GYPS: Beakes & Ford 1983) for 24 hours at room temperature (c. 20° C) before being induced to sporulate by transfer to sterile lake water. Formvar coated copper grids (3.0 mm 100 mesh) were placed in the bottom of each dish and left for 24 to 96 hours, depending on the species. The grids were checked for the presence of spores, then removed and excess water was carefully drawn off using hardened Whatman No. 8 filter paper. The dried grids were shadowed with gold palladium and coated in carbon in an Edwards Vacuum coating unit as described in Beakes (1983). The specimens were examined in a Kratos CORA electron microscope operated at 60kV with a 25 um aperture.

(3) Bacteriology

In September 1986 bacterial sampling was included in the protocol for 34 infected and 32 uninfected fish. These samples were taken with a flamed loop, streaked on DIFCO nutrient agar in a 90-mm petri dish and spread with a sterile swab dipped in sterile saline. If mycelia were present on a lesion part was lifted with sterilized forceps and the loop touched on the exposed tissue. If mycelia were

absent the ulcerated surface was sampled directly. A sample was taken also from unaffected skin on the mid-flank. The flank was then seared and opened along the swim-bladder using sterilized scissors. The liver and anterior kidney were incised with a sterilized scalpel and the incision sampled. A control plate also was streaked with the sterilized loop and spread as above.

Each infected fish was paired with a similar-sized uninfected fish caught at the same time and place; these were treated in the same way, excepting the lesion sample. The culture plates were stored for three days at about river temperature (12-18°C).

The primary culture plates were examined for oxidase-positive colonies using oxidase strips (Institute of Medical & Veterinary Science, Adelaide). These colonies were observed, counted, sub-cultured to blood agar and incubated overnight at 35°C. Sub-cultures were checked for purity before inoculation variously into peptone water (motility read at 4 h), OF medium and blood agar (incubated anaerobically).

Presumptive Aeromonas isolates were screened for oxidase reactivity, motility, fermentation at 24 h in an open OF tube, anaerobic growth at 24 h and colony morphology.

Verifications were made using the API 20E Identification System for Entero-Bacteriaceae and other Gram-Negative Rods (API Systems S.A., Montalieu-Vercieu, France).

(4) Virology

In September 1986 three healthy, three slightly infected and three severely infected fish were frozen on dry ice and sent to the Australian Fish Health Reference Laboratory at Benalla, Victoria. Kidney, liver, spleen and affected skin samples were inoculated onto rainbow trout gonad (RTG-2), redfin embryo (R Femb) and golden perch embryo (G Pemb) cell lines. A pass for cytopathic agents was made at 10 days and checks were continued for a further 10 days.

(5) Histology

Skin tissue samples from nine infected and three apparently uninfected specimens were embedded in paraffin, sectioned (2 um) and stained with haemotoxylin and eosin. Gridley's, periodic acid-Schiff (PAS), Grocott-Gomori and methenamine silver stains also were used to detect fungal elements.

Results

(1) Epizootiology

The epizootic typically begins in June-July, when

temperatures are minimal (Fig. 1A) and flooding has not commenced (Fig. 1B). This is two months before the annual low in the body Condition Factor (Fig. 2A), three months before there is a significant rise in GSI and five months before spawning (Fig. 2B). The peak monthly incidence of infection is correlated with the mean July-August water temperature (Spearman Rank Z = -2.08, n = 5, P <0.05), but not with mean daily discharge. Incidence reaches a peak in August and September and involves 10-64% of the adult population. Juvenile fish (TL <150 mm) are rarely affected (<0.4%). There is no significant difference in the incidence of infection in the two sexes (paired samples t-test). There are no differences in mean Condition Factor (log(X+1)-transformed) or mean GSI for infected and uninfected fish (paired samples t-test, n = 86 pairs), and no correlation between condition and area of the lesion (Spearman Rank).

The water quality data suggest that no heavy metals or organo-chlorine pesticides occurred in the lower Murray in toxic concentrations over the sampling period. Nor were there changes in pH, conductivity or dissolved oxygen likely to cause stress to the bony bream. No other fish species showed signs of infection.

(2) Gross Pathology

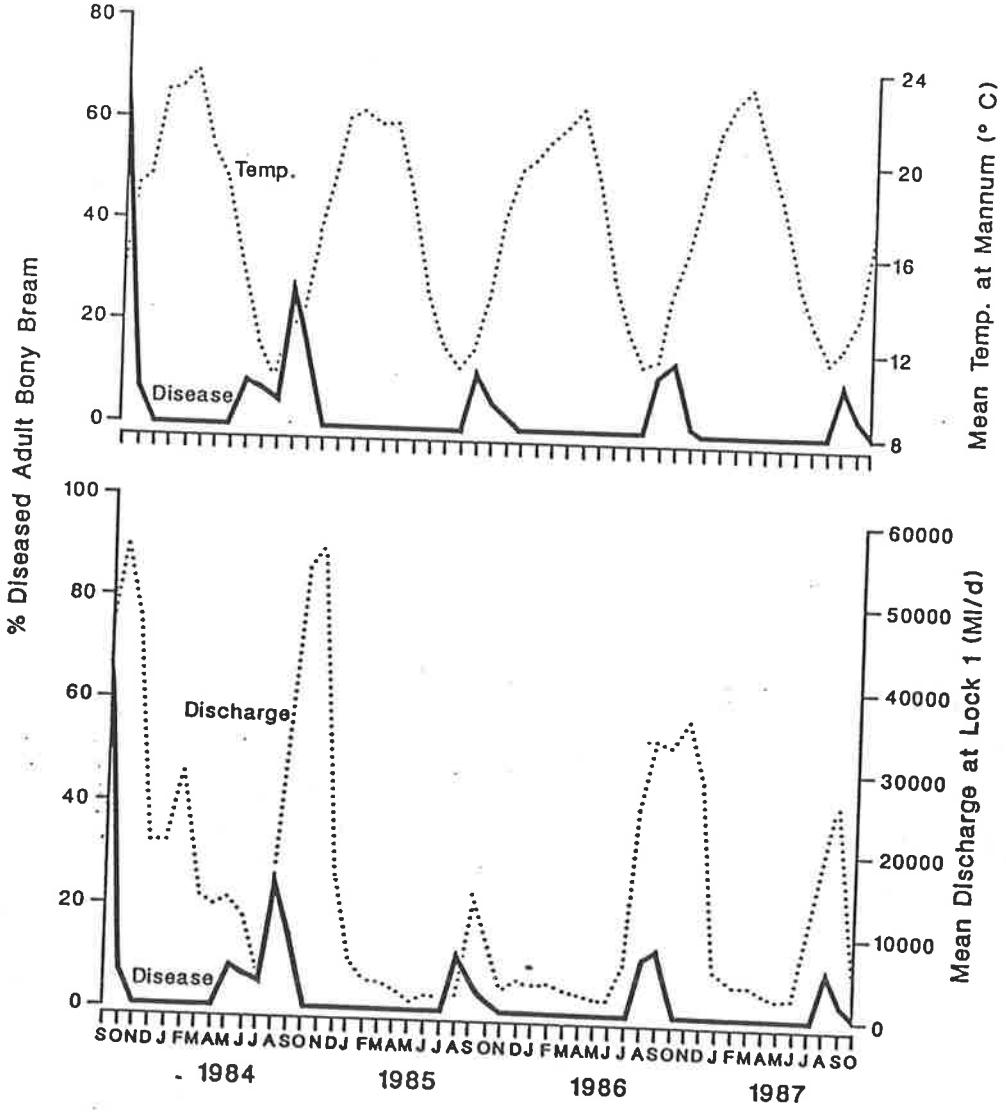


Fig. 1. Percentage incidence of dermatitis in adult bony bream 1983-1987 compared to (A) mean monthly water temperature and (B) mean daily discharge.

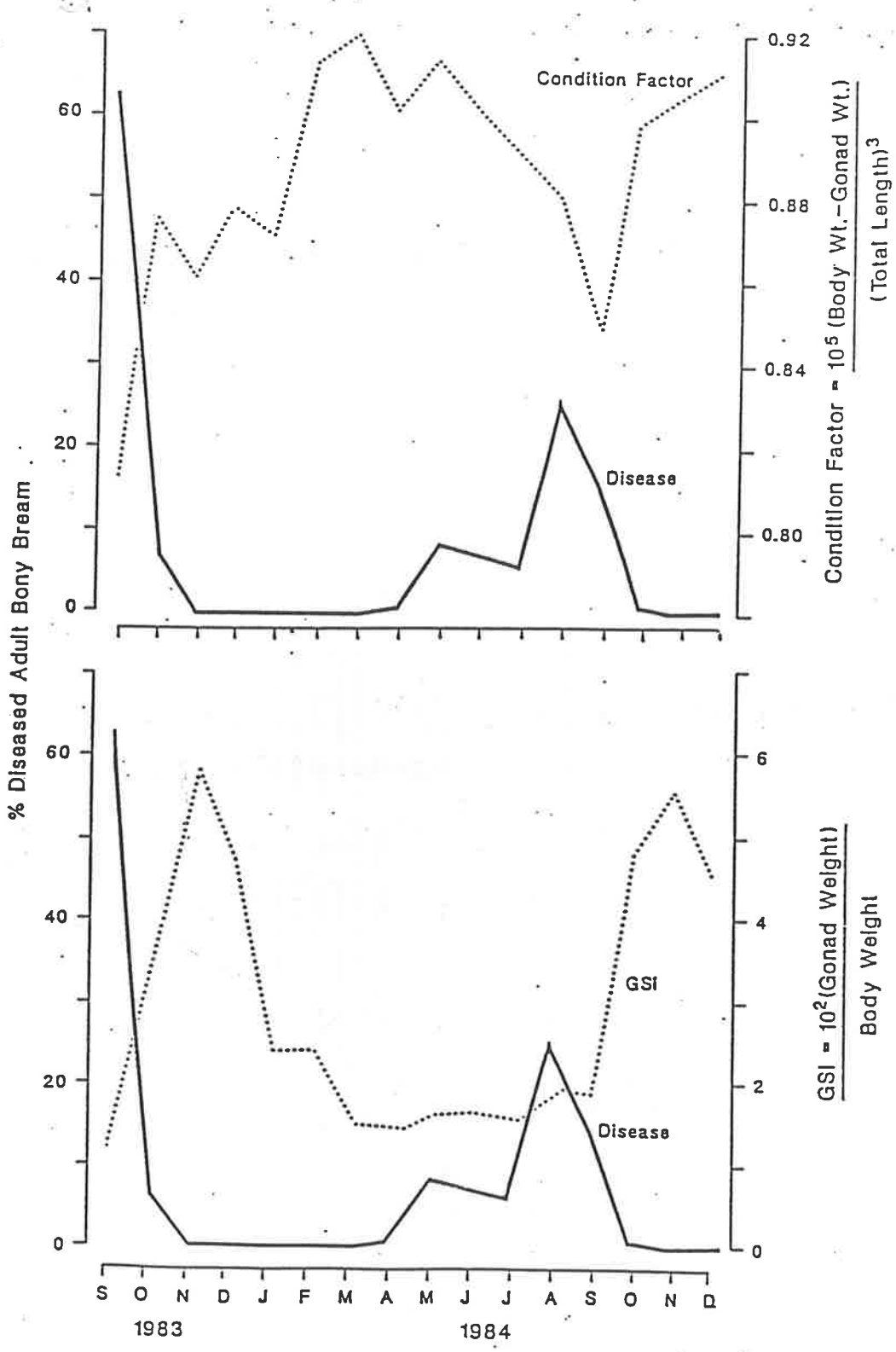


Fig. 2. Percentage incidence of dermatitis in adult bony bream in 1983-1984 compared to (A) mean monthly condition factor and (B) mean monthly GSI.

The proportions of infected grid cells (among 168 per fish) in four body regions (fins, head, mid-flank and posterior flank) on the left and right sides of the body were analyzed by Stepwise Logistic Regression (BMDP Statistical Software 1987, program LR). The potential effects due to fish, body region and side were included as main effects in the logistic model, together with a side vs region interaction. The lesion area is significantly higher on the anterior mid-flank ($P < 0.001$); the head and gills, fins and posterior flank are rarely affected. There is also a significantly greater mean area of infection on the left than the right flank ($P < 0.05$). There is no significant interaction between side and region. The mean area of lesion is 5.3% of the body surface (SD = 3.5%, n = 91) and the maximum is 16%.

The least severe and probably earliest lesions appear as a thin fuzz of mycelia over skin with no obvious haemorrhage or inflammation (Fig. 3A). Distinct lesions without fungus infection rarely were observed; there was occasional reddening of the skin and elevation or loss of scales, but this is typical of the reaction of bony bream to capture and handling.

Lesions progress by an increase in the density of

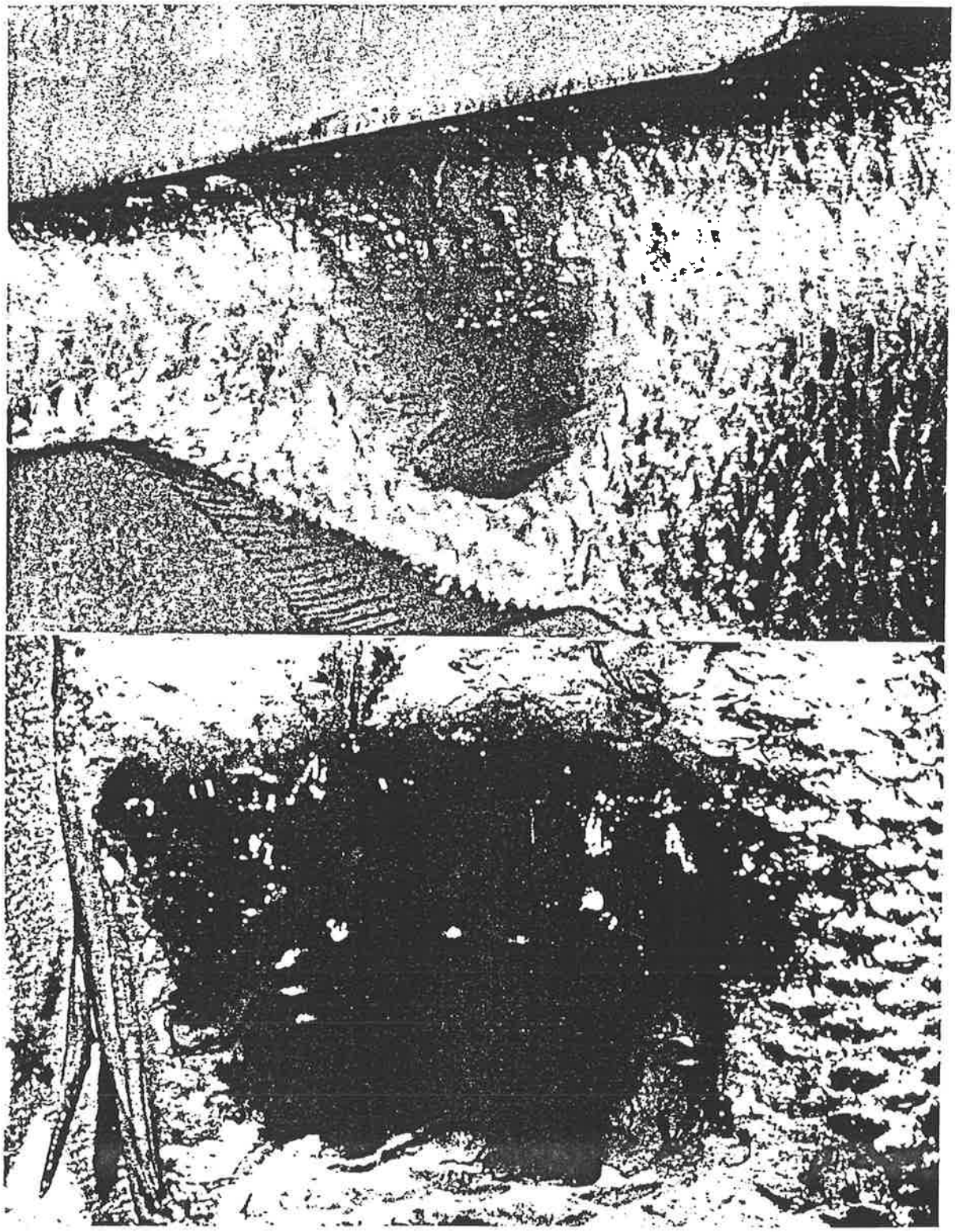


Fig. 3. Mycotic lesions on skin of bony bream: (A) slight, (B) moderate.

investing mycelia, erosion of the epidermis and protrusion then loss of scales, with increasing peripheral haemorrhage and erythema (Fig. 3B). Intermyotomal haemorrhage and myomalacia occur in advanced lesions. One specimen only was found with scar tissue and pigmentation suggestive of healing, and only two moribund specimens were captured. With few exceptions the internal organs of fish with external lesions appeared normal.

(3) Mycology

In mycelia transferred directly from a lesion to sterile water there were occasional hyphomycetes, but the dominant organisms were non-septate oomycetes and all, apart from one specimen of Leptomitus sp., belonged to the Saprolegniales.

In 60 of 62 isolates of Saprolegniales the primary zoospores cleaved within the zoosporangium and were released terminally. Ten isolates observed at the moment of zoospore release all demonstrated active dispersal of the primary zoospores from the sporangium mouth, as is typical of Saprolegnia. In only one of 61 isolates (Achlya sp.) were primary zoospores found encysting at the sporangial opening. Of 34 fish with lesions collected in 1986, 31 yielded Saprolegnia isolates.

Six of the 15 isolates (1289, 1356, 1376, 1431, 1494,

1570) maintained on hemp seed developed oogonia within six months. The isolate which most readily reproduced sexually (1494) was referred to S. ferax (Gruith.) Thuret and the other five to S. diclina Humphrey (after Seymour 1970).

The isolates examined by electron microscopy mostly fell into two groups. None of the first group (1323, 1358, 1372, 1380, 1408, 1428, 1504, 1512) produced oogonia and (except for 1380, for which hair number was not determined) all had bundles of 8-16 boathook hairs 8.0-15.0 um long on their secondary cyst coats (Fig 4E, Table 1). Where measured the primary cysts of these isolates (Fig 4B) had unusually long spines (Table 1, c.f. Fig 47, Beakes 1983). Isolate 4345, however, although non-sexual, had single moderately long boathook hairs on its secondary cyst coat (Table 1).

The second group (1289, 1376, 1494, 1570) produced oogonia and had single short boathook hairs on their secondary cyst cases (Fig. 4D, Table 1). In addition the simple tubular spines on their primary cyst cases were significantly shorter than those of the previous group (Table 1). Isolates 1356 and 1431 also produced oogonia and had similar primary cyst coat architecture to the second group (Fig. 4A), but differed in having a smooth secondary cyst coat (Fig. 4C).

Figure 4

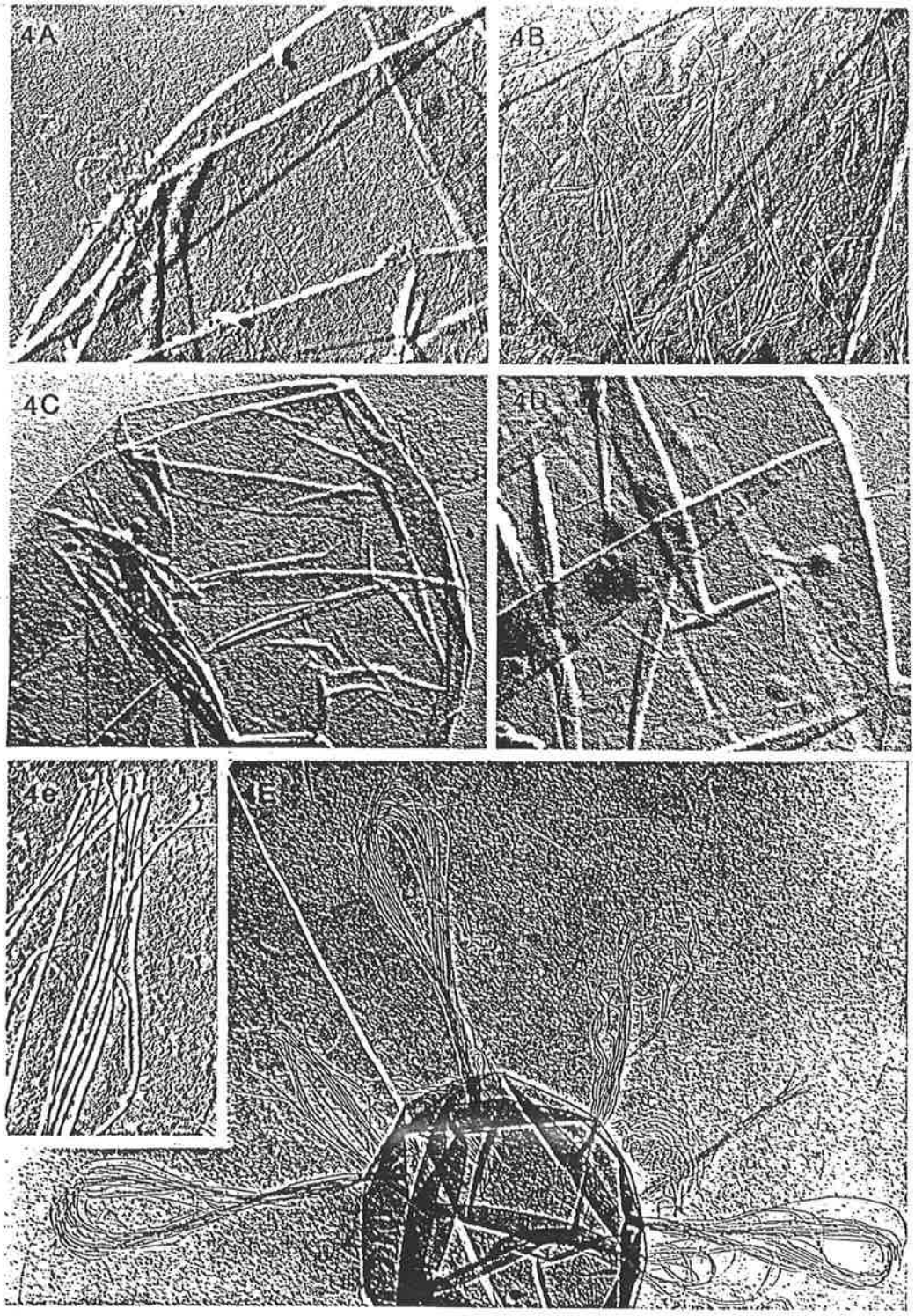
4A. Part of a primary cyst coat of isolate 1356, showing tufts of short primary spines, typical of oogonium-forming species. x 15,800.

4B. Part of a primary cyst coat of isolate 1372, showing groups of much longer primary spines typical of the non-oogonium-producing fish lesion isolates. x 15,800.

4C. Secondary cyst case of isolate 1356, showing smooth wall, without hooked hairs. x 9,600.

4D. Detail of part of secondary cyst case of isolate 1376, showing short single boathook hairs typical of oogonium-producing isolates (S. ferax and S. diclina). x 20,000.

4E, 4e. Part of secondary cyst of isolate 1372, showing characteristic bundles of hooped boathook hairs which distinguish the non-oogonium-producing isolates (S. parasitica). x 5,920.


4A
4B
4C
4D
4e
4E

(4) Bacteriology

The following groups were identified tentatively in isolates from diseased and healthy fish: Aeromonas, Pseudomonas, Alcaligenes, Flavobacterium, Chromobacterium and "oxidase-negative gram-negative bacillus". Aeromonas hydrophila was identified in 38/48 suspected Aeromonas isolates. A. hydrophila was isolated from 22/34 skin lesions and 3/31 skin samples from healthy fish. Only 4/34 fish with lesions yielded A. hydrophila from liver and/or kidney. Seven samples from uninfected skin areas of 34 diseased fish gave A. hydrophila isolates (Fig. 5). There was no difference between the incidence of Saprolegnia and A. hydrophila in lesions (Chi-squared, $P > 0.05$), but of the 3/34 fish that did not yield Saprolegnia, one did not yield A. hydrophila, one showed an integumentary infection of A. hydrophila and the third had obvious Aeromonas septicaemia.

(5) Virology

No cytopathic agents were isolated from the kidney, liver, spleen or skin lesions of diseased fish.

(6) Histopathology

The principal lesions involved erosion of the epidermis and loss of scales, hypodermal and muscular oedema, haemorrhage and myofibral degeneration. The earliest obvious infections

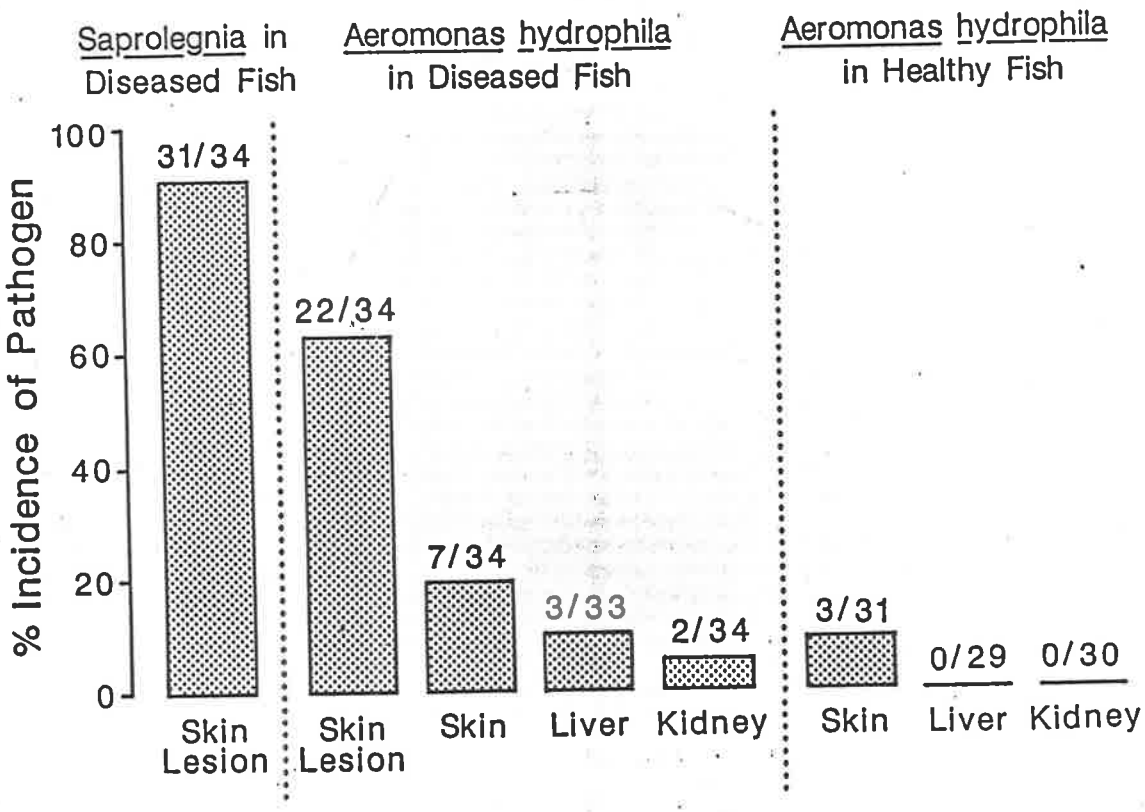


Fig. 5. Incidence of Saprolegnia and Aeromonas hydrophila isolates in infected and healthy tissues of bony bream.

consisted solely of fungal hyphae penetrating the epidermis and dermis, often in scale pockets (Fig. 6A). This progressed to erosion of the epithelium and hypodermal oedema, as previously described for saprolegniasis (Copland & Willoughby 1982). Bacterial invasion also was commonly observed at this stage in development of the lesion.

Extensions to the underlying musculature occurred in severe cases, inducing haemorrhage and oedema, particularly in the intermyotomal connective tissue. Fungal hyphae were not visible in sections stained with haematoxylin and eosin, but methenamine silver staining revealed one or more hyphae, but never numerous hyphae, within each haemorrhagic focus (Figs 6B, 6C). Individual myofibres often contained a single hypha within the sarcoplasm, with or without sarcoplasmic degeneration. Bacteria were rarely visible in these deeper lesions.

No stage of the lesions showed leucocytic inflammatory response to the fungal elements. Lesions with substantial bacterial involvement displayed mononuclear inflammatory infiltration at the sites of invasion.

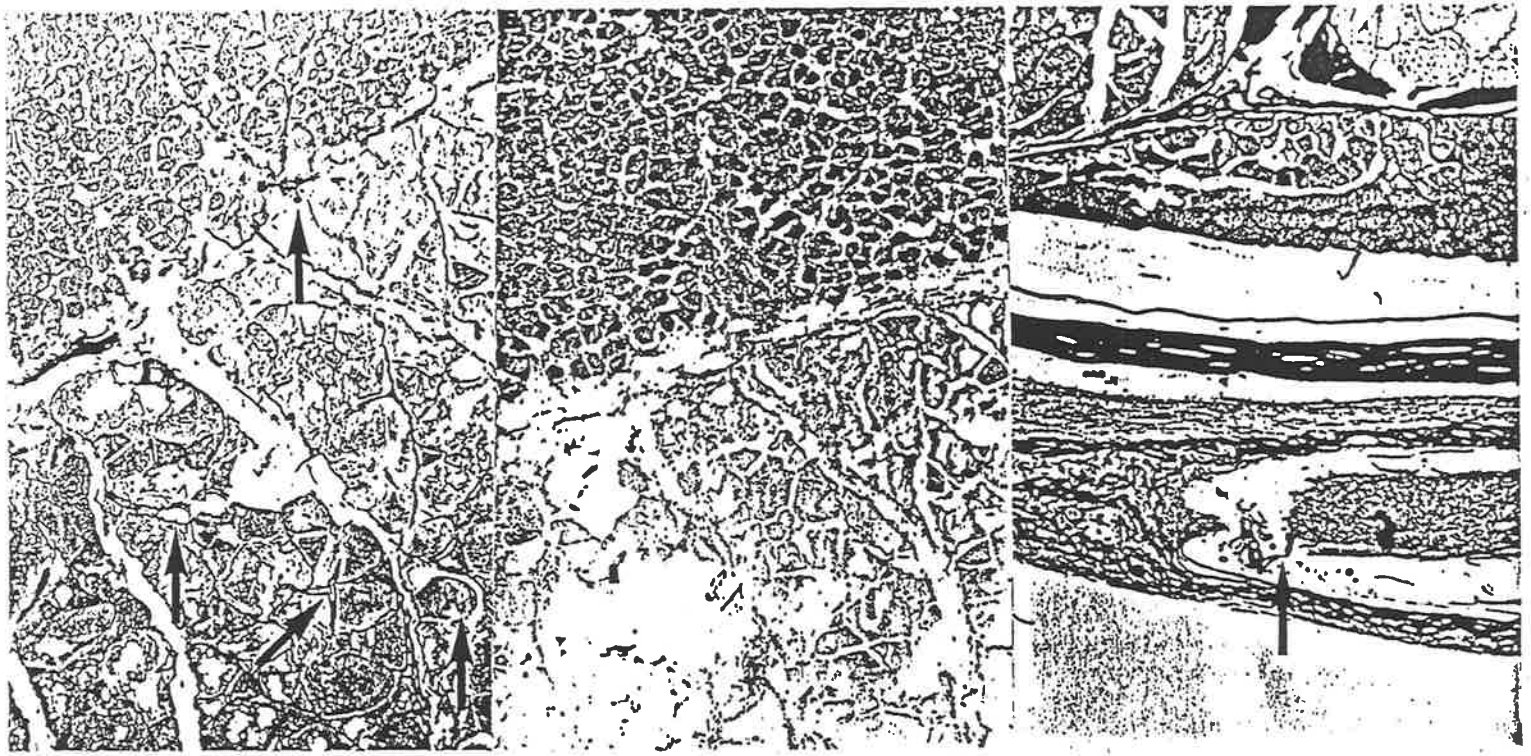
Discussion

Irruptions of Saprolegnia and Aeromonas hydrophila commonly occur in late winter and spring (Jester & Jensen

Fig. 6A. Fungal hyphae (arrow) in scale pocket in earliest stage of infection (methenamine silver, X130).

6B. Haemorrhage and oedema of intermyotomal connective tissues at junction of pink lateral and white muscle, and white myofibral degeneration (H+E, X100)

6C. Section similar to Fig. 5B but silver-stained to reveal fungal hyphae (arrows) within haemorrhagic foci and myofibres (methenamine silver, X130)



1972; De Figueiredo & Plumb 1977; Porak & Tranquilli 1981). It appears that low winter temperatures may act directly to lower antibody production and blood proteins and thereby immunity, or indirectly through inhibition of feeding (Roberts 1975; Cipriano, Bullock & Pyle 1984; Brenden & Huizinga 1986). The rise of temperatures in spring also may lower immunity through stress, particularly in the case of A. hydrophila infections (Esch & Hazen 1980; Nieto, Corcobado, Toranzo & Barja 1985). Sexual maturation may contribute but the mechanism is unclear (Pickering & Pottinger 1985, Pickering 1986).

The general preference of bony bream for warmer waters is consistent with the winter-stress model. Bony bream evidently are sensitive to low temperatures and low oxygen concentrations (Cadwallader 1977; Allen 1982), as these conditions are implicated in reported infections by bacteria and Saprolegnia (Johnston & Bancroft 1921), Chilodonella (Langdon, Gudkovs, Humphrey & Saxon 1985) and rhabdovirus and Aeromonas (Dept Ports & Fisheries 1986) in this species.

The timing of the epizootics in the lower Murray also is consistent with the winter-stress model. Peak incidence occurs after the winter temperature minimum in July, and the level of incidence is correlated with the mean July-August

water temperature. However, in 1984 the incidence of the lower Murray disease peaked before body condition reached its minimum. There was no difference between the condition of healthy and diseased fish, and condition was not significantly correlated with the area of lesions. If winter-cold stress is acting as an initiating factor in the disease cycle, it seems likely that it is doing so directly through suppression of immunity rather than by lowering body condition through inhibition of feeding.

Several authors have commented on the lack of a leucocytic inflammatory response to invading hyphae, and the presence of haemorrhage in the inflammation induced by the hyphae of Saprolegnia sp., particularly in deeper lesions (Bootsma 1973; Wolke 1975; Neish 1977; Copland & Willoughby 1982). The lack of such a response in bony bream could arise from temperature-mediated inhibition, or it may reflect the fungal species involved (there is some cellular inflammatory response to bacterial invasion). This contrasts with the marked granulomatous inflammatory response shown by fish infected by Aphanomyces sp. (Noga & Dykstra 1986), Phoma herbarium (Ross, Yasutake & Leek 1975), Aphanomyces piscicida (Hatai, Takahashi & Egusa 1984) and Exophiala pisciphila (Langdon & McDonald 1987).

The fact that adults, rather than juveniles, are affected

suggests that hormonal changes associated with sexual maturation may increase susceptibility to the disease. In fact pre-maturational changes are indicated because the infection becomes intense two months before the onset of vitellogenesis. Similarly, the annual flooding of the Murray usually occurs too late to affect the initiation of infection.

Captive salmonids and eels succumb rapidly to saprolegniasis (Copland & Willoughby 1982; Pickering & Willoughby 1982), and it is possible that wild populations also suffer high mortalities (White 1975). In the present case the scarcity of healing or dead bony bream is difficult to explain. The species remains extremely abundant in the lower Murray, as shown by commercial catches (South Australian Dept Fisheries 1988).

The specificity of the lower Murray disease for bony bream contrasts with the generality of a dermatitis attributed to Achlya sp. in fish of the Coongie Lakes region of Cooper Creek in central Australia, where bony bream, callop (Macquaria ambigua), desert rainbowfish (Melanotaenia splendida tatei) and goldfish (Carassius auratus) are affected (Puckridge & Drewien 1988).

The cyst coat architecture of non-sexual (group 1)

isolates from the Australian bony bream is similar to that found in *S. parasitica* from British and Japanese salmonids (Pickering, Willoughby & McGory 1979; Beakes 1983; Beakes & Ford 1983). This supports recent suggestions that *S. parasitica* should be retained for this group (Wood *et al.* 1980; Hatai *et al.* in press). Mean hair numbers per bundle and mean hair lengths of the group 1 isolates fit at the upper end of a regression of hair length against hair number based on an analysis of British fish lesion isolates (Pickering *et al.* 1979, Fig. 7). This provides evidence for the generality of this relationship. The only non-sexual isolate of doubtful identity is 4345, which has a cyst coat ornamentation similar to that of typical *S. hypogyna* isolates (Hatai *et al* in press). The remaining (sexual) isolates have cyst coat architecture consistent with their identification (as *S. diclina* or *S. ferax*) based on morphology of sexual structures. The cyst coats of isolates 1356 and 1431 resemble those of the 'filter-paper' isolates described by Pickering *et al.* (1979).

It seems likely that *S. parasitica* is the principal fungal pathogen involved in bony bream dermatitis, as it is in British salmonid saprolegniasis, but since in Japan *S. diclina* also has been implicated as a salmonid pathogen (Hatai *et al.* in press), a definite answer must await a

study of the pathogenicity of both taxa.

In saprolegniasis the localization of surface lesions is distinctive for particular host-pathogen relationships. The pattern seen in bony bream, favouring the mid-flank region, differs from that in salmonids (White 1975; Neish 1977; Richards & Pickering 1978) where head, dorsal surface and fins are affected. It differs also in being not consistent with initiation of infection by injury. The significant difference in infection area between the two sides of the fish is not simply explained, and may be an artifact of large sample size. However, there is evidence of assymetry in parasitic infections (Moser, Sakanari, Wellings & Lindstrom 1984; Rohde 1984), and bony bream have significant gonad assymetry (Puckridge & Walker in press).

The Chi-square analyses reported by Richards & Pickering (1978) are at best marginal indicators of environmental and biological effects, whereas the method employed here is multivariate in nature and has potential for comprehensive studies of the relations between such effects and the distribution and intensity of infection.

The task of establishing primacy in a complex disease process is difficult, and conflicting evidence is common. This is well-shown by work on Japanese "fungus disease" of

eels (Egusa 1965), UDN of salmonids (Carbery 1968) and the Asia-Pacific ulcer disease (Roberts, MacIntosh, Tonguthai, Bronyaratpalin, Tayapatch, Phillips & Millar 1986). In the present situation, it is possible that inconspicuous prefungal A. hydrophila lesions occur initially in the lower Murray disease, and that Saprolegnia invades these before they become obvious. However, there are several lines of evidence to suggest that this is a case of primary saprolegniasis:

(1) Saprolegnia occurs in the earliest detectable lesions.

(2) Gross haemorrhage and inflammation are absent in early lesions.

(3) External and internal symptoms of systemic bacteraemia are absent.

(4) The yield of A. hydrophila cells from internal organs is low (this is not uncommon (Thorpe & Roberts 1972; Snieszko 1974) and the slightly enhanced levels that occur in fish weakened by Saprolegnia lesions are to be expected).

(5) About a third of lesions (11/34) yielded Saprolegnia but not A. hydrophila.

(6) Few lesions (2/34) yielded A. hydrophila but not Saprolegnia.

(7) Saprolegnia hyphae occur in the scale pockets in early lesions, and deep in the muscle in advanced lesions.

(8) Viral agents were not apparent, at least in preliminary investigations.

The evidence presented here suggests that the lower Murray bony bream epizootic is an addition to the comparatively few known instances in fish of primary pathogenicity among aquatic fungi (Ross & Yasutake 1973; Ross, Yasutake & Leek 1975; Hatai, Takahashi & Egusa 1984; Noga & Dykstra 1986). In addition, this appears to be the first reported case of primary saprolegniasis in a wild, non-salmonid fish population.

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Figure Legends

Fig. 1. Percentage incidence of dermatitis in adult bony bream 1983-1987 compared to (A) mean monthly water temperature and (B) mean daily discharge.

Fig. 2. Percentage incidence of dermatitis in adult bony bream in 1983-1984 compared to (A) mean monthly condition factor and (B) mean monthly GSI.

Fig. 3. Mycotic lesions on skin of bony bream: (A) slight, (B) moderate.

Fig. 4. All figures of gold palladium shadowed whole mounts on formvar coated grids.

4A. Part of a primary cyst coat of isolate 1356, showing tufts of short primary spines, typical of oogonium-forming species. x 15,800.

4B. Part of a primary cyst coat of isolate 1372, showing groups of much longer primary spines typical of the non-oogonium-producing fish lesion isolates. x 15,800.

4C. Secondary cyst case of isolate 1356, showing smooth wall, without hooked hairs. x 9,600.

4D. Detail of part of secondary cyst case of isolate 1376,

showing short single boathook hairs typical of oogonium-producing isolates (*S. ferax* and *S. diclina*). x 20,000.

4E,4e. Part of secondary cyst of isolate 1372, showing characteristic bundles of hooped boathook hairs which distinguish the non-oogonium-producing isolates (*S. parasitica*). x 5,920.

Fig. 5. Incidence of *Saprolegnia* and *Aeromonas hydrophila* isolates in infected and healthy tissues of bony bream.

Fig. 6A. Fungal hyphae (arrow) in scale pocket in earliest stage of infection (methenamine silver, X130).

6B. Haemorrhage and oedema of intermyotomal connective tissues at junction of pink lateral and white muscle, and white myofibral degeneration (H+E, X100)

6C. Section similar to Fig. 5B but silver-stained to reveal fungal hyphae (arrows) within haemorrhagic foci and myofibres (methenamine silver, X130)

APPENDIX III

Table 1 Summary of cyst coat morphology for isolates selected for whole mount examination in the TEM.

ISOLATE NO.	PRIMARY CYST COAT Mean Spine Length (SE) um	SECONDARY CYST COAT Bundle/Hair Length (SE) um	Mean No. Hairs/ Bundle (SE) um
1289	0.34 (0.20)	0.41*	1
1323	1.84 (0.53)	9.18*	14*
1356	0.35 (0.09)	smooth	
1358	1.56 (0.42)	9.44 (0.84)	9(0.58)
1372	1.61 (0.17)	9.46 (0.60)	12(0.70)
1376	0.28 (0.02)	0.39 (0.04)	1
1380	2.01 (0.30)	10.00*	ND
1408	1.61 (0.10)	12.80 (0.85)	16
1428	2.14 (0.31)	11.20 (1.51)	14(1.05)
1431	c.0.30*	smooth	
1494	c.0.30*	0.40*	1
1504	ND	13.30*	12
1512	ND	8.41 (0.44)	12(1.07)
1570	c.0.30	0.40*	1
4345	ND	3.40*	1

Where the standard error of the mean (SE) is given each

measurement represents the mean of at least 20 measurements. Dimensions which are followed by a * are from data sets of less than 10 measurements or from poor preparations where accurate measurements were not possible. In some samples some of the spore stages were not observed and these are indicated as not determined (ND).

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